Deterioration of High-Moisture Corn

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Two small, leaky silos were filled with normal high-moisture corn (HMC), and two with HMC severely infested by Helminthosporium maydis. Counts of mesophilic bacteria, lactobacilli, coliforms, yeasts, and molds were made on corn samples as received and periodically thereafter during 220 days of storage. Temperature and gas levels also were monitored. Sequential changes in the populations of lactobacilli, yeasts, and molds were determined during spoilage of HMC. These population changes were compared on the basis of the variables encountered in the present study as well as with the results of previous studies conducted on normal HMC stored under adequate conditions. Heavy infestation by H. maydis had no appreciable effect on HMC preservation.

Numerous studies are available on the roles that fungi play in the deterioration of stored corn (2, 6, 19, 29), the prevalence of aflatoxins (21, 27, 30, 34), and other toxic fungal products (7, 8, 20; 32, 36) in infested corn, and changes occurring in fungal populations during quality loss in corn (4, 9, 31). Few data are available, however, on alterations of the bacterial and yeast flora during storage of shelled high-moisture corn (HMC), and only a few workers (4, 5, 11) have examined the complex interactions of molds, yeasts, and bacteria that occur during HMC storage. For some of these studies (4, 5), special attention was given to restricting gas interchange between the interior and exterior of the silos so that normal storage conditions, simulating those on the farm, could be duplicated. In one study (11), we attempted to induce spoilage of HMC by regulated periods of aeration, but the degree of aeration was insufficient to cause spoilage. Recently, we succeeded in obtaining deterioration of HMC at rates that approached those sometimes observed on the farm in defective storage units; these investigations are reported herein. In addition, comparisons were made of the genera and species recovered in the present study with those recovered in previous studies (4, 5, 11); the effects of heavy infestation by Helminthosporium maydis also were examined.

MATERIALS AND METHODS

Four metal storage structures were used (4); each was 1.8 m diameter by 3.4 m high. The structures had been damaged by high winds and contained numerous small air leaks. Two silos were filled (about 225 bushel/silo) on 6 October with 21.7% moisture, 9.9% damaged, grade 4 corn; the corn had been infested by the fungus causing Southern corn leaf blight (H. maydis). Two other silos were filled with 24.3% moisture corn of high quality.

Sampling protocols and temperature and gas measurements were performed as described previously (4). Viable cell counts were made on a plate count agar (Difco) for aerobic bacteria; deoxycholate agar (Difco) for coliforms; tomato juice agar special (Difco), supplemented with 0.1% sorbic acid, and LBS agar (BBL) for lactobacilli; and Littmns Oxgall agar (Difco), supplemented with 30 μg of streptomycin per ml, for yeasts and molds. Colonies were counted after incubation at 30 C for periods of 2 days for aerobic mesophilic bacteria and coliforms, 3 days for lactobacilli (incubated in CO₂ GasPaks, BBL), and 4 days for yeasts and molds.

Colonies for identification were randomly selected from plates representing alternate samples from the lower levels of each structure. Samples from the lower levels were used because they were more representative than samples from the upper levels of the microbial changes that would occur during storage of HMC in conventional silos. After purification on the isolation medium, lactobacilli were maintained in Micro Assay culture agar (Difco) deeps, yeasts on malt extract agar (Difco) slants containing 0.5% added agar, and molds on Czapek dox agar (Difco) slants. Cultures were incubated at 30 C for 48 h (5 days for the molds) and stored at 5 C. Coliforms were transferred to Trypticase soy agar (BBL) slants; fewer than half the cultures remained viable after storage.
for about 6 months, however, and identification of the coliforms to species was not attempted.

Extensive tests were conducted on 1,006 isolates: 308 lactobacilli, 357 yeasts, and 343 molds. For identification, lactobacilli were transferred to fresh Micro Assay culture agar deeps and then to 10 ml of brain heart infusion (Difco) broth in 15-ml dropping bottles, which were incubated for 48 h at 30°C and used as inocula. One drop of inoculum was used for each tube of medium; solid media in plates were inoculated (16 cultures/plate) with a capillary replicator (10). Data from 82 morphological, cultural, and physiological tests were scored according to the methods described by Lessel and Holt (18). The tests were largely routine (McMahon, M.S. thesis, Iowa State Univ., Ames, 1972), with the exception of the pH determinations in carbohydrate fermentation tests. Final pH determinations were made by using a semimicro-combination electrode; pH values of 5.0 or less, between 5.0 and 6.0, between 6.0 and 7.0, and above 7.0 were scored A, B, C, and D, respectively.

