# Reduction of Mycoflora and Control of In-Shell Weevils in Pecans Stored Under High Carbon Dioxide Atmospheres

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**ABSTRACT**

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High carbon dioxide ($CO_2$) atmospheres reduced the mycoflora and controlled in-shell weevils in pecans stored under accelerated storage conditions of 7 C and 65% relative humidity. After 5 mo in atmospheres of 21% oxygen ($O_2$) and 30% $CO_2$, pecan weevil mortality was 100%, total mycoflora isolated from kernels was significantly reduced, and off-flavors had not yet developed in the kernels. In atmospheres of 3 and 10% $CO_2$ (plus 21% $O_2$) or in 1% $O_2$ (with or without 30% $CO_2$), weevil mortality was less than 100%. Objectionable off-flavors developed in high $CO_2$ atmospheres after 6 mo. *Alternaria* and *Pestalotia* spp. were the fungi most frequently isolated from pecans held in air. After 5 mo in high $CO_2$ atmospheres, *Alternaria*, *Penicillium*, and *Fusarium* spp. were the most frequently isolated fungi.

Additional key words: *Carya illinoensis*, controlled atmospheres, *Curculio caryae*, modified atmospheres, storage fungi

In the southeastern United States, pecan (*Carya illinoensis* (Wang.) K. Koch) nuts are harvested from October to January. Much of the crop is refrigerated and stored to supply shelling plants with pecans throughout the year (11). In good crop years, refrigerated storage is an important means of holding excess supply until market conditions become more favorable to the producer. Since a heavy crop year is inevitably followed by a light crop year because of the alternate bearing nature of the pecan tree, long-term storage of pecans is also important in stabilizing the supply.

Pecans are semiperishable. High-quality pecans may be held at 0–2 C for 1 yr before detectable off-flavors or rancidity develop (12). Not all pecans are of high quality at the beginning of storage, however. Late-harvested nuts may have lost freshness of flavor and the desirable light amber kernel color. Pecans may also be infested with the pecan weevil (*Curculio caryae* (Horn)), which damages kernels and provides avenues of invasion for postharvest storage fungi (9).

Although refrigerated storage arrests the development of storage fungi and reduces damage caused by insects, it does not significantly affect survival of these organisms (5,8). Control is important, however, because of quarantine restrictions against the shipment of nuts infested with the pecan weevil (6) and because of the potential for toxin production by fungi commonly found on weevil-damaged nuts (8,9).

Modified atmosphere storage is used to maintain the market quality of perishable commodities (1,3). Darkening of kernel color is prevented in pecans held in low oxygen atmospheres (5). Controlled atmospheres are also being considered to control insect infestations in stored grain (7) and in almonds (2). Levels of oxygen less than 1% and carbon dioxide ($CO_2$) greater than 10% inhibit the growth of fungi that cause postharvest decays of perishable horticultural crops (10). No information is available, however, on the effects of modified atmospheres on insect and fungal infestations of pecan nuts in storage.

This report summarizes some effects of low oxygen and high $CO_2$ atmospheres on the flavor and appearance of pecan nuts in storage, the survival of in-shell and free pecan weevils, and the infestation of pecans by storage fungi.

## MATERIALS AND METHODS

Weevil-infested pecans cv. Stuart were harvested from trees in experimental orchards at Byron, GA. Nuts were shaken from the trees before normal drop, as they are in large commercial orchards. Samples were blended, subdivided into smaller lots, and sealed in 2.5-L jars equipped with gas inlets and outlets. Jars were flushed with 15 volumes of dry nitrogen and then connected to gas lines to cylinders containing gases of certified premixed compositions. The controlled atmospheres tested contained nitrogen and 21% $O_2$ and the mixtures listed in Table 1. Jars were stored in a chamber at 7 C to accelerate storage conditions and to encourage the development of storage fungi. Gas flows were maintained at about 0.6 L/hr as determined with a rotometer, and gas humidity was maintained at 65% by blending twin flows of saturated and dry gas at a ratio of 65:35.

Nuts from one jar from each atmosphere were removed for examination after 1 wk in storage and then once each month thereafter for 7 mo. At each sampling period approximately 250 (2 kg) nuts were shelled by machine, and kernel weights were determined for the sound and the weevil-damaged fractions. From 175 to 200 additional nuts from the same chambers were hand-shelled, and survival ratios were taken for in-shell weevils. Samples of weevil-damaged kernel pieces were surface-sterilized for 5 min with 0.5% sodium hypochlorite (10% Clorox) solution and rinsed in sterile distilled water; 2- to 4-mm sections were excised aseptically from each piece and

