A Stochastic Model of Horizontal Resistance Based on Frequency Distributions

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ABSTRACT

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Data were collected on the frequency distributions associated with sampling number of powdery mildew colonies and leaf area on individual leaves, and number of colonies formed from groups of 10 conidia on leaves in growth chamber experiments with seedlings of the wheat cultivar Chancellor. The frequency of colonies on leaves was found to approximate the negative binomial distribution in field and growth chamber studies. Additional studies indicated that leaf area could be described by the gamma distribution and number of conidia forming elongating secondary hyphae in groups of 10 conidia was binomial distributed. These results were used as the basis for development of a theoretical statistical model for colonies on leaves with parameters representing sporulation potential and infection efficiency. Possible application of this model for the detection of relative levels of these components of horizontal resistance is discussed.

Additional key words: Erysiphe graminis f. sp. *tritici.*

Plant breeders traditionally have sought a type of disease resistance typified by a hypersensitive reaction to the pathogen. This type of resistance can be categorized as vertical resistance sensu Vanderplank (9) when specific isolates interact with different host genotypes and is usually conditioned by one or a very few genes. Vertical resistance often is short-lived due to selection in the pathogen population of alternate genes for pathogenicity that are effective against specific vertical genes. A second form of resistance termed horizontal resistance (HR) sensu Nelson (6) is characterized by a reduction in the rate of disease increase with time. Although cultivars with HR have been identified, plant breeders have had difficulty selecting plant types with improved HR and suitable agronomic traits. The problem of how to effectively select for improved HR is particularly important in light of recent work demonstrating the potential of some pathogens to erode HR by selection for increased parasitic fitness (7). Horizontal resistance often is assumed to be polygenically inherited requiring quantitative procedures to separate small differences in plant selections for HR. It might be possible to identify the individual components of HR in the breeding nursery and, assuming they are independently inherited, make the appropriate crosses to combine individual components into a superior cultivar.

Some components of rate-reducing resistance include sporulation capacity (SC), the number of spores produced in the lifetime of a colony; infection efficiency (IE), the proportion of spores deposited on susceptible tissue that subsequently form colonies; latent period (LP), the time from deposition to subsequent sporulation by the resulting colonies; and lesion size (LS). Few rapid procedures exist for identifying plant selections with a high level of an individual component of HR. Umaerus (8) developed a laboratory assay using detached potato leaves to identify potato selections with increased LP or reduced SC or IE to *Phytophthora infestans.* Jones (5) studied several oat selections with adult plant resistance to *Erysiphe graminis* f. sp. *avenae* and suggested that visual selection for increased LP was possible on the third or higher leaf. Direct measurements of the components of HR are tedious and time consuming often to the point of being prohibitive to the plant breeder, and, as such, an alternative approach is needed to permit breeders to sample individual selections rapidly and precisely.

A previous mathematical approach to this problem compared rates of disease increase of cultivars using the rate parameter of the logistic growth model suggested by Vanderplank (9). This model may be inappropriate in some situations (7) and has not provided the statistical precision needed to resolve small differences between horizontally resistant selections. Moreover, the logistic model does not account for the effects of individual components of HR nor does it account for the spacial distribution of disease.

A different type of model, stochastic in nature, is presented herein and is based on the observed frequency distribution of lesions on leaves. Each component of HR is a random quantity represented by a random variable. For example, a sample of spores landing on leaves and inciting lesions would produce counts of colonies per leaf varying randomly around some mean value. Associated with each distribution are parameters with their own biological meaning estimable from a random sample of the population. The assumed distributions describing individual components of HR can be combined by standard statistical procedures (4) to produce a model with parameters representing biological elements in the system. The specific model presented has been constructed for application to breeding for HR to powdery mildew on cereals and represents one plausible example of a general approach not previously utilized as a tool in phytopathological research.

