Weibull Distribution of Lesion Size in the Stagonospora Leaf Spot of Orchardgrass

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I thank C. E. Antle, R. R. Hill, Jr., M. O. Westerhaus, and C. C. Bahler for helpful discussions and R. G. Pedersen, M. Tischler, W. McGraw, and M. R. Hoover for technical help.

Contribution 8605 of the U.S. Regional Pasture Research Laboratory.

Accepted for publication 10 October 1986.

ABSTRACT

Sherwood, R. T. 1987. Weibull distribution of lesion size in the Stagonospora leaf spot of orchardgrass. Phytopathology 77:715-717.

Five resistant and five susceptible genotypes of *Dactylis glomerata* were inoculated with *Stagonospora arenaria* in two replicated greenhouse tests. Distributions of lesion length and area were determined for populations of lesions on single leaves, using samples of 50 lesions per leaf. The two trials gave closely similar results. The null hypothesis that size was normally distributed could be rejected for resistant genotypes but not for suceptible, using the Shapiro-Wilk w statistic at $P = 0.01$. The sample of all genotypes showed positive skewness (tail to the right). The lognormal distribution could be rejected in favor of the Weibull distribution in all samples at $\alpha = 0.01$. All samples showed a good fit to the Weibull distribution with location (a) set at minimum spot size. Weibull maximum likelihood estimates for shape ($\hat{c}$) were significantly lower than c expected for a normal distribution; this further demonstrated positive skewness. Values for a, c, and slope ($\hat{b}$) were significantly lower for resistant genotypes than for susceptible genotypes. Two additional tests with other genotypes gave the same trends.

Additional key words: disease assessment, image analysis.

The size of lesions induced by pathogenic fungi is genetically and environmentally regulated. Lesion length responds to plant genotype (10,12); pathogen strain, temperature, and illuminance (6,7); and duration of wetness (12). Estimated area varies in relation to cultivar (9,19), temperature (5,11,19,20), moisture (5,11), and light (4). The size of individual lesions within a population of lesions initiated during one inoculation varies widely even in a single leaf or stem. This may reflect variable latent periods and expansion rates of colonies. Berger and Jones (2) noted that curves plotted of populations of lesions appearing over a period of time from synchronous infections may be linear, sigmoidal, monomolecular, or of another shape. If lesion size at a given time after initiation is related to latent period, it follows from the observation of Berger and Jones that different populations of lesions may have different size-distribution patterns.

Distribution affects statistical description and analysis of populations. Nonnormal data can lead to heterogeneous variance among treatments and necessitate data transformation before analysis (17). There are no previous descriptions of the distribution of lesion populations.

This study was undertaken to characterize distribution in the leaf spot of orchardgrass (*Dactylis glomerata* L.) caused by *Stagonospora arenaria* Sacc. Upward of 100 separate, elongate, dark purple spots can form on a single mature leaf. This study included only separated lesions initiated on one leaf at single infection loci from one inoculation, thus minimizing effects of leaf position, environmental variation, and colony crowding on lesion size. Visually rated size of Stagonospora leaf spot previously was a criterion in developing resistant genotypes (21,22), testing environmental stability (1), and discovering an illusory role for size and number in visual assessment (16).

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MATERIALS AND METHODS

Source of infected leaves. Infected leaves were saved from a study of stability of 60 genotypes described earlier (1). The five susceptible and five resistant genotypes sampled here were originally selected as individual plants and vegetatively cloned in clay pots. The 10 genotypes were randomized in four replicate blocks on a greenhouse bench in January 1981 (1).

Untillered plants with mature basal leaves were inoculated with conidia of *S. arenaria* in March 1981 (1). After 48 hr of incubation in a moist room at 22 ± 1 C, plants were returned to the bench. Four well-infected basal leaves per plant were collected 13 days after inoculation. The center 8-cm length of each leaf was fixed and cleared in ethanol:acetic acid, 3:1 (v/v), and stored in 50% ethanol. Top growth was removed in April and July. In October, regrowth leaves (all healthy) were inoculated to constitute a second trial.