**Table 1.** Fungal colonies isolated from weevil-damaged pecan kernels held in air or in modified atmospheres at 7 C and 65% relative humidity

| Storage atmosphere | No. of colonies[y] after storage (mo) | | | | | Total[z] |
|---|---|---|---|---|---|---|
| | 2 | 4 | 5 | 6 | 7 | |
| Air | 112 | 117 | 104 | 88 | 57 | 478 a |
| 1% $O_2$ | 106 | 114 | 115 | 80 | 44 | 459 a |
| 21% $O_2$ + 3% $CO_2$ | 72 | 60 | 57 | 55 | 12 | 256 b |
| 21% $O_2$ + 10% $CO_2$ | 69 | 67 | 42 | 61 | 34 | 273 bc |
| 21% $O_2$ + 30% $CO_2$ | 70 | 62 | 21 | 19 | 9 | 181 c |
| 1% $O_2$ + 30% $CO_2$ | 52 | 49 | 7 | 2 | 1 | 111 d |
| Totals[y] | 481 a | 469 a | 346 b | 305 b | 157 c | |

[y] Isolated from pieces of 100 kernels plated on malt-salt agar and incubated at 21 C for 3 wk.
[z] Totals followed by the same letter do not differ significantly ($P = 0.05$) as determined by Duncan's multiple range test.

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**Table 2.** Predominant genera of fungi isolated from weevil-damaged kernels stored in air or 21% $O_2$ + 30% $CO_2$ + $N_2$ at 65% relative humidity

| Genus | No. of colonies[x] after storage (mo) | | | | | | Total | Percent of all colonies |
|---|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 5 | 6 | 7 | | |
| Air | | | | | | | | |
| *Alternaria* | 74 | 62 | 67 | 72 | 46 | 15 | 336 | 56.0 |
| *Cladosporium* | 5 | 3 | 2 | 0 | 0 | 1 | 11 | 1.8 |
| *Penicillium* | 11 | 14 | 20 | 1 | 6 | 1 | 53 | 8.8 |
| *Fusarium* | 22 | 21 | 3 | 1 | 5 | 7 | 59 | 9.0 |
| *Pestalotia* | 10 | 10 | 25 | 18 | 22 | 27 | 112 | 17.8 |
| Miscellaneous[y] | 0 | 2 | 0 | 12 | 10 | 6 | 30 | 6.5 |
| Total[z] | 122 a | 112 a | 117 a | 104 a | 89 a | 57 a | 601 | |
| 21% $O_2$ + 30% $CO_2$ + $N_2$ | | | | | | | | |
| *Alternaria* | 74 | 42 | 31 | 5 | 6 | 3 | 161 | 53.4 |
| *Penicillium* | 11 | 8 | 12 | 15 | 13 | 5 | 52 | 18.9 |
| *Fusarium* | 22 | 7 | 4 | 0 | 0 | 0 | 43 | 14.3 |
| *Pestalotia* | 10 | 8 | 8 | 0 | 0 | 0 | 16 | 7.0 |
| *Cladosporium* and Miscellaneous | 5 | 3 | 4 | 1 | 0 | 3 | 19 | 6.3 |
| Total[z] | 122 a | 70 ab | 62 ab | 21 b | 19 b | 9 b | 301 | |

[x] Isolated from pieces of 100 kernels plated on malt-salt agar and incubated at 21 C for 3 wk.
[y] Includes *Aspergillus*, *Phoma*, *Eurotium*, *Trichothecium*, *Rhizopus*, and unidentifiable species.
[z] Monthly totals not followed by the same letter differ significantly ($P = 0.05$) as determined by Duncan's multiple range test.

**Table 3.** Mortality of in-shell pecan weevils after storage in modified atmospheres at 7 C and 65% relative humidity

| Storage atmosphere | Percent mortality[y] after storage (mo) | | | | | | Average[z] |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Air | 0 | 33 | 24 | 32 | 27 | 34 | 25.0 a |
| 1% $O_2$ + $N_2$ | 0 | 37 | 38 | 21 | 20 | 28 | 24.0 a |
| 1% $O_2$ + 30% $CO_2$ + $N_2$ | 8 | 81 | 74 | 86 | 90 | 90 | 71.5 b |
| 21% $O_2$ + 30% $CO_2$ + $N_2$ | 62 | 95 | 92 | 100 | 100 | 100 | 91.5 b |

[y] Based on average of 32 weevils found in each lot of 175–200 pecans.
[z] Averages not followed by the same letter differ significantly ($P = 0.05$) as determined by Duncan's multiple range test.

plated on malt-salt agar. Fungal colonies developing from sections after 3 wk of incubation at 21 C were classified and enumerated by genera. Those not readily identifiable were classed as miscellaneous. Samples of sound kernels from selected atmosphere treatments were tested by a panel of three tasters for flavor acceptability. Panelists were instructed to taste and rate each sample as acceptable (fresh, little flavor, but acceptable) or as unacceptable (no flavor, off-flavor, rancid).

