FUMONISIN: GROWNING PROBLEM IN CORN

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The existence of the mycotoxin fumonisin was discovered in 1988 by a group of researchers in southern Africa while investigating the cause of high esophageal cancer rates in that region. While the identification of the organism is fairly new, it has been suspected as the culprit of human and animal disease as far back as the early 1900's. In 1998, a call to the Camden County Extension Center from a producer that farmed in the northeast corner of the county indicated that he had problems with some corn that he was harvesting. The problem was diagnosed as Fusarium Ear Rot. The field appeared to have healthy fodder, strong tall stalk, good ear size, excellent yield but the ear and kernels appeared to be deteriorating and the corn shuck did not cover the end of the ear. Corn that had been harvested appeared to be rotten. Samples taken from these fields were heavily infected with fumonisin. While this problem was initially identified in Camden, other severe cases of fusarium ear rot were eventually found in other areas across eastern North Carolina during the 1998 harvest season.

Fusarium (*fusarium moniliforme*) fungi is found in the soil and affects many different crops. Fusarium can be transferred systemically into the plant through the roots, can enter the ear through the silks by air or rainfall, or it can enter through insect damage. Environmental conditions such as drought, high temperatures, and insect feeding can cause stress to the corn plant and can increase he likelihood that Fusarium will infect the corn kernel. This fungus can cause stalk and ear rots in corn and produces a byproduct known as fumonisin. Fumonisin is a mycotoxin that causes weight loss, abortions, and cancer in animals and humans. Due to a growing concern about the toxic effects of fumonisin on animals and humans, the Food and Drug Administration (FDA) is seeking to place limits on the amount of fumonisin allowed in samples of corn grain.

In 1999, the FDA published suggested regulations concerning the levels of fumonisin it would allow in grain. These levels are 5 ppm for human or equine consumption, 10 ppm for swine, and 50 ppm for cattle and poultry. Based on testing of grain samples from fields in eastern North Carolina that showed fumonisin levels over 20 ppm it quickly became clear that these regulations would pose serious problems for corn growers. It became important to find information on the infection of corn by *Fusarium moniliforme*, the development of fumonisin in the corn kernel, and how to avoid or eliminate fumonisin contamination of grain. Unfortunately, there was little or no information available on fumonisin development. Therefore, the North Carolina Extension Service took the initiative to conduct field tests to determine how fumonisin develops in corn and what management practices could be used to reduce or eliminate fumonisin contamination. This paper describes the results of these field tests.

FIELD TESTS CONDUCTED

Fumonisin Checks to Determine the Extent of the Problem

In 1999, 63 hybrids were planted in a strip plot. A tester was used in every third strip to measure variability with in the field. Just prior to harvest, five ears were selected at random from each hybrid and tester strip and visually evaluated for grain quality by six extension faculty trained in assessing ear rot damage. Visual evaluations were based on a scale from 1 to 10 with 1 designating a sample that was completely infested with fusarium ear rot and 10 designating a sample that was completely free of any damage. The six ratings were averaged and that score was used as the final score for that hybrid or tester strip. At harvest, grain samples from six hybrids were selected based on the visual grain quality scores and sent to the lab for testing to determine the actual levels of fumonisin present in the grain.

2000 Hybrid Evaluation for Fumonisin

Agents in five northeast North Carolina counties were surveyed concerning the hybrids being used in their counties and a list was developed to identify hybrids to use in a strip plot design replicated four times across different locations. Fourteen hybrids were selected based on this list. Ear and grain samples were pulled from the plots at physiological maturity (9-10 August) and on 18 September (simulating a late harvest) visual grain quality was scored as previously described and yield data was collected. The samples were sent to the mycotoxin lab at NCSU to check for fumonisin levels.

2001 Evaluation for Fumonisin Development and Relationships to Enviroment

Based on observations and the results of 2000, a study was designed to better describe the conditions that lead to fumonisin development in corn. Weather monitoring units were placed at three sites and collected site-specific rainfall, temperature, and relative humidity data on an hourly basis. Three hybrids (Pioneer 32Z18, Pioneer 34K77, and Syngenta N79-L3) were selected for the test based on differences in resistance noted in 2000 and company ratings. These hybrids were planted in a randomized complete block pattern. Grain samples were collected from the middle two rows of each plot at a weekly interval beginning at R2 (first sign of kernel) and ending on 9-September. Samples were hand shelled, dried and sent to the mycotoxin lab at NCSU for testing. Winston M. Hagler, Jr., PhD., Professor and Director of the lab assisted along with W.,Hunter Edwards, M.S. and Amanda S. Fairchild, M.S. in the testing at the lab. Labortory analysis was done using the following method: Bennett, G,, and J.Richard, 1994 Liquid chriomatographic methods for analysis of the naphthalene dicarboxaldehyde derivative of fumonisins, J.Assoc. Offic, Anal. Chem. 77(2):501-506.

