EFFECTS OF CROP SPECIES ON INDIGENOUS MICROFLORA AND OF SILAGE ADDITIVES ON THE MICROBIAL SUCCESSION DURING THE ENSILING PROCESS[1,2]

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Summary

This study considered the effects of crop species (alfalfa vs. corn) and silage additives on six categories of indigenous microorganisms (those naturally occurring on the crop) important to silage fermentation, and on the microbial succession during the ensiling process. The numbers of streptococci, Enterobacteriaceae, yeasts and molds, lactate-using yeasts, and carbohydrate-fermenting clostridial spores were higher on corn than on alfalfa. The lactic acid bacteria (LAB) comprised less than 2% of the total microbial populations on both crops.

Alfalfa treated with Biomate® inoculant and the combination of dextrose and Biomate showed higher LAB counts than the control and dextrose treatments at 1 day post-ensiling. Adding dextrose accelerated multiplication of LAB in the ensiled alfalfa. Adding 1174® inoculant to corn silages did not affect the microbial succession during the ensiling process. Development of Enterobacteriaceae, yeasts and molds, lactate-using yeasts, and clostridia on either crop during ensiling was not influenced by the additives.

(Key Words: Epiphytic Microflora, Alfalfa, Corn, Additive, Silage.)

Introduction

Indigenous (epithytic) microorganisms are important in silage preservation. Not only are they responsible for silage fermentation, but they influence the effectiveness of silage additives. The microflora involved in ensiling comprises mainly lactic acid bacteria (LAB), but 'bad' organisms, i.e., Enterobacteriaceae, clostridia, yeasts, and molds can also be present. Their frequencies on silage crops are quite variable and are affected by crop species, variety/hybrid, maturity stage, climate or soil, and mowing, field-wilting, or chopping processes. Dramatic changes in numbers and proportions of the epiphytic microflora also occur once the chopped forage is ensiled.

Stimulating silage fermentation by adding bacterial cultures has become a common practice, because these products are safe to handle and help establish homolactic fermentations (fermentations producing only lactic acid). This study investigated the effect of additives on microbial succession on alfalfa and corn during the ensiling process. The factors influencing the epiphytic microorganisms on alfalfa and corn in this study were reported last year (Rep. of Prog. 592; pp. 118-122).

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[1]Biomate® was provided by Chr. Hansen's Bio Systems, Milwaukee, Wisconsin and contains Lactobacillus plantarum and Pediococcus cerevisiae.

[2]1174® was provided by Pioneer Hi-Bred International, Inc., Des Moines, Iowa and contains Lactobacillus plantarum (multiple strains) and Streptococcus faecium.

Experimental Procedures

A second-year stand of Cody alfalfa was examined at 2nd, 3rd, 4th, and 5th cuttings and at late-bud, 10% bloom, and 50% bloom within each cutting. Three Pioneer corn hybrids (3377, 3379, and 3389) were grown under irrigation and were ensiled at the two-thirds milk line of kernel maturity.

Chopped alfalfa (2nd and 4th cuttings) received no additive (control) or three treatments: dextrose (applied at 2% of the crop dry matter), Biomate inoculant (to supply 1.5×10^5 colony-forming units (CFU)/g of fresh crop), and a combination of dextrose and Biomate. The corn was treated with 1174 inoculant to supply 1.5×10^5 CFU/g of fresh crop. Treated crops were ensiled in 4 × 14 in. laboratory silos. Three replicate silos were opened at various times up to 120 days post-ensiling.

Weather data were recorded on sampling days. By use of appropriate selective media, lactobacilli, pediococci, and leuconostoc (LPL); streptococci (Str); Enterobacteriaceae (Ent); yeasts and molds (YM); lactate-using yeasts (LUY); and lactate-fermenting clostridial spores (Clo) were enumerated. The LAB were calculated as the sum of LPL and streptococci. Other details of the procedures used were published in Rep. of Prog. 592.