Additional confirmatory tests were done on the effects of selected modified atmospheres at 21 C on the survival of free weevils. Weevils were collected on trays as they emerged from infested pecans held for 2 wk at 21 C. Seventy-five weevils per treatment were placed on moist filter paper pads in 54-mm petri dishes modified with gas inlets and outlets. Humidified, premixed gasses flowed through the jars at 0.2 L/min. Weevil mortality was recorded during a 2-wk treatment at room temperature. Identical tests were conducted in 1976, 1977, and 1978. Data (x) were transformed to square roots of (x + 0.5) and subjected to analysis of variance and Duncan's multiple range test.

## RESULTS

**Mycoflora.** The number of fungal colonies isolated from weevil-damaged pecans held in air or in modified atmospheres decreased as time in storage increased (Table 1). At 2 mo after storage, 481 colonies were isolated from pecans in all treatments, and the total was significantly reduced after 5 and 7 mo of storage.

Treatment atmospheres also affected total colony counts. High $CO_2$ concentrations generally reduced survival. Colony counts from kernels stored in $O_2$ plus 3% or more $CO_2$ were, overall, significantly lower than those from kernels stored in air (Table 1). Thirty percent $CO_2$ was more effective than 3% $CO_2$ in reducing survival. Low oxygen alone had no effect on survival of mycoflora compared with air, but combined with 30% $CO_2$, it significantly reduced survival compared with an atmosphere of 21% $O_2$ plus 30% $CO_2$.

The predominant genera of fungi isolated from pecans stored in air were *Alternaria*, *Pestalotia*, *Fusarium*, and *Cladosporium*; other fungi included *Aspergillus*, *Phoma*, *Eurotium*, *Trichothecium*, *Rhizopus*, and unidentified isolates (Table 2). *Alternaria* spp. predominated, making up 56% of the colonies counted, and *Pestalotia* was the second most frequently isolated genus.

In general, modified atmospheres did not affect the basic populations of fungi isolated, although percent occurrence of some genera differed (data not shown). Changes were most evident after 4 or 5 mo in storage. In 21% $O_2$ plus 30% $CO_2$, *Alternaria* spp. were dominant up to the fourth month, after which their occurrence was considerably reduced (Table 2). *Penicillium* became the dominant genus in the mycoflora after 5 mo. *Pestalotia* was seldom isolated from any high $CO_2$ treatment after 5 mo.

**Weevil mortality.** Low oxygen atmospheres alone had no effect on weevil mortality, but mortality was significantly higher in high $CO_2$ than in air (Table 3). Weevil mortality was 100% after 4 mo in 21% $O_2$ plus 30% $CO_2$. In an atmosphere of 1% $O_2$ plus 30% $CO_2$, mortality never exceeded 90%.

The insecticidal effect of high $CO_2$ was similarly demonstrated in a separate test with weevils out of the shells exposed to modified atmospheres for 2 wk. Average mortality of weevils in 30% $CO_2$ with 21% $O_2$ (79–85% mortality) was significantly higher than in atmospheres without $CO_2$ (30–42% mortality).

**Flavor stability of pecans.** The aroma and flavor of pecan meats stored in air or in any of the atmospheres tested were acceptable to panelists up to 3 mo of storage (data not shown). Loss of fresh flavor was detected in nuts held for 4 mo in low oxygen atmospheres with or without $CO_2$. On the fifth month, loss of flavor was detected in nuts held in 21% $O_2$ plus 30% $CO_2$, and by the sixth month unacceptable off-flavors were present in all high $CO_2$ treatments.

## DISCUSSION

The storage temperature selected for these tests (7 C) was designed to accelerate the process of evaluation rather than to be representative of optimum storage temperatures for in-shell pecans, which are 0–2 C. These evaluations determined that low oxygen atmospheres (1%) did not provide 100% control of in-shell weevils, did not significantly reduce the mycoflora, and caused relatively rapid flavor deterioration. This is consistent with the known tolerance of some stored-products insects (7) and fungi (10) to oxygen concentrations of 1% or more. Less than 1% $O_2$ concentration is also required to substantially extend the storage life of pecans (4). High $CO_2$ atmospheres were beneficial, however, in that they provided 100% control of in-shell weevils (after 4 mo), tended to reduce the survival of mycoflora, and preserved fresh flavor. Similar results have been obtained with high $CO_2$ storage of grains (7) and some horticultural crops (1).

At the high storage temperatures of these tests, 5 mo in high (30%) $CO_2$

atmospheres was the limit of flavor stability of pecans. Although objectionable off-flavors did not develop until the sixth month, detectable flavor loss occurred. At 5 mo, however, high $CO_2$ atmospheres controlled all weevils and significantly reduced the mycoflora. It is possible, therefore, to control in-shell weevils and reduce mycoflora with modified atmospheres within the tolerance of the commodity. Further tests are needed to determine maximum storage times in high $CO_2$ atmospheres at lower, recommended temperatures.

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