Contamination of Fresh and Ensiled Maize by Multiple *Penicillium* Mycotoxins

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ABSTRACT

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Toxins produced by *Penicillium* species are reported in maize silage and have been associated with health problems in cattle. Our objectives were to evaluate the prevalence and dynamics of patulin (PAT), mycophenolic acid (MPA), cyclopiazonic acid (CPA), and roquefortine C (ROC) in fresh and ensiled maize. To achieve these objectives we developed a high-performance liquid chromatography method coupled with mass spectrometry to detect all four toxins simultaneously in silage. In addition we collected weather data, information on agronomic practices, and silage fermentation characteristics for each study site. Silage was collected at harvest and after ensiling in 2001 and 2002 from 30 Pennsylvania dairies. The average concentration of toxins (range in parentheses) was: PAT 0.08 µg/g (0.01 to 1.21), MPA 0.16 µg/g (0.02 to 1.30), CPA

0.12 µg/g (0.02 to 1.43), and ROC 0.38 µg/g (0.01 to 5.71). ROC was the most frequently detected toxin (60%), followed by MPA (42%), CPA (37%), and PAT (23%). Of 120 samples tested, 15% contained no detectable levels of toxin, 25% were contaminated with one toxin, 32% with two, 18% with three, and 10% with all four toxins. All four mycotoxins were found in freshly harvested material, contradicting the belief that *Penicillium* toxin formation occurs exclusively during storage. We observed that weather conditions during specific growth stages of the crop affected the final concentration of toxins in freshly harvested maize. In ensiled material, PAT levels were affected by concentrations of propionic and isobutyric acids. Based on our data, *Penicillium* mycotoxins can form while the crop is in the field and after ensiling, suggesting that preventative measures should begin prior to ensiling.

Additional keywords: corn silage, dairy cattle toxicosis.

Within the last several decades, knowledge of fungal contaminants in agricultural products has increased dramatically, particularly for toxins produced by plant pathogens such as *Fusarium* species. Occasionally, mycotoxins produced by saprophytic or opportunistic fungi, such as *Penicillium* species, are also present in agricultural commodities (55). Several mycotoxins produced by *Penicillium* spp., including cyclopiazonic acid (CPA) (63), patulin (PAT) (10,37), mycophenolic acid (MPA) (52), and roquefortine C (ROC) (3) are known to occur in maize based feeds including silage. In addition, there are reports of serious health problems occurring when cattle are exposed to these toxins. The majority of toxin production is thought to take place during storage, as some mycotoxigenic *Penicillium* will grow in conditions similar to silage with low water activity, low pH, and oxygen concentration (6,15,26). For example, *P. roqueforti* and the closely related *P. paneum* are often present in silage and they and their toxins have been isolated from material that is not visibly molded (3,6).

Many toxigenic species of *Penicillium* are saprophytic on plant material and soil debris and *Penicillium* ear rot on maize can occur on ears damaged by birds and insects (8,23). Several studies have found that *Penicillium* are among the most commonly isolated filamentous fungi (1,11,14,22,24,44). The most frequently isolated *Penicillium* in silage is generally *P. roqueforti* (3,43,58,61), which has recently been divided into three species, *P. roqueforti*, *P. paneum*, and *P. carneum*, based on analysis of rDNA genes and chemotaxonomic profiles (6). Of the *P. roque-*

forti group, only *P. roqueforti* and *P. paneum* have been reported in silage (6,58). Both species produce ROC and *P. roqueforti* also produces PR-toxin and MPA, while *P. paneum* produces PAT (6,40,41). Other species reported in silage include *P. expansum*, which will tolerate low pH and oxygen and produce ROC and PAT, *P. crustosum* and *P. commune*, which both produce CPA acid and ROC (13).