Results and Discussion

Microorganism counts were higher on the standing corn than alfalfa ($P < .05$) (Figure 1). Enterobacteriaceae were predominant on alfalfa (10^6 CFU/g); yeasts and molds and Enterobacteriaceae were predominant on corn (10^6 CFU/g). The LAB comprised less than 2 percent of the total microbial population on both alfalfa and corn; the main LAB were streptococci. No clostridial spores were found on the 12 standing alfalfas, but they were present on two of the corn hybrids. The appearance of clostridial spores on the corn hybrids might be attributed to heavy rainfall prior to harvest.

Cutting number did not influence the epiphytic microflora ($P > .05$) on alfalfa, although the 3rd and 4th cuttings had numerically higher populations of LAB than the 2nd and 5th. Wilting of alfalfa decreased LAB counts slightly ($P > .05$) but increased Enterobacteriaceae ($P < .05$), yeasts and molds, and clostridial spores ($P > .05$). Once the forage crops went through the chopper, a dramatic increase ($P < .05$) occurred in LAB and Enterobacteriaceae on both alfalfa and corn. The 'chopping inoculation' phenomenon has been observed by others and has been an enigma to researchers. It has been attributed to harvester contamination, microbial growth, or both. However, recent studies have shown these earlier explanations to be neither adequate nor true. A new "somnicell" hypothesis, in which bacteria are assumed viable but in a non-culturable stage on standing crops, is proposed but still needs more investigation.

After the alfalfa was ensiled, LAB quickly proliferated and dominated the silage at one day post-ensiling (Figures 2 and 3). Note that the y axis is logarithmic, so differences that appear small can be large. Silage treated with Biomate and the combination of dextrose and Biomate showed higher LAB counts than either control or dextrose treatments ($P < .05$) at 1 day post-ensiling. Adding dextrose resulted in faster LAB growth up to 3 days post-ensiling, demonstrating the limiting nature of water soluble carbohydrates in alfalfa. The LAB counts were similar between treatments after 7 days post-ensiling, except that the Biomate-treated silage maintained a high level of LPL at the end of ensiling. Enterobacteriaceae and yeasts and molds decreased continuously as ensiling progressed (Figures 4 and 5). Both dextrose and combination treatments accelerated this decrease. Clostridia remained at very low levels and were not influenced by the treatments at any points during the ensiling process.

The 1174 inoculant did not affect microbial succession during the ensiling process of corn (P>.05) (Figure 6). Epiphytic LAB reached 10^7/g at ensiling, which was 60 times that provided by the 1174 inoculant. The LAB became predominant at 6 hours post-ensiling in both control and inoculated silages. Enterobacteriaceae declined slowly as ensiling progressed, but yeasts and molds did not decrease until after 42 days post-ensiling (Figure 7).