RESULTS AND DISCUSSION OF FUMONISIN RESEARCH STUDY

Analysis of data from three years of tests indicates that year and hybrid were significant factors influencing fumonisin levels in corn samples. There were no interactions between these factors. This fact is particularly important in assessing differences in fumonisin contamination among hybrids. This indicates that the hybrid with the highest levels of fumonisin at one location or in one year was also the hybrid with the highest levels at other locations or in succeeding years.

Differences in Fumonisin Levels across Years

The highest levels of fumonisin were found in 1999 with intermediate levels found in 2000 and low levels found in 2001 (Fig. 1). Previously, it had been thought that fumonisin levels were related to environmental conditions and were triggered by

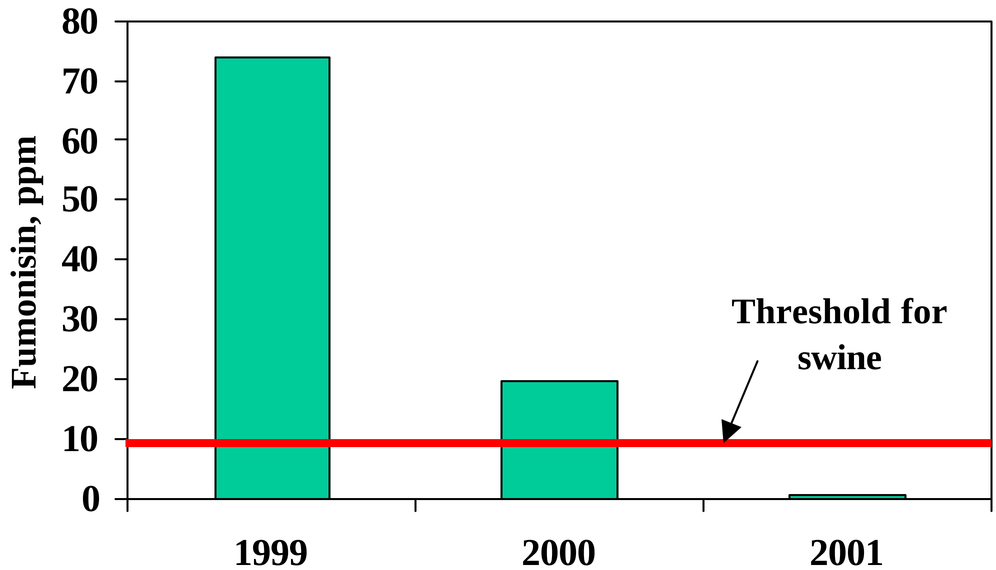

Fig. 1. Average fumonisin levels recorded for each year of the study.

droughty conditions during grainfill followed by rainfall and high humidity during the harvest period. This study indicates that this line of reasoning is not entirely accurate.
While fumonisin levels were highest in 1999 when drought occurred just prior to silking, damaging levels of fumonisin were found in 2000, a year with above average rainfall during the growing season. The lowest levels of fumonisin occurred in 2001 which was characterized by minimal but timely rains during the growing season followed by dry weather starting in late August (Fig. 2). It is clear that fumonisin levels differ by year based on the environment. It is still not entirely clear what combination of environmental

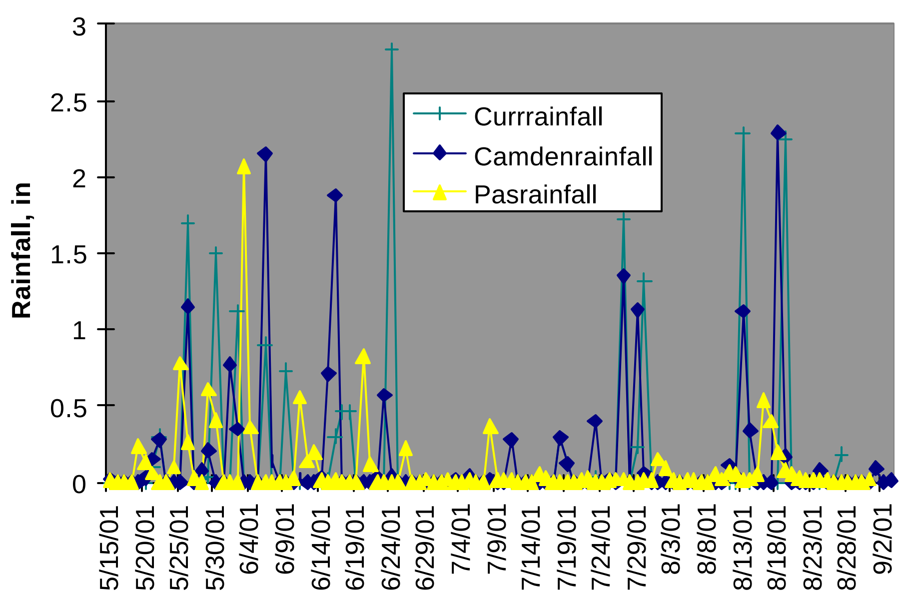

Fig. 2. Rainfall events at the field plot sites in Currituck, Camden, and Pasquotank Counties in 2001.

factors were most import in influencing fumonisin development in these studies. Drought during the growing season, though important, is clearly not the only or even the major cause of fumonisin development in corn. Further discussion of enviromental effects on fumonisin development based on data collected in 2001 can be found later in this paper.