MATERIALS AND METHODS

Distribution of colonies on leaves. Several hundred 8-day-old seedlings of the soft red winter wheat *Triticum aestivum* 'Chancellor' grown in 5-cm-diameter clay pots were inoculated with isolate 85 of *Erysiphe graminis* DC f. sp. *tritici* Marchal (Egt-85) by shaking leaves with actively sporulating Egt colonies over the plants. Inoculated plants were placed in a growth chamber at 18 C and 60% RH with a 12-hr photoperiod for 8 days. The numbers of colonies of Egt on the upper leaf surface of primary leaves were subsequently counted and the frequencies of the counts determined.

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Distribution of leaf size. Seedlings of the wheat cultivar Chancellor were grown in 5-cm-diameter clay pots in a growth chamber at 18 C as described above. The area of 140 primary leaves of 16-day-old seedlings were determined with a Numonics digitizer (Numonics Corporation, Hancock St. and Rt. 202, North Wales, PA 19454) located at the hybrid computer facility at The Pennsylvania State University.

RESULTS

Distribution of lesions with fixed n. Data from a previous study (7) for counts of the number of conidia forming elongating secondary hyphae (ESH) in groups of n = 10 were arranged into frequency classes for each of the isolate-cultivar combinations used in that study. Discrete frequency distributions were fitted to the data according to the FORTRAN IV program written by Gates and Etheridge (3). The Poisson distribution fitted all isolate-cultivar combinations tested, but the binomial distribution fit data only for cultivars infected with Egt-85. These results are summarized in Table 1 which gives the value for the probability of exceeding the observed chi-square. In a preliminary experiment using Egt-85 on the cultivars Blueboy and Redcoat, only the binomial distribution provided a significant goodness of fit to count data for the number of ESH. These results suggest that the binomial distribution best fit the count data for Egt-85 (Fig. 1) while the Poisson distribution fitted the data when Egt-112 was used.

TABLE 1. Discrete distributions associated with frequencies of conidia forming elongating secondary hyphae of two isolates of *Erysiphe graminis* f. sp. *tritici* on three winter wheat cultivars

	Isolate 85		Isolate 112	
Cultivar	Distribution	$P(\chi^2 > Obs.)$[a]	Distribution	$P(\chi^2 > Obs.)$[a]
Knox	Binomial (10, 0.14)[b]	0.1442	Binomial (10, 0.25)	0.0001
	Poisson (0.72)[c]	0.1126	Poisson (0.25)	0.2383
Redcoat	Binomial (10, 0.25)	0.2521	Binomial (10, 0.41)	0.0121
	Poisson (0.51)	0.5093	Poisson (0.63)	0.6331
Blueboy	Binomial (10, 0.37)	0.3739	Binomial (10, 0.29)	0.0228
	Poisson (0.80)	0.2400	Poisson (0.62)	0.6151

[a]The probability that the observed chi square value exceeds the expected value.
[b]Numbers in parentheses represent parameters in the binomial; the first number is n, the number of conidia examined per sample; the second number is π, the estimated probability that a conidia will form a colony.
[c]Numbers in parentheses represent the estimated parameter for the Poisson distribution which is the mean proportion of spores that form colonies.

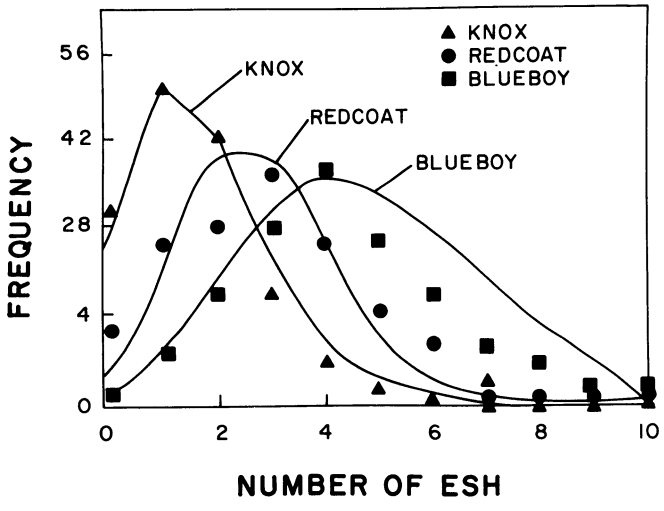

Fig. 1. Frequency of number of elongating secondary hyphae (ESH) of isolate 85 of *Erysiphe graminis* f. sp. *tritici* on three winter wheat cultivars grown in the growth chamber at 18 C and approximated by the binomial distribution.

