Comparing Characterization Methods of Fusarium Ear Rot Resistance in Corn

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Comparing Characterization Methods of Fusarium Ear Rot Resistance in Corn

By

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A creative component submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Agronomy

Program of Study Committee:
Dr. Gary P. Munkvold, Major Professor
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Abstract

Fusarium Ear Rot in corn is a disease that produces toxins known as fumonisins that are harmful to humans and other animals. Characterizing and improving the resistance to Fusarium Ear Rot in corn is important to corn seed producers like Corteva to reduce fumonisin exposure in humans and livestock. To characterize corn hybrids Corteva visually inspects ears each from a single corn variety and scores them based on observed symptoms. This experiment was intended to compare different methods of characterizing Fusarium Ear Rot resistance in order to improve the accuracy and efficiency of Corteva’s characterization methods. Four methods of characterization were compared. The first method was the traditional visual assessment. The second method was quantitative analysis of a grain sample for the amount of fumonisin toxins present. The third method was collecting images of ear piles and using a computer program to quantify the amount of symptomatic corn versus healthy corn based on color. The fourth method was collecting images of loose kernels and using a computer program to measure the amount of symptomatic corn versus healthy corn based on color. The third and fourth method use digital image analysis which is known as photometry. Statistical analysis of the data included Pearson’s correlation, linear regression and logistic regression analyses to compare the different methods. Correlation and regression statistics indicate a strong relationship between the four methods of characterization. Whole ear photometry performed equally as well as visual inspection at predicting the amount of fumonisin toxins present in a sample. Loose kernel photometry did not perform as well as the visual method or whole ear photometry, but still performed well enough at predicting fumonisin concentrations in a sample to potentially be used for characterization. Photometry shows potential as an effective characterization method for Fusarium Ear Rot resistance that could reduce the potential for human error and be more easily automated than visual inspections or laboratory analysis for toxins.
INTRODUCTION

Fusarium Ear Rot in corn (caused by *Fusarium verticillioides* and other *Fusarium* species) produces toxins known as fumonisins that are harmful to both people and livestock. Fusarium Ear Rot in a field can make the entire field unusable as human food, animal feed, or for ethanol production. The FDA recommends no more than four parts per million of fumonisin toxins in anything intended for human consumption. The maximum allowable fumonisin concentration for animal feeds can vary from 5 parts per million for equids and rabbits to 100 parts per million for some poultry, but the general recommendation for livestock and pet animals feed is no more than 10 parts per million total fumonisins (FDA, 2001).

Corteva’s Fusarium infection characterization and screening fields in Woodland, CA are some of the best in the company due to the environment and management practices. Woodland is a unique location because it gets almost no precipitation from mid-May to late September. This lack of precipitation and precision drip irrigation techniques allows significant control over field moisture and the field environment. Water is withheld to stress the corn at specific stages during its growth, which helps make the corn more vulnerable to Fusarium infection (Parsons and Munkvold, 2010). The Woodland research team is able to consistently get high disease pressure through natural infection uniformly across entire fields, which make this location ideal for characterizing Fusarium resistance.

Strong natural infection can be difficult to induce from year to year, so most other locations inoculate their corn with the disease. Inoculation provides consistent disease pressure but does not always accurately reflect how the corn acts when naturally infected. There are a number of ways to inoculate corn with Fusarium including spraying the ears with a suspension of Fusarium spores, and inserting toothpicks that have been colonized with Fusarium directly into the silk channel, but one of the more effective ways of inoculating corn with Fusarium is to directly inject a suspension of Fusarium spores through the ear husk leaves into the ear (Clemens et al., 2003). The drawback to direct injection is that the ear husk, the kernels pericarp, or other factors could be providing some natural resistance that is overcome by directly injecting Fusarium spores into the kernels.

Western flower thrips (*Frankliniella occidentalis*) are one of the prime natural vectors for Fusarium in corn (Farrar and Davis, 1991). In Woodland’s
Fusarium trials thrips are encouraged by not applying pesticides and by bringing in Two-Spotted Spider Mites (Tetranychus urticae) from other fields, which thrips feed on. Corteva is able to get very high Fusarium pressure while keeping most other diseases out, which makes it easier to assess the Fusarium infection on its own.