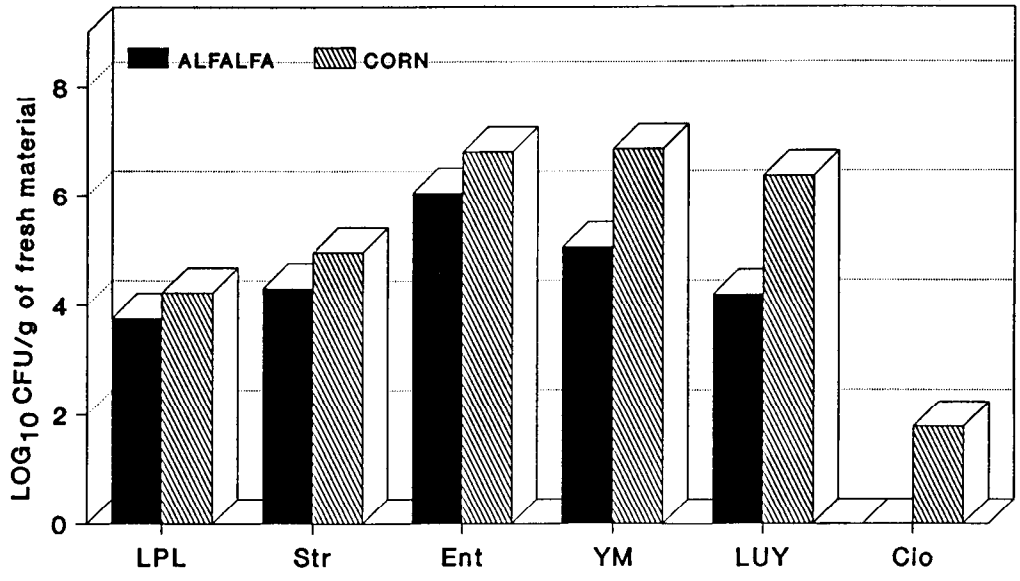

Figure 1. Effect of Crop Species on Epiphytic Microflora

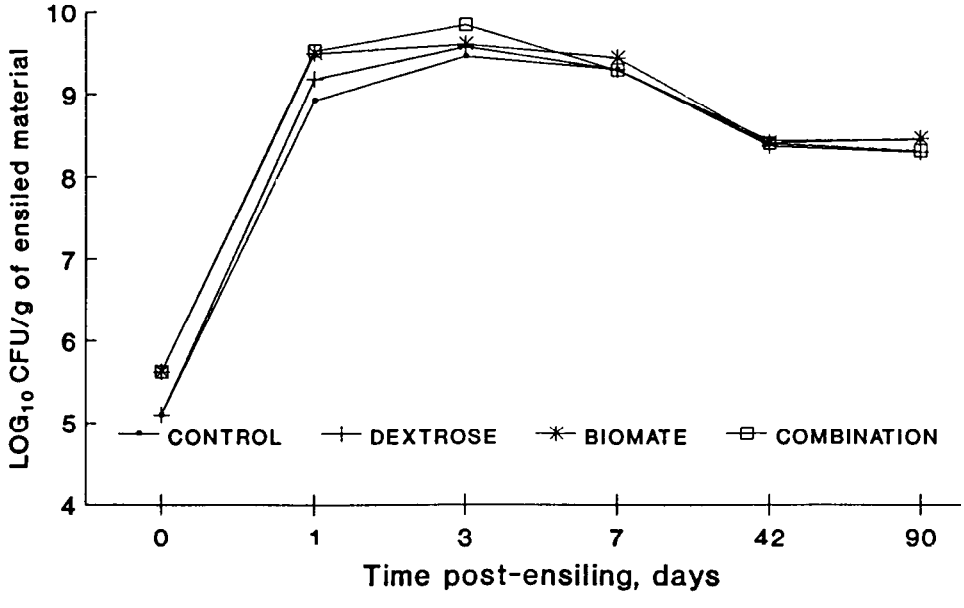

Figure 2. Effect of Silage Additives on LPL during the Ensiling Process of Alfalfa