Yeasts identification (55 different tests) was performed according to the classification of Lodder (23) and culture techniques of Wickerham (35), using the dropping-bottle and capillary-replication techniques where possible (McMahon, M.S. thesis, 1972). Carbon-assimilation tests were recorded as: (i) negative, if, after 2 weeks at 30°C, the broth was clear with no evident sediment; (ii) weak, if there was a slight turbidity or sediment; and (iii) positive, if definite turbidity or sediment was present. Molds were identified to genus by their morphological characteristics.

RESULTS AND DISCUSSION

Bacterial populations. Counts of aerobic, mesophilic bacteria increased from about 10⁴/g at the time of ensiling to about 10⁷/g by day 71 (Fig. 1). These increases in numbers were more gradual than those observed previously (4, 11). There were few differences between counts of aerobic bacteria made on samples of normal and blighted HMC taken from either level of the four silos, although counts made on samples from one of the silos of blighted corn generally were lower than counts made on the other corn samples. We will return to this point later.

In adequately preserved corn (4), numbers of coliforms increased 10- to 100-fold during a few days of storage and then declined to fewer than 10/g during the next week or two. In the present study, however, coliform numbers remained high in the upper levels (Fig. 2) or decreased slowly in the lower levels (Fig. 3) of the silos. Thus, the presence of appreciable numbers (more than 10⁴/g) of coliforms after 2 weeks of HMC storage at ambient temperature is suggestive of abnormal storage conditions; also, decreases in coliform numbers during storage cannot be used to ascertain adequate storage conditions.

![Fig. 1. Counts of aerobic, mesophilic bacteria obtained on samples of HMC taken from the lower level of each silo during the storage period. Symbols: (O) silo 1; (□) silo 2 (normal HMC); (●) silo 3; (■) silo 4 (blighted HMC).](image1)

![Fig. 2. Counts of coliform bacteria obtained on samples of HMC taken from the upper level of each silo during the storage period. Symbols same as Fig. 1.](image2)

Reliable estimates of lactic acid bacteria were not obtained until day 21 after ensiling because of mold overgrowth on tomato juice agar plates; molds subsequently were inhibited upon the addition of 0.1% sorbic acid. LBS agar also was effective in this regard, but it was not used until day 56 of ensiling. Because counts and sequences of lactobacilli were similar for both media, the data were combined for this presentation. Numbers of lactic acid bacteria fluctuated considerably during storage in both levels of the four silos (Fig. 4; only the lower levels are shown), in contrast to the results of Hesser et al. (11) who reported that lactobacilli maintained fairly constant levels of about 10⁷/g during adequate sealed storage of HMC.

The properties of lactic acid bacteria isolated
from the corn samples were compared with descriptions in Bergey's Manual of Determinative Bacteriology (3) and data obtained on strains of Lactobacillus spp. isolated from silages (1, 11, 12, 14, 15) and other materials (13, 26). Identification was aided by preliminary computer grouping (18, 22); each strain, however, also was examined feature by feature. Some computer groups were split into subgroups, and many individual strains that did not appear in the computer groupings at high similarity values were later assigned to the groups on the basis of key properties. Detailed analyses of the data are available elsewhere (McMahon, M.S. thesis, 1972).

Table 1 shows the numbers of lactic acid bacteria isolated after various times of storage, together with data reported by Hesser et al. (11). The types of lactobacilli isolated within treatment (normal versus blighted HMC) were similar, and the data from each type of treatment were pooled in the table. Lactobacillus plantarum was the most frequent isolate, although it was not as predominant in the normal corn as it was in a previous study (11). About 80% of the strains fermented arabinose strongly and may be designated as var. arabinosus; one culture (var. pentosus) fermented arabinose and xylose. Some variant strains did not coagulate milk, and raffinose was only occasionally fermented. Other strains varied in one or more specific carbohydrate fermentations.