The Corteva visual scoring system uses a 1-9 scale with 9 being the healthiest and 1 being the most diseased. A grower or producer is most concerned with whether they will be able to sell their crop, so the visual scoring system is focused on reducing risk for the grower rather than a fully linear scale. Providing scores to growers is not the only use of the data, it is also used to provide scores to corn breeders. At the low (most diseased) end of the visual scale everything gets a failing score, so it is difficult to tell from the score if a variety was just bad enough to fail or was the worst variety seen. This scale works well for growers avoiding risk, but corn breeders need a scale where they can make finer distinctions between varieties and to make statistical analysis more robust. Better data may help the breeders focus their efforts as they attempt to breed corn with better disease resistance.

Along with improving the scientific usefulness of the 1-9 visual scale, the company would also be interested in increasing efficiency through the use of technology. It currently takes a lot of skilled man-hours to characterize all the Fusarium ear rot plots but if there was a way to automate or increase the speed of scoring, the company could save resources and characterize a larger number of plots. The use of digital image analysis to characterize Fusarium Ear Rot resistance has a high potential for automation to enable higher throughput and faster data collection. This experiment is intended to compare several different ways of characterizing Fusarium ear rot resistance to assess and improve the current system.

**BACKGROUND**

**Fusarium Ear Rot**

Fusarium Ear Rot is a fungal disease caused primarily by *Fusarium verticillioides* as well as several other *Fusarium* species. The fungi overwinter on corn debris in the soil, the soil itself, and on nearby weeds and plants. The fungi spores can be seedborne, soilborne, or airborne. Airborne spores can infect individual kernels through the silk channels, but Fusarium often infects the ears through insect damage. “Starbursting” is caused when the kernel is
infected through the silk channel and hyphae grow within the pericarp of the kernel. In cases of severe infections, the fungus may completely consume and cover the ear, leaving a dry moldy husk.

Fusarium infections can produce the mycotoxins known as fumonisins, which are carcinogenic and known to be harmful to humans and fatal to horses and pigs. Fumonisin exposure in animals has been associated with a number of negative health effects. Many animals tested showed negative effects to the liver and kidneys after exposure. Fumonisin exposure was observed to affect the reproduction of pigs and rabbits, while birth defects were induced in mice. Horses exposed to fumonisin toxins can develop leukoencephalomalacia, which is the softening of brain tissue. In humans, fumonisin exposure has been shown to be associated with an increased risk of esophageal cancer, increased risk of neural tube defects in babies, and possible growth impairment in children. Because these studies show associations with fumonisin exposure and negative health outcomes, but have not established causation, further studies on the effects of fumonisin exposure in humans are needed (WHO, 2018).

**Current Characterization Method**

The majority of Fusarium trials are done using inoculation techniques (Mesterhazy et al., 2011). Most locations do not have adequate disease pressure for natural Fusarium infection characterization, but Corteva has successfully done this work in Woodland for decades. Many independent experiments have shown a strong correlation between visual symptoms and fumonisin toxin concentrations (Schaafsma et al., 2006; Afolabi et al., 2007; Parsons, 2008). Through lab testing for fumonisin toxin concentrations, and discussions with growers and breeders, Corteva developed a visual scoring system that they have been using for around 20 years with minimal changes. A 1 through 9 scale is used with a 9 being perfectly healthy corn and a 1 being completely mold infested by Fusarium Ear Rot. There are two main symptoms of Fusarium that are considered as each variety of corn is scored. Visible mold is the most important of the symptoms, and as soon as any mold is seen, the score is dropped to a 4 at best. A 4 is the lowest score that would be considered acceptable for a product, and a 4 could still have fumonisin accumulation issues if environmental conditions are favorable for Fusarium Ear Rot. The second and less critical symptom is called “starbursting”, which looks like streaks or lines radiating out under the pericarp from the top of each kernel where the silk was attached.
Image 1. Visible Mold on Corn Ears   Image 2. Starburst on Corn Kernels

Depending on how much starbursting is present, and if no mold is visible, a variety could score between a 4 and a 9. The Corteva scale is as follows:

**Score: Description**

9: No mold or symptoms.
8: Ears have less than 10% of kernels starbursted, mostly at tip of ear.
7: Ears have between 10-20% of kernels starbursted, mostly at tip of ear.
6: Ears have between 20-50% of kernels starbursted throughout the ear.
5: Ears have between 50-75% of kernels starbursted, no visible mold.
4: Ears have between 75-100% of kernels starbursted AND/OR between 0-25% of kernels are cracked. Any visible mold is 4 or lower.
3: Ears have between 25-50% of kernels visibly cracked or moldy.
2: Ears have between 50-75% of kernels visibly cracked or moldy.
1: Ears have over 75% of kernels visibly cracked or moldy.
Once the disease has fully developed in the fields, each plot is hand harvested and the ears are placed at the front of the plot. Some locations that screen Fusarium Ear Rot are able to use automated ear pickers to harvest, but at the Woodland location the stalks do not degrade enough and clog the machine. Because the disease can be affected by increased airflow, the two plants on each end of the plot are not harvested to avoid any border effect. After the corn has been harvested, one or two trained scorers kneel next to each ear pile and examine it. They then assign it a score from 1 to 9 using the visual scale previously described and enter this score into a handheld device. Comments may be collected as to why a plot scored a certain way, whether there is any insect damage or other diseases present, or any other things observed about the plot and related infection.

