Potassium sorbate reduces production of ethanol and 2 esters in corn silage¹

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ABSTRACT

The objective of this work was to evaluate the effects of biological and chemical silage additives on the production of volatile organic compounds (VOC; methanol, ethanol, 1-propanol, methyl acetate, and ethyl acetate) within corn silage. Recent work has shown that silage VOC can contribute to poor air quality and reduce feed intake. Silage additives may reduce VOC production in silage by inhibiting the activity of bacteria or yeasts that produce them. We produced corn silage in 18.9-L bucket silos using the following treatments: (1) control (distilled water); (2) *Lactobacillus buchneri* 40788, with 400,000 cfu/g of wet forage; (3) *Lactobacillus plantarum* MTD1, with 100,000 cfu/g; (4) a commercial buffered propionic acid-based preservative (68% propionic acid, containing ammonium and sodium propionate and acetic, benzoic, and sorbic acids) at a concentration of 1 g/kg of wet forage (0.1%); (5) a low dose of potassium sorbate at a concentration of 91 mg/kg of wet forage (0.0091%); (6) a high dose of potassium sorbate at a concentration of 1 g/kg of wet forage (0.1%); and (7) a mixture of *L. plantarum* MTD1 (100,000 cfu/g) and a low dose of potassium sorbate (91 mg/kg). Volatile organic compound concentrations within silage were measured after ensiling and sample storage using a headspace gas chromatography method. The high dose of potassium sorbate was the only treatment that inhibited the production of multiple VOC. Compared with the control response, it reduced ethanol by 58%, ethyl acetate by 46%, and methyl acetate by 24%, but did not clearly affect production of methanol or 1-propanol. The effect of this additive on ethanol production was consistent with results from a small number of earlier studies. A low dose of this additive does not appear to be effective. Although it did reduce methanol

production by 24%, it increased ethanol production by more than 2-fold and did not reduce the ethyl acetate concentration. All other treatments increased ethanol production at least 2-fold relative to the control, and *L. buchneri* addition also increased the 1-propanol concentration to approximately 1% of dry matter. No effects of any treatments on fiber fractions or protein were observed. However, *L. buchneri* addition resulted in slightly more ammonia compared with the control. If these results hold under different conditions, a high dose of potassium sorbate will be an effective treatment for reducing VOC production in and emission from silage. Regulations aimed at reducing VOC emission could be ineffective or even increase emission if they promote silage additives without recognition of different types of additives.

Key words: silage, potassium sorbate, volatile organic compound, ethanol

INTRODUCTION

Silage contains numerous volatile organic compounds (VOC), including organic acids, alcohols, esters, aldehydes, and ketones (Hafner et al., 2013). Volatile organic compounds present in silage can contribute to poor air quality (Howard et al., 2010) and reduce feed intake by livestock (Weiß and Auerbach, 2012; Gerlach et al., 2013). Both problems could be addressed through the use of silage additives if VOC production can be reduced by inhibiting the activity of the bacteria or yeasts that produce them. Both biological additives (usually consisting of lactic acid bacteria) and chemical additives are commonly used to reduce fermentation losses, improve silage quality, and improve aerobic stability (Dunière et al., 2013). In general, effects of these additives on VOC have not received much attention, but several studies have reported effects of additives on ethanol production during ensiling. Ethanol may be produced by at least 4 groups of microorganisms present within silage: lactic acid bacteria, enterobacteria, clostridia, and yeasts (Hafner et al., 2013). Excluding acetic acid, it is generally the most concentrated VOC present in corn silage (Hafner et al., 2013) and con-