L. brevis was the second most prevalent species (Table 1); similar proportions of this species were isolated previously from normal corn in sealed storage (11). In the present study, L. brevis was first isolated on day 35 and remained throughout the storage period with few observable differences in dominance among silos. The delayed appearance of L. brevis is in agreement with the report of Hesser et al. (11) for good quality corn ensiled under adequate conditions. About half the L. brevis isolates were typical, with the exception of failure to hydrolyze arginine. The remaining half were negative in one or more of the following characteristics: arabinose fermentation, growth at 25 and 37 C, and resistance to 60 C for 45 min.

In addition to L. brevis, another group of lactobacilli was not encountered until day 35 of storage. This group, intermediate between L. plantarum and L. casei, conformed to Keddie's group 2 (12), except that the cells were longer (up to 4.2 μm long) and strongly fermented arabinose. Because of the prevalence of such strains in some natural habitats, perhaps another species should be promulgated.

L. buchneri was recovered at all sampling dates. For some reason that we cannot explain, very substantial numbers were isolated from three of the four silos on day 33 of storage.

L. casei-like cultures (15), isolated mostly during the latter half of the storage period (Table 1), resembled L. plantarum in some respects, but arabinose and melibiose were not fermented. Furthermore, these strains did not ferment lactose, coagulate milk, or reduce litmus; they are reportedly characteristic of poor-quality silages (16, 17). L. curvatus strains, only a few of which weakly fermented maltose, and L. coryniformis both appeared late in the storage period. The L. coryniformis isolates presented carbohydrate fermentation patterns that did not resemble any Lactobacillus spp. described in Bergey's Manual of Determinative Bacteriology (3); the cells, however, were kidney
Table 1. Frequency of isolation of certain microorganisms from deteriorating normal (N) and blighted (B) corn after various storage times

| Microorganism                                    | Storage time (days) | % of total | % in reference 5, 11 |
|-------------------------------------------------|---------------------|------------|----------------------|
|                                                 | 0       | 3       | 7       | 21      | 35      | 56      | 93      | 143     | 199     | Total   | N+B  |
| Lactic acid bacteria*                            | N       | B       | N       | B       | N       | B       | N       | B       | N       | B      |      |
| *L. plantarum*                                   | 0       | 7       | 4       | 9       | 4       | 41      | 13      | 10      | 7       | 5      | 68    | 28    | 52    |
| *L. brevis*                                      | 0       | 0       | 2       | 5       | 9       | 4       | 13      | 10      | 7       | 7      | 10    | 5     | 41    | 23    | 34    |
| Lactobacillus sp., group 2                       | 0       | 0       | 0       | 2       | 8       | 2       | 4       | 3       | 6       | 6      | 0     | 2     | 18    | 15    | 11    | 0     |
| L. buchneri                                      | 1       | 0       | 2       | 2       | 3       | 0       | 7       | 8       | 0       | 4     | 3     | 2     | 16    | 16    | 10    | 3     |
| L. casei*                                        | 0       | 1       | 0       | 0       | 2       | 1       | 2       | 1       | 3       | 8     | 9     | 13    | 15    | 9     | 0     |
| L. sanfrancisco                                  | 1       | 8       | 6       | 0       | 0       | 0       | 0       | 0       | 0       | 0     | 0     | 0     | 7     | 8     | 5     | 0     |
| L. curvatus                                      | 0       | 0       | 0       | 0       | 2       | 0       | 0       | 1       | 2       | 3     | 3     | 6     | 5     | 4     | 0     |
| L. coryniformis                                  | 0       | 0       | 0       | 0       | 0       | 0       | 0       | 0       | 0       | 0     | 0     | 0     | 6     | 1     | 7     | 3     | 3     |
| Pediococcus cerevisiae                          | 5       | 0       | 1       | 0       | 0       | 0       | 0       | 0       | 0       | 0     | 0     | 0     | 6     | 0     | 2     | 0     |
| Unidentified lactobacilli                       | 2       | 0       | 0       | 0       | 0       | 1       | 4       | 1       | 0       | 2     | 2     | 2     | 0     | 8     | 6     | 5     |

