Fungal Susceptibility at Four Temperature-Moisture Combinations and Carbon Dioxide Kit Color Reader Evaluation

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ABSTRACT

The susceptibility of shelled corn to invasion by storage fungi was assessed using a carbon dioxide (CO_2) test kit. Shelled corn samples were rewetted to either 16 or 21% wb moisture content (MC) and incubated ≤80 hr at either 24 or 30°C in sealed glass jars. CO_2 concentration in the jars was quantified using the color number (0–5) of an indicator gel, which was determined either by visual comparison to a color card (VR) or using a Digital Color Reader (DCR). Plots of DCR color numbers versus time were smoother than those for VR color numbers. The DCR was better able to distinguish differences among samples, especially when color numbers were <2.0. The highest coefficients of determination (r^2) for linear regressions of color number versus ergosterol difference were 0.75 for the DCR readings and 0.73 for the VR readings. Rankings of fungal susceptibility were similar at all four temperature-MC combinations and many r^2 values for linear regressions comparing readings at other conditions with those at 21% MC and 24°C were statistically significant ($\alpha \leq 0.05$). Incubation conditions and time of prior storage had a noticeable yet relatively minor effect on which fungi infected the kernels.

Fungal invasion is a major source of quality deterioration in shelled corn during shipment and storage. It is affected by factors such as length of time in storage and previous storage conditions. Managers of elevators, storage facilities, or processing plants have little or no information about these factors and must assess the likelihood of spoilage by visually examining a sample of shelled corn and measuring its moisture content (MC). As stated previously (Moog et al 2008), we believe that a relatively simple carbon dioxide (CO_2) test kit can be used to assess susceptibility of shelled corn to fungal invasion. Fungal susceptibility, as it is used here, is defined as the likelihood that fungi will grow during subsequent storage or shipment if the shelled corn is subjected to conditions conducive to fungal growth. Measurement of fungal susceptibility would benefit the industry by allowing more susceptible corn lots to be utilized before fungal growth reaches unacceptable levels. Furthermore, information on susceptibility would be useful for selecting corn lots to be exported overseas to tropical or subtropical regions where temperatures and relative humidities are conducive to fungal growth. Even corn shipped to temperate regions may be exposed to high temperatures and relative humidities when the vessel passes through subtropical or tropical regions.

In an earlier study (Moog et al 2008), we used a test kit to measure CO_2 evolution from samples rewetted to 21% MC and incubated at 24°C for ≤80 hr. We assumed that fungal growth during the incubation period could be assessed on the basis of difference in ergosterol before and after testing and concluded that CO_2 evolution measured using the test kit was a reasonable measure of fungal susceptibility. The linear regression models relating ergosterol differences to test kit color number had coefficients of determination (r^2) of 0.46–0.60.

One source of uncertainty associated with using the CO_2 test kits is obtaining a consistent and reliable assessment of the color number from the paddles that are inserted into the incubation containers. Woods End Laboratories (Mount Vernon, ME) recently developed a digital color reader (DCR) to determine the paddle color number from 0 to 5. The DCR can determine the color number to within ±0.01 unit, which increases resolution by a factor of 5–10 compared with the current standard method of estimating paddle color by visual comparison of its color to numbered colors on a color card. The DCR is based on diode-array technology in which incident light at three wavelengths is reflected from the paddle and detected using a 16-bit micro-array panel (Woods End Laboratories 2006).

Jonsson et al (1997) stated that the most important factors affecting the colonization of fungi are grain MC and temperature. They found that maximum storage time was halved when the MC at harvest increased by 2–3%, or if storage temperature increased by 5°C.

Chitrakar et al (2006) used the CO_2 test kit to evaluate effect of moisture and time on mold growth for storage at 27°C and 18–22% MC. However, they used only one corn cultivar in their tests and did not evaluate the effect of temperature. Reed et al (2002) conducted storage experiments in which they used a temperature of 25°C to simulate highland tropical conditions and 30°C to simulate lowland tropical conditions. They found that relatively little deterioration occurred over a period of two months when the samples were stored at highland conditions, while mold biomass doubled and there was a significant increase in percentage of kernels with mold infection when samples from the same bulk grain lot were stored at lowland conditions. In the earlier study of fungal susceptibility (Moog et al 2008), we did not consider the effects of using a higher incubation temperature, similar to that found in tropical climates, or using a lower moisture, closer to typical storage moistures.

Temperature and MC also influence the species of fungi that is most likely to grow on shelled corn during storage. In general, *Eurotium* can grow at lower MC (15.5%) than *Aspergillus flavus* or *Penicillium* spp. Optimum temperatures for growth of several common storage fungi are *Eurotium* ≈25°C (Gonzales et al 1988) or 25–30°C (Abellana et al 1999); *Aspergillus flavus* 36–38°C (Stroshine et al 1986); for many *Penicillium* spp. 23°C (Stroshine et al 1986) or 20–24°C (Watson 1987); *P. citrinum* 30°C (Gonzales et al 1988); and *Fusarium* 25–30°C (Marin et al 1995). Perez (1982) reported an optimum temperature of 30°C for field *Penicillia* and a different optimum (24°C) for storage *Penicillia*.

