Image Analysis and Visual Estimates for Evaluating Disease Reactions of Corn to Fusarium Stalk Rot

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ABSTRACT

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Two field-grown hybrids were evaluated in three tests (1987–1989) for reaction to the stalk rot fungi *Fusarium moniliforme, F. graminearum*, and *F. proliferatum*, using a visual rating scale (0–4) and image analysis (0–100%). Both rating methods indicated that hybrid W153R × A619 had more stalk discoloration than A632 × A619 when inoculated with any of the three *Fusarium* species. The difference between hybrids was greater when an image analyzer was used. Significant differences ($P = 0.05$) between the disease reactions caused by the three pathogens were found among ratings made with the image analyzer in all 3 yr of testing. *F. moniliforme*, compared with the other *Fusarium* spp., caused significantly more ($P = 0.05$) stalk discoloration in the 1987 and 1988 tests when measured by the image analyzer. Significant differences among discoloration caused by *Fusarium* species were found only in the 1988 test using the visual rating scale. The image analyzer provides a more precise means of evaluating corn for susceptibility to Fusarium stalk rot as compared to visual ratings.

Fusarium stalk rot is a disease that is found worldwide wherever corn (*Zea mays* L.) is grown. The disease is caused by several species of *Fusarium* that occur singly or as part of a complex. Incidence and severity of stalk rot are greatly influenced by environmental factors such as plant population, tillage methods, soil temperature, soil moisture, hail, leaf blights, crop rotations, nutrition, and insect damage (1,4,6,7,9,10,13). Genetic factors such as disease resistance, stalk strength, and senescence patterns also affect disease (1,11,12).

Because environmental factors greatly affect disease severity, there is considerable variation in the amount of disease from year to year, from location to location, and among replicates in a single field, even in inoculated test plots. Environmental variation interferes with the identification of resistance in the corn plant and of virulence in stalk-infecting species of *Fusarium*. The difficulty of identifying genetic differences in host resistance or pathogen virulence is compounded by the relatively insensitive rating scale typically used to assess the reaction of corn to infection by stalk rot pathogens.

A common method used to rate stalk rot is a visual rating of the amount of discoloration in the inoculated internode using a rating scale with four categories, each category covering 25% of the total 100% (4). Presumably, the utilization of a scale with few classes, each of a large range, facilitates disease rating for the scorer and increases the precision of assessment as compared to direct visual estimates of percent tissue. A broad-based scale, however, decreases the precision of the rating.

In addition to the variation caused by environmental factors, error in disease assessment is introduced by the scorer. Disease may be overestimated, especially at low disease incidence, and estimates may be inconsistent among scorers (14). This type of error makes it difficult to distinguish differences either in disease resistance among corn genotypes or in virulence levels of the pathogen. One method thought to increase the precision in disease assessment is image analysis (2,8), which measures the percentage of discolored internodal tissue. The objective of this study was to compare ratings of corn stalk discoloration caused by *Fusarium* using the standard visual rating scale with ratings made by the image analyzer.

MATERIALS AND METHODS

Inoculations. Plants were inoculated by the toothpick method (16). Toothpicks were soaked in water, rinsed, and placed on end in a glass jar filled with 25 ml of Difco potato-dextrose broth. Plugs of culture medium from the edges of 9- to 10-day-old fungal cultures were transferred to the sterilized growth medium and allowed to grow under cool-white fluorescent lamps at 24 C for 24 hr for 2 wk. Toothpicks were removed from the jars and air-dried. At approximately 3 wk after silking of corn ears, one toothpick was placed in the middle of the second internode above the soil line in each cornstalk.

Experimental design. Two dent corn hybrids (A632 × A619 and W153R × A632) were inoculated with three *Fusarium* species obtained from the *Fusarium* culture collection at the Department of Plant Pathology, University of Minnesota: *F. moniliforme* J. Sheld., *F. proliferatum* (T. Matsushima) Nirenberg, and *F. graminearum* Schwabe. Each plant was inoculated with only one

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of the three *Fusarium* species. Controls included a mock inoculation with a sterile toothpick and noninoculated plants (except for the 1987 test, which contained only a toothpick control). The experiment used a randomized block design with four replicates of 10 plants per treatment and was repeated three times—at a location in Prescott, Wisconsin, in 1987 and 1988 and at the University of Minnesota farm in St. Paul in 1989.

Analysis of variance was done on each of the three experiments (15). Results of the statistical analysis revealed no significant interactions between any of the treatments. Data are presented as the mean rating as averaged over all other treatments.

