ASSESSING THE SUSCEPTIBILITY OF SHELLED CORN TO INVASION BY STORAGE FUNGI

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ABSTRACT

Moog, Dale Jude P. PhD., Purdue University, May 2006. Assessing Susceptibility of Shelled Corn to Invasion by Storage Fungi. Major Professor: Dr. Richard L. Stroshine.

Shelled corn samples taken from different storage environments were rewetted and evaluated for susceptibility to fungal invasion (SFI) using ergosterol measurements and a test kit that measured CO_2 evolution from 100 g samples placed in 0.473 liter air-tight containers. The kit uses a gel that changes color as ambient percent CO_2 changes. Sample attributes measured included kernel damage, percent germination, electrolyte leakage from soaked kernels, and percent kernel infection. Using linear regression attribute measurements were compared to the difference in ergosterol (EGD) before and after rewetting and incubation for three days (21% mc, 24°C). Differences in CO_2 kit measurements were consistent with expectations regarding SFI and CO_2 kit measurements were well correlated with EGD ($0.44 < r^2 < 0.57$). These results indicate the test kit effectively measures SFI. Except for percent kernel infection and percent fines (6.35 mm sieve), coefficients of determination (r^2) for regressions of EGD with the sample attributes were statistically significant and varied from 0.47 for percent germination to 0.12 for percent fines (4.76 mm sieve). Correlations between the attributes and CO_2 kit measurements were statistically significant ($0.13 < r^2 < 0.49$) with the exception of percent fines (6.35 mm sieve) and percent kernel infection. A fungal susceptibility damage index (FSDI) was defined that was significantly correlated with EGD ($r^2 = 0.28$). The

performance of a digital color reader (DCR) for determining gel color was also investigated. The DCR was better able to distinguish differences among samples and the plot of DCR color number versus time was smoother than the plot for color numbers determined visually (VR). The r^2 for EGD versus DCR color number was slightly higher (0.75) than the r^2 for EGD versus VR (0.73). The DCR was used to assess the effect of incubation temperature (24°C versus 30°C) and moisture content (16% versus 21%) on CO_2 kit readings for five samples representing a wide range of SFI. The 75th hour color number rankings for both moistures were similar at 24°C, but were more evident at 21%. Incubation at 30°C changed the rankings slightly. Kernel plating revealed differences in fungal growth that explained these changes.

CHAPTER 1. INTRODUCTION

This dissertation describes research on several methods of assessing stored shell corn susceptibility to invasion by storage fungi. This chapter describes the importance of the problem, defines fungal susceptibility, and discusses the methods that will be used to determine the susceptibility of shelled corn to invasion by storage fungi. It concludes with a statement of the specific objectives of this research.

1.1. The Corn Industry

A better appreciation for the importance of developing tests for measuring susceptibility of corn to fungal invasion can be gained by considering the quantity of corn produced in the U.S. and around the world along with quantity of corn that is stored. World production of corn for 2005 was 706 million metric tons (MMT). The United States produced 280 MMT, which is 40% of the world production (USDA, 2005). The value of this production was $21 billion. The total amount of corn traded in world markets was 75.4 MMT with 47 MMT (62%) coming from the U.S. The leading markets for U.S. corn are Japan, Mexico, Canada, Taiwan, Egypt and South Korea. The total grain storage capacity of the US, as reported by USDA in January 2005 was 11.19 billion bushels (282 MMT) for on-farm storage and 8.50 billion bushels (214 MMT) for off-farm storage. Considering only the states in the Midwest (NASS, 2005), on farm corn stocks in June of 2005 were estimated as 2.38 million bushels (60 thousand MT) and off farm stocks were estimated as 1.66 million bushels (42 thousand MT). In March of 2004, there were 5.27 million bushels (0.132 MMT) of corn in storage of which 58% was stored on farm. By June, inventory had dropped to 2.97 million bushels (75 thousand MT) and in September, it dropped to 968 thousand bushels (24 thousand MT). In each case, approximately half of the corn was in on-farm storage. The amount of corn in storage peaked in December, with 9.4 million bushels (237 MMT) available of which 65% was stored on farms (NASS, 2005). Based on 30 year data, the inventory reaches its maximum in December, at the end of the harvest season, and then by September of the following year it drops by 80%. Generally, the majority of the corn is stored on farm except during September when the majority, about 60%, is found in elevators, terminals, mills and at processing facilities.