The overall objectives of the study were to evaluate the newly developed DCR and to determine effects of temperature and moisture on the CO_2 test kit fungal susceptibility measurements.

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The specific objectives were to 1) evaluate the use of DCR for determining the CO_2 test kit paddle color and assessing shelled corn fungal susceptibility; and 2) determine the effects of incubation at 24 and 30°C and corn MC of 16 and 21% on fungal growth and CO_2 evolution.

Fungal susceptibility was determined by rewetting the samples to moistures at which fungi can grow. This is similar to the moisture change that occurs when water condenses on the kernels, there is a leak in the storage structure that allows water intrusion, or the corn is exposed to high humidity air for several weeks. Rewetting has previously been used in storability testing of shelled corn. Wilcke et al (2001) harvested corn at 23.5% and dried a portion to 16.5% by spreading it on trays in the laboratory. When the corn was rewetted to 23.5% using distilled water, the storability measurements on the rewetted and fresh corn gave nearly identical results. For this entire study, the difference in ergosterol levels before and after rewetting was used as an alternate measure of fungal growth to which CO_2 test kit results were compared.

MATERIALS AND METHODS

A total of 18 samples of shelled corn were used for these tests. They were selected from the 68 samples used in the study by Moog et al (2008) and had a wide range of storage histories. The samples were stored in a walk-in cooler (2°C) or refrigerator (6°C) after they were collected and until fungal susceptibility tests were conducted. All had MC values <14% when they were placed in storage. Subsamples were removed from the refrigerated samples when they were needed for testing. Sixteen of the samples were taken from bins at Purdue's Postharvest Education and Research Center (PHERC), one set was hand-harvested at a Purdue farm, and one set was obtained from a grain elevator.

CO_2 Test Kit

The CO_2 evolution of the samples was determined using Solvita test kits (Chitraka et al 2006; Moog et al 2008) manufactured by Woods End Laboratories (Mt. Vernon, ME). Subsamples (320 g) from each of the samples of shelled corn were rewetted with deionized water to either 16 or 21% MC and equilibrated in sealed plastic bottles with screw-on caps for 24 hr, either in the laboratory at 24°C ± 1.0 or in a forced convection laboratory oven at 30°C ± 0.5. Each subsample was divided into three 100-g portions that were placed in three separate 472-mL glass jars. The remainder of the sample was used for MC determinations by means of the 72 hr whole kernel oven method (ASABE 2006). Metal canning jar lids consisting of a metal lid held in place by a screw-on band (or ring) were used to seal the jars. The gasket around the edge of the lid forms a tight seal when the band is screwed onto the jar. The sealed jars were placed in the laboratory at 24°C ± 1.0 or in a convection oven at 30°C ± 0.5 for another 24 hr.

When the total time after rewetting reached 47 hr, the lids were removed for 1 hr to allow the CO_2 content in the jar to equalize with ambient conditions. At 48 hr, plastic test paddles (referred to as such because they have a shape similar to tiny paddles) were inserted and the lids secured on the jars. A strip of gel indicator is affixed to the paddle and it changes color in response to increases in the CO_2 concentration of the surrounding air. Throughout this procedure, the containers remained at the specified temperature of either 24 or 30°C. For the next 8 hr, the paddle color number was determined hourly by visually comparing the gel indicator color to the numbered colors (1, 2, 2.5, 3, 3.5, 4, or 5) on a card provided by the manufacturer (Moog et al 2008) or using the digital color reader (DCR). At 71 hr after rewetting, the lids were again removed for 1 hr and at 72 hr new CO_2 test paddles were inserted. The jars were sealed and hourly readings were recorded for 8 hr.

Upon completion of the CO_2 test, triplicate 15-g samples were taken from each jar for MC determination using the whole kernel oven technique described above. The remainder of each sample was placed in a wire basket and dried to 14% wb moisture using a convection oven set at 40°C. The weight loss needed to reduce MC from test moisture to 14% was calculated, and drying progress was monitored by periodically removing the samples from the oven, weighing them, and returning them to the oven until the weight decreased to the target value.

DCR Evaluation of CO_2 Paddle Color Change

The temperature effect tests were planned and initiated before the DCR became available for evaluation. After six of the tests reported here were completed, the prototype DCR became available and it was used along with VR for all subsequent tests. The paddle-shaped test strips were inserted into a slot in the DCR that displayed either the color number or the estimated %CO_2 of the air in the jars. Therefore, the paddles had to be removed from the jars once each hour. When both DCR and visual readings were taken on a sample, VRs were determined first, before the paddle was removed.