Differences in Fumonisin Levels across Hybrids

Statistical analysis of a limited number of hybrids across years (three hybrids) and of a large sampling of hybrids in 2000 indicates that there were differences in fumonisin levels among the hybrids used in this study. When tested from 1999 to 2001, the hybrid Pioneer '32Z18' had significantly higher levels of fumonisin in the grain than did either Pioneer '34K77' or Syngenta 'N79-L3'. In 1999, fumonisin levels ranged from 10 ppm to 250 ppm. The levels of fumonisin for the individual hybrids utilized for the test were DeKalb 'DK595 BTY' with 30 ppm, Pioneer 32Z18 with 250 ppm, Mycogen '2832 IMI' with 36 ppm, Pioneer '34B23' with 90 ppm, Pioneer '34T14 PDR' with 10 ppm and Syngenta '4640 bt' with 26 ppm.

At the early harvest date (9 Aug.) in 2000, Pioneer 32Z18 and DeKalb DK595 had significantly higher levels of fumonisin than ten of the fourteen hybrids tested (Fig. 3). Grain samples from both of these hybrids had fumonisin contamination that would have prevented feeding these hybrids to swine. Two other hybrids, Syngenta 4640bt and DeKalb 'DK585' had intermediate levels of fumonisin. At the late harvest date (18 Sept.), only Pioneer 32Z18 had fumonisin levels that were significantly higher than the remaining thirteen hybrids tested (Fig. 4). Of these remaining thirteen hybrids only three, Pioneer '34A55', Pioneer '33K81', and Syngenta N79-L3 had fumonisin levels below the

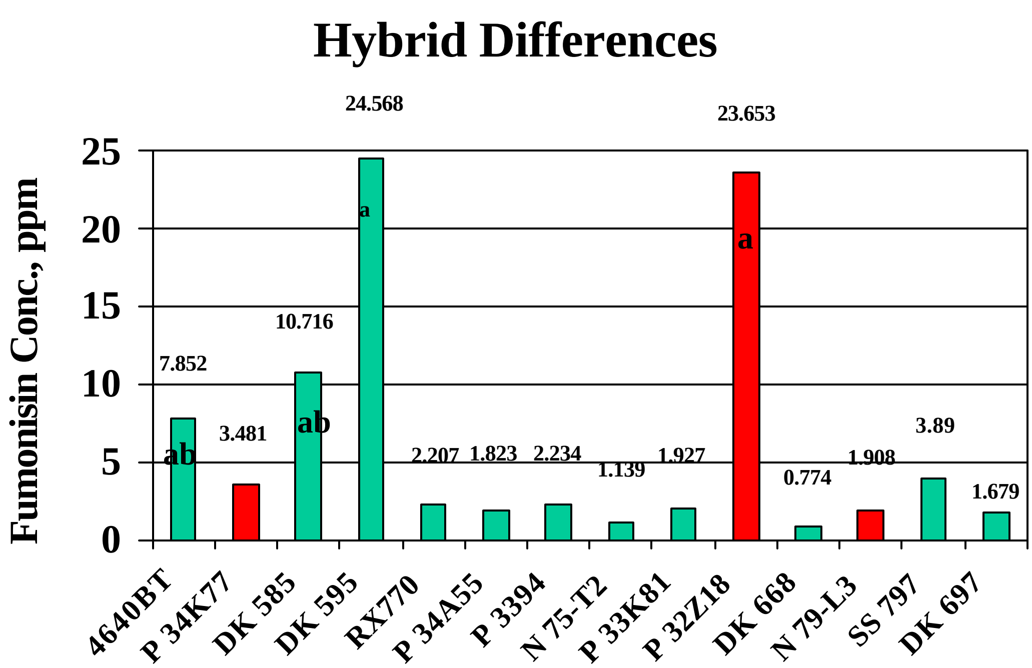

Fig. 3. Fumonisin levels for fourteen corn hybrids arranged in order of maturity measured on samples collected on 9-10 August from sites in Camden, Currituck, Perquimens, and Beaufort Counties.