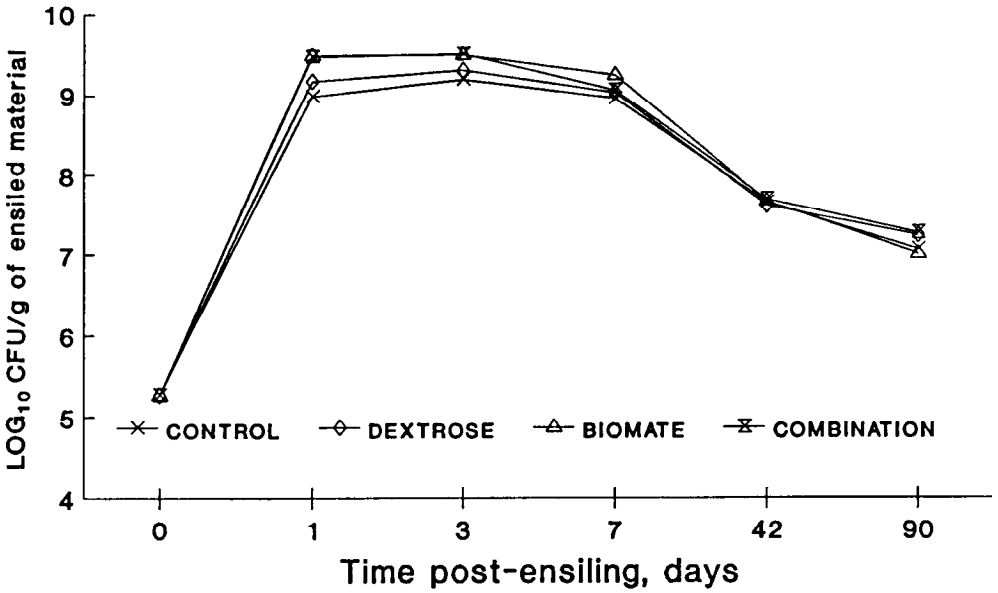

Figure 3. Effect of Silage Additives on Streptococci during the Ensiling Process of Alfalfa

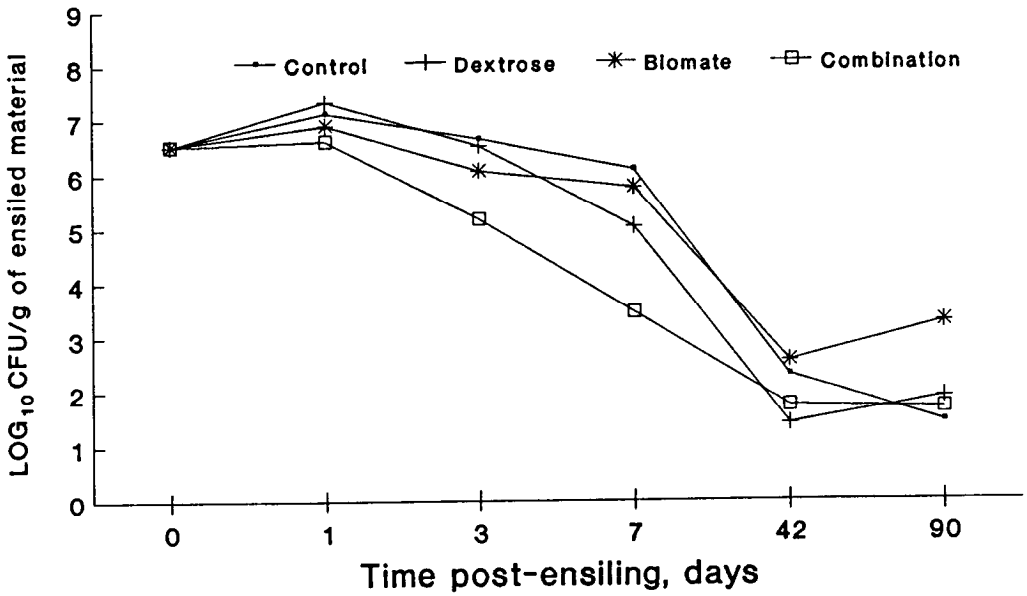

Figure 4. Effect of Silage Additives on Enterobacteriaceae during the Ensiling Process of Alfalfa