TABLE II
Number of Samples Evaluated at Each Temperature and Moisture

Set-Up		Visual Reading	Digital Color Reader	Ergosterol Difference
Temp (°C)	Moisture (%)			
24	21	14	12	12
30	21	12	12	12
24	16	12	10	10
30	16	12	10	10

TABLE I
Samples Used for Comparisons in Figs. 1, 3, 4, and 5

Sample No.	Initial MC	Description[a]
68	13.2	Low fungal susceptibility. Corn sample from the top of aerated bin used for long-term storage study. Corn was stored at least 1 year.
84	13.8	High to moderate fungal susceptibility. Corn sample from nonaerated bin for long-term storage study. Corn was treated with spinosad (an insect control agent) and stored at least 1 year. Most kernels had blue eye mold.
85	13.5	High to moderate fungal susceptibility. Corn sample from bin for long-term storage study. Bin was untreated and corn was stored at least 1 year.
86	14.1	High to moderate fungal susceptibility. Sample from a nonaerated bin for a study of insect control during storage. A composite of samples from several locations in the bin. Many corn kernels had blue eye mold.
87	13.6	Moderate fungal susceptibility. Corn sample from bin for long-term storage study. Treated with actellic and stored at least 1 year.
88	11.4	Low fungal susceptibility. Corn sample harvested by hand from a field at Purdue's Agronomy Center for Research and Education, shelled by hand, and then placed in storage.
99	14.0	Low quality corn from an outdoor pile at a commercial elevator. Almost all kernels had mold damage and sample had an unpleasant odor.

[a] Bins used in long-term storage study located at Purdue's Post Harvest Education and Research Center with a capacity of 35 m^3 (1,000 bu). Some bins were treated with insect control agents.

When the paddles were removed for the DCR reading, air exchange between the outside atmosphere and the air in the jars was minimized. This was accomplished by removing and reinserting the paddle through a small slot (≈4 mm wide × 45 mm long) that had been cut in the lid. This slot was only slightly larger than the thickness and width of the paddle. A piece of thin (80 gauge) stainless steel wire 15-mm long was bent in a "U" shape and the two free ends were attached to the plastic test strip using transparent tape. The wire formed a harness that could be used to pull the plastic strip from the jar through the slot. After the plastic paddle was lowered into the jar, the slot was sealed with a strip of transparent tape. This tape also secured the base of the U-shaped paddle harness that remained outside the jar. Once each hour, the tape was peeled back and the plastic strip was pulled from the jar using the wire harness. The slot was quickly sealed by replacing the tape while the paddle was being inserted into the DCR. As soon as the reading was obtained, the tape was once again peeled back, the plastic paddle was inserted into the jar, and the end of the wire was again fixed in place by fastening the tape over the slot. This entire procedure took ≤18 sec.

Plots of color number versus time were used to compare VR and DCR readings versus time. Plots of VR versus time were used to visualize the effects of MC and temperature. Descriptions of the samples used for these comparison plots are given in Table I. The samples were selected because they exhibited a wide range in CO_2 kit readings in previous tests and therefore would provide a good basis for comparing the performance of the DCR with the VR and evaluating the effects of temperature and moisture. Samples 68, 87, and 88 had relatively low fungal susceptibility while the remainder of the samples were moderately or highly susceptible. A summary of all the tests conducted is given in Table II.

Ergosterol Analysis

Soon after the samples were collected, a 50-g representative subsample (MC < 14%) was removed from each sample, placed in a doubled plastic bag (one within the other), and sent to the USDA/ARS Grain Marketing and Production Research Center (GMPRC) in Manhattan, KS, for ergosterol analysis. The relatively low MC of the samples prevented mold growth during shipment and before ergosterol testing. For all 44 samples on which DCR readings were taken (Table II), 50-g subsamples were removed from the portion that was dried in the convection oven (as described previously) after CO_2 kit testing. The sample was placed in a doubled plastic bag and sent to GMPRC for ergosterol analysis. The initial ergosterol content (μg/g) that was previously determined for each sample was subtracted from this final value of ergosterol content to give the difference in ergosterol (μg/g). This difference was used as an alternate quantitative measure of the fungal growth in the shelled corn samples during incubation.

The ergosterol content (μg/g) was determined using an HPLC with UV detection at 282 nm. Details of the procedure are given in Moog et al (2008). It was a slight modification of the method described by Seitz et al (1979). The HPLC was fitted with a Luna-C18 reverse-phase column (Phenomenex, Torrance, CA) that was 150 mm long, with an inside diameter of 4.6 mm, and a particle size of 5 μm. The flow rate was 1.2 mL/min, the mobile phase was 93.3:6.7 methanol-to-water and the column temperature was 60°C.

Kernel Infection

Either 50 or 100 kernels were removed from the samples that were dried in the convection oven after the CO_2 tests had been completed and the percent of kernels infected with fungi was determined. Table I includes descriptions of samples for which kernel infection was determined. Kernels were surfaced-sterilized with 6% NaOCl (bleach) and washed with deionized water. Ten kernels were placed in each of 20 petri dishes that contained 6% malt salt agar (Tuite et al 1985). The dishes were placed in an incubator at 29°C and after seven days the number of kernels invaded by fungi was determined. Although no attempt was made to identify the specific species of fungi that had invaded the kernels, the genus of the colonies growing on the plated kernels and the number of kernels with each genera of fungi were determined for *Eurotium, Penicillium, Fusarium,* or "other." The "other" category included but was not limited to *Alternaria* spp., *Aspergillus candidus, A. niger,* and *A. flavus.*

Statistical Analyses

The data were analyzed using the statistical software (v.9.1, SAS Institute, Cary, NC). The mean values, standard deviations, minimum and maximum values of each attribute were calculated using the MEANS procedure. The linear regression parameters were calculated using the REG procedure and the significance of each regression of color number versus ergosterol level was determined at the 95% confidence level using the CORR procedure.