Both PAT and ROC have been implicated in cattle toxicoses, although clinical data on the affects of these toxins on cattle are not available, which is also true for MPA and CPA. ROC has been reported as a suspected causal agent in several cases of paralysis, abortion, and placental retention in cattle (3,16,43,57). However, as the strains of *P. roqueforti* isolated in these cases may have been producing other mycotoxins as well, including PR-toxin, it is difficult to determine if ROC was the sole toxin responsible for the observed symptoms. In a somewhat conflicting study, Tuller et al. (60) found that ROC is not acutely toxic to sheep at levels generally encountered in silages. In several cases of suspected ROC toxicosis in dogs, animals that ingested food colonized by *P. roqueforti* suffered paralysis, tremors, and convulsions (7,39,62), suggesting a neurotoxic effect. However, in several of these cases another toxin, penitrem A, was also present. Caution should also be used in interpreting the affects of these toxins as there may be significant differences in toxin sensitivity from dogs to ruminants and other species.

PAT has also been implicated in cattle health disorders, where several beef herds experienced tremors, paralysis, and death (51). In this particular case the PAT-producer was not a *Penicillium* sp., but rather *Aspergillus clavatus*. Autopsy of the affected animals revealed serious damage to the nervous system and PAT was detected from fungal cultures and in the feed. In another case, cattle that consumed *P. roqueforti*-infested silage experienced a

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loss of appetite, gastrointestinal hemorrhaging, and abortion (61). In this incident the suspected agents were PR-toxin and PAT, although no tests were performed on the feed to verify this hypothesis. PAT has also negatively impacts the metabolism of rumen microorganisms, suggesting that PAT exposure may result in a subtle decrease in overall animal health (12,59).

Unlike PAT and ROC, MPA does not appear to be acutely toxic although it is a potent immunosuppressive agent and is used in anti-rejection therapy for organ transplant patients (4). In a study conducted with maize silage, MPA was reported in 38 of 135 samples and in some samples, the authors stated that the concentration of MPA was equivalent to 10% of the dose given to patients undergoing immunosuppressive therapy (52). In a recent study with sheep, Mohr et al. (2007) found that animals fed varying concentrations of MPA for 44 days suffered no significant effects from exposure (36). Although exposure to MPA may not result in an obvious decline in health, the immunosuppressive action of this toxin has been suggested to render animals more susceptible to infectious diseases and more sensitive to the presence of other mycotoxins (47).

In contrast to the toxins discussed above, there is no anecdotal or clinical information regarding the affects of CPA on cattle or other ruminants. In poultry, CPA exposure resulted in tremorgenic activity and lesions on the liver, kidney, and gastrointestinal tract (9). In addition to the direct health hazard that CPA may present, this toxin can also be excreted in milk and remains stable following pasteurization, and for extended periods of storage (46).

In general, our knowledge of the potential dangers presented by *Penicillium* mycotoxins in feed is fairly limited, as much of the information regarding the sensitivity of cattle to these toxins is anecdotal. Although some studies suggest that exposure to toxins such as ROC and MPA present a minor threat to ruminants, there is virtually no information regarding synergistic or additive effects when more than one toxin is present. Also, the few clinical studies performed on animal subjects have been strictly limited to the affects of acute, rather than chronic, toxin exposure. However, as there have been a limited number of studies addressing the natural prevalence of these toxins in silage, or factors affect their presence in silage, it is unclear whether costly animal studies are warranted. With these concerns in mind, our objective was to better understand the frequency, levels, and factors that affect contamination of silage by CPA, MPA, PAT, and ROC. These four toxins were selected to represent *Penicillium* species isolated in a previous work, including *P. roqueforti*, *P. paneum*, *P. commune*, *P. crustosum*, and *P. expansum* (30).

To achieve our objective, we developed an analytical method that coupled high-performance liquid chromatography (HPLC) with mass spectrometry, to detect and quantify these four toxins from fresh and ensiled maize collected over a 2-year period from Pennsylvania dairies. Using toxin data, information regarding the farms' agronomic practices, and site-specific weather data, our goal was to better understand the overall prevalence of *Penicillium* mycotoxins and identify factors that affect mycotoxin contamination of silage.