Yeast*                                            | N       | B       | N       | B       | N       | B       | N       | B       | N       | B      |      |
| H. anomala                                       | 0       | 1       | 2       | 0       | 0       | 0       | 4       | 12      | 4       | 19     | 5     | 18    | 10    | 20    | 13    | 18    | 15    | 20    | 53    | 108   | 45    | 52    |
| C. guilliermondii                                | 1       | 9       | 4       | 8       | 9       | 12      | 16      | 3       | 15      | 1      | 15    | 2     | 10    | 0     | 7     | 2     | 4     | 4     | 0     | 81    | 37    | 33    | 0     |
| C. parapsilosis                                  | 20      | 7       | 7       | 10      | 7       | 0       | 0       | 0       | 0       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 18    | 17    | 14    |
| T. candida                                       | 8       | 0       | 0       | 2       | 3       | 0       | 0       | 1       | 0       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 11    | 3     | 4     |
| C. pelliculosa                                   | 0       | 0       | 0       | 0       | 0       | 0       | 0       | 0       | 2       | 1     | 0     | 1     | 0     | 1     | 2     | 3     | 0     | 3     | 4     | 9     | 4     | 0     |

Mold*                                             | N       | B       | N       | B       | N       | B       | N       | B       | N       | B      |      |
| Fusarium spp.                                    | 35      | 41      | 17      | 8       | 9       | 8       | 15      | 8       | 6      | 0     | 9     | 2     | 0     | 1     | 1     | 0     | 0     | 0     | 0     | 92    | 68    | 47    |
| Aspergillus spp.                                 | 0       | 0       | 3       | 0       | 0       | 10      | 2       | 4       | 7     | 4     | 8     | 4     | 12    | 14    | 9     | 10    | 8     | 0     | 49    | 46    | 28    |
| Penicillium spp.                                 | 1       | 4       | 0       | 2       | 10      | 2       | 3       | 6      | 2      | 7     | 0     | 1     | 1     | 1     | 0     | 3     | 1     | 1     | 3     | 11    | 14    | 28    | 40    | 20    |
| Mucor spp.                                       | 2       | 7       | 0       | 3       | 0       | 0       | 0       | 0       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 2     | 10    | 3     |
| Cladosporium spp.                                | 3       | 0       | 0       | 0       | 0       | 0       | 0       | 1       | 0     | 0     | 0     | 0     | 0     | 1     | 0     | 0     | 0     | 0     | 0     | 0     | 2     | 1     | 1     |
| Alternaria spp.                                  | 2       | 1       | 0       | 0       | 0       | 0       | 0       | 0       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 2     | 1     | 1     |

*Includes 11% L. fermenti, not observed in the present study (11).
*Both typical and atypical strains were included; see text.
*Includes 22% C. krusei, 4% C. intermedia, 3% P. membranaceae, and 2% C. tropicalis; none of these were observed in the present study (5).
* A single Trichoderma sp. was also identified.
shaped and occurred in pairs, similar to cells described by Abo-Elnaga and Kandler (1).

Another group of isolates that did not match some definitive descriptions of lactobacilli (1, 3, 11, 12, 14, 15), resembled _L. sanfrancisco_ (13, 26). Maltose, and sometimes xylose, were the only carbohydrates fermented. Other exacting nutritional requirements observed by Kline and Sugihara (13) were absent, and our isolates occasionally were 30 to 40 μm long and did not produce CO₂. _L. sanfrancisco_ was isolated only during the early storage period (Table 1), as were a few strains of _Pediococcus cerevisiae_ that resembled the group 4 of Langston and Bouma (14).

When the data obtained in the present study are compared with those of Hesser et al. (11), it is apparent that a more diverse range of species may be characteristic of ensiled HMC in the process of deterioration, relative to less diversity in good-quality ensiled HMC. In both types of HMC, strains of _L. plantarum_ and _L. brevis_ become predominant with time of storage. In deteriorating HMC, _L. casei_-like strains also seemed to compete favorably; these and several other _Lactobacillus_ spp. were not observed by Hesser et al. (11) and seemed to have been replaced in Hesser’s study by _L. fermenti_ (Table 1).

