Survival of Aspergillus flavus and Fusarium moniliforme in High-Moisture Corn Stored Under Modified Atmospheres

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Freshly harvested high-moisture corn with 29.4% moisture and corn remoistened to 19.6% moisture were inoculated with Aspergillus flavus Link ex Fr. and stored for 4 weeks at about 27 °C in air (0.03% CO₂, 21% O₂, and 78% N₂) and three modified atmospheres: (i) 99.7% N₂ and 0.3% O₂; (ii) 61.7% CO₂, 8.7% O₂, and 29.6% N₂; and (iii) 13.5% CO₂, 0.5% O₂, and 84.8% N₂. Kernel infections by A. flavus, Fusarium moniliforme (Sheld.) Syd. et Hans., and other fungi were monitored weekly. The modified-atmosphere treatments delayed deterioration caused by A. flavus and F. moniliforme, but their growth was not completely stopped. A. flavus survived better in the remoistened than in the freshly harvested corn. F. moniliforme survived in both. A. flavus and F. moniliforme were the dominant fungi in corn removed from the modified atmospheres and exposed to normal air for 1 week.

Harvesting high-moisture corn frequently causes damage to the kernels, making the corn subject to rapid fungal deterioration. Corn at 30% moisture can be held for only 2.6 days at 24 °C before a reduction of quality is observed (1). Aspergillus flavus Link ex Fr. is frequently associated with moldy corn in the southeastern United States (2, 9), and infestation may lead to aflatoxin contamination.

Marasas and Smalley (7) isolated 42 different species of fungi and actinomycetes from moldy corn. Cladosporium herbarum (Pers.) Link ex S. F. Gray and Fusarium moniliforme (Sheld.) Syd. et Hans. were initially present in large numbers, but after heating, Aspergillus fumi-gatus Pres. and A. flavus were among the dominant species. F. moniliforme grown in pure culture at 25 °C was toxic to chicks and rats (7). One toxic compound produced by F. moniliforme is moniliformin, the potassium salt of 1-hydroxycyclo-1-ene-3,4-dione (10).

Aflatoxin production in peanuts inoculated with A. flavus could be decreased by storage in 99% N₂ or 60 to 80% CO₂ (5, 8). Wilson and Jay (11) found that modified-atmosphere storage of high-moisture corn did not allow an aflatoxin accumulation of over 20 μg/kg as long as the corn was exposed to the modified atmospheres. Lilleyhoj et al. (6) found that penicillic acid could not be detected in high-moisture corn inoculated with Penicillium martensii Bourge incubated for 4 weeks at 5 and 10 °C in a 60% CO₂ atmosphere.

Since high-moisture corn deteriorates rapidly, modified-atmosphere storage of corn was tested to find whether undesirable fungal deterioration could be delayed. Kernel infection by A. flavus, F. moniliforme, and other fungi was monitored weekly in freshly harvested and remoistened corn after storage in modified atmospheres.

MATERIALS AND METHODS

Freshly harvested, shelled corn with an initial moisture content of 29.4% and corn remoistened to 19.6% were used for these experiments; damaged kernels were not removed from the initial samples. The corn was inoculated with 150 ml of a spore suspension of A. flavus isolate NRRL 5520, giving about 780,000 conidia/liter of corn. The corn was put in 3.8-liter jars that were placed in water baths at 26.9 ± 1 °C and continuously exposed to 40 ml of one of four different atmospheres per min: (i) air (0.03% CO₂, 21% O₂, and 78% N₂); (ii) N₂ (99.7% N₂ and 0.3% O₂); (iii) CO₂ + low O₂ (61.7% CO₂, 8.7% O₂, and 29.6% N₂); (iv) output from a controlled-atmosphere (CA) generator previously pumped into cylinders with about 13.5% CO₂, 0.5% O₂, and 84.8% N₂. Details of the treatment method, temperatures, moistures, aflatoxin contents, and atmosphere changes in the corn were described by Wilson and Jay (11).

Two 0.4-liter samples were taken from each jar after 1, 2, 3, and 4 weeks of exposure to each atmosphere. One sample was exposed for 1 week to the atmosphere in a room maintained at 26.7 ± 1 °C at 60 ± 5% relative humidity. The other sample was tested within 2 days for mycoflora. Two replicates of the freshly harvested corn were held for 4 weeks in the CA atmosphere and sampled only once.
Fungi growing from kernels disinfested by immersion in 0.5% aqueous sodium hypochlorite for 3 min were observed in two ways. First, 25 kernels from each replicate were plated and incubated at 25 C on M3S1B agar medium (3). Four to six days after plating, hyphal tips of fungal colonies were transferred to malt extract agar. Identifications were made by growing the fungi on malt extract, Czapek-Dox, or Dodge corn meal agars (4). Second, 100 kernels from each replicate were placed on M3S1B agar, 10 kernels per plate, incubated at 28 C, and observed by using a binocular microscope 3 to 4 days later.

