Evaluation of Inoculation Techniques and Rating Dates for Fusarium Ear Rot of Opaque-2 Maize

T. J. Gulya, Jr., C. A. Martinson, and P. J. Loesch, Jr.

Former graduate research assistant; associate professor, Department of Botany and Plant Pathology; and research geneticist (deceased), U. S. Department of Agriculture, Science and Education Administration, and professor, Department of Agronomy, Iowa State University, Ames 50011. Present address of senior author: Department of Plant Pathology, USDA-SEA-AR, North Dakota State University, Fargo 58105.

Journal Paper J-9456 and Project 2194 of the Iowa Agriculture and Home Economics Experiment Station, Ames.
Appreciation is expressed to Clyde Black and Sons, Hybrid Seed Farms, Ames, IA, for donation of seeds of some of the inbred lines.
Accepted for publication 19 May 1980.

ABSTRACT

GULYA, T. J., Jr., C. A. MARTINSON, and P. J. LOESCH, Jr. 1980. Evaluation of inoculation techniques and rating dates for Fusarium ear rot of opaque-2 maize. Phytopathology 70:1116-1118.

Two commonly used inoculation techniques were compared for efficacy in screening resistance to maize ear rot incited by *Fusarium moniliforme*. Fusarium ear rot severity was greater and resistant genotypes were more easily distinguished when opaque-2 maize inbreds were inoculated by the ear-puncture method compared with the silk-spray inoculation technique. Ear rot severity, rated on a linear 1–100 scale followed by logarithmic transformation, was more efficient and statistically valid than subjective evaluation of ear rot on nonlinear scales. Ear rot development, as measured on ears incubated in moisture chambers for 3 days after collection, reached a maximum 6 wk after inoculation. Thus, evaluation of ear rot resistance may be done before normal harvest of seed in breeding lines.

Additional key words: Fusarium moniliforme, Gibberella fujikuroi.

Fusarium moniliforme Sheld. [conidial stage of *Gibberella fujikuroi* (Saw.) Ito] is a common ear rot pathogen of maize (8) and is one of the major factors limiting the utilization of opaque-2 (high-lysine) maize (4,13,19,21). Effective screening of maize germplasm depends on a reliable inoculation technique coupled with a statistically valid rating system. Numerous methods with various degrees of efficiency and accuracy have been used to artifically inoculate maize with ear-rotting pathogens (1,8,15,18,21,24). Inoculation of stalks, leaves, or ear shanks was unsuccessful in producing Fusarium ear rot (8). Silk inoculation also was found ineffective with both opaque-2 (14) and normal maize inbreds and hybrids (8,15), but this method is still widely used (13,17,18). Tip-of-ear inoculation, involving pulling back the husk and covering the inoculated ear with a glassine or plastic bag, has been more successful (1,8), but is time consuming and may not be the most reliable method (1,15). The toothpick method of Young (24) has been used successfully with *F. moniliforme* (14,15) and other ear-rotting pathogens (17).

Various methods have been used to evaluate ear rot severity, including whole-row ratings (17), ear ratings (1,8,14,15,18,19), kernel sorting (5,8,21), and kernel plating (14,21). In addition, the rating scales used in ear evaluations vary widely; many are ill-defined and probably statistically invalid.

The objectives of the present study were: to compare the efficacy of the two most common inoculation techniques for producing Fusarium ear rot; to develop an easily used and statistically valid scale for rating ear rot damage; and to determine the optimum time after inoculation for rating ear rot. A preliminary report has been published (3).

MATERIALS AND METHODS

A split-plot design with three replications was employed in the field experiment conducted at the Iowa State University Ross Farm in 1976. Treatment combinations (inbred line × inoculation method) were randomly assigned to whole plots, and sampling dates constituted the subplots. Nine opaque-2 (o_2) inbred lines were used, representing a cross section of the current germplasm used in commercial Corn Belt hybrids. They included A632o_2, B14Ao_2, B37o_2, H84o_2, Mo17o_2, N6o_2, N28o_2, Oh43o_2, and W64Ao_2. Fifty seeds were planted per 5.3 m two-row plot, with rows spaced 76 cm apart. The field was isolated from the nearest known source of normal maize pollen by 800 m and was surrounded by a 12-row border of an opaque-2 hybrid. After thinning, the final stand was 37,000 plants per hectare. Inoculation treatments were: silk spray with *F. moniliforme* conidia, silk-spray check (distilled water only), ear puncture with a fungus-encrusted toothpick, ear-puncture check (sterile toothpick), and uninoculated control.