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tributes to the production of additional VOC: ethyl esters (Weiß et al. 2009; Weiß and Auerbach, 2012, 2013) and possibly acetaldehyde (Hafner et al., 2013). Biological additives have been reported to increase or decrease ethanol production in silage, or even have no effect (see review in Hafner et al., 2013). However, a small number of studies have reported large reductions in ethanol production with the addition of potassium sorbate. Teller et al. (2012) found that 0.1% addition of potassium sorbate (fresh-mass basis) reduced ethanol production in corn silage by at least 70%. Kleinschmit et al. (2005) reported that 0.1% of a 1:1 mixture of potassium sorbate and EDTA (fresh-mass basis) reduced ethanol production in corn silage by 80%. Weiß and Auerbach (2012) reported a 70% reduction in ethanol by addition of a commercial mixture of sodium benzoate and potassium sorbate [21.9% sodium benzoate and 13.2% potassium sorbate, applied at 0.2% (all additive doses are given on a fresh-mass basis); K. Weiß, Humboldt Universität Berlin, Berlin, Germany, personal communication] to corn silage. Furthermore, production of 2 esters (ethyl lactate and ethyl acetate) was reduced by at least 45%. In another study, Queiroz et al. (2013) found that 0.1% addition of sodium benzoate reduced ethanol production in corn silage by 68%. Auerbach and Nadeau (2013) found reductions of 73 to 85% in ethanol production in corn silage treated with 2 commercial products containing potassium sorbate, sodium benzoate, and, in one product, sodium nitrite (potassium sorbate application ranged from 130 to 300 mg/kg, whereas sodium benzoate application ranged from 250 to 515 mg/kg). Most recently, Bernardes et al. (2014) evaluated 2 doses of potassium sorbate or sodium benzoate (0.1 and 0.2%). All treatments reduced ethanol by at least 54%, and the high dose of potassium sorbate was most effective, reducing ethanol production by 85%.

The protonated form of the sorbate ion, sorbic acid, is toxic to many microorganisms (Lambert and Stratford, 1999). This uncharged molecule diffuses through cell membranes and may acidify the cytosol, which would interfere with the proton gradient used for ATP production and with other cellular processes (Ter Beek et al., 2008). Natural acidification of silage increases the ratio of sorbic acid to sorbate and, therefore, would be expected to increase this inhibitory effect. Yeasts, molds, and most gram-negative bacteria are generally sensitive to sorbic acid, but lactic acid bacteria are not (Emard and Vaughn, 1952; Woolford, 1975). Because yeasts and enterobacteria may be responsible for production of many silage VOC, their inhibition would be expected to reduce VOC production.

Confirmation of the effects of potassium sorbate on ethanol and ester production is needed. Furthermore, it

is important to determine the effect of potassium sorbate on other important VOC. In the present study, our objective was to evaluate the effect of potassium sorbate and other additives on the production of 3 alcohols and 2 esters: methanol, ethanol, 1-propanol, methyl acetate, and ethyl acetate. These compounds were selected because they are among the most significant silage VOC from an air-quality perspective (Hafner et al., 2013), and are relatively easy to measure. Ethanol is generally the single most important compound emitted from corn silage, based on its relative effect on air quality (Hafner et al., 2013). However, other compounds that may have a significant effect on air quality under some conditions, such as some aldehydes, were not included.

MATERIALS AND METHODS

Silage Production

Corn silage was made from a single batch of forage collected from a commercial dairy farm. Each silage additive was applied to a single subsample of forage that was divided among 6 replicate buckets after mixing. The process of applying additives and mixing was done by the same people using the same method all on a single day to minimize confounding handling effects, so differences were expected to have a negligible effect on measured variables. Corn forage, at approximately 25% DM, was harvested from a commercial dairy farm in Elk Grove (California) on September 22, 2012, and chopped in the field to a nominal length of 10 to 15 mm. Treatments were applied in 1.0 L of distilled water applied to 75 kg of wet forage using spray bottles, and the forage was thoroughly mixed using shovels and rakes on the top of plastic tarps. To avoid cross-contamination, tarps were either new or disinfected with a 10% bleach solution, thoroughly washed, and then dried for each treatment.

Treatments were as follows: (1) control (distilled water only); (2) *Lactobacillus buchneri* 40788 (**LB**; Lallemand Animal Nutrition, Milwaukee, WI), with 400,000 cfu/g of wet forage; (3) *Lactobacillus plantarum* MTD1 (**LP**; Ecosyl Products Ltd., Stokesley, UK), with 100,000 cfu/g; (4) a commercial buffered propionic acid-based preservative (**PA**; 68% propionic acid, containing ammonium and sodium propionate and acetic, benzoic, and sorbic acids; total concentration of the acids was 82% by mass; Kemin Americas, Des Moines, IA) at a concentration of 1 g/kg of wet forage (0.1%); (5) a low dose of potassium sorbate (**PSL**) at a concentration of 91 mg/kg of wet forage (0.0091%); (6) a high dose of potassium sorbate (**PSH**) at a concentration of 1 g/kg of wet forage (0.1%); and (7) a mixture (**MIX**) of *L. plantarum* MTD1 (Ecosyl Products Ltd.; 100,000