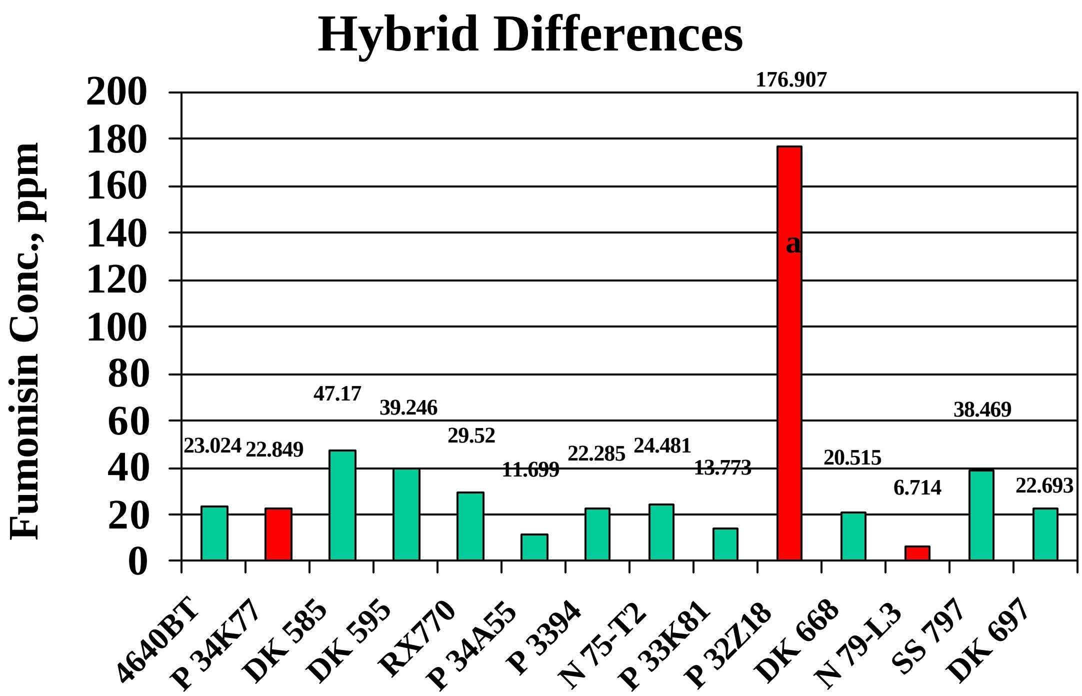

Fig. 4. Fumonisin levels for fourteen corn hybrids arranged in order of maturity measured on samples collected on 18 September from sites in Camden, Currituck, Perquimens, and Beaufort Counties.

threshold established for swine (10 ppm).

Comparisons of fumonisin levels with grain yield found that there was a relationship between fumonisin contamination and yield. Pioneer 32Z18 had significantly higher levels of fumonisin and had one of the lowest yield records in 2000 (Fig. 5). On the other hand, Syngenta N79-L3 had the lowest level of fumonisin contamination but was one of the highest yielding hybrids. The result was a significant difference in yield between these two hybrids that was similar to the difference in fumonisin levels. The most likely explanation for the relationship between fumonisin levels and grain yield is that the Fusarium ear rot fungus reduces kernel weight and yield as it infects and destroys the kernel. It appears that high levels of fumonisin are linked to reductions in grain yield.

These data indicate that hybrids differ in their susceptibility to fumonisin contamination. While there is no way to determine whether these hybrid differences were due to the physical characteristics of the plant or to resistance to the fusarium fungus, based on the consistent differences across years and upon the lack of a relationship to hybrid maturity it is clear that there are inherent differences that could be manipulated through plant breeding.