1.2. Storage Losses

Unfortunately, as is true for all agricultural commodities, storage losses occur during trading and marketing of corn. The World Health Organization estimated worldwide postharvest losses of grains and seeds and of oleaginous and leguminous plants as 5-10%

of world production. Small losses of only 1 to 2% occur in countries employing advanced technologies, although even these small percentages represent substantial quantities. By contrast, in less developed countries losses can reach 30%, which is also considerable even though the total production is smaller (Richard-Molard, 1988). Storage losses of corn can be caused by macro-organisms such as rodents, birds, and insects. However, deterioration can also be brought about by proliferation of molds during storage. Losses due to mold growth (Wilcke, 1998) can sometimes reach 10% of the value of the stored grain.

Corn infected with fungi is lower in quality and commands a lower price because it is thought to have a lower nutritive value. The maximum allowable percentage of discolored kernels for U.S. grade 2 corn is 5% or less. In the U.S., corn having a commercially objectionable foreign odor, is considered to be sample grade. Such corn lots cannot be mixed with good quality corn because the infected kernels may contain mycotoxins harmful to both humans and domesticated animals. One author estimated that annual losses to the feed and livestock industry in the US and Canada due to only the impact of mycotoxins were $5 billion (FAO, 2001 citing Miller). Donley (2006) stated that annual market losses worldwide due to rejection of corn, could increase by $200 million if countries using a standard of no more than 2 mg of fumonosin per kg were to change this standard to the lower level of 0.5 mg/kg used by many countries.