RESULTS

Percentage of infection of the freshly harvested corn by A. flavus after storage in modified atmospheres is given in Fig. 1. In the normal air treatment the corn was covered with mycelium by the end of the first week and many of the kernels had germinated. The percentage of infection by A. flavus was 89 to 90% in the air treatment by the end of the second week. Infection by A. flavus was highest after a 1-week exposure to the modified atmosphere treatments and declined in subsequent weeks. F. moniliforme was recovered from 21% of the kernels initially and after 4 weeks from 100% of the kernels from the CO₂ + low O₂ treatment, 48% from the N₂ treatment, and 25% from the CA treatment; other fungi were recovered from 16, 2, and 1%, respectively, of the kernels. The CO₂ + low O₂ product had an unpleasant odor and was visibly overgrown by an unidentified yeast. The N₂ and CA products appeared sound, but an aromatic odor was detected. In the subsamples exposed to modified atmospheres for 4 weeks and then held for 1 week in normal air, 100% of the kernels from all three treatments contained F. moniliforme, whereas A. flavus was recovered from 18, 5, and 8%, respectively.

In the remoistened corn the number of kernels infected by A. flavus did not decrease in any of the treatments (Fig. 2A). Kernel infection by A. flavus increased in the N₂ and CA treatments between the first and third weeks. Infection by A. flavus was lowest in the CO₂ + low O₂ treatment. The incidence of F. moniliforme was initially 67%, and kernel infection increased in all modified-atmosphere treatments (Fig. 2B). Kernel infection by Eurotium rubrum Konig, Spiek. & Bremer survived the high CO₂ + low O₂ treatment but was not present in the N₂ or CA treatments after 4 weeks (Fig. 2C). Penicillium citrinum Thom (Fig. 2D), Mucor spinosus v. Tieg., and Aspergillus tamarii Kita populations increased only in the air treatment. Mucor hiemalis Wehmer and Rhizopus oryzae Went et Geerlings were present in more than one treatment, but the percentage of infection by these fungi was never high. The other fungi recovered in low numbers were Penicillium granulatum Bain., Absidia ramosa Lindt, Penicillium variable Sopp, Cephalosporium acremonium Corda, Aspergillus repentens d By., Penicillium cyclopium Westling, Rhizopus arrhizus Fischer, Mucor racemosus Fres., Penicillium roqueforti Thom, Penicillium citrinum series, and Candida sp. After 4 weeks of exposure to the CO₂ + low O₂, N₂, and CA treatments there was 0, 1, and 1% infection by Penicillium species, 11, 0, and 1% infection by Aspergillus species other than A. flavus, and 2, 1, and 3% infection by members of the Mucorales, respectively.

DISCUSSION

Modified-atmosphere storage of high-moisture corn could be used for temporary holding before drying or for longer-term storage. The main advantages of CA storage would be residue-free insect control and the retardation of undesirable fungal deterioration. Nitrogen or CA generator-produced atmospheres can contain less than 1% O₂ and control insects and the growth of many fungi.

Survival and infection of A. flavus were dramatically different in the freshly harvested and
FIG. 2. Percentage of infection of kernels by (A) Aspergillus flavus, (B) Fusarium moniliforme, (C) Eurotium rubrum, and (D) Penicillium citrinum in remoistened high-moisture corn inoculated with A. flavus and stored in modified atmospheres. Gas mixtures were the same as in Fig. 1. Values are averages of three replications, 25 kernels per replication.

remoistened corn, although the temperatures were held fairly constant. A. flavus did not survive well in the freshly harvested corn but survived in the remoistened corn. No visible A. flavus was seen, and less than 20 μg of aflatoxins per kg was produced in any of the modified-atmosphere treatments (11). A. flavus in naturally infested corn would probably survive better in freshly harvested high-moisture corn stored in modified atmospheres than in our experiments because it would be established and probably have less competition.

In contrast, F. moniliforme survived in both the freshly harvested and remoistened corn. The incidence of F. moniliforme recovered from the kernels increased in the remoistened corn and rapidly invaded freshly harvested corn upon removal from the modified atmospheres. Since F. moniliforme is capable of being toxigenic, feeding studies would be necessary be-
fore it could be determined whether this is a potential problem.

Little growth of any fungus was apparent in the corn from the N₂ and CA treatments. In the CO₂ + low O₂ treatment the corn was visibly damaged by a yeast. The CO₂ + low O₂ treatment was the least desirable because of the yeast, the survival of *E. rubrum*, and the unpleasant odor of the corn.

The high-moisture product from the N₂ or CA treatments may be useful if dried immediately or used immediately for feed, although in practice the temperature would not be controlled and the fungi may be different. The product is no more stable than untreated high-moisture corn and may deteriorate very rapidly upon exposure to the normal atmosphere.

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LITERATURE CITED