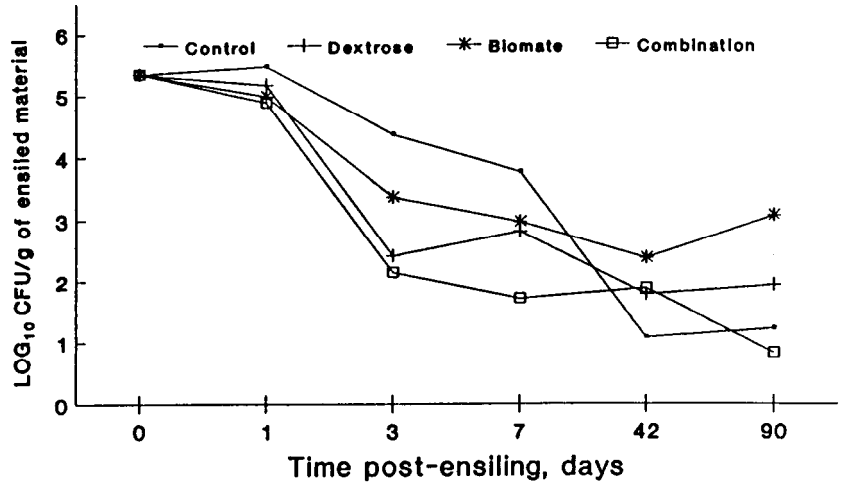

Figure 5. Effect of Silage Additives on Yeasts and Molds during the Ensiling Process of Alfalfa

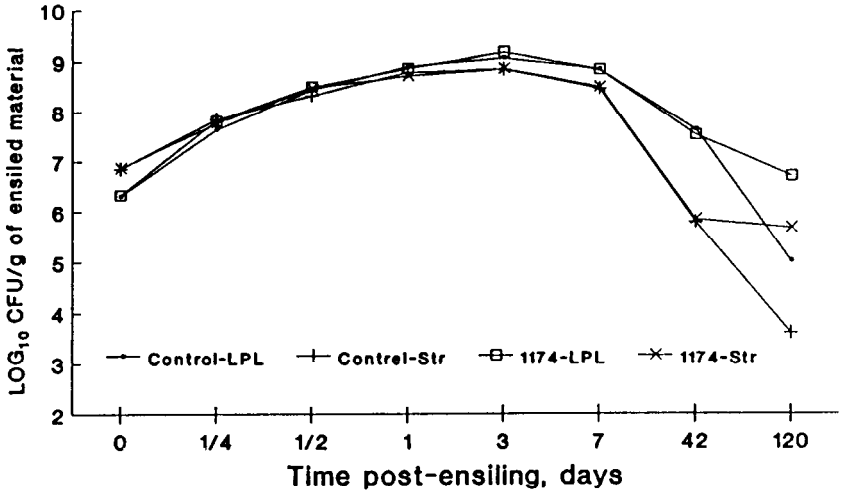

Figure 6. Effect of 1174 on LAB during the Ensiling Process of Corn

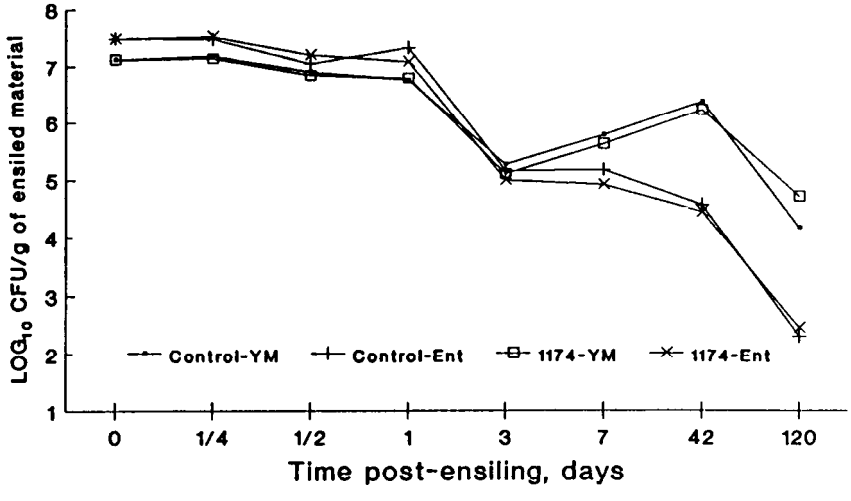

Figure 7. Effect of 1174 on Enterobacteriaceae, Yeasts, and Molds during the Ensiling Process of Corn