Distribution of colonies on leaves. Data from the work of Rouse (7) indicated that the distribution of colonies of Egt on leaves in the field was highly aggregated, particularly early in the season. The negative binomial distribution (NBD), while not a significant fit to the data, $P = 0.01$, did produce the lowest chi-square value of the distributions tested. In the growth chamber experiment the FORTRAN IV program of Gates and Etheridge (3) was used to fit the Poisson, Poisson-binomial, Thomas double Poisson, Neyman type A, binomial, negative binomial, Poisson with zeros, and logarithmic with zeros distributions to the frequency data for number of colonies on primary leaves of Chancellor. The negative binomial distribution gave a significant fit with a probability of exceeding the chi-square value of 0.771 (Fig. 2). The other distributions did not give a significant fit.

Distribution of leaf size. Leaf size is a continuous variable indicating that a continuous distribution should be fitted to the data. The gamma distribution was fitted to the leaf size data using a trial and error method (Fig. 3). The normal distribution also gave a significant fit, but because the chi-square was higher and the data appeared slightly skewed the gamma distribution was assumed appropriate for description of the data.

THEORETICAL BASIS OF THE MODEL

This model represents an explanation of how the negative binomial distribution might arise from counts of lesions on leaves. Results from other research (7) are used as supporting evidence for some of the assumptions made herein.

It is assumed that the number of spores deposited per unit area of leaf at a given time is proportional to the number of spores produced at a previous time by existing colonies or, alternatively, were deposited by artificial inoculation. The random variable N denoting number of spores landing on a leaf is assumed to be Poisson distributed with mean $a\lambda$. It is assumed for the present that leaves have a constant area a. This assumption will subsequently be modified in the next portion of the model. The distribution is stated as

$$P(N = n) = \frac{(a\lambda)^n e^{-a\lambda}}{n!}, \quad n = 0, 1, 2, \ldots \qquad (1)$$

The parameter λ represents the average number of spores landing on unit areas and reflects sporulation as measured by a conventional spore trap.

Spores are assumed to act independently on the leaf surface and to possess the same potential for infection. Let the random variable X denote the number of spores subsequently forming colonies

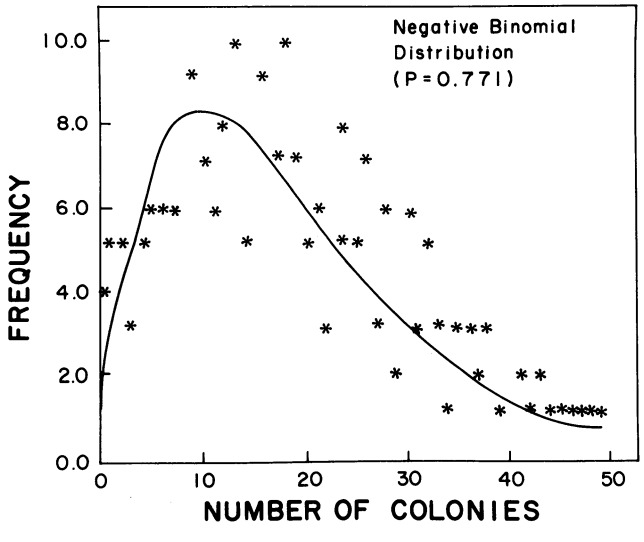

Fig. 2. Frequency of number of colonies of *Erysiphe graminis* f. sp. *tritici* on 16-day-old seedlings of the winter wheat cultivar Chancellor grown in a growth chamber at 18 C and approximated by the negative binomial distribution (solid line).

given n spores had landed per unit area of leaf. This random variable is assumed to be binomial with parameters n = number of spores per unit area and π= the probability that a spore will form a colony. The distribution has the form

$$P(X = x \mid N = n) = \binom{n}{x} \pi^x (1-\pi)^{n-x}, \; x = 0, 1, \ldots, n \qquad (2)$$

In another study (7), actual numbers of conidia germinating and forming elongating secondary hyphae (ESH) were counted out of groups of 10 conidia randomly selected on leaves 48 hr after inoculation. That data presented in Fig. 1 as the frequency of ESH was described by the binomial distribution given in equation 2 above.