cfu/g) and the low dose of potassium sorbate (91 mg/kg), added separately. Potassium sorbate was 99.0% pure (Sigma-Aldrich, St. Louis, MO). Twelve kilograms of each treated forage was then manually compressed in 18.9-L buckets. Buckets were covered with tight-fitting lids, which were installed with silicone caulk to ensure a gas-tight seal. Excess silage gas accumulated in 5-L Tedlar gas bags (SKC-West Inc., Fullerton, CA) attached to the bucket silo lids using a plastic through-wall connector sealed with a rubber o-ring. Each treatment was replicated 6 times and all bucket silos were stored inside an unheated building with air conditioning in summer months. The temperature inside the building was not monitored during the entire trial but based on limited measurements ranged from approximately 15 to 27°C. The outside temperature during this period ranged from 3 to 43°C.

Bucket silos were opened after 303 d, and the top 10 cm of silage was removed and discarded as a precaution in case any air infiltration had occurred. Samples (approximately 100 g) were collected from a depth of 10 to 20 cm and quickly (within 5 min) vacuum packed in polyethylene/nylon bags (FoodSaver, Sunbeam Products; Jarden Consumer Solutions, Boca Raton, FL) where they remained until analyzed. Vacuum-packed samples were stored under refrigeration (about 4°C), apart from 1 wk at about 20°C due to an equipment failure. Five of the 6 replicates were analyzed for VOC over a period of 66 d after opening the silos, and the last set of replicates was analyzed for VOC 170 d after opening the silos. A second set of vacuum-packed samples were shipped on ice to a commercial laboratory where all other analyses were completed within 4 wk of opening the silos.

Silage Analysis

Concentrations of methanol, ethanol, 1-propanol, methyl acetate, and ethyl acetate were measured using a headspace gas chromatography procedure. About 2 h before analysis, vacuum-packed bags were removed from refrigeration, N₂ gas was added using a needle, bags were resealed with tape, and the samples were allowed to warm to room temperature. Then, a 1.0-mL gas sample was removed using a gas-tight syringe and manually injected in a Varian CP 3800 gas chromatograph with a 0.53-mm i.d. capillary column with a 0.5- μ m SPB-1000 coating (Supelco Analytical, Bellefonte, PA). Split injection was used, with a split ratio of 5:1 and an injector temperature of 75°C. Carrier gas was N₂ at 10 mL/min. The oven temperature was 35°C, and the flame ionization detector temperature was 250°C. Standards were produced by mixing a stock mixture of pure compounds in water, and were equilibrated with

an air phase in 125-mL jars with a septum in the cover (I-Chem Septa Jars; Fisher Scientific, Hampton, NH) alongside bagged silage samples. Standard solutions were made each day measurements were made by adding a stock mixture of compounds (methanol, ethanol, 1-propanol, and methyl acetate were $\geq 99.9\%$ pure; ethyl acetate was 99.8% pure; all from Sigma-Aldrich Chemie GmbH) in 18 M Ω -cm deionized distilled water. The stock mixture contained no water and was stored below -18°C. Headspace samples from the standards were removed and injected as with silage samples. Compounds were identified based on retention time relative to ethanol. Retention times within 2.5% of expected values were accepted (based on results from the standards), although 6% was allowed for 2 injections where carrier gas flow appeared to differ slightly from the standards), and were quantified using peak height to minimize the influence of overlapping peaks. Typical relative standard deviation for the method was 2 to 10%, depending on the compound and the sample.

The underlying mechanism of this headspace method is a fixed aqueous-gas partitioning coefficient (i.e., concentration ratio) for each individual compound at a given temperature. If the partitioning coefficient is identical in solution standards and silage solutions, a calibration curve determined from headspace samples taken from aqueous standards can be used with the flame ionization detector response from silage samples to directly calculate aqueous-phase concentrations without determining gas-phase concentrations. These aqueous phase concentrations can be converted to a dry mass basis using the corrected DM. Evaluation of this headspace method is described in the data supplement (<http://dx.doi.org/10.3168/jds.2014-8537>).