For silk-spray inoculations, *F. moniliforme* was grown on 750 g of millet (*Panicum miliaceum* L.) in 1-L Erlenmeyer flasks for 2–3 wk. The millet was boiled in excess tap water, drained, and autoclaved twice on successive days. The inoculum used to seed the flasks was a composite of several isolates of *F. moniliforme*, obtained directly from infected maize seed, and plated onto water agar to verify purity. This prevented loss of virulence, often noted during prolonged culture of *F. moniliforme*, and also circumvented the variability between isolates (11). The contents of the flasks were agitated in water to dislodge the microconidia, strained through several layers of cheesecloth, and the concentration was adjusted to 5,000 conidia per milliliter. The conidial suspension was sprayed onto the silks until runoff with a low-pressure hand sprayer 10 days after the 50% silk date of each inbred.

For ear-puncture inoculation, round wooden toothpicks were boiled or autoclaved four to six times in excess water to remove fungitoxic compounds. The toothpicks were arranged vertically in 250-ml beakers and covered to one-third their length with potato broth. The beakers were capped with aluminum foil and autoclaved (121 C for 30 min.). The contents of the beakers were seeded with *F. moniliforme* and were incubated at room temperature for 2–3 wk. Preceding inoculation, the fungus-encrusted toothpicks were removed from the beakers and allowed to air-dry overnight. Ears were inoculated 10 days after the 50% silk date of each inbred by inserting a single toothpick through the husk perpendicular to the ear axis and midway between the butt and ear tip. The toothpicks remained in the ears until harvest.

Ears were harvested at 2, 4, 6, and 8 wk after inoculation. At each harvest, five ears were picked from a plot, husked, and rinsed in 1% sodium hypochlorite for 2 min. After being rinsed in running tap water, the ears were individually placed in moisture chambers and allowed to incubate at room temperature for 3 days. The moisture

chamber consisted of an alcohol-rinsed plastic cup (11 cm diameter × 14 cm high) covered with a transparent polyethylene bag. An autoclaved "stand," consisting of a 4-cm square piece of plywood pierced with a 5-cm nail, was used to hold the ear upright without touching the sides of the cup. Incubation of the ears in the moisture chamber allowed development of the fungus in infected, but as yet symptomless, kernels.

Ear rot severity was rated by two methods. The first, a modified Horsfall-Barratt scale (6), consisted of rating the percentage of rotted area on a nonlinear 1–5 scale (1 = 0–1%, 2 = 1–10%, 3 = 10–25%, 4 = 25–50%, 5 = 50–100% of the ear rotted). The second method consisted of rating the percentage of rotted area on a linear 1–100 scale (1 = 0–1%, 10 = 1–10%, 25 = 10–25%, 50 = 25–50%, and 100 = 50–100% of the ear rotted). The increments within the latter scale were thus proportional to the actual percentages they represented. In addition, the radius of the ear rot lesion was measured on ears that had received the ear-puncture treatment.

RESULTS

Ear rot scores based on the linear 1–100 scale produced a data distribution highly skewed toward the lower end of the scale; ie, resistant genotypes. Transformation of the data to the natural logarithm of the row means [$\log_e$ ((ear rot × 10) + 1)] effectively corrected the skewness toward a more normal distribution.

Significant differences in ear rot scores were found among the inoculation methods and the rating dates (Table 1). Both the ear-puncture and silk-spray inoculations produced more ear rot than did their respective controls. The ear-puncture method, however, consistently produced higher ear rot scores at every sampling date than did the silk-spray inoculation method. The ear-puncture check, which consisted of implanting a sterile toothpick, resulted in as much disease as spraying the silks with inoculum (Table 1). Kernel infection was observable before the ears were placed in the moisture chambers, as early as 2 wk after inoculation. Ear rot severity always was greatest on ears that received the ear-puncture treatment. The highest ear rot scores were observed 6 wk after inoculation (Table 1).