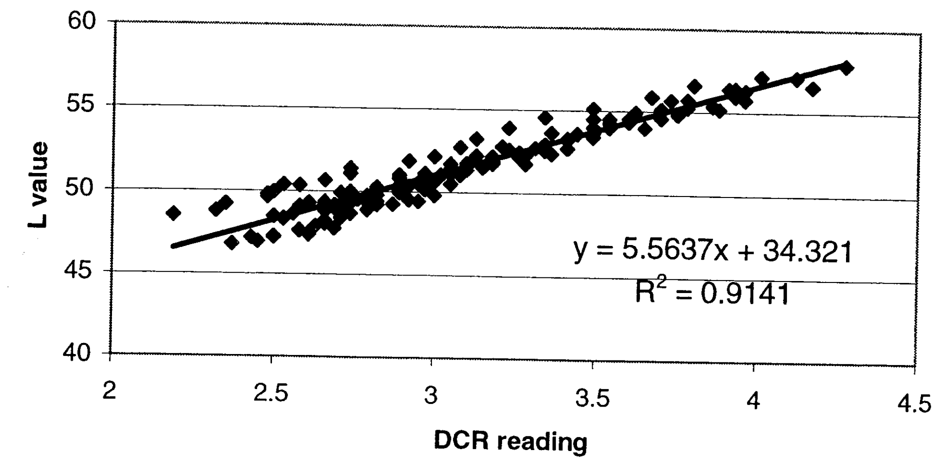

Figure 4.31. Comparison of DCR reading with L value of spectrophotometer

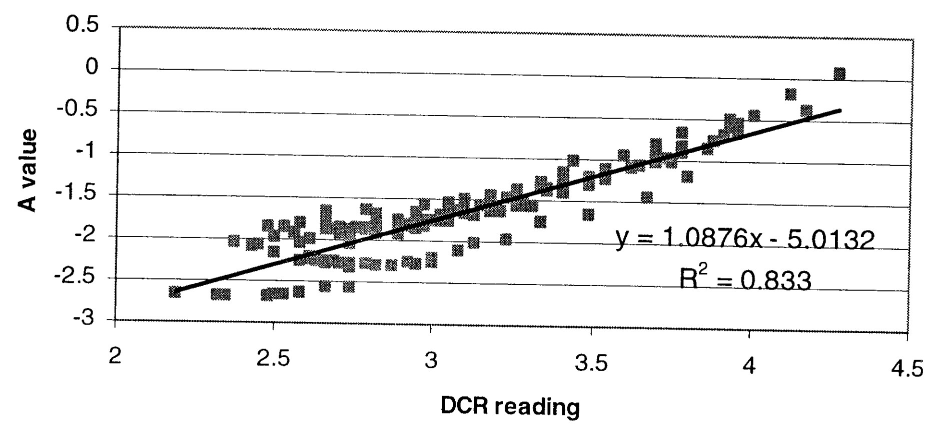

Figure 4.32. Comparison of DCR reading with a value of spectrophotometer

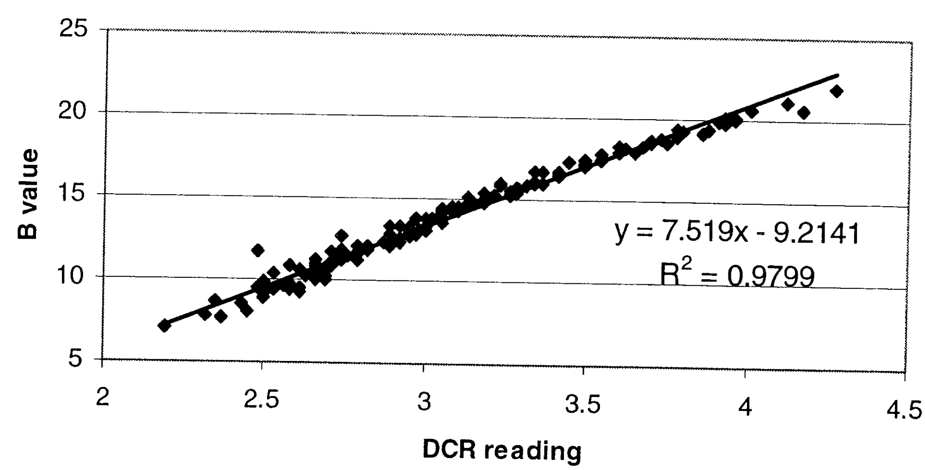

Figure 4.33. Comparison of DCR reading with b value of spectrophotometer

CHAPTER 6. RECOMMENDATIONS

This chapter enumerates three groups of recommendations for further work related to: 1) improving the procedures used in this study or developing new procedures; 2) developing better methods for assessing mold growth, and 3) more accurately measuring the CO_2 evolved by the fungi. Recommendations on the use and the application of the CO_2 kit by the grain industry are also discussed.

6.1. Improving Procedures used in this Study and Developing New Procedures

a. Additional studies could be conducted for the purpose of identifying kernel damage measurements that indicate susceptibility of the kernels to fungal invasion. The Fungal Susceptibility Damage Index (FSDI) discussed in section 4.1.7.3 could be further evaluated by conducting more tests and comparing the FSDI to measurements of ergosterol difference, the CO_2 produced by the sample, or the CO_2 kit color number. This should reveal whether the FSDI or a similar type of index can be used for screening samples to identify those that may be highly susceptible to invasion by storage fungi. It should also be used in multiple regression analyses to determine whether the FSDI can be combined with one or more other factors to give a better indication of the susceptibility of the sample to fungal invasion.

b. Additional samples should be evaluated using the NIR and FT-NIR to determine whether either of both can be used to rapidly determine the ergosterol content of shelled corn. A separate set of validation samples should be prepared so that the accuracy with which NIR can measure ergosterol content can be determined. NIR and FT-NIR should also be evaluated for their potential in determining other quality factors such as kernels viability and the extent of mold damage to kernels.

c. The electrolyte leakage of the samples used for the CO_2 kit tests could be determined both before and after the samples are rewetted and incubated. If mold growth affects electrolyte leakage (conductivity), then the change in conductivity could be compared with other methods of quantifying mold growth including the CO_2 test kit readings and the difference in ergosterol before and after the sample is rewetted and incubated.

d. Since percent germination had one of the best correlations with ergosterol difference, methods of rapidly measuring the percent germination of a sample of shelled corn should be investigated. One test that should be investigated is the tetrazolium test.

e. In this research, several different factors thought to influence the susceptibility of shelled corn to invasion by storage fungi were studied. The extent to which these factors are independent, measuring distinctly different attributes, should be clarified and additional factors influencing fungal susceptibility should be identified and evaluated.