MATERIALS AND METHODS

Sample collection and preparation. Maize silage was collected from four geographically diverse regions in Pennsylvania (northeast [NE], northwest [NW], southeast [SE], and south central [SC]) at dairies with a history of cattle health problems within the previous 5 years (Fig. 1). Samples were collected at harvest and 3 to 6 months after ensiling, to determine if toxin levels changed during storage (28,29). Harvest samples consisted of freshly chopped maize that was collected from a mechanical harvester as it made several passes within a field. Ensiled samples were removed either from several locations at the face of a structure (bunker or trench silo), or removed as the silage was

emptied from the silo during feed out (upright silo). The sampling frequency of ensiled material was determined by the amount of silage fed per day at each farm. Samples were collected either every other day for 14 days for sites feeding 1 to 2 tons per day and every day for 7 days for sites feeding 3 to 4 (or more) tons per day. After initial sample collection, both harvest and ensiled samples were mixed by hand and subsampled to obtain a representative sample of 1 to 3 kg. All samples were stored at -80°C until toxin analysis. Prior to extraction, silage was dried at 55°C for 5 to 7 days, milled to a flour-like consistency in a Thomas-Wiley Mill (Thomas Scientific, Inc., Swedesboro, NJ), and 500 g was removed for analysis.

Toxin extraction and cleanup. The method used to extract CPA, MPA, PAT, and ROC was modified from that of Rundberget and Wilkins (50). All solvents used for extraction were of HPLC grade. For each sample, 10 g of silage was extracted for 1 h with 60 ml of acetonitrile-water (9:1, vol/vol) (Burdick & Jackson, Muskegon, MI; Milli-Q UV Plus System, Millipore, Billerica, MA) with 0.1% formic acid (EM Science, Gibbstown, NJ) on an automatic shaker at 300 rpm. Samples were centrifuged at $6,000 \times g$ for 10 min and 10 ml was decanted for cleanup. Samples were defatted twice with 10 ml of hexane (EM Science, Gibbstown, NJ) and the lower aqueous phase was removed for analysis. Defatted samples were transferred to an 11-ml vial and de-solvated to dryness under a stream of nitrogen at 65°C . The residue was redissolved in 1 ml acetonitrile, filtered with a pre-wet nylon filter (Acrodisc GHP 0.2 μm filter, Pall Life Science, West Chester, PA), de-solvated a second time, and resuspended in 500 μl of acetonitrile. Samples were stored in 2-ml amber vials at 4°C until HPLC-mass spectrometry analysis.

Standards. Standards for CPA, MPA, PAT, and ROC (all toxins from Sigma-Aldrich, St. Louis, MO) were prepared by dissolving toxins in HPLC-grade acetonitrile to a final concentration of 100 $\mu\text{g/ml}$.

HPLC-MS analysis. Immediately preceding analysis, samples were de-solvated to dryness and resuspended in 350 μl of methanol (Mallinckrodt-Baker, Phillipsburg, NJ) and 150 μl of 0.1% acetic acid (EM Science, Gibbstown, NJ). The solvent delivery system consisted of a Shimadzu LC-20AD HPLC with a Shimadzu SIL-5000 autosampler (Shimadzu Scientific Instruments, Columbia, MD) coupled to a Waters LCT Premier mass spectrometer (Waters Corp., Milford, MA) for detection. Chromatographic separation was achieved using a reverse phase Thermo Betasil C-18 column (150 $\times$ 2.1 mm; 5 μm particle size, pore size 60 $\text{\AA}$) (Thermo, Bellefonte, PA). Solvents consisted of the following: A, water amended with 0.05 M ammonium acetate and B, methanol with 0.05 M ammonium acetate. The method began with A at 100% with a linear gradient program moving to 100% B from time 0 to 7.5 min, holding at B from 7.5 to 12.5 min, and

Fig. 1. Map of the relative locations of dairies where silage samples were collected. Symbols represent the four major dairy production regions North-east, ★; Northwest, ▲; Southeast, ■; and South central, ●.

then re-equilibrating with A from 12.5 to 17.5 min before the next injection. The flow rate was constant at 0.3 ml solvent per minute. The toxins were detected with an atmospheric pressure chemical ionization probe in the positive mode (APCI+). The instrument was set for selective ion detection for masses between 100 to 450 m/z where the masses of the toxins (the parent compound plus H^+) were as follows: PAT – m/z 155, MPA – m/z 321, CPA – m/z 337, and ROC – m/z 390 (Fig. 2). Data analyses were performed using Mass Lynx version 3.5 mass spectrometry software (Milford, MA) where toxin quantification was determined by comparing sample peaks to an external mixed standard of each of the four toxins.