Not only did the ear-puncture method produce higher ear rot scores than the silk-spray method, but also the relative ranking of the inbred lines differed with the two methods (Table 2). For example, A632o_2 was significantly more susceptible to ear rot than B37o_2 when inoculated with the ear-puncture method; while with the silk-spray method A632o_2 was rated significantly more resistant than B37o_2.

Ear rot scores, measured either by subjectively estimating the percentage or by measuring the radius of the diseased area from the point of inoculation, were significantly correlated (r = 0.90, P = 0.01). Both methods were highly efficient for distinguishing resistant genotypes, and the relative ranking of the lines did not differ between the two methods (Table 3).

TABLE 1. Effect of rating date, inoculation method, and date × method interaction on amount of ear rot averaged over nine inbred lines of opaque-2 maize[a]

Rating date[b] (wk)	Ear puncture	Ear puncture check	Silk spray	Silk spray check	Control	Date mean
2	4.4[c]	4.2	4.1	3.8	3.5	4.0
	(8)	(7)	(6)	(4)	(3)	(5)
4	5.3	4.5	4.4	4.3	4.3	4.6
	(19)	(9)	(8)	(7)	(7)	(10)
6	5.8	5.1	5.3	5.0	5.0	5.2
	(32)	(16)	(19)	(15)	(15)	(18)
8	5.4	4.4	4.4	4.0	4.0	4.4
	(22)	(8)	(8)	(5)	(5)	(8)
Method mean	5.2	4.6	4.5	4.3	4.2	
	(18)	(10)	(9)	(7)	(7)	

LSD (P = 0.05) = 0.17 for date means and 0.15 for method means.

[a] Maize ears were inoculated at 10 days after 50% silking.
[b] Ears were rated 2, 4, 6, or 8 wk after inoculation.
[c] Log percent ear rot and percent ear rot (in parentheses) from indicated treatment.

DISCUSSION

Nonlinear rating scales, such as the 1–5 scale, are popular because the increments are easily discernible and data recording is simplified, but they have two inherent drawbacks: row means are essentially logarithms of the geometric mean and thus cannot easily be converted to arithmetic means (10); and misleading comparisons often are made, as illustrated in Table 4. Mean ear rot scores of hypothetical rows I and II are the same, whereas row I has nearly twice as much ear rot; conversely, rows I and III have the same amount of ear rot, yet these scores are not the same. Both of these disadvantages are eliminated by using the linear 1–100 scale in which the categories are referred to by their upper limits.

Nonuniformity among ear rot rating scales precludes accurate comparisons of data from different sources. Kernel separation (5) was proposed by Ullstrup et al (20) as a standardized method, but it has not been widely used because it is too time-consuming. In later experiments, we found a highly significant correlation (r = .81) between ear rot assessments made by grain separation and those made by visually estimating rot percentages on the linear 1–100 scale. Use of the upper limit of each infection class in the 1–100 scale may elevate the rot rating, but that was considered justifiable because healthy-appearing kernels may be infected (4). Measurement of the rotted area (when the ear puncture method is used) would be less subject to reader bias than estimating ear rot severity on the 1–100 scale, and would thus be more accurate when evaluations are made by more than one person.

The decline in ear rot scores at 8 wk probably was a reflection of kernel maturity (ie, lower moisture). Ears collected 8 wk after

TABLE 2. Relative rankings of inbred lines of opaque-2 maize based on ear rot ratings from ears inoculated by either the ear-puncture or silk-spray inoculation method

Ear Puncture		Silk spray	
Inbred	Rating[a]	Inbred	Rating[a]
Oh43	4.2 a[b]	Oh43	3.6 a
N6	4.7 ab	A632	3.9 a
B37	4.9 bc	N6	4.6 b
H84	5.0 bcd	H84	4.6 b
W64A	5.3 bcde	N28	4.6 b
A632	5.5 def	W64A	4.8 b
B14	5.7 ef	Mo17	4.8 b
N28	5.8 ef	B37	4.8 b
Mo17	5.9 f	B14	5.1 b

[a] Averaged over four sampling dates and three replications. Ear rot rated on a linear 1–100 scale and corrected by logarithmic transformation.
[b] Ratings not followed by a common letter are significantly different, P = 0.05.