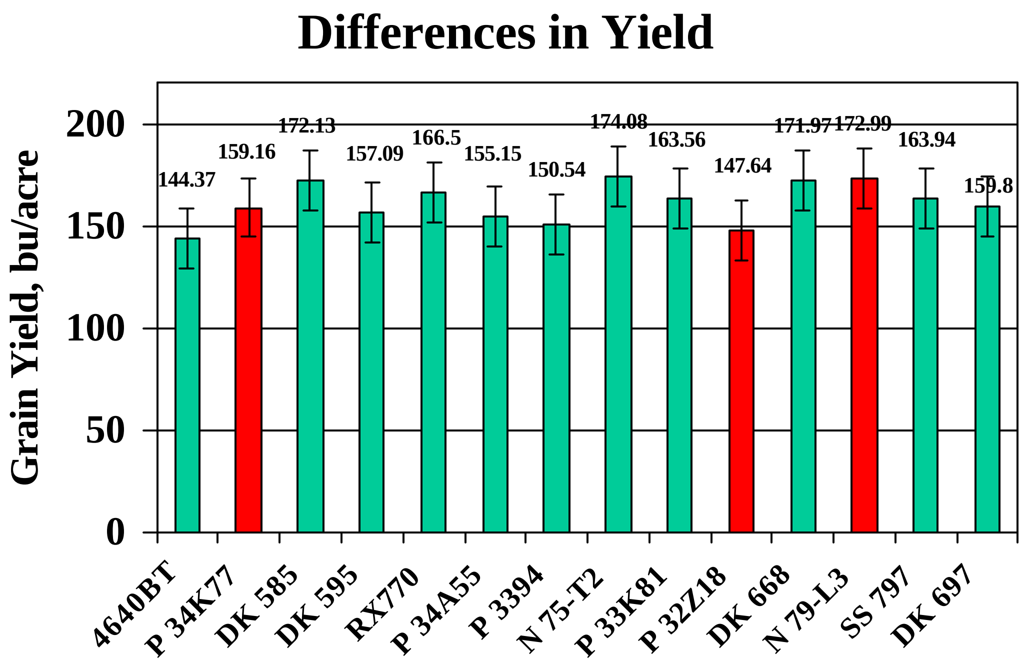

Fig. 5. Average yield of fourteen hybrids tested in Currituck, Camden, and Pasquotank Counties in 2001.

Relationships Between Kernel Moisture, Environmental Conditions, Harvest Date, and Fumonisin Levels

In an effort to find management practices that could be used to reduce or eliminate fumonisin contamination the main study in 2001 focused on the relationships between kernel moisture, environmental conditions throughout the growing and harvest period, harvest date, and fumonisin levels. The two key questions were: 1) Could a grower harvest early enough to avoid or reduce fumonisin contamination and 2) What environmental factors, rainfall, temperature, or relative humidity (RH), tend to trigger a rapid increase in fumonisin levels. Sequential harvests of grain samples found that across hybrids fumonisin levels began to increase between 6 August and 13 August (Figs. 6 and 7). Although the fumonisin levels differed by hybrid, all hybrids showed some fumonisin contamination beginning on 13 August. The increase in fumonisin levels after 13 August was rapid and continued until approximately 27 August before leveling off or, in some cases, decreasing. Unfortunately, a comparison between grain moisture and the date when fumonisin levels begin to increase rapidly shows that for all three hybrids grain moisture was between 35 and 40%. Since blacklayer usually occurs at a grain moisture level of 30 to 33%, this shows that fumonisin accumulation begins at the time that the blacklayer or physiological maturity occurs. Corn at this stage is too wet to harvest efficiently or economically. Therefore, there is very little or no chance that growers could have harvested early enough to avoid fumonisin contamination entirely. Since fumonisin levels increased rapidly over the next 7 to 14 d, it would have been difficult for growers with substantial acreage to even use early harvest to reduce fumonisin levels. However, if growers could determine which fields would be prone to develop fumonisin

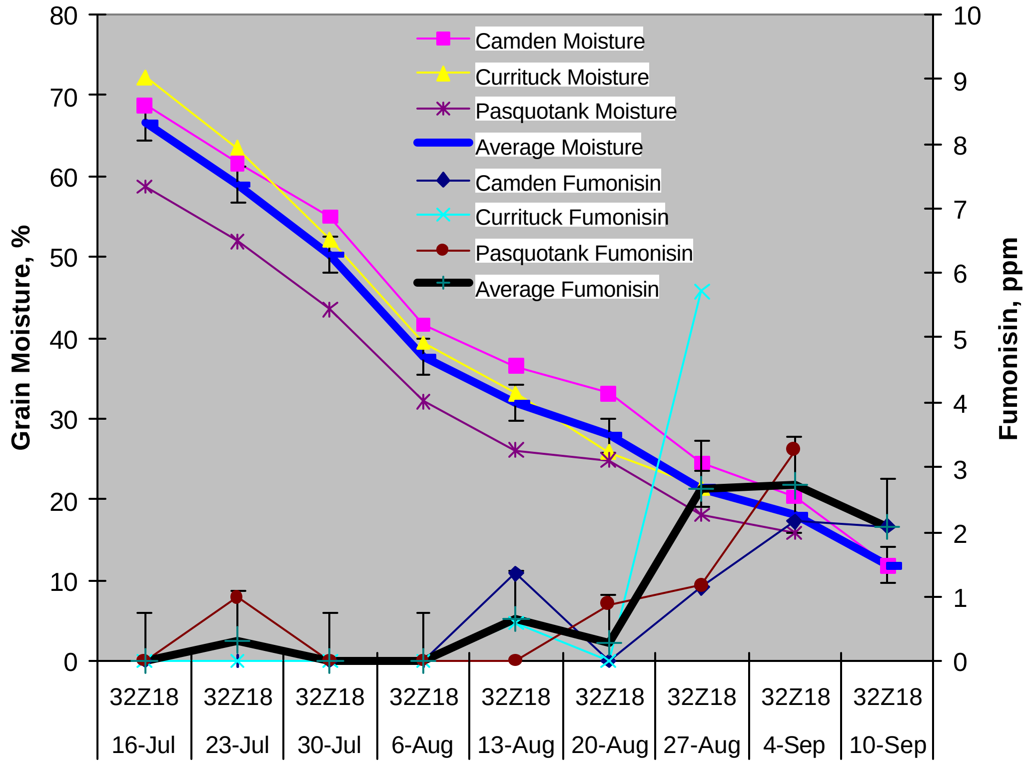

Fig. 6. Changes in grain moisture and fumonisin levels over time for Pioneer 32Z18 at three locations in eastern North Carolina.