Image 3. Ear piles harvested and ready for visual assessment

**Issues with Current Characterization Method**

One of the main drawbacks to this scoring system is that it is subjective and at times there can be a lot of confounding issues that make scoring each ear pile difficult. One issue is that while the Woodland fields are relatively clean of foliar and stalk diseases besides Fusarium when compared to other Fusarium
locations Corteva manages, common smut (*Ustilago zeae*), *Penicillium* and *Aspergillus* infection occur when conditions in the Woodland fields are conducive for these diseases. These diseases can compromise the ear and allow the Fusarium to colonize the ear much more severely than it would have been able to otherwise. Excessive insect damage also compromises an ear and allows the disease to develop to a greater degree than natural infection. Scorers must take these factors into account when scoring an ear pile with these other factors present and try to assign a score based off the Fusarium alone.

Scorers must mentally subtract the amount of damage they feel is due to other diseases exacerbating the Fusarium. Another confounding issue encountered when scoring is inconsistent piles of ears. Researchers attempt to give a single score that indicates the resistance of the entire pile of ears, but when the pile is a mix of healthy ears and diseased ears it can increase the subjectivity when determining the score. Because scoring an ear pile is a subjective process, a large challenge in getting good data is making sure everyone scoring is doing so in a similar manner. All the scorers receive extensive training to ensure consistency in scoring. Currently at the Woodland station, only the research team manager and one veteran research associate score the ear piles in order to maximize consistency. At the beginning of each season, the manager and associate score a large block of varieties together to make sure they are consistent with each other. The first block scored consists of the “Check” experiments, which contain varieties with well-established resistance scores. These Check experiments allow the researchers to assess the disease pressure and calibrate scoring. Corteva plants Check experiments in each field to allow assessment of disease pressure in each location. The purpose of this experiment is to explore and compare some different methods of characterization with the hope of improving the accuracy and efficiency of Corteva’s characterization method.

**Methods**

**Characterization Methods**

Four different methods of characterizing Fusarium Ear Rot in corn were chosen.

Method 1 – Current Visual Method

As described above, this method required examination of every ear from a plot and the plot was assigned a score of 1 through 9. This method is
expensive and time consuming as it requires a controlled number of highly skilled scorers.

Method 2 – Fumonisin B1 and B2 testing

The ears from each plot were shelled together into one bag. A representative sample of grain (approximately 25g) was taken from each plot and sent to a lab for Fumonisin testing. At the lab, a mass spectromic method was used to determine the amount of the toxins Fumonisin B1 and B2 that were present in the sample (in parts/million). This method is expensive and slow. It does not require skilled labor to collect and ship samples, but shipping and lab costs can be expensive and getting lab results returned can be slow.

Method 3 – Whole Ear Photometry

Each pile of ears was collected and photographed in a special camera box. A Canon Rebel T2i 18.0 megapixel camera was used to take the images. To reduce errors the images were cropped to eliminate areas without corn to analyze. Final images averaged 2400 pixels wide by 1600 pixels high. The program Assess 2.0 was used to analyze the images. Currently, this method is slow and expensive per plot as each pile must be hand collected and the ears put in the camera box. However, if this method is proven effective it could be automated in ways that would reduce cost and increase speed of characterization.

Method 4 – Loose Kernel Photometry

The ears from each pile was shelled into one bag, and a sample of loose kernels was photographed on a black tray. A Canon Rebel T2i 18.0 megapixel camera was used to take the images. To reduce errors the images were cropped to eliminate areas without corn to analyze. Final images averaged 2000 pixels wide by 2000 pixels high. Each sample was a full 100ml cup of kernels. The program Assess 2.0 was used to analyze the images. Similar to whole ear photometry, this method is currently slow and expensive but could be automated for decreased costs and increased efficiency.