RESULTS AND DISCUSSION

The results include both a comparison of DCR and VR readings and observations on the effects of temperature and MC on CO_2 kit measurements. The DCR-VR reading comparison, conducted on five samples that had a wide range of fungal susceptibility, is presented first and that is followed by the MC-temperature study. Four combinations of MC and temperature were used for this second set of tests. For five of these samples, the percentages of kernels infected with different genera of mold were also determined.

Digital Color Reader (DCR)

CO_2 kit paddle color numbers for five samples, determined hourly using both the DCR and visual comparison with a color card are shown in Fig. 1. The five samples were selected from those tested at 24°C and 21% MC and they represented a range of fungal susceptibility.

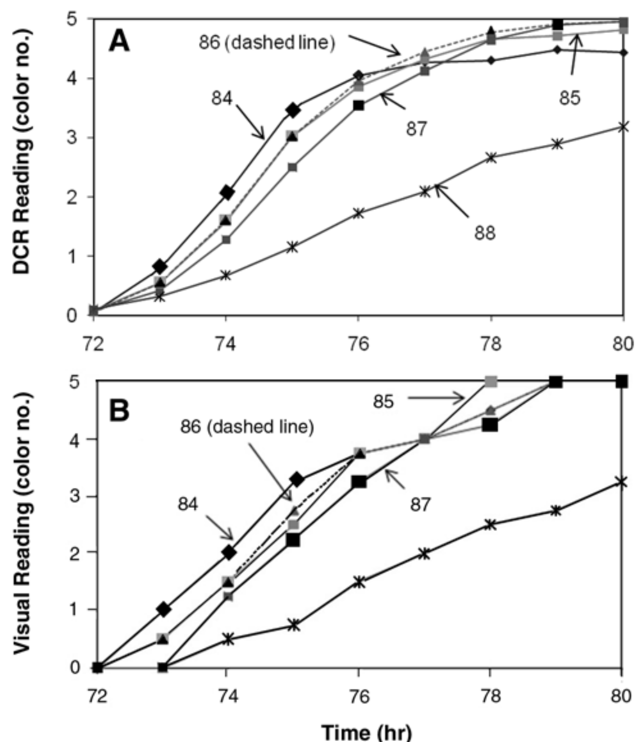

Fig. 1. Color number vs. time for the carbon dioxide kit tests. Digital Color Reader **(A)** and visual readings **(B)** based on comparison with a color card. Tests conducted at 21% MC and 24°C.

In addition to giving a much smoother curve, the DCR was better able to differentiate subtle differences in paddle color. Note that both the DCR and visual numbers for sample 84 are highest up to 76 hr. After 76 hr, the DCR readings indicated that fungal activity in sample 84 slowed. This distinction could not be made using VR, where results were identical for samples 84 and 86 at 76–80 hr. Even though VR evaluation of the gel color is more difficult, both methods gave the same ranking of the four samples at 75 hr. With experience, it is sometimes possible to estimate color number within 0.25 using visual comparison to the color card. However, greater precision can be attained using the DCR.

Linear regression was used to compare ergosterol differences with the VR and DCR color numbers of 12 samples tested at 21% MC and 24°C. The coefficients of determination (r^2) for these regressions are summarized in Table III. These r^2 values are similar to those reported for VR readings in Moog et al (2008), which were 0.46–0.60. Ergosterol differences of these samples were 0–8.6 ppm. Seven of the 10 r^2 values for color numbers determined with the DCR were greater than the r^2 values when color numbers were determined visually. Several of the DCR r^2 values were substantially greater (49, 50, 73, 74). For 51–53 hr, the r^2 values for VR were only slightly greater than those for the DCR. At 50 hr, DCR (Fig. 2A) gave the best correlation with differences in ergosterol (r^2 = 0.75) and was slightly greater than the best correlation for the VR, which was for 51 hr (Fig. 2B, r^2 = 0.73).

The DCR could detect slight color changes when the color number was ≤2.0, which often occurs during the first 2 hr after the paddles are inserted. Most of the time, the best accuracy that could be achieved between color numbers 0 and 2.0 using VR was ±0.5, although a very skilled reader could sometimes read color numbers to ±0.25 at 1.0–2.0. In one test, the DCR reading increased by 0.38, while no changes were discerned in the VR. In another test, the VR changed by 0.25, while the DCR reading changed by 0.47.

The noticeably higher r^2 values for the DCR readings (Table III) at shorter incubation times, when all color numbers were <2.1, is another indication of the greater precision for determing-ing color number in this range. For example, in samples at 49 and 73 hr, 1 hr after insertion of the paddles, the r^2 values for VR were < 0.01 and the r^2 values for the DCR readings were >0.3.

Effect of Temperature and Moisture Content

VR readings from five of the samples rewetted to either 21 or 16% MC and incubated at 24°C are shown in Fig. 3. Differences among the five samples are more evident for the test conducted at the higher MC, when mold growth is usually faster. Although the rankings of the readings for 75 hr are similar for both moistures, the differences among the five samples are less apparent at 16% MC. For example, samples 84 and 86 had the same color number at 75 hr and there were only slight differences among the other samples.