contamination. They could harvest these areas first and have a reasonable chance of reducing fumonisin levels in the grain.

When the timeline of changes in fumonisin levels (Figs. 6 and 7) is compared to daily rainfall (Fig. 2), temperature (Fig. 8), and RH (Fig. 9) records for the three locations (Camden, Currituck, and Pasquotank Counties) the most striking environmental event as it relates to fumonisin development is the rainfall that occurred on 12 and 13 August at all locations. These rainfall events were accompanied by increases in RH and short term decreases in temperature. Immediately prior to these rainfall events temperatures had increased dramatically reaching their highest point for the summer early August. It is clear from these weather data that the key factor in fumonisin development in 2001 was rainfall accompanied by warm temperatures following physiological maturity of the crop. These factors were sufficient to increase fumonisin levels even though little or no drought or temperature stress occurred during the growing season. These results may explain why fumonisin levels were so high in 1999 when hurricanes brought an increase in rainfall during the harvest period and in 2000 when rainfall levels were high during late August and early September.

From a management standpoint, it appears from this data that very little can be done to avoid fumonisin contamination by harvesting early. It would also be difficult to adjust planting practices to avoid stress or rainfall during harvest. Hopefully, this information could be used to screen hybrids to determine if genetic differences in

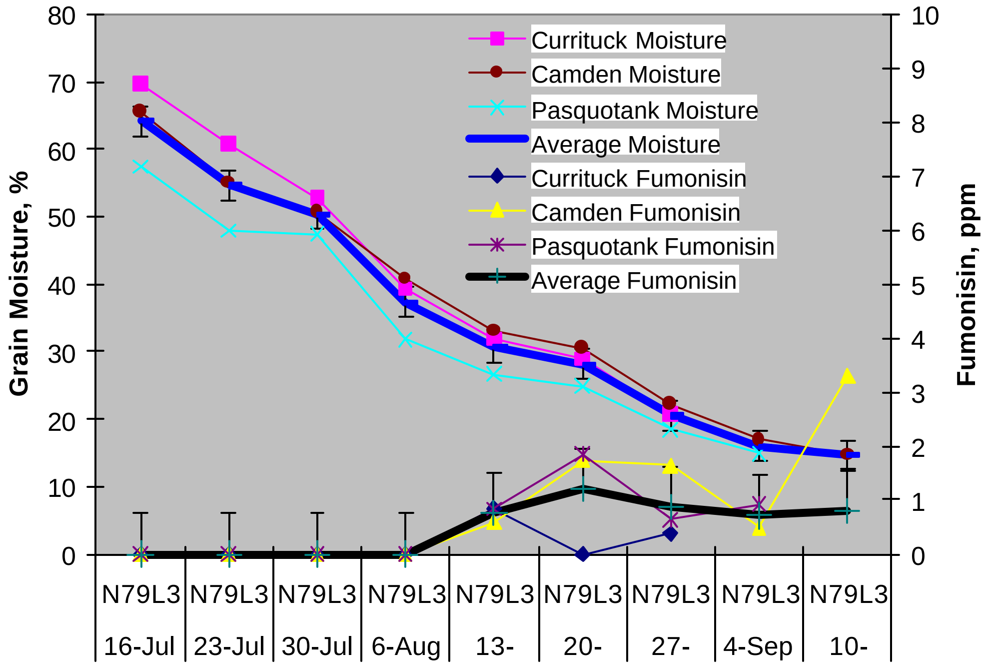

Fig. 7. Changes in grain moisture and fumonisin levels over time for Syngenta N79-L3 at three locations in eastern North Carolina.

physical characteristics or disease resistance could be manipulated to reduce fumonisin development.