**Yeast populations.** Yeasts play a significant role in the preservation of ensiled HMC. Huxsoll (M.S. thesis, Purdue Univ., Lafayette, Ind., 1961) reported that marked increases of yeasts occurred in deteriorating HMC at moisture contents of 18 to 23%; less marked increases were observed in corn of 23 to 28% moisture. The yeasts grew well at O₂ concentrations as low as 0.5%, and at O₂ concentrations of about 8%, molds competed with the yeasts. Our data (Fig. 5) showed that, initially, there were about 10⁴ viable yeast cells/g of corn in the lower levels of the storage units. Numbers of yeasts increased during the days 14 to 50 of storage to levels of about 10⁵ to 10⁶/g. The blighted corn contained as few as 0.01 of the numbers of yeasts contained in the undamaged corn, yet the O₂ levels in the silos containing the blighted corn were much less than those in the silos containing the normal corn. Thus, contrary to the findings of Huxsoll (M.S. thesis, 1961), the yeasts competed favorably even in structures containing 10 to 15% O₂, O₂ and moisture levels were not the primary determinants for yeast growth in HMC.

The yeast microflora of stored HMC may be divided into two groups: field species and storage species. Of the five species that predominated in our HMC samples (Table 1), _Candida parapsilosis_ and _Torulopsis candida_ were isolated early in the study and were largely replaced after the first week of storage by _Hansenula anomala-Candida pelliculosa_ and _C. guilliermondii_.

_H. anomala_- _C. pelliculosa_ accounted for 49% of the isolates. _C. pelliculosa_ is essentially a biotype of _H. anomala_, differing by the production of matted colonies and failure to produce ascospores. _C. guilliermondii_ constituted a substantial proportion of the yeasts present in the damaged corn at the time of ensiling; this yeast became predominant later (during about 1 to 12 weeks of storage) in the normal corn. In both instances _C. guilliermondii_ was gradually replaced by _H. anomala-C. pelliculosa_; this occurred more rapidly in the damaged corn where the O₂ levels were lower than in the normal corn. _C. parapsilosis_ comprised about the same percentage of the yeast flora as was observed previously (11). In contrast to Lodder’s description of this species (23), our isolates, except for two strains, all assimilated cellobiose and salicin. Only 4% of the yeasts were identified as _Torulopsis candida_; none assimilated raffinose or cellobiose. A single strain of _C. albidos_ was identified.

Four yeast species identified in a previous study (4) were not encountered in the present study; these seem to have been replaced by three other species (Table 1).

**Mold populations.** Initial mold counts (probably consisting mostly of mold spores) were about 10%g of corn (Fig. 6). Since initial numbers of molds were approximately equal in both normal and _H. maydis_-infested corn, _H. maydis_ and secondary fungal invaders did not appreciably alter the total mold (spore) population at
the time of ensiling; this is contrary to the findings of some other workers (33).

In the present study, mold counts reached between 10^4 and 10^9/g of corn in three silos during storage. Counts from one silo of *H. maydis*-infested corn fluctuated widely and were low during winter storage. The differences in total mold counts between the two silos containing blighted corn (Fig. 5) could not be explained, but a similar occurrence for yeast numbers in these two silos (Fig. 4) suggests that some factor(s) restricted growth of both molds and yeasts in the lower level of the same silo. These results are in contrast to those obtained on adequately ensiled HMC, in which mold counts increased from 0 to 2 weeks and then rapidly declined to less than 10^3/g within 30 days of storage (4).

Mold microflora sequences followed similar patterns in both normal and *H. maydis*-infested corn (Table 1) and were similar to those observed by other investigators (see 6, 29). Initially, the field (6) fungi (*Fusarium*, *Mucor*, *Cladosporium*, *Alternaria*, and *Trichoderma*) predominated. At this time, storage (6) fungi comprised no more than 4% of the isolates. *H. maydis* was not isolated from the blighted corn, probably because it was greatly outnumbered by *Fusarium* spp. (28); in addition, sporulation of *H. maydis* often is poor, and its isolation is difficult unless infected kernels are surface-sterilized before plating (33).