Evaluation of extraction and detection method. When test silage samples ($N = 12$) were spiked with each of the four toxins (range of 0.5 to 5 μg of toxin per g of silage), extracted, separated, and detected with the methods described above, the percent recoveries were as follows: PAT = 82 to 89%, MPA = 86 to 94%, CPA = 80 to 87%, and ROC = 88 to 92% with a limit of detection of 10 ng/g for each toxin.

Evaluation of sample characteristics. From each participating dairy, information was collected on how the crop was managed. Characteristics included crop rotation with a broadleaf crop (yes or no), the tillage system used (moldboard, no-till, variety of systems used), whether an inoculant or acid additive was added prior to ensiling (yes or no), and what type of silo the material was stored in (upright, bunker/trench, or bag silo). Ensiled material was also evaluated by Cumberland Valley Analytical Services

(Hagerstown, MD) for percent dry matter, pH, NH_4 content, and the concentration of volatile fatty acids (lactic, acetic, propionic, butyric, and isobutyric) (53).

Site-specific weather data collection. For each of the study sites, estimated daily weather data for the minimum, maximum, and average temperature, total precipitation, average relative humidity, wetness hours, growing degree day (base 10°C), precipitation minus evaporation (day 1 planting date), and a hybrid growth prediction (based on planting date) from tasselling (VT) through maturity (R6), were provided by ZedX Inc. (Bellefonte, PA) (2,27,48). Prior to statistical analysis, daily weather variables were averaged to a single value for each growth stage.

Statistical analyses. All statistical analyses were performed using a 95% confidence interval (64). Toxin occurrence in silage from both seasons (2001 and 2002) and from harvest and ensiled samples were compared using the Mann-Whitney test (Minitab version 13.32, Minitab Inc., State College, PA). Differences between toxin levels in these samples were evaluated with the Student's paired t test. The occurrence of toxins between the four regions was evaluated with the Kruskal-Wallis test, while differences in levels were evaluated with a general linear model. To determine if agronomic practices, silage fermentation characteristics, or weather variables during each crop growth stage had a relationship to toxin concentration in silage, we used Pearson's correlation. Kendall's correlation (PROC CORR procedure in SAS software, SAS Institute Inc., Cary, NC) was used to understand the relationship between these variables and toxin occurrence in silage.

RESULTS

Levels and occurrence of PAT, CPA, MPA, and ROC in fresh and ensiled maize. The average concentration of toxins (with the range in parentheses) in maize silage from both seasons ($N = 120$) was: PAT 0.08 $\mu g/g$ (0.01 to 1.21), MPA 0.16 $\mu g/g$ (0.02 to 1.30), CPA 0.12 $\mu g/g$ (0.02 to 1.43), and ROC 0.38 $\mu g/g$ (0.01 to 5.71). The frequency of toxin contamination in the same samples was: ROC 60%, MPA 42%, CPA 37%, and PAT 23%. Of 120 samples, 15% had no detectible amount of toxin, 25% were contaminated with a single toxin, 32% with two, 18% with three, and 10% with all four toxins. In terms of toxin co-occurrence, ROC was the most frequent co-contaminant, occurring in 83% of samples with more than one toxin present. ROC occurrence was positively correlated to the presence of MPA (Kendall's correlation) where 77% of the samples positive for MPA also contained ROC.

All four mycotoxins were detected in freshly harvested maize silage samples: 17% contained PAT, 27% MPA, 30% CPA, and 50% ROC ($N = 60$). The average (range in parentheses) amounts of toxin in fresh samples were as follows: PAT 0.05 $\mu g/g$ (0.01 to 0.91), MPA 0.06 $\mu g/g$ (0.08 to 0.60), CPA 0.05 $\mu g/g$ (0.02 to 0.38), and ROC 0.20 $\mu g/g$ (0.02 to 1.10).

There were no differences detected between the 2001 and 2002 growing seasons in terms of average toxin concentration or occurrence and as such, data for both seasons was considered a single dataset. All four toxins were present in both harvest and ensiled samples and MPA, CPA, and ROC were present at significantly higher average levels in ensiled material (Fig. 3A). Although there were no significant differences between levels of PAT in harvest and ensiled maize, this toxin was found more often in ensiled samples (60%) than in those that were freshly harvested (8%) (Fig. 3B).