Silage DM content, fiber fractions, CP, organic acids, 1,2-propanediol, pH, and yeast counts were determined by a commercial laboratory (Cumberland Valley Analytical Services, Cumberland, MD). Dry matter content was determined by oven drying at 60°C for 4.5 h in a forced-air oven, followed by grinding and then additional drying for 2 h at 105°C. Dry matter values were corrected for loss of volatile compounds by assuming that 8% of lactic acid and 95% of acetic acid [based on Weißbach and Strubelt (2008)], 100% of NH₃ [based on Porter and Murray (2001)], and the mass of all VOC [based on the 100% estimate made by Weißbach and Strubelt, (2008) for alcohols] was volatilized during drying. For determination of pH, ammonia-N, organic acids, and 1,2-propanediol concentration, a 25-g sample of silage was mixed with 200 mL of deionized water. The sample mixture sat overnight and then was blended for 2 min and filtered through coarse filter paper (20–25 μ m particle retention). Sample pH was measured using a 30-mL subsample. Ammonia-N was measured

by distillation and titration. L-Lactic acid was measured with a YSI 2700 Select Biochemistry Analyzer (YSI Inc., Yellow Springs, OH), and multiplied by 2 for an estimate of total lactic acid. For determination of 1,2-propanediol and acetic, propionic, and butyric acids, 3 mL of extract was filtered through a 0.2- μ m filter membrane and a 1.0 μ L subsample was injected into a PerkinElmer AutoSystem gas chromatograph (PerkinElmer Inc., Shelton, CT) using a Restek column packed with Stabilwax-DA (Restek Corp., Bellefonte, PA) and a flame ionization detector. Helium was used as the carrier gas, and injector, oven, and detector temperatures were 225°C, 150°C, and 250°C, respectively. Nitrogen content was determined by total combustion of the sample using a Leco CNS 2000 Analyzer (Leco Corp., St. Joseph, MI) and was multiplied by 6.25 to obtain CP content. Soluble protein content was determined using the borate-phosphate buffer procedure by Krishnamoorthy et al. (1982). Samples were analyzed for NDF using sulfite and amylase (Van Soest et al., 1991) and ADF content was determined using the method of AOAC International (2000a; method 973.18). Starch was measured using the procedure described by Hall (2009). Yeast and mold counts were measured using the method AOAC International (2000b; method 995.21). The detection limit for yeast and mold was 1,000 cfu/g (wet-mass basis).

Data Analysis

Linear regression with dummy variables to represent the treatments was used in the R programming language (v. 3.02; R Core Team, 2013) for data analysis. Treatment coding was used with the control group as the baseline. Each treatment was compared with the control response using separate *t*-tests with a single pooled estimate of the standard error of the difference (calculated using the “summary” method for “lm” objects). The type I error rate α was set at 0.05, and the Bonferroni adjustment was applied for the comparison, resulting in an α of 0.0083. All VOC concentrations were logarithmically transformed to account for error distributions closer to log-normal than normal and to eliminate heteroscedasticity. Means of VOC concentrations presented below are back-transformed values, and standard errors were also back-transformed and expressed as a relative value, using the formula $10^{SE_i} - 1.0$, where SE_i is the standard error of logarithmically transformed data. Standard errors were not expressed as a percentage to avoid confusion with units of percentage of DM. Because of values below the detection limit for yeast count, these results were analyzed using a non-parametric approach, the Wilcoxon rank sum test, with the Bonferroni correction. Analysis of covariance was

used to assess the effect of storage duration at 4°C on VOC concentrations. The lm function was used as above for this analysis, but storage duration was included as a covariate. For each compound, both the overall effect of duration and separate effects for each treatment were evaluated using *t*-tests, with $\alpha = 0.05$ and no adjustment for multiple comparisons. Last, multiple linear regression (also with the lm function) was also used to assess correlation between esters, alcohols, and acetic acid.

RESULTS

Typical silage properties are summarized in Table 1. Dry matter content was lower than recommended values for corn silage (typically 30–40%). Silage pH, lactic acid, and acetic acid were within the range of typical corn silage with DM content of 30 to 40% (Kung and Shaver, 2001), with some exceptions: lactic acid was above 7% in the control (7.87%) and PSL (7.18%), and acetic acid was well above 3% in LB (5.73%). Additionally, propionic acid was above 0.1% in PA (0.18%), presumably due to addition of the compound, and also very high in LB (1.3%). The LB treatment markedly increased acetic acid production, markedly reduced lactic acid, and increased pH ($P < 0.001$ for each). All other treatments moderately reduced lactic acid production, and apparently did not change acetic acid production, resulting in a decline in the lactic acid:acetic acid ratio for all treatments relative to the control. The LB treatment slightly increased production of ammonia (by 0.026% of DM; $P < 0.001$). Additives did not affect the concentrations of total or soluble CP, fiber fractions, or starch.