TABLE 3. Ear rot scores of nine inbred lines of opaque-2 maize rated by visually estimating percentage rotted area or by measuring the radius of the rotted area

Estimated rot		Measured rot	
Inbred	Score[a]	Inbred	Score[a]
Oh43	4.2 a[b]	Oh43	4.5 a[b]
N6	5.3 b	H84	5.3 b
H84	5.3 b	N6	5.4 b
B37	5.4 b	W64A	5.6 bc
W64A	5.6 bc	B37	5.7 bc
B14	5.8 bc	B14	5.7 bc
A632	5.8 bc	A632	5.8 bc
Mo17	6.4 c	Mo17	6.1 c
N28	6.4 c	N28	6.1 c

[a] Averaged over three replications and two sampling dates. Scores were corrected by logarithmic transformation.
[b] Ear rot scores not followed by a common letter are significantly different, P = 0.05.

TABLE 4. Ear rot scores of three hypothetical rows, using the nonlinear 1–5 scale (corresponding linear 1-100 scale ratings are in parentheses)

Row	Individual ear scores					Row mean	Actual rot percentage
I	1	1	3	5	5	3	
	(1)	(1)	(25)	(100)	(100)		45
II	3	3	3	3	3	3	
	(25)	(25)	(25)	(25)	(25)		25
III	4	4	4	4	3	3.8	
	(50)	(50)	(50)	(50)	(25)		45

inoculation undoubtedly had as much ear rot as those collected at 6 wk, but the decreased moisture content limited sporulation of internally-infected kernels. Koehler et al (9) show that ear infection by *F. moniliforme* reaches a maximum level at the "mature" stage (kernel moisture ~ 28%), with no increase by the "husking" stage (moisture ~ 22%). Theoretically, ear rot evaluation could be made at, or slightly preceding physiological maturity, and thus only the desirable genotypes need be harvested at a considerable savings of time and labor.

Ear-puncture inoculation produces a highly localized infection since the inoculum is deposited at a single point. The resultant lesion size is thus dependent on kernel resistance alone, and is not affected by passive movement of spores, as when a spore suspension is used with either the silk-spray or syringe inoculation techniques. The single lesion produced by the ear-puncture technique also allows one to rate ear rot by measuring the spread of infection from the point of inoculation.

The higher ear rot ratings obtained with the ear-puncture method are due partly to circumvention of physical barriers that exclude the pathogen. This would explain why the ear-puncture checks developed as much rot as the silk-spray inoculated ears. A tight husk, completely covering the tip of the ear, would tend to reduce the amount of inoculum that could passively reach the kernels (8), either under natural conditions or with the silk-spray method. Thus, lower ear rot severity with the silk-spray method may be due primarily to the klendusic effect of the husk and not to the innate resistance of the kernels.

The primary advantage cited for the silk-spray inoculation method is that it supposedly approximates the natural mode of infection (21). Although Koehler (7) shows that airborne conidia falling on the silks and ear tips is a primary means of kernel infection, it is well substantiated that any injury to the ear will result in increased rot. Damage to the ears by hail (12), birds (22), and insects such as the European corn borer (2), corn earworm (16), and picnic beetle (23) all result in increased ear rot. The ear-puncture method, although it does circumvent the protective nature of a tight husk, insures that a standardized amount of inoculum will reach the kernels and thus allows evaluation of kernel resistance to infection apart from other klendusic factors.