6.2. For Assessing Mold Growth

a. For some of the samples tested in this research project, the ergosterol content at the end of the incubation period was lower than the ergosterol content prior to incubation. Sampling and testing procedures should be modified to eliminate this discrepancy or to reduce the frequency with which it occurs. In this project, two readings were taken on each sample and the results were averaged. It may be necessary to conduct three or four ergosterol measurements on each sample. An alternative would be to grind a larger sample and then take a representative sub sample from this ground sample. This may reduce the impact of one or two moldy kernels in the sample.

b. The samples used for the CO_2 test could be evaluated and graded by a licensed grain inspector before and after the CO_2 kit tests are conducted. This would provide an additional quantification of mold growth during the three day incubation period.

c. Additional procedures for evaluating mold growth in the samples should be investigated. These include but are not limited to the use of machine vision to assess the extent of mold invasion and the use of an electronic nose for measuring volatiles related to mold growth that are released by the sample. Those that can be measured rapidly should be identified. The methods could be evaluated by examining correlations of their results with the CO_2 kit measurements.

6.3. CO_2 Kit Measurements

a. The relative contributions of insects, respiration of the germ, and mold growth to the CO_2 production by the sample should be determined. .The effects of sample temperature and moisture on these contributions should also be determined.

b. Methods of improving the accuracy of the CO_2 kit determinations of fungal susceptibility and reducing the time required to complete the test should be studied. These could include adjusting the ratio of the sample weight to the volume of the incubation chamber or developing a method of adjusting the results to account for seed respiration. Another goal should be to reduce the time required to complete the CO_2 kit test to 48 hours or less. One approach would be to develop a better understanding of how CO_2 production during the first 24 to 48 hours relates to CO_2 production between hours 48 and 80.

c. The consistency of the CO_2 kit paddle readings should be investigated by inserting two or three paddles in the same jar and observing the change in color number to determine whether all the paddles change color at the same rate. In addition, the response of the paddles could be checked by placing the paddles in an environment containing a known concentration (percent by weight) of CO_2. The effects of storing the paddles at different temperatures for different periods of time should also be determined. This would provide the user with guidelines for storing paddles and information on how long the paddles can be stored before their effectiveness decreases.

d. Samples of shelled corn should be tested with the CO_2 kit and then placed in storage at a prescribed temperature and relative humidity. The results could be used to evaluate the recommendations for allowable storage time (AST) developed in section 4.1.8 of this thesis.

f. The CO_2 kit tests could be applied to other cereal grains and oilseeds such as rice and wheat, for which susceptibility to fungal invasion during storage and shipment is a concern.

6.4. Use of the CO_2 Test Kit by the Grain Industry

Farmers, grain elevator managers, managers of processing facilities, grain exporters and those who purchase grain should be able to utilize the CO_2 test kit for making decisions about where and when grain should be processed or sold to domestic and foreign buyers. This relatively simple and inexpensive kit could reduce losses caused by mold growth in stored grain. Even though three days are required to complete the test, there are some scenarios in which this would be feasible. For example, corn residing in on-farm storage or stored at an elevator could be sampled with a probe or automatic sampling device and evaluated using the CO_2 test kit. Results could be used when making decisions about when the grain should be marketed. Processors with storage facilities could test samples from in-bound shipments and identify which shipments are best suited for storage at their facility prior to utilization. Exporters intending to ship corn to tropical or sub-tropical locations could use the test results to identify lots of corn currently in storage that would be least likely to deteriorate after arrival at their destination.