Mold counts were below the detection limit (10^3 cfu/g) for every sample. Additives appeared to have effects on yeasts, but it was difficult to conclusively detect differences using the control group in comparisons, as 1 of the 6 replicates had a high yeast count and the remaining 5 replicates were at or below the detection limit. Counts in both LB and PSH were below the detection limit (10^3 cfu/g) for almost all samples, and it was not possible to determine if these results were different from the control treatment. But all other treatments appeared to increase yeast counts by at least 2,800-fold whether compared with the control samples with the high value omitted or to LB and PSH samples.

The most concentrated VOC present in the silages (Table 2) was ethanol, which ranged from 1,440 to 10,100 mg/kg (0.14 to 1.0% of DM) based on mean values. The LB samples, where 1-propanol reached 10,200 mg/kg (1.1% of DM), were an exception. Silage additives had clear effects on the production of some VOC, but in many cases additives actually stimulated their

Table 1. Characteristics of corn silage treated with biological and chemical additives^{1,2}

Item	Treatment ³							Pooled SE
	CON	LB	LP	PA	PSL	PSH	MIX	
DM	27.5	26.7	26.8	27.4	26.6	27.5	27.0	0.255
pH	3.68	4.08***	3.70	3.69	3.68	3.77*	3.75	0.022
CP	7.66	7.67	7.67	7.83	7.67	7.45	7.67	0.127
NH ₃ ⁴	0.134	0.160***	0.129	0.145	0.128	0.127	0.126	0.00342
ADF	30.7	31.2	30.5	30.9	31.7	30.6	32.4	0.545
NDF	45.6	47	46.8	46.6	47.6	46.9	48.2†	0.708
Starch	22.3	21.0	22.2	22.1	20.0	21	19.8	0.846
Yeasts ⁵	<3.70	<3.00	6.88*	6.46*	6.67*	<3.00	6.73*	0.278
Lactic acid	7.87	1.80***	6.87**	5.97***	7.18*	6.57***	6.41***	0.173
Acetic acid	1.14	5.73***	1.22	1.26	1.26	1.31	1.25	0.105
Lactic:acetic	6.95	0.324***	5.64***	4.76***	5.74***	5.05***	5.18***	0.255
Propionic acid	ND ⁶	1.28	ND	0.179	ND	ND	ND	ND

¹The number of replicates was 6 for all groups. All results are given as percentage of DM, except pH and yeasts.

²1,2-Propanediol was detected only in silage treated with *L. buchneri*, where it was 1.01% of DM.

³CON = control; LB = *Lactobacillus buchneri* 40788, with 400,000 cfu/g; LP = *Lactobacillus plantarum* MTD1, with 100,000 cfu/g; PA = buffered propionic acid-based preservative; PSL = potassium sorbate at a concentration of 0.0091% of wet forage mass; PSH = potassium sorbate at a concentration of 0.1%; MIX = *L. plantarum* MTD1 with 100,000 cfu/g, plus potassium sorbate at a concentration of 0.0091%.

⁴Ammonia is expressed as percentage of DM as N.

⁵Units are log₁₀ (cfu/g) on a wet-mass basis, and the detection limit was 3.0. All replicates for PSH and all but 1 replicate for CON and LB were below the detection limit. Asterisks show results of comparisons to CON, with the single high value excluded, or LB or PSH (identical results for each).

⁶ND = no detection.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (statistical significance is based on comparisons of each group with the control with the Bonferroni adjustment).

production (Table 2). *Lactobacillus buchneri* stimulated production of all 3 alcohols and both esters relative to the control treatment. The largest relative increase due to an additive was seen in this treatment, where 1-propanol was approximately 400-fold the control mean ($P < 0.001$). Additionally, 1,2-propanediol was about 1% of DM in these samples, but was not detected in any others. All additives except PSH substantially increased the production of ethanol. The increase ranged from 2.0-fold ($P < 0.001$) by LB to 2.9-fold ($P < 0.001$)

by PA. Conversely, all treatments except LB and PSH reduced methanol production, albeit slightly—the largest reduction was 24% by PSL ($P < 0.001$).