Between the four study regions (Fig. 1) there were differences in the levels of CPA and ROC (Fig. 4A). In general, it appears that the southern regions had higher concentrations of these toxins, with SE having an average of 0.15 $\mu g/g$ CPA and 0.50 $\mu g/g$ ROC and SC with 0.26 $\mu g/g$ CPA and 0.46 $\mu g/g$ of ROC. For MPA and PAT, there were no significant differences observed among the four regions (Figs. 4A and B).

Fig. 2. Liquid chromatography-mass spectrometry chromatograms showing A, total ion chromatogram for a mixed standard of four mycotoxins and reconstructed ion chromatograms showing silage samples contaminated with B, patulin (PAT) m/z 155, C, mycophenolic acid (MPA) m/z 321, D, cyclopiazonic acid (CPA) m/z 337, and E, roquefortine C (ROC) m/z 390. Mass is at the upper right of each chromatogram and corresponds to the gain of H^+ compared with the parent compound, the total ion count (TIC) is given for the standard. The number at the lower right is absolute signal intensity corresponding to the 100% level indicated on the axis.

Relationship between toxin contamination in maize silage, agronomic practices, fermentation characters, and seasonal weather conditions. There was no relationship between toxin levels and occurrence in maize silage and agronomic practices. However, there was a positive correlation between PAT levels in ensiled maize and propionic ($R = 0.287$, $P \leq 0.026$) and isobutyric acid ($R = 0.252$, $P \leq 0.046$). Therefore, it appears that higher PAT levels occurred in samples with higher concentrations of these acids.

Patterns of toxin concentration with some of the seasonal weather variables as summarized in Table 1. In general, toxin concentration appeared to be most strongly impacted by temperature and precipitation variables during tasselling (VT) and early kernel development (R1 to R3; silking through milk stages). Levels of MPA toxin were higher in samples where the average minimum temperature and relative humidity were higher during R2 or blister stage. Levels of CPA were lower in samples where the maximum temperature, average temperature, and precipitation minus evaporation values were higher than average during tasselling. However, toxin levels were higher in samples where precipitation was higher than average during silking (R1) and the precipitation minus evaporation, relative humidity, and wetness hours were higher during the blister stage (R2). Levels of PAT were higher in samples where there were more wetness hours than average during silking (R1) and in samples where the precipitation and precipitation minus evaporation values were higher than average. ROC levels appeared to be affected later in plant development than MPA, CPA, or PAT. ROC concentrations were higher in samples at blister (R2) when temperatures were higher

than average and the relative humidity lower than average. Levels were also higher in samples where at the milk and dough stage (R3 and R4) temperatures were higher than average and in samples where at physiological maturity there was less moisture than average present.

DISCUSSION

The natural occurrence of *Penicillium* toxins in maize silage has been addressed by several other researchers, primarily located in Europe. As far as we are aware, this is the first study to comprehensively address contamination by several *Penicillium* toxins in fresh and ensiled North American maize silage. In 2000, Schneewis et al. (52) found that 32% of the silage samples in Germany contained MPA at a range of 0.2 to 350 $\mu\text{g/g}$ with an average of 14 $\mu\text{g/g}$ of toxin. MPA was slightly more prevalent in our samples (42%) but the average amount of toxin was comparable, however, it was detected at a lower range. One explanation for their observations is that they purposely selected samples that were highly infested with *Penicillium* species, whereas we randomly selected our samples and observed no visible contamination.

In a laboratory silo, Muller and Amend (37) observed that MPA and PAT were produced in silage at concentrations of 3.56 and 3.06 $\mu\text{g/g}$, respectively. In this case, silage was deliberately inoculated with a strain of *P. roqueforti* known to produce these toxins in culture. Interestingly, they observed that production of MPA and PAT in silage was preceded by an increase of pH, which is somewhat in contrast to our observations where PAT contamina-

Fig. 3. A, Average concentration of *Penicillium* mycotoxins in maize silage. Asterisks over bars indicate samples (harvest or ensiled) with significantly ($P \leq 0.05$) higher toxin concentration. **B,** Frequency of *Penicillium* mycotoxins in fresh and ensiled maize samples. Patulin (PAT), mycophenolic acid (MPA), cyclopiazonic acid (CPA), and roquefortine C (ROC).