Assess 2.0

Ear photometry works by using an image taken of each pile of ears and then using a computer program to analyze the colors in the image. Currently, other groups in the company use a Corteva program to scan for yellow grain
versus white cob and then estimate yield based on that information. The intention of this experiment was to use a program to differentiate between healthy yellow grain and Fusarium infested grain, which has mold or starbursting. After inspecting Corteva’s program, it was determined that Assess 2.0 was better suited to the needs of this experiment. Assess 2.0 is a well-known plant pathology program made available from the American Phytopathology Society that was designed to quantify disease lesions in pictures of leaves (Lamari, 2008). It has a number of adjustable filters (based on the RGB - Red/Green/Blue or HSI - Hue/Saturation/Intensity spaces) available to highlight and specify which parts of a picture are the whole ears and which parts are diseased. It then counts the number of pixels in each category and calculates a “Percent Sick”.

Image 4. Original Whole Ear picture seen in Assess 2.0 work window
Using a portable Fumonisin assays as a fifth method was considered because they are commonly used in the corn industry. Ultimately, they were not included because while the tests themselves are cheap, the instruments to read the tests are expensive. Additionally, many portable tests only have a narrow test range (1-10 parts per million), which is adequate for identifying the presence
of fumonisin toxins but would not give the differentiation needed to breed corn for Fusarium Ear Rot resistance.

**Experiment Design and Planting**

The experimental design included 8 reps of 8 corn varieties (labeled Hybrid A through H) for a total of 64 plots. The experiment was planted in two separate fields in both 2015 and 2016 for a total of 256 plots. The two fields were planted 7-14 days apart each year, which helped ensure at least one planting had the proper stress to encourage Fusarium. Varieties were chosen with a wide range of Fusarium resistance scores that have been well established through many years of characterization. Twenty-five seeds were planted in each plot with the expectation of a minimum of 18 plants germinating. The experiment was mapped as eight Randomized Complete Blocks per field (4 plots wide by 2 plots deep). Each block had a minimum of four rows of corn surrounding it to prevent any border effect. The plot length was 9.5 feet with 2.5 feet alleys in between. A 4-row cone planter was used to plant single row plots. Plots were open pollinated.

**Plot Care and Harvest**

Immediately after planting, the field was irrigated using a drip tape system to initiate germination. The field was continuously well-watered until mid-way through vegetative growth, when watering was stopped and the corn was allowed to become slightly stressed, at which point watering was resumed. Watering was also paused to stress the corn shortly before flowering. It has been shown that drought stress predisposes corn to Fusarium infections (Parsons and Munkvold, 2010). Drought stress levels were determined by observing leaf rolling in the late afternoon and recovery the following morning. It has also been shown that thrips vector Fusarium and that thrips feed on mites (Parsons and Munkvold, 2010). In order to encourage a strong mite and corresponding thrip population, early in the season, mite-infested corn leaves were collected from neighboring fields and spread throughout the Fusarium fields. Bringing mites into the field early in the season increases the mite population which results in an increased thrip population. After the ear is infected with Fusarium, it needs adequate moisture for the disease to fully develop. To overcome Woodland’s dry environment and ensure there was
sufficient moisture available in the plant canopy, after black-layer, water was applied using an overhead gun.

The plots were harvested in mid to late October of each year. Each plot was hand harvested and the first and last two plants of each plot were skipped to account for any edge effect. The ears were placed on the ground at the front of their respective plot in the field. At this point data collection began.

**Data Collection**

This experiment tested four different ways of scoring Fusarium resistance against each other. Each of the plots was scored four times, once using each method. This included the visual 1-9 method, ear photometry (both whole ear and shelled grain); and lab testing for toxins produced by Fusarium.

Plots were hand harvested and placed on the ground in front of their respective plots, examined and assigned a score based on the visual 1-9 scale. The ear piles were then collected from the field in labeled bags and brought into the lab for further characterizing. Whole ears were then photographed in a camera box designed to photograph corn ears. The box can photograph 5 ears at a time so each plot had 2-4 pictures taken. Then the ears were shelled and samples of the loose kernels were photographed. Two sample images for each plot were taken. A subsample of the loose kernels was then sent to the lab for fumonisin testing. The data from fumonisin testing is labeled “FUSFB1”.