Quick Methods for Determining Fumonisin Levels in the Field

Visual ratings of hybrids in 1999 and 2000 were made to determine if fumonisin contamination could be detected and levels determined through simple visual evaluation. When fumonisin levels (Figs. 3 and 4) for fourteen different hybrids were compared to a visual rating of ear samples (Fig. 10) there was a clear relationship between fumonisin levels and visual rating within each sample date. It was possible to determine the relative difference in the amount of fumonisin in the grain by visual score. However, the visual scores were not useful in determining the absolute level of fumonisin in the grain sample. Therefore, to determine if the levels of fumonisin in a grain sample exceeded a threshold value a laboratory analysis would have to be done on a test sample and the visual scores for the remaining samples would have to be compared with that sample taken at the same time and location. This makes this method impractical for determining if grain can be sold for a given purpose. However, this method would allow growers to determine which fields have a problem and need immediate harvest.

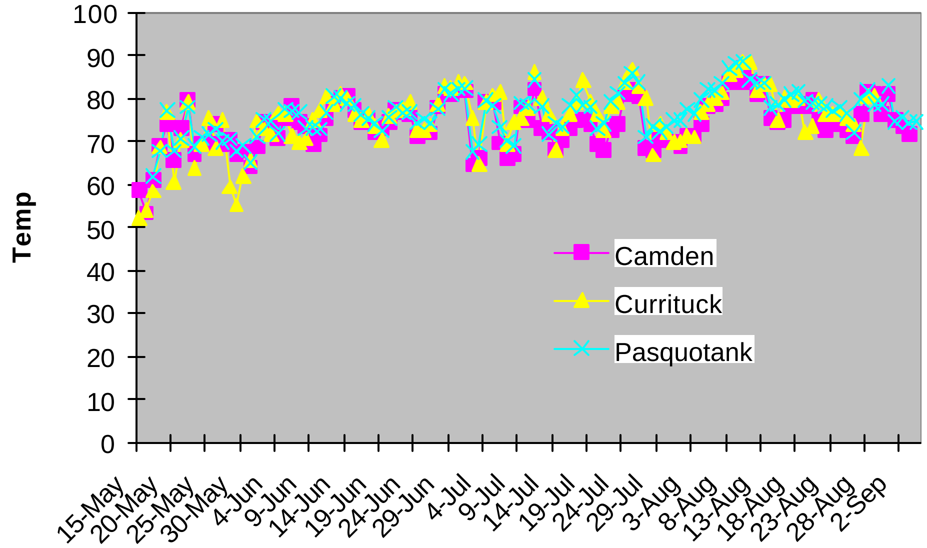

Fig. 8. Daily average temperatures at each field site over the corn growing season in 2001.

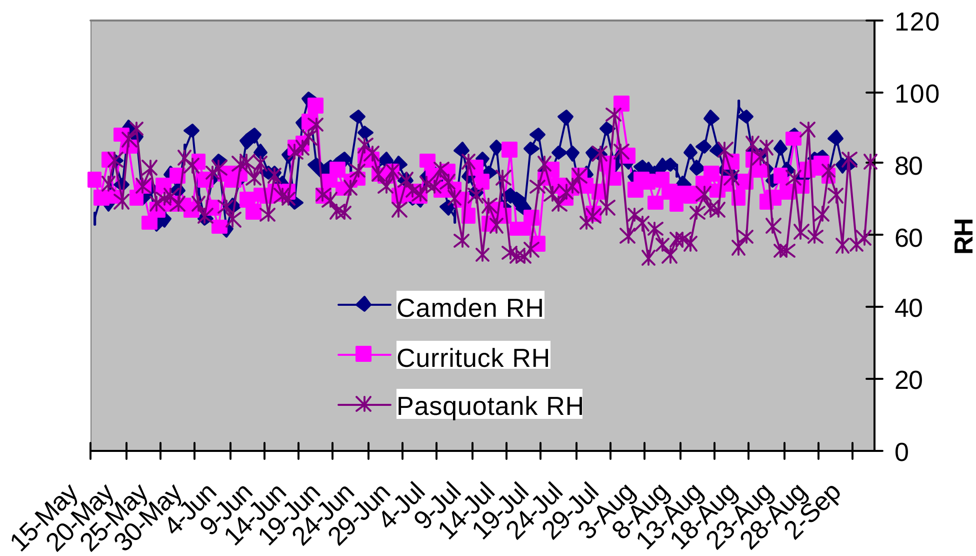

Fig. 9. Daily average relative humidity for each field site over the corn growing season in 2001.