The PSH was the only treatment that reduced concentrations of multiple VOC: it reduced ethanol by 58% ($P < 0.001$), ethyl acetate by 46% ($P = 0.0014$), and methyl acetate by 24% ($P = 0.0068$). Conversely, the PSL treatment increased ethanol production (2.25-fold) and did not reduce the ethyl acetate concentration (42% increase; $P = 0.052$). Ethanol concentrations

Table 2. Mean concentrations of volatile organic compounds measured using a headspace gas chromatography method (mg/kg, DM basis) in corn silage treated with biological and chemical additives¹

Item	Treatment ²							Pooled SE ³
	CON	LB	LP	PA	PSL	PSH	MIX	
Methanol	538	696***	415***	450**	409***	470†	402***	0.0368
Ethanol	3,450	6,810**	8,400***	10,100***	7,780***	1,440***	9,420***	0.138
1-Propanol	25.6	10,200***	43.3	47.7	42.3	14.1	38.6	0.309
Methyl acetate	9.91	28.9***	8.52	8.63	7.83	7.48*	8.85	0.0713
Ethyl acetate	20.5	168***	38.5**	61.8***	29.1	11.1**	47.7***	0.132

¹The number of replicates was 6.

²CON = control; LB = *Lactobacillus buchneri* 40788, with 400,000 cfu/g; LP = *Lactobacillus plantarum* MTD1, with 100,000 cfu/g; PA = buffered propionic acid-based additive; PSL = potassium sorbate at a concentration of 0.0091% of wet forage mass; PSH = potassium sorbate at a concentration of 0.1%; MIX = *Lactobacillus plantarum* MTD1 with 100,000 cfu/g, plus potassium sorbate at a concentration of 0.0091%.

³Pooled SE was calculated from logarithmically transformed values, and the values here are relative values calculated by $10^{SE_i} - 1.0$, where SE_i is the standard error of the logarithmically transformed values.

† $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (statistical significance is based on comparisons of each group with the control with a *t*-test using the pooled SE and the Bonferroni adjustment).

were more variable in the control samples than in most other groups, which affected the precision and power of all estimates of relative effects on production of this compound. The control sample with the highest ethanol concentration (9,460 mg/kg, about 2.7-fold the mean value) was also the one with the most yeasts ($\log_{10}$ cfu/g = 7.20), but other variables were similar to mean values. To determine if the apparent reduction in ethanol by PSH was caused by a small number of particularly high samples in the control treatment, a comparison was made without this highest or the sample with the second-highest ethanol concentration (7,740 mg/kg, about 2.2-fold the mean value). In this case, the mean concentration in the control group was 2,230 mg/kg, for an apparent reduction by PSH of 36% instead of 58% (95% CI: 15 to 53% from a 2-sample *t*-test).

It is possible that potassium sorbate can reduce the production of other alcohols as well. The mean methanol concentration for PSH was slightly lower than the control, but the *P*-value (0.014) was above the critical value. However, PSL did reduce methanol (described above). For 1-propanol, the PSH mean was about 45% smaller than the control mean, but some overlap existed between the 2 groups, and the comparison *P*-value (0.13) was much higher than the critical value. Additional experiments will be needed to assess effects on these compounds. Importantly, there was no evidence that PSH increased production of any VOC.

Ester concentrations were strongly correlated with their respective alcohols and acids. Based on least-squares regression using results from individual samples, the concentration of ethyl acetate could be related to ethanol (*e*) and acetic acid (*a*) concentrations (all in mg/kg) by $-32.4 + 0.00464e + 0.00292a$ (adjusted $R^2 = 0.96$; $P < 0.001$ for each term). Similarly, methyl acetate could be related to methanol (*m*) and acetic acid by $-4.22 + 0.0194m + 0.000340a$ (adjusted $R^2 = 0.95$; $P < 0.001$ for each term).

Only 1 bucket showed signs of air infiltration: mold was present at the surface of a PSL replicate, but measured variables for this silage were similar to the other replicates and it was not excluded. In general, little evidence existed that storage duration of vacuum-packed samples at 4°C affected VOC concentrations. Only ethyl acetate in the control ($P = 0.007$) and PSL ($P = 0.024$) treatments, and ethanol in the control ($P = 0.031$) treatment showed significant responses to storage duration [all positive; 0.96%/d or less (% of concentration, not % of DM)]. An overall effect of storage duration was detected for ethyl acetate only ($P = 0.0014$), but this appeared to be due to the apparent responses in the control and PSL samples. No clear trends existed with storage duration for other treatments.

