J. Dairy Sci. 97:1877–1884
http://dx.doi.org/10.3168/jds.2013-7545

Hot topic: Brown marmorated stink bug odor compounds do not transfer into milk by feeding bug-contaminated corn silage to lactating dairy cattle

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ABSTRACT

Brown marmorated stink bug (BMSB; *Halyomorpha halys*) is an emerging invasive species of grave concern to agriculture as a polyphagous plant pest with potential negative effects on the dairy industry. The purpose of this study was to determine the risk of including BMSB-contaminated silage in lactating dairy cow rations. First, 6 dairies, either highly infested (n = 3; 30 to 100 bugs per stalk) or not infested (n = 3), were sampled to assess the prevalence of bug secretion compounds tridecane (major component) and *E*-2-decenal (stink odor component) in silage and milk. Second, using wild BMSB, a mini-silo dose-response experiment (adding 100, 50, 25, 10, and 1 freshly crushed bugs/0.5 kg of chopped corn) was conducted to assess the effect of ensiling on BMSB stink odor compounds. Finally, synthetic BMSB stink odor compounds (10 g of tridecane and 5 g of *E*-2-decenal) were ruminally infused twice daily over 3 d, and samples of milk, urine, and rumen fluid were collected to evaluate disposition. Bug stink odor compounds were sampled by solid-phase microextraction (SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS). Milk production and feed composition were unaffected when BMSB-contaminated silage was fed. Moreover, no *E*-2-decenal was detected in silage or milk (detection threshold = 0.00125 μg/mL). The dose-response of tridecane in mini-silo samples exhibited a linear relationship ($R^2 = 0.78$) with the amount of BMSB added; however, *E*-2-decenal was completely decomposed and undetectable in spiked mini-silos after ensiling. Both synthetic secretion compounds infused into rumen were undetectable in all milk and urine samples. *E*-2-Decenal was not detectable in rumen fluid, whereas tridecane was detected only at 15 min postinfusion but not present thereafter. Feed intake was unaffected by infusion treatment and BMSB secretion compounds (*E*-2-decenal and tridecane) were not observed in milk. *E*-2-Decenal and tridecane from the metathoracic gland of BMSB are not able to contaminate milk either due to the ensiling process or because of metabolism within the rumen. Concern over BMSB stink odor compounds contaminating the fluid milk supply, even on highly infested farms, is not warranted.

Key words: dairy cow, brown marmorated stink bug, milk, stink bug odor

Hot Topic

The brown marmorated stink bug (**BMSB**), *Halyomorpha halys*, is an invasive insect pest introduced into the United States from its native range in Japan, Korea, and China. The BMSB was first identified in 2001 in Allentown, Pennsylvania, but is believed to have been introduced as early as 1996 (Hoebeke and Carter, 2003; Hamilton, 2009), possibly through the movement of bulk containers (Hamilton, 2009). Since that time, it has spread to multiple states in the continental United States and Canada (Wermelinger et al., 2008). Currently, BMSB has established populations on both coasts but is most widely spread in the mid-Atlantic region (Leskey et al., 2012).

In addition to the economic impact of this pest, BMSB is a nuisance pest for businesses and homeowners. It overwinters as an adult, aggregating in high numbers when seeking shelter (Hoebeke and Carter, 2003), usually on the sides of buildings, garages, and other structures (Hamilton, 2009). The population size of BMSB has steadily grown in the United States since its introduction. Although lower levels of economic damage were observed as early as 2002 in Pennsylvania (Bernon, 2004), documentation of greater damage appeared in 2006, when BMSB first caused major economic damage (Nielsen et al., 2008; Hamilton, 2009). During the 2009 growing season, serious economic injury to several crops, including peach, apple, and Asian pear, was reported due to large BMSB populations (USDA-ARS, 2010).

Received September 30, 2013.
Accepted January 25, 2014.
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During the 2010 growing season, significant damage occurred throughout the mid-Atlantic area to the crops listed above as well as field corn, sweet corn, soybeans, brambles, and grapes. Populations of BMSB as high as 100 per corn stalk were reported in the Middletown and Burkittsville areas of Maryland. Harvested corn silage had the distinctive BMSB odor, and dairy producers were concerned about the effect on feed quality and the subsequent milk produced from this feed. Milk contaminated with BMSB off-flavors could have a devastating effect on the fluid milk industry. A literature review found no published information on the effect of stink bugs in milk. If dairy feed is contaminated with BMSB odor-causing compounds, it could result in contaminated milk. Therefore, the objective of these studies was to assess if the odor-causing compounds secreted by BMSB were stable enough to transfer through the harvesting, processing, ensiling, feeding, and digestion processes and, ultimately, to contaminate milk.