TABLE III
Coefficients of Determination (r^2) for Regressions of Visual and DCR Readings[a]

Time After Rewetting (hr)	Visual Reading	DCR Reading
49	0.010	0.532*
50	0.416*	0.751*
51	0.730*	0.724*
52	0.706*	0.682*
53	0.593*	0.575*
73	0.009	0.343*
74	0.295*	0.537*
75	0.415*	0.551*
76	0.476*	0.546*
77	0.448*	0.505*

[a] Ergosterol difference (µg/g) at selected hours after rewetting for tests at 21% MC and 24°C (n = 12). *, Significant at α = 0.001.

Figure 4 shows the color readings for the five samples incubated at 30°C and either 21 or 16% MC. A comparison of Fig. 4A with 4B reveals the effects of the MC difference at the higher temperature. Although the rankings of the samples are the same for both moisture levels, higher color numbers were attained at 21% MC. For example, the 75 hr readings were 2.8–5.0 at 21% MC, and 0.5–2.2 at 16% MC.

Similarly, a comparison of Figs. 3A with 4A and 3B with 4B indicates the effect of temperature on test kit VR readings at the respective moistures. Most of the rankings of the hourly readings were the same at the two temperatures for 75–78 hr. However, the relative position of sample 99 changed. Sample 99 had the highest color number at the higher temperature, while its readings were in the middle at the lower temperature. This indicates that a test conducted at 24°C may give a less accurate assessment of the risk of spoilage of a corn lot exported to a tropical region or exposed to tropical conditions during shipment overseas. However there was obvious kernel discoloration in this sample before incubation and it is unlikely that corn in that condition would be exported.

As noted above, both MC and temperature can affect the type of fungi that grow. Percentages of kernels internally infected by the four types of mold at the two temperatures and moistures are shown in Fig. 5. *Penicillium* infection was observed in samples 68 and 86 rewetted to 16% MC but none was found when they were rewetted to 21% MC. The minimum moisture for growth of *Penicillium* is 16.0–16.5% and its optimum growth temperature is 20–24°C (Watson 1987). This, combined with competition from other fungi, may explain the absence of *Penicillium* in corn sample 68 when it was incubated at 30°C and in all of the 21% MC samples.

Although *Fusarium* is occasionally found growing on high-moisture corn stored at 19% MC (Stroshine et al 1986; Watson, 1987), in these tests, it grew in samples incubated at both moistures and temperatures. As expected, the growth was greater at 21% MC in every sample except 86, where growth at 30°C was the same at the two MC levels. No *Fusarium* grew on sample 99.

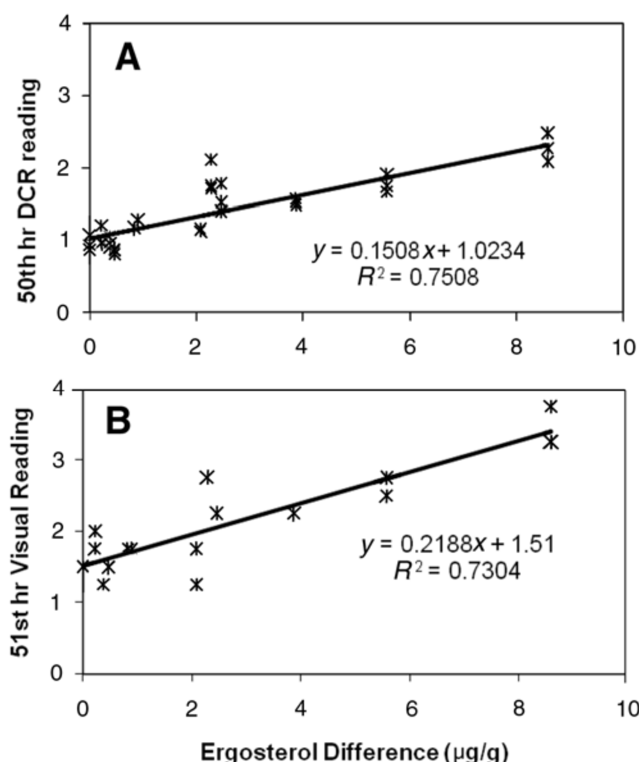

Fig. 2. Correlation of ergosterol difference with 50th hr Digital Color Reader readings **(A)** and 51st hr visual readings **(B)**. Tests conducted at 24°C and 21% MC.

This sample had been stored for at least several months and it had visible mold. *Fusarium* would not have been able to survive in that environment. Sample 88, the good quality hand-shelled sample, showed the highest growth of *Fusarium* at 30°C. Very few kernels were invaded by other genera and there was little competition to retard *Fusarium* growth. Marin et al (1995) reported that *Fusarium* grew over a water activity range (a_w) of 0.90–0.994 at 20–35°C. Corn at 19% MC and 25°C has an a_w of ≈0.90. There should have been very little growth of *Fusarium* in the 16% MC samples. Most of the kernels may have been infected before harvest. Apparently, *Fusarium* colonies that survived during the interim storage began to grow when the kernels were plated.