DISCUSSION

With the exception of PSH, the additives evaluated here were not effective at reducing VOC production under these ensiling conditions. It is useful to understand why these additives increased VOC production. Ethanol is the single most important VOC, but understanding effects of additives on production of this alcohol can be challenging because it is produced by at least 4 groups of microorganisms present in silage: lactic acid bacteria, enterobacteria, clostridia, and yeasts (Hafner et al., 2013). Increases in ethanol production by LP, PA, PSL, and MIX are difficult to explain. Yeast activity may have played a role because, except for LB, all treatments resulted in higher yeast counts than PSH. But why yeasts may be stimulated, or less inhibited, when these additives are used is not clear. Alternatively, the ethanol increase due to LP and MIX treatments could be due to direct production through the facultative heterolactic fermentation by *L. plantarum*. An increase in ethanol production due to addition of bacterial additives is not uncommon (Steidlová and Kalač, 2003; Kleinschmit et al., 2005; Tabacco et al., 2009; Queiroz et al., 2013). Increased ethanol production has also been reported in response to addition of a formic and propionic acid mixture (Weiß and Auerbach, 2012), but the mechanism is not clear.

The strong correlations between the acetate esters and alcohols and acetic acid suggest that reducing alcohol production will reduce ester production also, and so effects of additives on esters can largely be explained by effects on alcohols and acids. Correlation between ethyl esters and ethanol has been observed before and has been cited as evidence of abiotic esterification reactions in silage (Weiß et al., 2009; Weiß and Auerbach, 2012, 2013). However, PSH reduced methyl acetate production without a clear change in methanol production, which is not consistent with this explanation. Limited statistical power for detecting an effect on methanol may underlie this apparent inconsistency. Implications of alcohol conversion to esters for air quality are probably insignificant. The tendency of these 2 esters to form ozone is less than for alcohols (on both a mass and mole basis; Carter, 2009), but their volatility is higher, so production of esters at the expense of alcohols could increase or reduce effects on air quality. However, the low concentrations of esters compared with alcohols, shown here for just 2 esters but for other esters for corn silage in general (Hafner et al., 2013; Weiß et al., 2009; Weiß and Auerbach, 2012, 2013; Gerlach et al., 2013) limit the effect of this conversion.

Concentrations of alcohols measured in these silage samples are within the wide range of values reported in earlier studies, as summarized by Hafner et al. (2013).

But ester concentrations measured in this study are lower than those reported in other studies summarized in Hafner et al. (2013) and reported since then (Gerlach et al., 2013). Gerlach et al. (2013) found mean ethyl acetate concentrations of 138 to 400 mg/kg in corn silage made with different chopping lengths and densities. In our measurements, the mean methyl acetate concentrations ranged from 7.5 to 29 mg/kg, and mean ethyl acetate concentrations ranged from 11 to 170 mg/kg. Variability in VOC concentrations among silages can be very high; based on empirical tolerance intervals for a set of silage samples from within the United States, Hafner et al. (2013) estimated that 25% of silage samples will have ethanol and ethyl lactate concentrations more than a factor of 2.4 away from the median value. The differences between the concentrations we measured and those reported in previous studies may be the result of this variability, but may also be due to biases of the measurement techniques.

The primary sources of methanol in silage have not been identified, but this compound may be produced from pectin demethylation catalyzed by plant enzymes (see review by Hafner et al., 2013). It is not clear why additives would affect this process, which can occur after harvest before additives are added.

In contrast with the other additives, the effects of LB on silage composition are consistent with the current understanding of this bacterium. *Lactobacillus buchneri* is added to silage to improve aerobic stability, which is thought to be a result of the higher acetic acid concentrations resulting from conversion of lactic acid to acetic acid (Oude Elferink et al., 2001) but could be due to other substances as well. However, *L. buchneri* is not recommended for wet silages, where it grows particularly well and can consume so much lactic acid that pH is substantially elevated (Nishino and Touno, 2005; Schmidt et al., 2005). This additive was probably not appropriate for the wet silage used in this work. *Lactobacillus buchneri* can produce ethanol directly through heterolactic fermentation of carbohydrates (Oude Elferink et al., 2001). Additionally, it produces 1,2-propanediol (Oude Elferink et al., 2001), which can result in propionic acid and 1-propanol production by *Lactobacillus diolivorans* (Krooneman et al., 2002). Therefore, the increases in ethanol, 1,2-propanediol, propionic acid, and 1-propanol compared with the control group, along with the effects on lactic and acetic acid described above, are consistent with high activity of *L. buchneri*. However, some of these effects are not typically observed for this inoculant. Conversion of lactic acid to acetic acid is generally more limited, propionic acid increases are typically not observed, and ethanol is not typically elevated when *L. buchneri* is