Fig. 4. A, average concentration of *Penicillium* mycotoxins in maize silage from the four different geographical sampling regions. NE – northeast, NW – northwest, SE – southeast, and SC – south central. Bars of the same toxin followed by a different letter are significantly different at $P \leq 0.05$. **B,** Frequency of *Penicillium* mycotoxins in maize silage samples from the four different geographical sampling regions. Patulin (PAT), mycophenolic acid (MPA), cyclopiazonic acid (CPA), and roquefortine C (ROC).

tion was positively correlated to levels of propionic and isobutyric acids. Propionic acid is naturally present in silage and has a strong inhibitory affect on fungal growth and toxin production (17,38,56) and therefore, it is unclear why its presence would positively affect that of PAT. Higher levels of isobutyric acid are associated with fungal spoilage in silage, so it is somewhat unsurprising that its presence is correlated with PAT levels. However, the levels of both of these acids in our silage samples were well within acceptable ranges delineated for quality silage (49). As far as we are aware, this is the first report of maize silage naturally contaminated with PAT as previous studies have focused on PAT production by isolates recovered from silage (12,51) or from a laboratory silo (37).

Yu et al. (63) observed CPA in 87% of samples of mixed feed and silage with an average of 0.34 µg/g of toxin at a range of 0.12 to 1.82 µg/g. The average levels and range of CPA in this study is comparable to what we observed, although we found CPA contamination at lower frequency in ensiled samples (40%).

ROC was the most frequently occurring toxin in our samples and the most common co-contaminant with other toxins, particularly MPA. This was expected as *P. roqueforti* produces both of these toxins and in an earlier work (30) we isolated *P. roqueforti*, *P. paneum*, *P. commune*, *P. crustosum*, and *P. expansum* from a subset of freshly harvested and ensiled maize samples included in this study. By far *P. roqueforti* was the most prevalent *Penicillium* species, which likely explains the prevalence of ROC and MPA in the silage samples. Auerbach et al. (3) detected ROC in 54% of the silage samples assessed at an average of 8.5 µg/g and a range of 0.2 to 36.0 µg/g. The concentrations of ROC in this study are significantly higher than what we found, however, these authors also selected some samples that were visibly infested with *Penicillium* species.

One of the most significant findings of this work may be that *Penicillium* toxins were present in the freshly harvested maize samples as well as those that were ensiled. In general, the levels and frequencies of the four toxins in fresh material were lower than that of ensiled samples (Fig. 3) however, many of the fresh samples were also co-contaminated with *Penicillium* toxins. The presence of these toxins in fresh samples is not completely surprising as several toxigenic *Penicillium* species were isolated and detected in freshly harvested material (30). In that same study, the majority of *Penicillium* isolated from fresh maize survived the

ensiling process and were isolated from the corresponding ensiled sample, which is likely why all four toxins were present in ensiled samples. The presence of mycotoxigenic *Penicillium* species on freshly harvested maize grain and plant material has been well documented (19,23,26,34). There have also been a significant number of studies where toxin production by *Penicillium* species in vitro is dependent on water activity, temperature, relative humidity, and other factors (15,19,20,26,31,32,34,45,54). Therefore, it is not surprising that we observed that temperature and water availability during the growing season affected the presence of the four toxins. Similar to in vitro studies, toxin production was positively affected by higher temperatures and water availability (26,31,35,45,54,56,58) except in the case of ROC where higher toxin levels were present in samples with lower moisture availability during physiological maturity. As this is the first study to look at seasonal weather variables and how they affect the presence of *Penicillium* mycotoxins in maize, it is unclear as to why temperature and water availability, particularly during reproductive and early kernel development, had an effect on toxin levels. One possibility is that conditions that stress the plant (low water availability and higher than average temperatures) are also more favorable for colonization by fungi, such as *Penicillium* species. Fungal colonization is more likely on maize that is stressed or damaged (42), and Blaney et al. (5) observed a high incidence of fungi (including *Penicillium* spp.) and toxins in corn that was drought stressed early in the growing season. Both the 2001 and 2002 growing seasons (April to October) had higher than average temperatures and lower precipitation values where 2001 ranked 53rd (out of 108 recorded seasons) as warmest and 28th as driest, and 2002 sixth as warmest and 24th as driest (National Climatic Data Center, Ashville, NC). Therefore, our observations may be primarily related to conditions that stress the plant in addition to favoring fungal growth and toxin production.