Measurement of lesion size. Only leaves with darkly pigmented purple spots were used. The few leaves that included some diffuse tan spots (described in [15]) were not used because a test showed that their inclusion resulted in heterogeneous error mean squares in the analyses of variance.

One cleared leaf per genotype per replicate was immersed in 50% ethanol and covered with a glass sheet measuring 5 × 10 cm in a large flat dish. A video image of the leaf was projected on a television screen at ×20 linear magnification. Outlines of the first 50 separate spots encountered per leaf were traced with ink on a sheet of transparent acetate measuring 22 × 28 cm. The diagram was photocopied and the photocopied spots were filled in with black ink. The length of each diagrammed spot was measured with a ruler. To measure area, diagrammed spots were projected on a video monitor at ×1,600 and the area of each was determined by computerized analysis of the digitized video image. Preliminary tests compared this procedure with methods involving various combinations of photography (4), gravimetry (16), and regression analysis (11) or with image analysis of each individual spot directly from the leaf at ×1,600. The method selected was equal or superior to the others in accuracy and technical convenience. Measurements were converted to unmagnified (×1) length or area before analysis.

Statistical analysis. Statistics were computed for length and area of each sample of 50 spots. (i) The SAS (13) PROC UNIVARIATE NORMAL was used to compute mean spot size, the Shapiro-Wilk (14) w statistic, and the statistic for skewness. (ii) The FORTRAN program of Dumonceaux and Antle (3) was used to compute ratio of maximized likelihoods (RML) for discrimination between the lognormal and Weibull distributions and to calculate maximum likelihood estimators $\hat{b}$ and $\hat{c}$ for slope (b) and shape (c) of the Weibull distribution. (iii) The method of Thoman et al (18) was used to calculate confidence intervals for $\hat{c}$. (iv) The maximized likelihood estimate of the mean for the Weibull distribution was calculated as $\widehat{\Sigma[w]} = a + \hat{b}\ \Gamma(1 + \hat{c} - 1)$ (8) using the gamma function (Γ) provided by SAS (13).

When estimating $\hat{b}$ and $\hat{c}$, the location parameter a was fixed at minimum spot length minus 0.01 mm or minimum spot area minus 0.001 mm^2 of the particular sample. That value was subtracted from each spot of the sample before $\hat{b}$ and $\hat{c}$ were computed. Analyses of variance were conducted using SAS PROC GLM.

RESULTS AND DISCUSSION

Statistics for distribution of lesion length of two genotypes in trial 1 are shown in Table 1. The data are representative of results for length and area of all genotypes in both trials.

The null hypothesis that input data were a random sample from a population with a normal distribution was tested using the Shapiro-Wilk (14) w statistic (Table 1). The null hypothesis was rejected for length and area of each resistant genotype in each trial

TABLE 1. Statistics for distribution of lesion length in one trial of a resistant and susceptible genotype

Genotype	Group	Replicate	w[a]	Skewness[b]	RML[c]	Weibull $\hat{c}$	96% confidence interval for $\hat{c}$	$\widehat{\Sigma[w]}$[d]	Observed mean length (mm)
5	Res	1	0.944	0.48	1.187	1.73	1.33–2.12	0.78	0.78
		2	0.900	0.82	1.160	1.35	1.04–1.65	0.71	0.71
		3	0.846	1.41	1.057	1.30	1.00–1.59	0.50	0.50
		4	0.884	0.79	1.117	1.22	0.94–1.49	0.61	0.61
28	Sus	1	0.919	1.02	1.194	1.67	1.28–2.04	1.74	1.74
		2	0.952	−0.05	1.373	1.54	1.18–1.88	1.59	1.61
		3	0.949	0.84	1.260	1.96	1.51–2.40	1.44	1.44
		4	0.958	0.32	1.336	2.01	1.54–2.46	1.37	1.38

[a] With $n = 50$ lesions, values of $w < 0.955$ and 0.931 lead to rejection of the null hypothesis that distribution was normal at the 0.1 and 0.01 levels, respectively (13,14).
[b] See (13).
[c] Ratio of maximizol likelihoods. With $n = 50$ lesions, values of RML > 1.054 lead to rejection of lognormal distribution in favor of Weibull distribution at $\alpha = 0.01$ (3).
[d] Maximum likelihood estimated mean of the Weibull distribution.