For each variety, 5-10 images that showed good examples of all the symptoms were used to determine filters that would best highlight the ears and the disease symptoms using the Assess program. Settings were quickly determined to highlight the entire ear, but highlighting the diseased portion of the ear was a greater challenge. Often to highlight all the symptomatic parts of the ear, the program would include some healthy parts, or if all of the healthy parts were excluded, it was missing some symptomatic portions. Settings were selected that did an acceptable job of highlighting the symptomatic portions of the ears with a small amount of error. These settings were then used to analyze all the images from each variety. The data produced from these analyses is labeled “%Sick Ear.”
Table 1. Assess 2.0 settings for each Hybrid used for analysis of whole ear photographs

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Ear Threshold</th>
<th>Disease Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrid A</td>
<td>HSI - Saturation 105-255</td>
<td>RGB - Blue 105-255</td>
</tr>
<tr>
<td>Hybrid B</td>
<td>HSI - Saturation 50-255</td>
<td>RGB - Blue 85-95</td>
</tr>
<tr>
<td>Hybrid C</td>
<td>HSI - Saturation 95-255</td>
<td>RGB - Blue 95-255</td>
</tr>
<tr>
<td>Hybrid D</td>
<td>HSI - Saturation 95-255</td>
<td>RGB - Blue 95-255</td>
</tr>
<tr>
<td>Hybrid E</td>
<td>HSI - Saturation 65-255</td>
<td>RGB - Blue 100-255</td>
</tr>
<tr>
<td>Hybrid F</td>
<td>HSI - Saturation 70-255</td>
<td>RGB - Blue 95-255</td>
</tr>
<tr>
<td>Hybrid G</td>
<td>HSI - Saturation 90-255</td>
<td>RGB - Blue 90-255</td>
</tr>
<tr>
<td>Hybrid H</td>
<td>HSI - Saturation 100-255</td>
<td>RGB - Blue 90-255</td>
</tr>
</tbody>
</table>

Some of the symptoms of Fusarium infection are more easily seen when the ear is broken in half and kernels are examined from all sides, which is why the ears were shelled and the loose kernels were photographed to see if additional accuracy could be gained by exposing more surface area. A number of experiments to examine different factors relating to Fusarium Ear Rot were done by Corteva at the Woodland station around 2003. To characterize Fusarium resistance these trials examined the amount of symptomatic grain in a sample as related to fumonisin toxin concentrations present. A high correlation between the number of symptomatic kernels and the amount of fumonisin toxins present was observed (Parsons, 2008). However, it was more difficult to highlight the kernels and the disease compared to the whole ear images when using Assess 2.0 to examine the images. The whole ear images were much more uniform since they showed the kernels from the top and from the same angle.

Loose kernel images showed the kernels from all angles and exposed the germ of the kernels. The kernels germ was often a similar color to some of the Fusarium symptoms the program was trying to highlight, making it difficult to separate the germ from the disease symptoms based on color. The 2003 trials that had success examining loose kernels individually examined each kernel in the sample under a microscope to look for symptomatic kernels (Parsons, 2008). While this microscopic observation was successful it is not practical for large scale screening. Acceptable filter settings were determined for the loose kernel images, but the margin of error was larger for the loose kernel images compared to the whole ear images. After testing the filter settings on the loose kernel images, the data it produced underestimated the disease in the least healthy
varieties and overestimated the disease in the healthier varieties compared to the whole ear photometry data. The data from these analyses is labeled “%Sick LK.”

Table 2. Assess 2.0 settings for each Hybrid used for analysis of loose kernel photographs

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Ear Threshold</th>
<th>Disease Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrid A</td>
<td>HSI - Saturation 45-255</td>
<td>RGB - Blue 205-255</td>
</tr>
<tr>
<td>Hybrid B</td>
<td>HSI - Saturation 50-255</td>
<td>RGB - Blue 85-95</td>
</tr>
<tr>
<td>Hybrid C</td>
<td>HSI - Saturation 30-255</td>
<td>RGB - Blue 190-255</td>
</tr>
<tr>
<td>Hybrid D</td>
<td>HSI - Saturation 30-255</td>
<td>RGB - Blue 190-255</td>
</tr>
<tr>
<td>Hybrid E</td>
<td>HSI - Saturation 30-255</td>
<td>RGB - Blue 185-255</td>
</tr>
<tr>
<td>Hybrid F</td>
<td>HSI - Saturation 30-255</td>
<td>RGB - Blue 185-255</td>
</tr>
<tr>
<td>Hybrid G</td>
<td>HSI - Saturation 30-255</td>
<td>RGB - Blue 165-255</td>
</tr>
<tr>
<td>Hybrid H</td>
<td>HSI - Saturation 30-255</td>
<td>RGB - Blue 150-255</td>
</tr>
</tbody>
</table>

Some plots were dropped from the experiment because there was missing data. A small number of the pictures were not in focus and could not be analyzed, and two of the lab samples were contaminated and were not processed. If any data was missing for a plot, the whole plot was dropped from the experiment.