used for corn silage (Kleinschmit and Kung, 2006). Effects on ethanol and the organic acids are probably due to the stimulation of *L. buchneri* by high moisture. Whether the high moisture also affected the activity of *L. diolivorans* and, therefore, production of propionic acid and 1-propanol, or the population of this or related bacteria was particularly high for this forage is unknown. Effects of *L. buchneri* addition on 1-propanol have generally not been reported, but in at least 2 studies, addition of $\geq 100,000$ cfu/g of *L. buchneri* led to large increases in production of the compound, up to 7,160 mg/kg (Kristensen et al., 2010), and 14,200 mg/kg (Driehuis et al., 2001), which encompass the mean observed in the LB samples here. The reactivity of 1-propanol is higher than for ethanol (Carter, 2009), so effects of *Lactobacillus buchneri* on air quality will depend on 1-propanol production. For the LB samples here, the potential effect of 1-propanol on air quality is about twice that of ethanol [based on the product of concentration and equal benefits incremental reactivity from Carter (2009)]. At least for the wet silage used here, *L. buchneri* appears to be a very poor additive for the purpose of reducing VOC production. Additionally, the effects of this additive provide an example of potential complexities of additive effects on air quality. If 1-propanol were not measured, the potential effect of this treatment on air quality would be substantially underestimated, and in cases where ethanol production was suppressed, the direction of the estimated effect could be wrong.

The only treatment we evaluated that could reduce VOC emission under conditions of this study was PSH. The 58% reduction in ethanol based on all data are comparable with other studies summarized in the introduction (Kleinschmit et al. 2005; Teller et al., 2012; Weiß and Auerbach, 2012; Auerbach and Nadeau, 2013; Queiroz et al., 2013; Bernardes et al., 2014), although smaller. The response of ethyl acetate to PSH was almost identical to the result from Weiß and Auerbach (2012) in response to a sodium benzoate and potassium sorbate mixture. Our results provide further evidence that a sufficient dose of potassium sorbate can reduce ethanol production and the production of 2 esters, even under wet conditions. The mechanism behind the reduction in ethanol and ethyl acetate is probably inhibition of yeasts, although inhibition of enterobacteria could also play a role. It is surprising that the effect of a lower dose of this additive (PSL), which approximates the concentration most commonly used in practice, appeared to stimulate ethanol production.

It will be important for future research to evaluate the effect of PSH on production of these, and, ideally, other VOC. Certain features of this study may have af-

affected VOC production and so other studies would find different VOC concentrations and possibly different effects of silage additives. The DM content, chemical composition, and composition of the native microbial population all could influence VOC production. In this particular experiment, the ensiling period (303 d) was relatively long, which could influence both total production of different VOC and also relative effects of additives, as at least ethanol can be produced by multiple groups of microorganisms, and so production may vary over time. The type of water used to dilute the microbial additives could affect their activity—here, we used distilled water, whereas tap water is typically used in the field. Additionally, microbial activity could have continued in vacuum-packed samples after the bucket silos were opened. The small amount of oxygen present in the samples immediately after vacuum packing could have stimulated growth of yeast or other microorganisms, which might have increased or decreased VOC concentrations through partial or complete oxidation, and also increased yeast counts. Gerlach et al. (2013) reported that ethanol and ester concentrations increased during storage of corn silage at room temperature in vacuum-packed samples, but increases were only observed for samples initially exposed to air for more than 4 d (K. Gerlach, University of Bonn, Bonn, Germany, personal communication). The samples in this work were exposed for less than 5 min. Some oxygen exposure usually occurs during full-scale silage production, so these conditions were not unlike typical silage production, but a more complete evaluation of these additives would include different ensiling periods and removal conditions.

CONCLUSIONS

Silage additives can reduce production of volatile organic compounds in corn silage, but not all additives are equivalent. Addition of potassium sorbate at 0.1% (fresh forage mass basis) can substantially reduce production of ethanol, methyl acetate, and ethyl acetate, but a lower dose (91 mg/kg of fresh weight) can increase VOC production. A commercial propionic acid-based product and the biological additives evaluated here can stimulate production of ethanol and ethyl acetate (and possibly 1-propanol) in corn silage. Regulations aimed at reducing VOC emission could be ineffective or even increase emission if they promote silage additives without recognition of different types of additives. Future work should be carried out to evaluate the effectiveness of potassium sorbate under different conditions and at different doses. Additionally, it will be important to evaluate the effect on the production of aldehydes.

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