No field fungi were recovered on day 7 after ensiling, excluding *Fusarium* spp. (consistently isolated through day 56 in two silos) and an occasional *Cladosporium* isolate. Storage species (*Aspergillus* and *Penicillium* spp.) were isolated on a regular basis by day 7 of ensiling. In three of the silos, *Penicillium* spp. generally appeared in large numbers only during the earlier and later stages of ensiling, while *Aspergillus* spp. predominated during winter storage. In the fourth silo, both species were present in approximately equal ratios during winter storage; in this silo, which also had lower mold counts and internal temperatures of 4 to 10 C lower than the other three silos, *Aspergillus* spp. did not multiply rapidly during winter storage.

Three morphologically distinct groups of aspergilli and three groups of penicillia were observed, but these were not characterized further. The three groups of penicillia may represent the three species described by Mislievec and Tuite (24, 25) in stored corn.

Numbers of lactic acid bacteria and yeasts, and the sequences of species, in deteriorating HMC were similar to those reported for good-quality ensiled HMC (4, 11). Numbers of molds, especially aspergilli and penicillia, were significantly greater, however, in corn undergoing spoilage. Therefore, as we suspected, molds are the primary factors in deteriorating HMC, even under conditions of low oxygen-high CO_2_ tension. Bacteria and yeasts play a relatively minor role in deterioration (except as scavengers of O_2_ in sealed structures) because bacterial and yeast populations were similar for both deteriorating and adequately stored HMC. Damage of corn by *H. maydis* does not substantially alter the fermentation pattern or susceptibility of the corn to spoilage during sealed storage.

**LITERATURE CITED**

1. Abo-Elnaga, I. G., and O. Kandler. 1965. Zur Taxonomie der Gattung Lactobacillus Beijerinck. I. Das Subgenus Streptobacterium Orla Jensen. Zentralb. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. II 119:1–36.
2. Barron, G. L., and R. W. Lichtwardt. 1959. Quantitative estimations of the fungi associated with deterioration of stored corn in Iowa. Iowa State J. Sci. 34:147–155.
3. Buchanan, R. E., and N. E. Gibbons (ed.). 1974. Bergey’s manual of determinative bacteriology, 8th ed. Williams and Wilkins Co., Baltimore.
4. Burmeister, H. R., P. A. Hartman, and R. A. Saul. 1966. Microbiology of ensiled high-moisture corn. Appl. Microbiol. 14:31–34.
5. Burmeister, H. R., and P. A. Hartman. 1966. Yeasts in ensiled high-moisture corn. Appl. Microbiol. 14:35–38.
6. Christensen, C. M., and H. M. Kaufmann. 1969. Grain storage; the role of fungi in quality loss. University of Minnesota Press, Minneapolis.
7. Ciegler, A., S. Kadas, and S. J. Ajl (ed.). 1971. Microbial toxins, vol. 6. Academic Press Inc., New York.
8. Curtin, T. M., and J. Tuite. 1966. Emesis and refusal of feed in swine associated with Gibberella zeae-infected corn. Life Sci. 5:1937–1944.
9. Danziger, M. T., M. P. Steinberg, and A. I. Nelson. 1973. Effect of CO_2_ moisture content and sorbate on safe storage for wet corn. Trans. Am. Soc. Agric. Eng.
22. Hartman, P. A., and P. A. Pattee. 1968. Improved capillary-action replicating apparatus. Appl. Microbiol. 16:679–682.

10. Hartman, J. M., P. A. Hartman, and R. A. Saul. 1967. Lactobacilli in ensiled high-moisture corn. Appl. Microbiol. 15:49–54.

12. Keddie, R. M. 1969. The properties and classification of lactobacilli isolated from grass and silage. J. Appl. Bacteriol. 22:403–416.

13. Kline, L., and T. F. Sugihrara. 1971. Microorganisms of the San Francisco sour dough bread process. II. Isolation and characterization of undescribed bacterial species responsible for the souring activity. Appl. Microbiol. 21:459–465.

14. Langston, C. W., and C. Bouma. 1960. A study of the microorganisms from grass silage. I. The coci. Appl. Microbiol. 8:212–222.

15. Langston, C. W., and C. Bouma. 1960. A study of the microorganisms from grass silage. II. The lactobacilli. Appl. Microbiol. 8:223–234.