Eurotium infected most of the samples incubated at 30°C and half of the samples incubated at 24°C (Fig. 5). The maximum infection (92%) occurred in sample 99, the poor quality corn. *Eurotium* can grow in corn with moistures as low as 14% when the temperature is 30–35°C, which is also the optimum temperature range for its growth (Watson 1987). Samples 68 and 88, which had low fungal susceptibility, were not invaded by *Eurotium* at 16%. Some of the "other" genera were present in all the samples incubated at both temperatures, with the exception of sample 88, which had only slight levels of invasion at 30°C. No trends were observed for invasion by "other" genera. The CO_2 production by the samples could be influenced by the variations in percentages of kernels infected by different genera of mold and the degree of infection by each genera. In studies with wheat starch, White et al (1982) reported fluctuations in cumulative CO_2 at different MC levels, which they related to variations in percentages of kernels infected by *Penicillium, Eurotium* (*A. glaucus*), *A. flavus,* and *Alternaria*. They also noted that bacteria competed with the fungi and had a negative effect on cumulative CO_2 production. For the tests reported here, the variations appeared to have only a minor effect on CO_2 kit measurements.

As noted previously, fungal susceptibility tests on corn lots shipped to tropical regions may be more accurate if samples are tested at 16% MC. However, the lower MC slows the rate of mold growth and this could increase the time required to complete a fungal susceptibility test. Differences that become apparent after 48 hr of incubation at 21% MC and either 24 or 30°C may not be evident until the third or even fourth day, when samples are incubated at 16% MC. The effect of moisture and temperature on the fungal susceptibility was investigated using linear regression analysis. Hourly readings obtained at 21% MC and 24°C were compared to corresponding hourly readings at each of the other three conditions. Coefficients of determination (r^2) for the comparisons of DCR readings at six different hours are given in Table IV. DCR rather than VR readings were used because, as demonstrated previously, they are more precise. At the hours used for comparison, DCR readings were only available for seven samples at all four MC-temperature combinations.

TABLE IV
Coefficients of Determination (r^2) for Regressions of Paddle Readings[a]

Temp and MC	Hours					
	51	52	54	74	75	77
16% 24°C	0.583* (0.046)	0.633* (0.032)	0.457 (0.095)	0.294 (0.208)	0.438 (0.106)	0.384 (0.138)
21% 30°C	0.814** (0.005)	0.914** (0.001)	0.872* (0.002)	0.847** (0.003)	0.890** (0.001)	0.790** (0.007)
16% 30°C	0.701* (0.019)	0.654* (0.028)	0.484 (0.084)	0.678* (0.023)	0.798* (0.041)	0.881** (0.002)

[a] Readings at indicated temperature and moisture content vs. corresponding readings at 21% MC and 24°C (n = 7).
[b] *,**, Statistically significant at α = 0.05 and α = 0.01. Probabilities in parentheses.

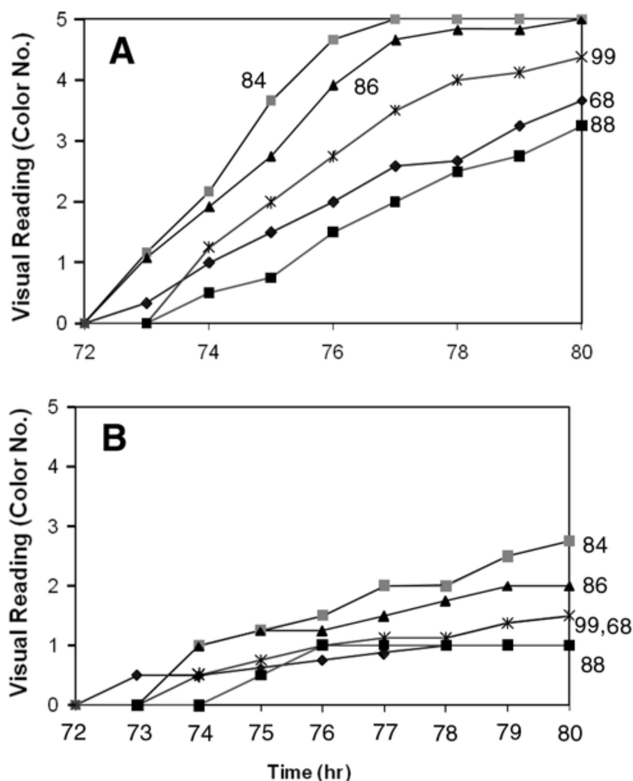

Fig. 3. Visual carbon dioxide kit paddle readings at 21% MC and 24°C (**A**) and 16% MC and 24°C (**B**).