Equations 1 and 2 can be combined into a single expression for the distribution representing the number of colonies per leaf with parameter $a\lambda\pi$. This well known statistical mixture (4) of the random variables X and N has a Poisson distribution as follows:

$$P(X = x) = \frac{e^{-a\lambda\pi}(a\lambda\pi)^x}{x!}, \; x = 0, 1, 2, \ldots \qquad (3)$$

The parameter "a" representing leaf area could be thought of as a realization of the random variable A representing leaf areas. Leaf area "A" is assumed to vary according to the gamma distribution stated as

$$f(A = a) = \frac{1}{\Gamma(k)} e^{-a} a^{k-1}, \; 0 < a < \infty \qquad (4)$$

Evidence for this distribution as reasonably describing leaf size variation was presented in Fig. 3.

Equations 3 and 4 can be combined as another well-known statistical mixture (1) to represent the distribution of colonies on leaves accounting for variation in size of leaves. This distribution of "X" is negative binomial and can be stated as

$$P(X = x) = \binom{x+k-1}{x} \left(\frac{\lambda\pi}{\lambda\pi+1}\right)^x \left(\frac{1}{\lambda\pi+1}\right)^k, \; x = 0, 1, 2, \ldots \qquad (5)$$

DISCUSSION

In two independent experiments (one from field plots the other from the growth chamber) the negative binomial distribution had been found to best describe the frequency of powdery mildew colonies on wheat leaves. The above model, which is based on that experimental evidence, suggests a plausible explanation for these results. The parameter $\lambda\pi$ is a measure or index of HR due to the confounded effects of sporulation and IE. In this sense it is analagous to other relative indices of HR such as the logistic rate parameter, "r". The rate of disease progress "r" implicitly includes time-dependent components of HR while $\lambda\pi$ does not. Additionally, this model provides a possible method of estimating IE and a measure of SC by sampling the numbers of colonies on leaves and fitting their frequencies to the negative binomial distribution described by equation 5. The negative binomial distribution has two parameters, $\lambda\pi$ representing the product of the number of spores landing on a unit area of leaf more than one latent period hence and IE, and k representing a parameter of leaf size.

The value of the parameter λ reflects the quantity of spores produced more then one latent period ago, and is assumed to be related to the sporulation capacity of powdery mildew. This assumption is made from the formal definition of λ as the mean number of spores deposited in a unit area times a constant (1/a) as presented in equation 1. The affect of time on this interpretation is relative since the sum of several Poisson random variables is still Poisson distributed with $\lambda = \lambda_1 + \cdots + \lambda_i$.

The parameter $\lambda\pi$ can be estimated from knowledge of the first and second moments of equation 5 from which the mean and variance of the distribution can be stated as

$$\mu = k(\lambda\pi) \text{ and } \sigma^2 = k(\lambda\pi)(1 + \lambda\pi).$$

The solution of these two equations for $\lambda\pi$ eliminating k is

$$\lambda\pi = (\sigma^2 - \mu)/\mu \qquad (6)$$

This equation relates $\lambda\pi$ to the mean and variance. Estimation of the mean and variance for a sample of counts of numbers of colonies on leaves allows the calculation of $\lambda\pi$ from equation 6.

The model makes two assumptions which must be considered if the model is to be applied to practical situations. The parameter $\lambda\pi$ is estimated from the assumption that the number of spores on a leaf surface at a given time is proportional to the number of spores produced on that plant selection at a previous time or times. Thus, individual plant selections must be isolated to avoid external inoculum affecting the distribution of colonies within individual plots. The model further assumes that initial levels of inoculum are equivalent in each test plot since differences in inoculum might affect the shape of the distribution over several cycles of disease increase.