16. Langston, C. W., and C. Bouma. 1960. Types and sequence change of bacteria in orchardgrass and alfalfa silages. J. Dairy Sci. 43:1575–1584.

17. Langston, C. W., H. G. Wiseman, C. H. Gordon, W. C. Jacobson, C. G. Melin, L. A. Moore, and J. R. McCalmon. 1962. Chemical and bacteriological changes in grass silage during the early stages of fermentation. I. Chemical changes. J. Dairy Sci. 45:396–402.

18. Lessel, E. F., and J. G. Holt. 1970. Presenting and interpreting the results, p. 50–58. In W. R. Lockhart and J. Liston (ed.). Methods for numerical taxonomy. American Society for Microbiology, Washington, D.C.

19. Lichtwardt, R. W., G. L. Barron, and L. H. Tiffany. 1966. Mold flora associated with shelled corn in Iowa. Iowa State J. Sci. 33:1–11.

20. Lillehoj, E. B. 1973. Feed sources and conditions conducive to production of aflatoxin, ochratoxin, Fusarium toxins, and zearalenone. J. Am. Vet. Med. Assoc. 163:1261–1284.

21. Lindenfelser, L. A., and A. Ciegler. 1970. Studies on aflatoxin detoxification in shelled corn by ensiling. Agric. Food Chem. 18:640–643.

22. Lockhart, W. R., and P. A. Hartman. 1963. Formation of monothetic groups in quantitative bacterial taxonomy. J. Bacteriol. 85:68–77.

23. Lodder, J. (ed.). 1970. The yeasts. North-Holland Publishing Co., Amsterdam.

24. Mislivec, P. B., and J. Tuite. 1970. Species of Penicillium occurring in freshly-harvested and in stored dent corn kernels. Mycologia 62:67–74.

25. Mislivec, P. B., and J. Tuite. 1970. Temperature and relative humidity requirements of species of Penicillium isolated from yellow dent corn kernels. Mycologia 62:75–88.

26. Ng, H. 1972. Factors affecting organic acid production by sourdough (San Francisco) bacteria. Appl. Microbiol. 23:1153–1159.

27. Richard, J. L., and S. J. Cysewski. 1971. Occurrence of aflatoxin producing strains of Aspergillus flavus Link in stored corn. Mycopathol. Mycol. Appl. 44:221–229.

28. Seerley, R. W., D. M. Baird, R. S. Lowrey, and T. L. Huber. 1972. Effect of Helminthosporium maydis infection on feeding value of corn. J. Animal Sci. 34:132–136.

29. Semeniuk, G. 1984. Microflora, p. 77–151. In J. A. Anderson and A. W. Alcock (ed.). Storage of cereal grains and their products. American Association of Cereal Chemists, St. Paul.

30. Shotwell, O. L., C. W. Hesseltine, M. L. Goulden, and E. E. Vandegraft. 1970. Survey of corn for aflatoxin, zearalenone, and ochratoxin. Cereal Chem. 47:700–707.

31. Tuite, J. F., C. G. Haugh, G. W. Issacs, and C. C. Huxsoll. 1967. Growth and effect of molds in stored high-moisture corn. Trans. Am. Soc. Agric. Engr. 10:730–732, 737.

32. Vesonder, R. F., A. Ciegler, and A. H. Jensen. 1973. Isolation of the emetic principle from Fusarium-infected corn. Appl. Microbiol. 26:1008–1010.

33. Vojnovich, C. R. A. Anderson, and J. J. Ellis. 1972. Microbial reduction in stored and dry-milled corn infected with Southern Corn Leaf Blight. Cereal Chem. 49:346–353.

34. Watson, S. A., and K. R. Yahl. 1971. Survey of aflatoxins in commercial supplies of corn and grain sorghum used for wet-milling. Cereal Sci. Today 16:153–155, 163.

35. Wickerham, L. J. 1981. Taxonomy of yeasts. U.S. Dept. Agric. Tech. Bull. 1029:1–19.

36. Wogan, G. N. (ed.). 1965. Mycotoxins in foodstuffs. Massachusetts Institute of Technology Press, Cambridge.