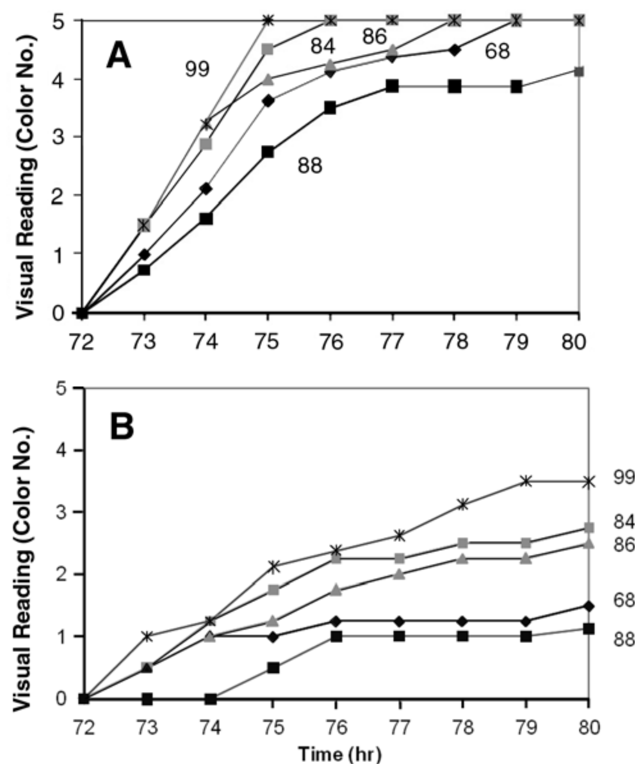

Fig. 4. Visual carbon dioxide kit paddle readings at 21% MC and 30°C (**A**) and 16% MC and 30°C (**B**).

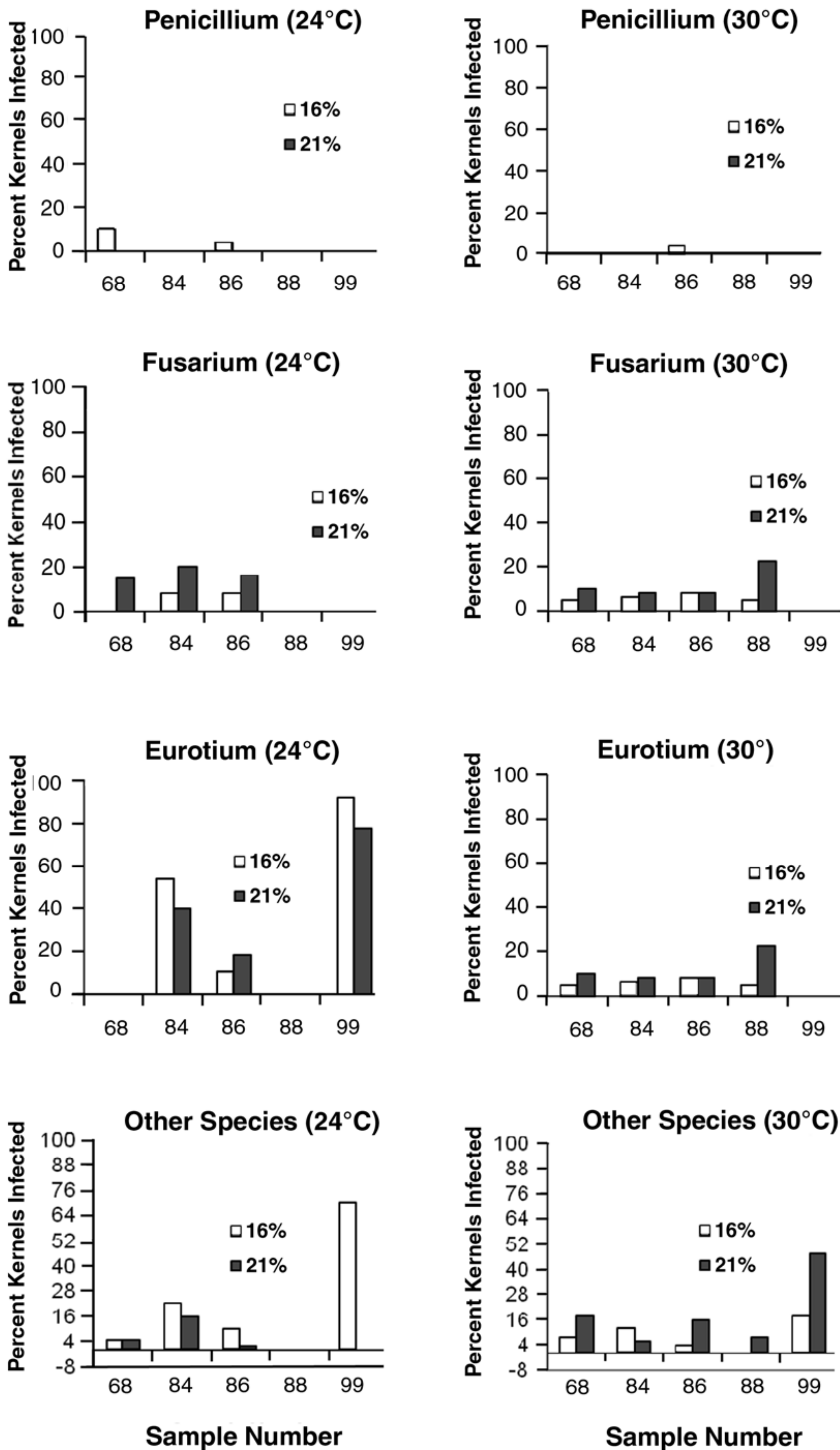

Fig. 5. Percentages of kernels (% of total kernels) internally infected with various genera of fungi for samples incubated at either 24 or 30°C and either 16 or 21% MC.