Analysis and Discussion

JMP (a statistical analysis program developed by the SAS Institute) was used to examine the data. The visual scoring method assigns a higher number to healthier corn (a 1 is the least healthy and a 9 is the healthiest) and the other three methods of characterization assign a lower number for healthier corn, so a “FUSERS ADJ” visual score was created where the order of visual scores was reversed (in the FUSERS ADJ visual score a 9 is now the most diseased and a 1 is now the healthiest). This change enabled uniform comparison of disease values without having to make any further adjustments. A Student’s t-test (t=1.97008, alpha=.05) was used to rank and separate each Hybrid into statistically similar classes for each characterization method.
The simple ranking showed that each method was reasonably consistent with each other. Hybrid C, Hybrid D, Hybrid G, Hybrid H and Hybrid B did not change rank using any of the four methods. Hybrid F, Hybrid E, and Hybrid A changed ranks within the 5-7 ranks. Hybrid A was a unique case in the experiment because it became co-infected with Penicillium as well as Fusarium. In accordance with the normal visual scoring methods, the Penicillium was ignored when scoring, and it scored as the second healthiest of all the entries with this method. Assess 2.0 was not able to fully isolate the Penicillium from the Fusarium, so the Whole Ear photometry (% Sick Ear) and Loose Kernel photometry (% Sick LK) data included some Penicillium symptoms and was skewed high, or more diseased.

A regression analyses was run using JMP and fit lines were created to see how well FUSERS ADJ, % Sick Ear, and % Sick LK could predict the Lab Analysis (FUSFB1) data. These three methods were chosen to try and predict FUSFB1 because FUSFB1 is the most quantifiable and actionable of the four methods and is a direct measurement of the fumonisin toxin that breeders are trying to prevent from occurring and accumulating in the crop.
Figure 1. Linear regression and fit line analysis of FUSFB1 (PPM) by FUSERS Adjusted and FUSFB1 (PPM) by Assess %Sick Ear
RSquare ($r^2$) shows the strength of the linear relationship between the response (FUSFB1) and each of the three predictors (FUSERS ADJ, %Sick Ears, %Sick LK). The closer $r^2$ is to 1, the stronger the linear relationship, but often disease data is found to be more variable than many other types of data, so an $r^2$ value as high as 1 was not expected in this experiment. FUSFB1 by FUSERS ADJ (Fig. 1) shows an $r^2$ value of 0.54, FUSFB1 by %Sick Ears (Fig. 1) $r^2$ value is 0.56, and FUSFB1 by %Sick LK (Fig. 2) has an $r^2$ value of 0.46. Aspects of the visual scoring method could be improved, but because it is a well-tested method that has proven its value, it is accepted that an $r^2$ value of 0.54 for FUSFB1 by FUSERS ADJ (Fig. 1) is an acceptable value for this limited data set. With an $r^2$ value of 0.56, FUSFB1 by %Sick Ears (Fig. 1) has a slightly stronger relationship than FUSFB1 by FUSERS ADJ (Fig. 1). This shows that by using pictures of whole ears and Assess 2.0 the FUSFB1 values of each plot were able to be predicted with more accuracy than using the visual scoring.
system by a small amount. Data from Figure 2 shows that the %Sick LK photometry analysis was not as accurate, and this was supported by the fact that FUSFB1 by %Sick LK (Fig. 2) had a lower $r^2$ value than either of the other methods. However, the graph for FUSFB1 by %Sick LK (Fig. 2) separated the worst samples from the best in an acceptable range. Zero to 20 %Sick LK appears to be acceptable material, 20 to 40 %Sick LK appears to be questionable material, and above 40 %Sick LK appears to be unacceptable material.

Next, the same regression and fit line analysis were performed to see if FUSERS ADJ (visual method) outcomes were predictable with the other three methods. All the methods were compared against FUSERS ADJ because this is the method currently used by Corteva.
Figure 3. Linear regression and fit line analysis of FUSERS Adjusted by FUSFB1 (PPM) and FUSERS Adjusted by Assess %Sick Ear
The FUSERS ADJ by FUSFB1 (Fig. 3) analysis produced an $r^2$ value of 0.54 (this is the same analysis as FUSFB1 by FUSERS ADJ (Fig. 1)). FUSERS ADJ by %Sick Ear (Fig. 3) had an $r^2$ value of 0.78 and FUSERS ADJ by %Sick LK (Fig. 4) had an $r^2$ value of 0.70. This showed that both %Sick Ear (whole ear photometry) and %Sick LK (loose kernel photometry) had a strong linear relationship with FUSERS ADJ.