To identify the BMSB effluvial odor, volatiles were collected using 3 groups of males, females, and nymphs (12 insects per group, 40-d-old males and females, 4 to 5 stages of nymphal instars) at room temperature. The bugs were separately introduced into three 1-L, 4-necked glass containers (Zhang et al., 1994). Air was drawn into the container through 6- to 14-mesh activated charcoal (Fisher Scientific, Pittsburgh, PA) and out of the container through 2 traps (15 cm × 1.5-cm o.d.) containing Super Q adsorbent (200 mg each; Alltech Associates Inc., Deerfield, IL) by vacuum (~1 L/min). Insects were fed with organic green beans and aerated continuously for 24 h at room temperature with a 16 h light:8 h dark photoperiod. Adsorbents were eluted with methylene chloride (4 × 0.5 mL); then, the eluates (2 mL/sample) were concentrated to ~200 μL under a nitrogen stream and stored at −30°C for future analysis. The headspace collection result showed that adult male BMSB released 4 compounds as major volatile emissions (Figure 1). The volatiles from females and nymphs were very similar to the adult male emission. The major compound, tridecane (peak 3 in Figure 1), is a chemically stable compound that is odorless and may simply serve as a solvent or spreading agent for the more irritating compounds. Another major compound, *E*-2-decenal (peak 2 in Figure 1), is the major irritant and responsible for the potent stink odor from BMSB. However, being an extremely unstable compound it can easily break down, and the degradation products do not have the distinctive BMSB odor. These 2 major components make up more than 70% of the total volatile emission and therefore were selected as target molecules to evaluate the effect of BMSB on feed quality and the subsequent milk produced. In this study, 3 independent approaches were used to assess the potential for an effect on (1) fluid milk quality in conventional production setting with or without contaminated corn fields, (2) resilience of the bug stink odor compounds through ensiling, and (3) the extent to which compounds directly infused ruminally can be observed in body fluids.

In the first approach, a farm survey using 6 farms self-reporting either high or low BMSB infestations in corn crops at harvest were selected, and monthly samples of silage and milk were collected from May to October 2011. Silage and milk samples from these farms were submitted to a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown MD) for standard composition analysis. In addition, raw, fresh milk samples were collected and analyzed for the presence of *E*-2-decenal and tridecane using solid-phase microextraction (**SPME**) and GC-MS.

The second approach assessed the effect of ensiling on odor compounds in silage. Adult and late instar BMSB were collected by hand from a sweet corn crop in Burrittsville, Maryland, over a 3-d period (August 30 to September 1, 2011). Insects were collected and kept with sweet corn as a feed source in a 23.2 cm × 16.8 cm × 15.24 cm plastic pet keeper (Small Petco Pet Keeper, International Pet Supplies and Distributors, San Diego, CA). Fresh corn silage for the mini-silo experiment was collected from a farm in Middletown, Maryland, at the time of corn chopping and trench silo filling. The field was scouted and no BMSB were found before or during chopping. Plant population was 38,768 plants/acre. Using an average plant population of 29,000 plants/acre, and a 16 ton/acre average yield (Maryland Ag Statistic 5-yr average; Maryland Department of Agriculture, 2004–2008), the number of BMSB required to equal 1, 10, 25, 50, and 100 BMSB per stalk of corn was determined to be 1, 10, 25, 50, and 100 BMSB per 500 g of chopped material. Pet keepers used to hold bugs were placed into a chest freezer for 60 s to slow movement of the bugs for counting and distribution of treatments into the mini-silo bags. Using a stainless steel mortar and pestle (model 8395, Amco Houseworks, Vernon Hills, IL), BMSB were crushed and mixed in a shallow aluminum pan with 500 g of corn silage. Subsequently, the mixture was placed into a 20-cm × 38-cm, 3-mil polyethylene vacuum bag and sealed in a single chamber vacuum packaging machine (Koch Ultravac 500, Koch Equipment LLC, Kansas City, MO) set at 96% vacuum. Zero-time bags for each dose were immediately frozen. All other bags were placed into black plastic bags and allowed to ferment for 90 d at room temperature (22°C) before freezing at −20°C until analyzed by SPME GC-MS (Rasmussen et al., 1997; Zhang et al., 1999).

In the third and final approach, 5 ruminally fistulated Holstein dairy cows in mid-lactation were directly