Disease assessment. Plants were harvested 4 wk after inoculation. Ten plants were selected randomly from each replicate, and the leaves were stripped from the stalk. Pruning shears were used to excise the inoculated internode from each plant. The samples were bundled together and brought into the laboratory for disease rating. Stalks were stored at 4 C until processed. All samples were rated within 24 hr of harvest.

Visual ratings. Each of 10 stalks of each treatment was split lengthwise with a knife and rated visually on a scale of 0–4, where 0 = no or a trace amount of discoloration around the wound made by the toothpick, 1 = 1–25% of inoculated internode discolored, 2 = 26–50% of inoculated internode discolored, 3 = 51–75% of inoculated internode discolored, and 4 = 76–100% of inoculated internode discolored.

Image analysis. One half of the split stalk was placed on a light table positioned under the camera of an area meter (Delta T Devices Ltd., Cambridge, England). The total area of the split corn stalk was determined, followed by the area of the discolored zone, if any, in the internode. The ratio of the two values multiplied by 100 resulted in the percentage of area discolored.

RESULTS

Comparison of *Fusarium* spp. *F. moniliforme* induced a slightly greater amount of discolored tissue in stalks than did the other two species for all 3 yr of testing, and *F. proliferatum* produced the least amount of discoloration as measured visually (Table 1). *F. graminearum* was not significantly different from *F. moniliforme* in causing discoloration in any of the 3 yr of testing.

In measurements made by image analysis, *F. moniliforme* caused the greatest amount of discoloration, from 10.2% in 1987 to 48.5% in 1988 (Table 1). *F. graminearum* and *F. proliferatum* were consistently lower in percentage of discoloration compared to *F. moniliforme*. The percentage of discolored tissue associated with *F. moniliforme* was statistically greater than that associated with *F. graminearum* in one of the 3 yr (1988) but was statistically greater than that associated with *F. proliferatum* in all 3 yr of testing.

Comparison of hybrids. Visual ratings indicated a greater amount of discoloration in the hybrid W153R × A619 than in A632 × A619 in 1987, whereas the opposite was true in 1989 (Table 2). The ratings were not statistically different in 1988.

Image analysis indicated significantly more tissue discoloration in W153R × A619 than in A632 × A619 in 1987 (Table 2). In 1989, a significantly greater amount of discoloration was detected in A632 × A619 than in W153R × A619. The amount of discoloration in the two genotypes in 1988 was not significantly different.

Comparison of the two methods. Small differences in the amount of stalk discoloration between fungal treatments were more apparent with use of the image analyzer than by the visual rating system. In 1988, the visual method revealed a small, nonsignificant difference between *F. moniliforme* and *F. graminearum* (3.5 vs. 3.4). With the image analyzer, however, the difference between *F. moniliforme* and *F. graminearum* (48.5% vs. 38.2%) was statistically significant. Moreover, by the visual method, *F. proliferatum* was only significantly different in pathogenicity from the other two species in 1988. The image analyzer indicated that this species, when compared with the other two species, produced significantly less stalk discoloration in all 3 yr. In all three tests, image analysis resulted in the detection of more significant differences among the treatments than did visual ratings (Table 1).

The two rating methods identified a similar degree of resistance in the two hybrids, as evidenced by the amount of stalk discoloration induced by inoculation. In 1988, neither method detected significant differences in stalk discoloration between W153R × A619 and A632 × A619, whereas both methods revealed differences in 1987 and 1989.

When treatments are ranked according to the amount of discoloration without regard to statistical differences, both

Table 1. Comparison of two methods of analysis of tissue discoloration in corn stalks inoculated by the sterile toothpick method with three species of *Fusarium*

	Infection rating per year and method per treatment[w]					
	1987		1988		1989	
Treatment	Visual[x] (0–4)	Image[y] (%)	Visual (0–4)	Image (%)	Visual (0–4)	Image (%)
F. moniliforme	1.1 a[z]	10.2 a	3.5 a	48.5 a	1.6 a	24.0 a
F. graminearum	1.1 a	9.6 ab	3.4 a	38.2 b	1.4 a	21.8 a
F. proliferatum	1.0 a	8.0 b	2.3 b	26.3 c	1.4 a	19.1 b
Inoculated control	0.8 b	5.5 c	2.3 b	23.2 c	1.1 a	13.0 c
Noninoculated control			0.9 c	7.0 d	0.4 b	0.0 d
CV	20.0	15.5	15.6	33.8	41.0	8.2

[w] Values are based on an average of four replicates each for two hybrids, A632 × A619 and W153 × A619.
[x] Visual rating is based on a scale of 0–4, where 0 = no or a trace amount of discoloration around inoculation wound, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% of inoculated internode discolored.
[y] Image analysis is based on percent discolored tissue in the inoculated internode as measured by the Delta T image analyzer.
[z] Means in a column for each year followed by the same letter(s) are not significantly different at $P = 0.05$ using Duncan's multiple range test.