Most of the filter settings used in Assess 2.0 for each entry overlapped. In order to further check the ability to characterize Fusarium using images, the images were analyzed using a single set of Assess 2.0 filter settings. This data
is titled “All %Sick Ear”. Hybrid B is a white corn variety and for analysis it had very different Assess 2.0 settings from all the other entries, so it was excluded from this analysis.

Table 4. Assess 2.0 settings used for analysis of whole ear photographs of all yellow Hybrids

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Ear Threshold</th>
<th>Disease Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Yellow</td>
<td>HSI - Intensity 90-255</td>
<td>RGB - Blue 95-255</td>
</tr>
</tbody>
</table>

Figure 5. Linear regression and fit line analysis of FUSFB1 (PPM) by All %Sick Ears and FUSERS Adjusted by All %Sick Ears
The same fit line analysis was run comparing the new “All %Sick Ear” data against the FUSFB1 (lab toxin analysis) and FUSERS ADJ (visual method) data. FUSFB1 by All %Sick Ear (Fig. 5) had an $r^2$ value of 0.57. This $r^2$ value of 0.57 was compared to the $r^2$ value of 0.56 for FUSFB1 by %Sick Ears (Fig. 1), or an $r^2$ value of 0.54 for FUSFB1 by FUSERS ADJ (Fig. 1) from the previous analyses. FUSERS ADJ by All %Sick Ear $r^2$ equaled 0.72 (Fig. 5), compared to FUSERS ADJ by %Sick Ear (Fig. 3) $r^2$ at 0.78 and FUSERS ADJ by FUSFB1 (Fig. 3) $r^2$ at 0.54. The Assess 2.0 filters selected did equally well at predicting FUSFB1 values as the traditional FUSERS ADJ visual method which is supported by the FUSFB1 by All %Sick Ear (Fig. 5) $r^2$ value of 0.57. The set of Assess 2.0 filters predicted the visual FUSERS score with an acceptable margin as shown by the FUSERS ADJ by All %Sick Ear (Fig. 5) $r^2$ value of 0.72.

### Table 5. Pearson’s correlation between data types

<table>
<thead>
<tr>
<th></th>
<th>FUSFB1 (PPM)</th>
<th>FUSERS ADJ</th>
<th>Assess %Sick Ear</th>
<th>Assess %Sick LK</th>
<th>ALL Assess %Sick Ear</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUSFB1 (PPM)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FUSERS ADJ</td>
<td>0.735</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assess %Sick Ear</td>
<td>0.75</td>
<td>0.885</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assess %Sick LK</td>
<td>0.677</td>
<td>0.838</td>
<td>0.855</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ALL Assess %Sick Ear</td>
<td>0.762</td>
<td>0.879</td>
<td>0.981</td>
<td>0.816</td>
<td>1</td>
</tr>
</tbody>
</table>

A Pearson’s correlation analysis was run as an additional method to compare the results. Pearson’s correlation is another way of examining the linear relationship between two variables. Pearson’s correlation values can range from -1 for a perfect negative linear relationship to +1 for a perfect positive linear relationship. A Pearson’s correlation value of 0 means that there is no linear relationship between the two variables (Clewer and Scarisbrick, 2001). A strong positive linear relationship between each pair of data collection methods was shown by all the Pearson’s correlation values from the analysis (Table 5).

Although the linear regression and correlation analyses indicated significant relationships among the variables, the scatter plots relating FUSFB1 to other variables (Figs. 1,2,5) indicated non-linear relationships. Nonlinear regression analyses were conducted to determine if a better fit could be found for the FUSFB1 (PPM) data. In Table 5, FUSFB1 (PPM) had the weakest Pearson’s correlation with all the other data. JMP was used to compare several different kinds of linear and nonlinear regressions including linear, quadratic, logistic, and exponential regressions. The logistic regression model matched
the data the best. The logistic regression model was used to compare FUSFB1 (PPM) to FUSERS Adjusted, Assess %Sick Ears, Assess %Sick LK, and All Assess %Sick Ears. Non-linear regressions use an adjusted RSquare value ($R^2$).