TABLE 2. Observed mean and Weibull distribution parameters for lesion length and area of 10 genotypes and combined analysis of variance of two trials

		Length				Area			
Genotype	Group[a]	Mean (mm)	a	$\hat{b}$	$\hat{c}$	Mean (mm^2)	a	$\hat{b}$	$\hat{c}$
				Trial 1					
11	Res	0.44	0.22	0.24	1.42	0.11	0.04	0.08	1.28
13	Res	0.60	0.28	0.36	1.56	0.18	0.06	0.14	1.46
5	Res	0.65	0.24	0.45	1.40	0.21	0.05	0.16	1.14
47	Res	0.74	0.28	0.51	1.53	0.22	0.06	0.16	1.24
10	Res	0.76	0.39	0.41	1.49	0.23	0.07	0.18	1.47
4	Sus	1.30	0.49	0.90	2.01	0.61	0.15	0.50	1.58
29	Sus	1.49	0.72	0.86	2.13	0.70	0.27	0.50	1.61
28	Sus	1.54	0.74	0.89	1.80	0.82	0.25	0.61	1.40
25	Sus	1.59	0.74	0.95	1.86	0.78	0.26	0.57	1.64
56	Sus	1.82	0.65	1.29	1.78	0.85	0.19	0.70	1.48
				Trial 2					
11	Res	0.51	0.24	0.30	1.50	0.16	0.06	0.11	1.38
13	Res	0.65	0.26	0.42	1.60	0.21	0.06	0.16	1.32
5	Res	0.66	0.25	0.44	1.43	0.20	0.05	0.16	1.24
47	Res	0.78	0.32	0.51	1.47	0.28	0.09	0.21	1.20
10	Res	0.85	0.35	0.55	1.52	0.35	0.09	0.28	1.29
4	Sus	1.20	0.62	0.65	1.91	0.57	0.21	0.38	1.65
25	Sus	1.29	0.63	0.73	2.12	0.60	0.22	0.42	1.79
28	Sus	1.40	0.65	0.84	1.76	0.67	0.21	0.50	1.49
56	Sus	1.54	0.59	1.05	1.98	0.85	0.19	0.72	1.68
29	Sus	1.54	0.70	0.93	2.17	0.91	0.30	0.67	1.75
Source	df			F ratios and error mean squares					
Replicate	3	2.01	0.66	1.77	0.43	3.35*[b]	0.56	3.99*	0.96
Res vs. Sus	1	1,445.43**	346.16**	459.67**	115.03**	1,731.04**	306.89**	1,560.04**	52.50**
Genotype in Res vs. Sus	8	17.98**	3.78**	12.31**	2.47*	18.16**	4.47**	25.76**	2.21
Error mean square	27	0.0090	0.0079	0.0105	0.0368	0.0031	0.0017	0.0020	0.0354
Sampling error mean square	40	0.0164	0.0067	0.0166	0.0730	0.0075	0.0019	0.0068	0.0361

[a] Res = resistant and Sus = susceptible.
[b] * and ** indicate that differences were significant at the 0.05 and 0.01 levels, respectively.

at $P = 0.01$. The susceptible genotypes gave probabilities ranging from 0.01 to 0.50 with a mean of about 0.20. Thus the null hypothesis could not be rejected for the susceptible genotypes using the Shapiro-Wilk test.