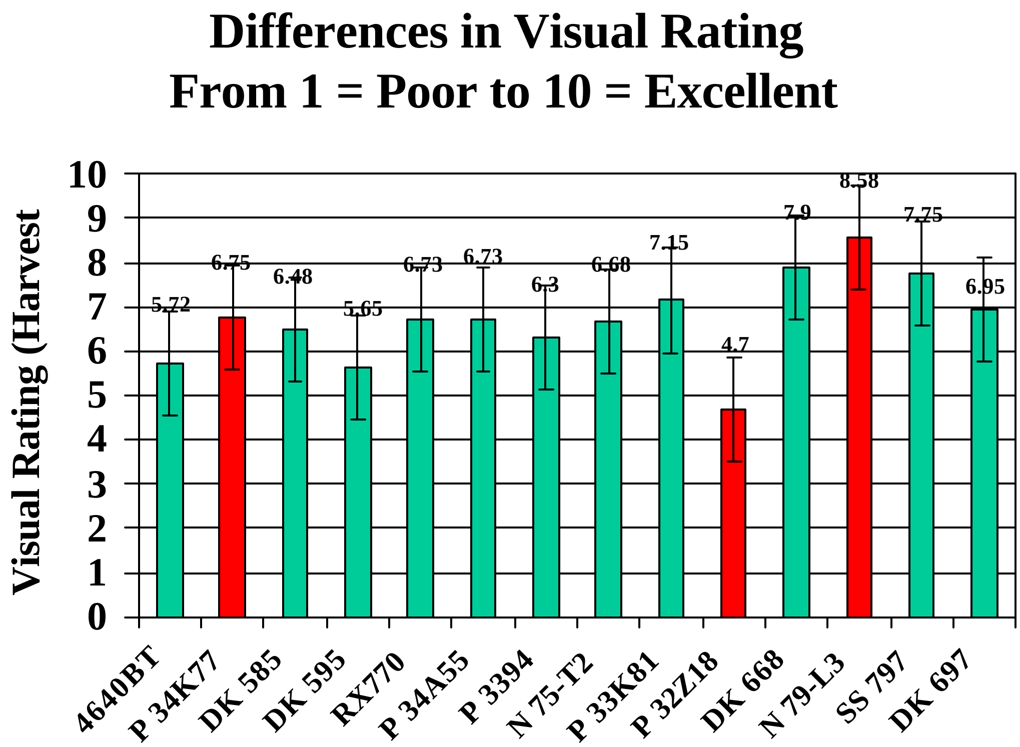

Fig. 10. Visual ratings for fusarium for fourteen corn hybrids done prior to harvest on 9 August.

SUMMARY

1. It is clear that given the levels at which the FDA proposes to regulate fumonisin contamination in corn, fumonisin will be a problem for North Carolina corn growers almost every season. There was no case in this study where fumonisin was entirely absent from corn grain grown in eastern North Carolina. In two of the three years, grain from most of the hybrids tested would have been over the FDA limit for swine, equine, and human consumption. The best that can be achieved is the reduction of fumonisin levels below thresholds for animal and human consumption.

2. There are differences among hybrids in susceptibility to the development of fumonisin. Hybrids such as Pioneer 32Z18 appear to always have higher levels than hybrids such as Pioneer 34K77 or Syngenta N79-L3. While no hybrid in these tests was entirely free from fumonisin, breeders should be able to find materials with better tolerance. While these studies did not indicate the mechanism for hybrid resistance, based on the rapid response of fumonisin to rainfall in 2001 it appears that resistance is gained through physical attributes of the plant such as better shuck coverage or a thicker shuck.

3. Fumonisin development is primarily caused by warm, moist conditions following the formation of the blacklayer in the corn kernel. Therefore, rainfall and warm weather during August and early September stimulate fumonisin development. Environmental conditions such as drought are not necessary for fumonisin development but do help increase the level of fumonisin in the grain.

4. Growers are limited in the management tools available for reducing fumonisin contamination. While early harvest could help reduce fumonisin levels, it would be difficult to harvest the entire crop early enough to avoid fumonisin. Once ideal environmental conditions are achieved, fumonisin develops rapidly. This will make it difficult to use early harvest as the primary tool in reducing fumonisin levels. The best option would be for growers to identify fields that are at risk and then harvest those first. The best management option for reducing fumonisin is to select hybrids with some resistance. Unfortunately, there is little reliable information that can be used to make a good hybrid selection for fumonisin resistance. The information gained from this study is a good first step, but must be followed up with subsequent tests. Seed corn providers must be encouraged to provide hybrid ratings for fusarium.

5. Visual inspection could be used to identify fields where fusarium is developing and where fumonisin is a potential problem. To make practical use of this tool biweekly inspections would be required beginning at physiological maturity and continuing until the crop is harvested. These inspections would be useful in targeting harvest and could be triggered by alerts from county extension offices based on weather conditions.

6. Future work should focus on four areas: 1) a study of the mechanisms of hybrid resistance, 2) the development of breeding programs based on knowledge of resistance mechanisms aimed at reducing fumonisin levels in corn, 3) a better understanding of the effects of fumonisin on livestock performance and health that would lead to realistic thresholds for fumonisin in grain, and 4) a survey of grain samples taken over several years from across North Carolina to determine where fumonisin is a likely problem from year-to-year and to develop a strategy for marketing grain which will enhance the value of North Carolina corn (For instance: corn from areas prone to fumonisin contamination could be directed to ethanol production).