The r^2 values for regressions between readings for the 21% MC samples at the two different temperatures, 24 and 30°C (line 2, Table IV), were ≥0.79 and all were statistically significant at the $\alpha = 0.01$ level. For incubation at 16% MC and 30°C, five of the six regressions with samples at 21% MC and 24°C were statistically significant (line 3, Table IV) at the 0.05 level and their r^2 values were ≥0.65. By contrast, only two of the correlations for 16% MC and 24°C were statistically significant ($\alpha = 0.05$).

The lower correlations for the 16% MC and 24°C samples may be at least partially explained by the slower growth of the samples at the lower moisture and temperature. This possibility was investigated by comparing results for longer incubation times at 16% MC and 24°C with shorter incubation times at 21% MC and 24°C (results not shown in Table IV). When color number readings from 55 and 78 hr of the 16% MC samples were compared with color number readings at 52 and 74 hr for the corresponding 21% MC and 24°C samples, the r^2 values increased relative to comparison at the same hours (Table IV). For samples incubated at 16% MC and 24°C, the r^2 and probability values (in parentheses) for 55 hr (at 16% MC) versus 52 hr (at 21% MC) were 0.846 (0.027) and for 78 hr (at 16% MC) versus 74 hr (at 21% MC) they were 0.922 (0.002). (Note: the 78 hr vs. 74 hr comparison was made with only six samples because a measurement for one of the samples at 16% MC and 24°C was not available at 78 hr). The averages of the color numbers for the 21% MC and 24°C samples at 52 hr and 74 hr were 2.58 and 1.27, respectively, and ranges were 1.78–3.31 and 0.61–2.45. At 52 hr and 74 hr, the averages of the color numbers for the 16% MC and 24°C sample were 0.86 and 0.51, respectively, and ranges were 0.40–1.63 and 0.27–1.04. The values at 16% MC increased at 54 hr and 78 hr where the averages were 1.38 and 1.48, respectively, and ranges were 0.55–2.97 and 0.68–2.90, respectively. Growth was slower at this lower MC and therefore the longer incubation time gave higher color numbers and made the differences in fungal susceptibility more evident.

The ranges of the color numbers (maximum reading minus minimum reading) were examined at each hour at which readings were taken. The difference reached its maximum value sooner when conditions were more favorable for mold growth.

For example, for incubation conditions of 21% MC and 30°C, the maximum first occurred at 50 hr, while the maximum was attained at 51 hr, 55 hr, and 56 hr, respectively, for 21% MC and 24°C, 16% MC and 30°C, and 16% MC and 24°C. These observations, along with the correlations discussed above, reveal the importance of taking into account incubation conditions when choosing the best incubation time for evaluating fungal susceptibility. Ideally, the time should be such that highly susceptible samples attain color numbers >2 or 3, while samples with low fungal susceptibilities have color numbers >1 or 2.

SUMMARY AND CONCLUSIONS

CO_2 test kit measurements on shelled corn rewetted to either 16 or 21% moisture and incubated at either 24 or 30°C indicated that the Digital Color Reader (DCR) improved the accuracy of the color number measurement compared to determination of color number by visual comparison (VR) with a color card. The plot of color number versus time was noticeably smoother when the DCR was used. Furthermore, the DCR was able to differentiate slight changes in color that could not be discerned visually. It was particularly useful for color numbers <2, where achieving accuracy of visual readings is more difficult. For incubation at 21% MC, differences in fungal susceptibility could be detected after 53 hr of incubation when the DCR reader was used.

The effects of shelled corn incubation temperature and MC on fungal susceptibility measurement were also determined. The color numbers increased more rapidly at higher temperature and higher MC, reflecting the effect of these factors on the rate of mold growth. Rankings based on color number at a given incubation time were similar regardless of which combination of temperature (24 or 30°C) and MC (16 or 21%) was used. Rankings at 30°C were the same for both MC levels, while the ranks of two of the less susceptible samples were interchanged at 24°C. A heavily invaded sample went from an intermediate susceptibility to the highest susceptibility when temperature was increased from 24°C to 30°C.

Plating of the kernels taken from five of the samples after the completion of the CO_2 kit tests revealed that some kernels in most samples had been invaded by *Eurotium* with higher infection rates in samples incubated at 30°C. Many of the samples were infected by *Fusarium* (<22% kernel infection) and there were higher infection rates in samples rewetted to 21% MC. *Fusarium* was not found in the sample of very poor quality corn and *Eurotium* was not found in the sample of high quality hand-shelled corn. Internal infection by *Penicillium* was observed in only three of the samples and those were incubated at 16% MC.

In general, a sample highly susceptible to mold growth at 21% MC and 24°C would also be susceptible at 30°C and either 16% or 21% MC. For these temperature-MC combinations, temperature had a greater effect than MC on fungal susceptibility measurements, possibly because it influenced the growth of *Eurotium*. If time and resources are available, it would be best to adjust incubation temperature and possibly moisture to more closely match conditions to which the shelled corn will be exposed during shipment or storage. When these factors are adjusted, incubation times should be selected to ensure color number readings are within the correct range (low susceptibility samples 1–2 and susceptible samples 4–5).

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