Figure 6. Nonlinear logistic regression and fit curve analysis of FUSFB1 (PPM) by FUSERS Adjusted and FUSFB1 (PPM) by Assess %Sick Ear
Figure 6. Nonlinear logistic regression and fit curve analysis of FUSFB1 (PPM) by Assess %Sick LK and ALL Assess %Sick Ear

Comparing the linear regression lines to the logistic regression lines (Figures 1 and 2 to Figures 5 and 6) shows that the logistic regression line does a better job of describing the data. Using the linear regression model, the $r^2$ values average 0.53, while using the nonlinear logistic model the average $R^2$ value was 0.59, which indicates that the nonlinear logistic model is a better fit for

### Table 6. Comparison of RSquare values and ranking from linear analyses and nonlinear logistic analyses

<table>
<thead>
<tr>
<th>Y by X</th>
<th>Linear Analysis Rsquare</th>
<th>Linear Analysis Rank</th>
<th>Nonlinear Logistic Analysis Rsquare Value</th>
<th>Nonlinear Logistic Analysis Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUSB1 (PPM) by FUSERS Adjusted</td>
<td>0.54</td>
<td>3</td>
<td>0.61</td>
<td>2</td>
</tr>
<tr>
<td>FUSB1 (PPM) by Assess %Sick Ear</td>
<td>0.56</td>
<td>2</td>
<td>0.62</td>
<td>1</td>
</tr>
<tr>
<td>FUSB1 (PPM) by Assess %Sick LK</td>
<td>0.46</td>
<td>4</td>
<td>0.53</td>
<td>4</td>
</tr>
<tr>
<td>FUSB1 (PPM) by ALL Assess %Sick Ear</td>
<td>0.57</td>
<td>1</td>
<td>0.6</td>
<td>3</td>
</tr>
</tbody>
</table>
This data. Either regression model shows the $r^2$ and $R^2$ values for FUSFB1 (PPM) by FUSERS ADJ, FUSFB1 (PPM) by Assess %Sick Ear, and FUSFB1 (PPM) by ALL Assess %Sick Ear differ by .04 or less (Table 6). This shows these methods all have a very similar relationship to the FUSFB1 (PPM) data. Whole ear photometry did an equally good job as the visual scoring method when related to fumonisin concentrations.

**Conclusion**

After comparing four methods of characterizing Fusarium infection, the data shows that photometry could currently be used to separate corn into 3 different health classes: clearly diseased, clearly healthy, and questionable health. With this limited number of entries photometry was not accurate enough to create 9 classes like the visual system, but the FUSERS ADJ compared to the FUSFB1 data showed that the visual method is not separating things into 9 statistically different classes in this experiment. The Students t-test for FUSERS ADJ (Table 3) indicated 7 separate classes but there was not a Hybrid that averaged a FUSERS ADJ score of 9 in this experiment, so that could account for the lesser number of classes. The Students t-test for FUSFB1 (Table 3) indicated that there were actually only 3 distinct classes based on FUSFB1, so the photometry data is matching that well.

Whole ear photometry worked best in this experiment, but there could be situations where loose kernel photometry would be preferable. Some advantages for loose kernel photometry is that it could allow people to combine multiple ears into one sample to get an average score or to take grain samples off a combine or from storage and assess disease presence. While loose kernel photometry performed the worst of the methods tested, it still had a reasonable correlation to FUSFB1 concentration. Considering that, and the success previous studies have had, visually inspecting grain samples and relating them to fumonisin toxin concentration, loose kernel photometry could be useful (Parsons, 2008). A basic consumer grade camera was used to take the images and the program used to analyze the images was not the newest or most sophisticated program. A more advanced camera and a newer program could likely do a better job at identifying Fusarium symptoms. A multispectral or hyperspectral imaging system could also improve the use of photometry to identify Fusarium.

Automating photometry could allow quicker screening of a larger number of hybrids and faster determination if the hybrids are clearly diseased, clearly
healthy, or of questionable health. Screening more hybrids at earlier stages in product development would be beneficial to Corteva. Product development is expensive and by eliminating Fusarium Ear Rot susceptible hybrids earlier in the process, a significant amount of resources could be saved. Even though photometry shows promise as a characterization method it cannot currently replace the visual scoring method. Using photometry, it would still be necessary to visually inspect and score hybrids that photometry classified as questionable or healthy. Hybrids that photometry classified as healthy would need to be verified by the visual method of scoring because these hybrids would be more likely to become products for sale, and Corteva would want to confirm this data before selling a product to growers. However, as the technology improves, photometry could be used to screen more and more hybrids early in the breeding process leaving a much smaller subset of hybrids that would have to be evaluated by trained scorers. The results of this study demonstrates there is potential for using photometry in Fusarium Ear Rot characterization, and I would recommend developing methods to integrate photometry into Corteva’s current characterization efforts.
References