The means of four replicate determinations for skewness were always positive (Table 1). Only eight individual replicates among the 160 total determinations (10 genotypes, 4 replicates, 2 traits, 2 trials) showed negative skewness (see example in Table 1). Positive skewness is characterized by a tail to the right; a few large values strongly influence the calculation of the mean. The skewness of these distributions is consistent with the departure from normality indicated by the Shapiro-Wilk test. Nonnormally distributed populations cannot be completely described by mean and standard deviation. Shape, location, and slope parameters are also needed (8).

Lognormal and Weibull distribution models are often considered for situations in which a skewed distribution of a nonnegative random variable is implicated (3). Dumonceaux and Antle (3) demonstrated that the RML provides a good test for selecting one of these alternatives. I applied the RML test for the hypothesis H_0:lognormal and H_1:Weibull. In each of the 160 determinations, the lognormal was rejected in favor of the Weibull at $\alpha = 0.01$.

When Weibull distribution values for shape (c) are near 3.6, the Weibull distribution is similar in shape to a normal distribution (8). Weibull c values lower than 3.6 reflect positive skewness. The degree of skewness is proportional to distance from 3.6. To test whether lesion size distributions were less than 3.6, a 96% confidence interval was calculated for each of the 160 $\hat{c}$ values, using the methods of Thoman et al (18). The upper limit of all 160 confidence intervals was substantially below 3.6; most were in the range of 1.5–2.5 (Table 1). This indicated that $\hat{c}$ for lesion size differed significantly from that of a normal distribution. For each of the 160 determinations, the Weibull maximum likelihood estimated mean agreed very closely with the observed mean (Table 1). This showed that the program of Dumonceaux and Antle (3) was reliable for estimating $\hat{b}$ and $\hat{c}$. Error mean squares for groups were homogeneous within trials when Bartlett's test (17) was applied. The data did not need to be transformed before analysis even though they were nonnormal.

The actual values for means a, $\hat{b}$, and $\hat{c}$ in trial 2 agreed closely with those in trial 1 (Table 2). Error mean squares were homogeneous between trials. Accordingly, a combined analysis of variance was conducted (Table 2). The major source of variation in size of means a, $\hat{b}$, and $\hat{c}$ was accounted for by susceptibility vs. resistance ($P > 0.01$). There were lesser but significant differences among genotypes within groups of all traits except $\hat{c}$ area.

The location parameter, a, closely approximated the size of the minimum lesion, having been set 0.01 mm below minimum length or 0.001 mm^2 below minimum area. Resistant genotypes had significantly lower a than did susceptible genotypes. Lesion size had a lower limit that did not extend to the limit of visibility. Smallest lesions of susceptible genotypes were larger than smallest lesions of resistant genotypes. The group of resistant genotypes showed significantly smaller slope, $\hat{b}$, than the susceptible group. This reflected the smaller mean lesion size of resistant genotypes. Shape parameter values, $\hat{c}$, were significantly lower for the resistant group than for the susceptible group, which reflected the greater skewness of the resistant group. Paired sample t-tests indicated that $\hat{c}$ area was significantly less than $\hat{c}$ length.

Additional genotypes were evaluated for distribution in two other tests. One test analyzed leaves from 20 plants that displayed a continuous range of mean lesion sizes from resistant to susceptible. All distributions were positively skewed, the Weibull distribution fit better than lognormal, $\hat{c}$ was below 3.6, and $\hat{c}$ was significantly correlated with lesion size. In the other test, two genotypes of intermediate susceptibility were inoculated and sampled in March, June, October, and December (1). Distributions fit the Weibull, and $\hat{c}$ was significantly less than 3.6. Date and genotype by date did not affect $\hat{c}$. Thus all tests indicated positive skewness and a good fit to the Weibull distribution.

The combination of size and frequency of Stagonospora lesions influences visual perception of the total area infected (16). Positive skewness that differs with resistance may cause some illusions in visual assessment of the disease.

The finding that a, $\hat{b}$, and $\hat{c}$ are significantly lower for resistant genotypes indicates that these traits are heritable. It would be interesting to learn the biological basis for skewed distribution of lesion size.

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