LITERATURE CITED

1. BOLING, M. B., C. O. GROGAN, and J. W. BROYLES. 1969. A new method of artificially producing epiphytotics of Fusarium ear rot of maize. Plant Dis. Rep. 47:315-317.
2. CHRISTENSEN, J. J., and C. L. SCHNEIDER. 1950. European corn borer (*Pyrausta nubilalis* Hbn.) in relation to shank, stalk, and ear rots of corn. Phytopathology 40:284-291.
3. GULYA, T. J. 1978. Evaluation of inoculation and rating methods for Fusarium ear rot of maize. (Abstr.) Phytopathol. News 12:87.
4. GULYA, T. J. 1978. Evaluation of *Fusarium moniliforme* ear rot resistance in opaque-2 maize and interaction with various kernel characters. Ph.D. Dissertation, Iowa State University, Ames. 128 pp.
5. HOPPE, P. E., and J. R. HOLBERT. 1936. Methods used in the determination of relative amounts of ear rots in dent corn. Agron. J. 28:810-819.
6. HORSFALL, J. G., and R. W. BARRATT. 1945. An improved grading system for measuring plant diseases. (Abstr.) Phytopathology 35:655.
7. KOEHLER, B. 1942. Natural mode of entrance of fungi into corn ears and some symptoms that indicate infection. J. Agric. Res. 64:421-442.
8. KOEHLER, B. 1959. Corn ear rots in Illinois. Ill. Agric. Exp. Stn. Bull. 639. 87 pp.
9. KOEHLER, B., G. H DUNGAN, and W. L. BURLISON. 1934. Maturity of seed corn in relation to yielding ability and disease infection. Agron. J. 26:262-274.
10. LARGE, E. C. 1966. Measuring plant disease. Annu. Rev. Phytopathol. 4:9-28.
11. LEONIAN, L. H. 1932. The pathogenicity and variability of *Fusarium moniliforme* from corn. W. Va. Agric. Exp. Stn. Bull. 248. 16 pp.
12. LITTLEFIELD, L. J. 1964. Effects of hail damage on yield and stalk rot infection in corn. Plant Dis. Rep. 48:169.
13. LOESCH, P. J., Jr., D. C. FOLEY, and D. COX. 1976. Comparative resistance of opaque-2 and normal inbred lines of maize to ear rotting pathogens. Crop. Sci. 16:841-842.
14. OOKA, J. J., and T. KOMMEDAHL. 1977. Kernels infected with *Fusarium moniliforme* in corn cultivars with opaque-2 or male-sterile cytoplasm. Plant Dis. Rep. 61:162-165.
15. SALAZAR, F. F., and E. G. VARGAS. 1977. Methods of evaluation and levels of resistance in corn to ear rot caused by *Fusarium moniliforme*. Agron. Costarric. 1:93-99.
16. SMELTZER, D. G. 1959. Relationship between Fusarium ear rot and corn earworm infestation. Agron. J. 51:53-54.
17. SMITH, F. L., and C. B. MADSEN. 1949. Susceptibility of inbred lines of corn to Fusarium ear rot. Agron. J. 41:347-348.
18. ULLSTRUP, A. J. 1970. Methods for inoculating corn ears with *Gibberella zeae* and *Diplodia maydis*. Plant Dis. Rep. 54:658-662.
19. ULLSTRUP, A. J. 1971. Hyper-sensitivity of high lysine corn to kernel and ear rots. Plant Dis. Rep. 55:1046.
20. ULLSTRUP, A. J., C. ELLIOT, and P. E. HOPPE. 1945. Report of the committee on methods for reporting corn disease ratings. Div. Cereal Crops Dis., Beltsville, Md. USDA 23CC. 5 pp.
21. WARREN, H. L. 1978. Comparison of normal and high-lysine maize inbreds for resistance to kernel rot caused by *Fusarium moniliforme*. Phytopathology 68:1331-1335.
22. WHITNEY, N. J. 1954. Ear rots in hybrid corn in Essex County, Ontario, in relation to damage by birds. Plant Dis. Rep. 38:384-387.
23. WINDELS, C. E., M. B. WINDELS, and T. KOMMEDAHL. 1976. Association of *Fusarium* species with picnic beetles on corn ears. Phytopathology 66:328-331.
24. YOUNG, H. C. 1943. The toothpick method of inoculating for ear and stalk rots. (Abstr.) Phytopathology 33:16.