The model has potential application for the plant breeder searching for components of HR and for the plant pathologist and/or plant breeder attempting to identify why a cultivar possesses HR.

Even in the absence of adequate isolation between selections, a relative ranking of the lines under evaluation could be obtained by counting colonies on leaves and fitting the negative binomial distribution to the data to estimate the parameter $\lambda\pi$. The gradient of dispersal from one plot to another would cause a significant amount of inoculum to come from within the plot (2). A scheme of relative ranking may be enhanced by taking counts of colonies on leaves early in the season to minimize the impact of external inoculum. The success of a relative ranking approach with this model would depend upon minimization of the cryptic error between plots so that a large proportion of inoculum deposited on leaf tissue in a plot containing a single selection would have originated from that plot. Selections suspected to possess HR from the relative ranking study could be evaluated further in isolated plots to more precisely determine their actual degree of HR. Visual estimates of differences in LP and lesion size could be combined with this procedure. Another approach to obtaining data on the level of HR using this model might be to count colonies on leaves or newly emerged leaves, for example the flag leaf, after a period of time between one and two latent periods of the fungus. Existing colonies on a newly emerged leaf at that time would be the result of sporulation of existing colonies in the plot over a very limited time interval. This approach would avoid the possible effect on the observed distribution of counting colonies produced from several cycles of sporulation.

This model also may be used for identifying components of HR in cultivars possessing rate-reducing resistance. Laboratory and greenhouse experiments have been needed in the past to identify quantitatively the individual components of HR. A more rapid estimation of IE and a parameter related to SC of a cultivar under

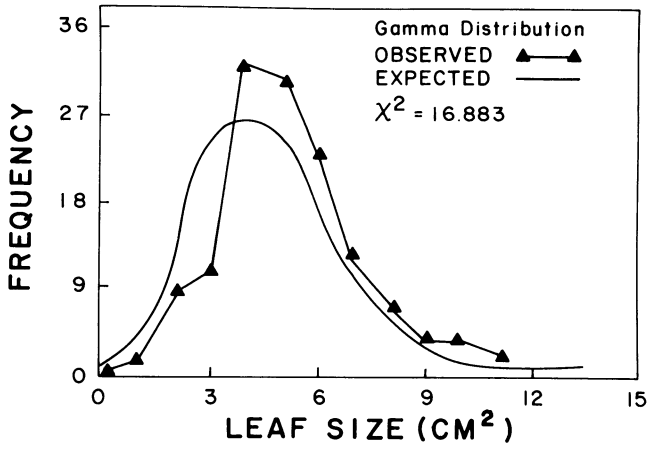

Fig. 3. Frequency of leaf areas of 16-day-old primary leaves of the cultivar Chancellor grown in a growth chamber at 18 C (△——△) approximated by the gamma distribution (smooth line).

field conditions is possible with this model. The observed distribution of colonies on leaves provides an estimate of $\lambda\pi$ the product of an index of SC and IE, making it necessary to evaluate only one of these components of HR. This could be accomplished with an artificial inoculation allowing IE to be estimated from counts of the resulting initial colonies.

There are few data in the phytopathological literature describing the frequency distributions of the sampled units. The possible usefulness of this approach in describing data for the extraction of biological information should be considered. A stochastic model describing the sampling units of interest in a biological experiment offers a flexible approach to modeling based on estimated sampling distributions having a biological interpretation. This particular model, although based on experimental results, has not yet been tested thoroughly in the field, but it is proposed herein as a potentially valuable technique for research on HR.

Inasmuch as this paper attempts to provide a theoretical explanation of why the observed frequency of number of lesions on leaves is negative binomial, it should be noted that statistical models based on discrete frequency distributions can be developed in several other ways, some of which might offer alternative interpretations of the biological meaning of the parameters. For example, the negative binomial distribution may arise as a birth and death process as well as a mixture of two distributions as used in this paper (1). The approach of statistical modeling is a general one, suggesting that useful models could be developed to obtain biological information for many host-pathogen systems.

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