Given that there has been little work addressing the overall toxicity of PAT, MPA, CPA, and ROC to cattle, it is unclear if the levels we observed in silage should be a health concern. Certainly the presence of multiple toxins in the same samples is concerning due to the additive or synergistic affects they may cause. Also of concern is the fact that formation of these toxins begins in the field and therefore, future management strategies should not exclusively focus on sanitation practices during ensiling and storage. It is hoped that the data presented here will motivate

TABLE 1. Relationships between weather variables and *Penicillium* mycotoxin concentrations in freshly harvested silage

Toxin	Stage ^a	Variable ^b	Average ^c	Range (N = 60)	Corr. coeff. ^d	P value
MPA	R2	TMIN	16.6	11.2 to 21.4	0.274	0.034
		RHUM	71.2	60.2 to 77.1	0.266	0.040
CPA	VT	TMAX	28.8	24.8 to 32.8	-0.253	0.051
		ATEMP	23.1	18.7 to 26.4	-0.265	0.041
		PVAP	-0.51	-0.91 to 0.76	-0.286	0.026
		PREC	0.05	0 to 0.26	0.268	0.039
	R1	PVAP	-0.46	-0.15 to -0.76	-0.270	0.028
	R2	RHUM	71.2	60.2 to 77.1	0.290	0.024
PAT	R1	WHR	9.8	3.2 to 16.2	0.283	0.029
		WHR	8.4	0 to 17.5	0.293	0.023
	R3	PREC	0.08	0.02 to 0.17	0.304	0.018
		PVAP	-63.0	-82.0 to -46.2	0.292	0.023
ROC	R2	TMAX	27.6	23.1 to 31.8	0.357	0.005
		ATEMP	22.1	17.4 to 26.0	0.355	0.009
		RHUM	71.2	60.2 to 77.1	-0.265	0.040
		TMAX	25.7	19.0 to 32.9	0.269	0.038
	R3	ATEMP	20.0	13.7 to 27.0	0.279	0.031
		ATEMP	18.1	8.6 to 27.5	0.285	0.041
	R4	PREC	0.20	0.0 to 0.71	-0.431	0.011
		PVAP	-0.23	-0.84 to 0.43	-0.395	0.021
	R6	PREC	0.20	0.0 to 0.71	-0.431	0.011
		PVAP	-0.23	-0.84 to 0.43	-0.395	0.021

^a Growth stages are as follows: VT – tassel, R1 – silking, R2 – blister, R3 – milk, R4 – dough, R5 – dent, and R6 – maturity (47).

^b Weather variable abbreviations: RHUM – relative humidity, WHR – wetness hours, TMAX – maximum temperature, TMIN – minimum temperature, ATEMP – average temperature, PVAP – precipitation minus evaporation, and PREC – precipitation.

^c Average for variables have the following units: RHUM as a percent, WHR as hours, temperature in degrees Celsius, and PREC as precipitation in centimeters.

^d Pearson's correlation coefficient.

others to determine the affects of cattle exposure to these toxins and potentially develop preventative strategies against toxin contamination. Although we saw no reduction in toxin frequency or levels during ensiling, there have been two reports of silage remediation from toxin contamination. Levy et al. (25) observed that toxigenic *Penicillium* isolates and PAT production was inhibited or reduced in the presence of various yeast isolates. Using oxidative degradation, McKenzie et al. (33) reduced in vitro amounts of CPA and PAT. Strategies such as these may be adapted in the future to combat mycotoxin contamination of silages and provide more high-quality feeds to improve animal health. Some of the observations we have made in this study, particularly those of weather variables and their affect on preharvest mycotoxin contamination, may provide direction for data collection and model development to predict when conditions are favorable for toxin formation. Such strategies have been quite successful in predicting favorable conditions for the formation of other mycotoxins (18) and may be applied to warning systems and better control strategies for *Penicillium* toxins.

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