Table 2. Comparison of two methods of analysis of tissue discoloration in two corn hybrids inoculated by the sterile toothpick method with three species of *Fusarium*

	Infection rating per year and method per hybrid[w]					
	1987		1988		1989	
Hybrid	Visual[x] (0–4)	Image[y] (%)	Visual (0–4)	Image (%)	Visual (0–4)	Image (%)
A632 × A619	0.9 a[z]	5.5 a	2.4 a	29.9 a	1.5 a	23.5 a
W153R × A619	1.1 b	11.1 b	2.3 a	28.5 a	0.8 b	13.4 b
CV	20.0	15.5	15.6	33.8	41.0	8.2

[w] Values are based on an average of four replicates for each hybrid.
[x] Visual rating is based on a scale of 0–4, where 0 = no or a trace amount of discoloration around inoculation wound, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% of inoculated internode discolored.
[y] Image analysis is based on percent discolored tissue in the inoculated internode as measured by the Delta T image analyzer.
[z] Means in a column for each year followed by the same letter are not significantly different at $P = 0.05$ using Duncan's multiple range test.

rating methods consistently rank *F. moniliforme* equal to or higher than *F. graminearum* and *F. graminearum* equal to or higher than *F. proliferatum* (Table 1). When evaluated by either system, stalk discoloration ratings of hybrid W153R × A619 were higher than those of A632 × A619 in 1989, whereas the opposite was true in 1987. Virtually no difference in the amount of tissue discoloration was found by either of the rating methods in 1988 (Table 2).

The effect of season on severity of stalk rot induced by the three *Fusarium* species is obvious using both rating methods. Visual ratings varied from 1.0 to 1.1 in 1987 to 2.3 to 3.5 in 1988; whereas ratings were intermediate (1.4-1.6) in 1989. These differences are more startling when the image analyzer is used. Percent discoloration of infected stalks varied from 8 to 10% in 1987 to 19 to 24% in 1989 to a range of 26 to 48% in 1988 (Table 1). Similarly, the severity of stalk rot on the two hybrids varied each year with the highest severity in 1988 and the lowest in 1987 (Table 2).

DISCUSSION

Disease assessments made using the four-category rating scale in this study overestimated the amount of disease compared with the assessments made by the image analyzer. When visual ratings were converted to percent by multiplying the rating by the midpoint in percent of that rating (3), the same differences held but did not show the accuracy in readings that could be obtained with the image analyzer. For example, in 1988, *F. moniliforme* had a visual rating of 3.5, which becomes 69% when converted to a percent scale (3). This value is 42% greater than the corresponding image analysis rating of 48.5%. Numerical conversions of the remaining treatments indicate a similar amount of overestimation—78% for *F. graminearum* and 60% for *F. proliferatum*. It has been noted (5) that the visual assessment of disease follows the Weber-Fechner law, which states that visual discrimination is a function of the logarithm of the intensity of the stimulus. Disease scales and disease assessment diagrams depicting logarithmetically increasing proportions of diseased tissue have been used to compensate for the poor ability of the eye to accurately assess midrange disease severities. Even when pictoral assessment keys are used, however, disease can be overestimated (14).

The data indicate, however, that the two disease assessment methods generally agree, and the image analyzer method confirms the dependability of the visual rating to determine relative differences among treatments. The visual rating may be useful for experiments requiring the ranking of plants according to disease severity. The use of an image analyzer is appropriate when sensitivity to slight differences in tissue discoloration is needed, as, for example, in heritability studies where small differences in resistance can be cumulative in different crosses and used to develop genotypes with greater resistance to stalk rot. Similarly, studies that require an exact determination of pathogenicity of different isolates of a pathogen would benefit from the greater accuracy of the image analyzer.

There are disadvantages of the image analysis method for routine evaluations of corn genotypes to stalk rot. It is easier and quicker to read plants in the field than to collect, bag, and label samples, then set up equipment in the laboratory and make the readings there. The labor and equipment costs may be prohibitive.

In conclusion, the selection of either rating method depends upon the objectives of any given experiment. For experiments that require field evaluations of many genotypes, or where the determination of relative differences among treatments is adequate, the visual method is satisfactory. For experimental studies of host resistance or isolate pathogenicity, or for experiments that call for the determination of the actual amount of disease, the image analyzer is a more effective method of disease assessment.

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