Techniques

Probits for Analyzing Latent Period Data in Studies of Slow Rusting Resistance

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ABSTRACT

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Probit analysis was applied to latent period data of wheat leaf rust. Analysis of data from monocyclic infection experiments in the greenhouse revealed a linear relationship between probit percent of uredinia erupted and days after infection. Coefficients of determination from linear regression analysis were greater than 0.91 for 32 of 33 sets of data analyzed. T_{50}, the day by which 50% of the uredinia have erupted, was calculated from the linear regression coefficient and y-intercept. T_{50}, which is analogous to the LD_{50} of toxicology, characterizes the latent period of the disease and can be used to compare host genotypes for degree of slow rusting resistance.

Latent period curves (percent uredinia erupted vs days after infection) generated from probit analysis statistics were quite similar to the original curves. The advantage of using T_{50} over calculating a weighted latent period or directly estimating the day on which 50% of uredinia erupt, is that plants need not be inspected each day for percent uredinia erupted in order to calculate T_{50}. Alternate day inspections are sufficient and greatly reduce the time and effort associated with studying slow rusting or using it in a breeding program.

Additional key words: Puccinia recondita, Triticum aestivum, modeling, computer simulation, general resistance, horizontal resistance, durable resistance.

Latent period, the time between infection and production of secondary inoculum from that infection, is one of the main elements that control the rate of development of wheat leaf rust. Consequently, latent period is an important part of slow rusting resistance (4,8–10). Applied to a single lesion, the definition of latent period is clear, but in most cases latent period refers to the time required for a population of lesions on a leaf, plant, or group of genetically similar plants growing under the same conditions, to become infectious. Although latent period for leaf rust is often given as the number of days between infection and production of spores, not all of the infections that occurred on a single night on a leaf commence sporulation on the same day (10). This variation in latent periods among infection sites on a leaf may be considerable, and it seems to be an inherent feature of the disease and not due to experimental error because it does not diminish under rigorously

controlled conditions. Moreover, variances as well as means for latent period differ among host genotypes (10).

Some workers have used the first day after inoculation on which any lesions produce secondary inoculum to characterize latent period (5,11,13). Others have used the day by which 50% of the lesions are producing secondary inoculum (4,8). Neither of these approaches uses all the available data. Two cultivars may differ by only one day in minimum latent period but the time required for the same proportion of lesions to sporulate may differ by two or three days (9). Likewise, the day on which at least 50% of the lesions are sporulating tells nothing about the minimum latent period nor the rate at which lesions become infectious. This rate is often highest at the time 50% of the lesions become infectious. That is, on one day only 20% of the lesions on a leaf may have formed uredinia but by the next day 70% of the lesions may have done so. The time required for 50% of the infection sites to become infectious must be calculated by linear interpolation or simply interpreted as the whole number of days required for at least 50% of the lesions to become infectious. There is imprecision in either of these methods that increases error variance when this statistic is used to compare cultivars.

A modification of expressing latent period as the time 50% of infection sites become infectious is the weighted average latent period, LP (7,10). It is calculated as: $LP = \sum_{i=0}^{n} P_i t_i$ in which P_i is the proportion of sporulating lesions (in relation to the final number of sporulating lesions) that appear on the ith day after inoculation, t_i is the ith day after inoculation and n is the number of days after inoculation when the maximum number of lesions are sporulating (10). This method of calculating latent period uses all the latent period data—time of first appearance of secondary inoculum and subsequent rate of commencement of sporulation by lesions. However, there are difficulties with its use. Leaves must be examined each day. Omitting observations on one or more days causes LP to be greater than when daily observations are used. When large numbers of plants must be examined in genetic studies or in screening lines for breeding, daily examination of all plants becomes very time-consuming. If plants could be examined every two or three days, work could be done more efficiently.

Plotting the percentage of *Puccinia recondita* Rob. ex Desm. infection sites that are sporulating on a wheat leaf against days after infection yields a sigmoid curve (9). A transformation of percent uredinia erupted that would change this curve to a straight line would facilitate comparison of treatments and permit interpolation of values, eliminating the need for daily inspection of plants. In this paper the possibility of the probit transformation for this purpose is examined. The probit transformation is designed to straighten the sigmoid cumulative normal probability curve (3).

MATERIALS AND METHODS

Data from three previously published experiments (10) were used to test the validity of probits for analyzing latent period data. In these experiments, fast-rusting wheats (*Triticum aestivum* L. em Thell 'Monon' [CI 13278] and 'Suwon 92' [CI 12666]), and slow-rusting wheats Suwon 85 (PI 157600) and Purdue breeding line P6028A2-5-9-6-1 (P6028) were inoculated with urediniospores of *P. recondita* at densities ranging from 77 to 1,023 spores per square

millimeter. Following inoculation, numbers of uredinia per square centimeter of leaf were counted each day until all had erupted. In the present study, the mean number of uredinia per square centimeter of leaf on four replicate leaves was used for probit analysis. There were 33 sets of data representing the various cultivar–inoculum level combinations among the three experiments.

Equations relating the probit of relative number of uredinia (relative to the final number of uredinia for each treatment combination) to days after inoculation were calculated by linear regression. The relative number of uredinia at the final observation time was 1, for which the probit $= \infty$, so this final relative number was omitted from the calculation of the regression line. The computations were made with a computer program that first calculated probits by using the rational approximation of the inverse normal integral given by Abramowitz and Stegun (1) and then calculated the linear regression statistics. Thus, for each day of observation, only the day and number of uredinia had to be entered into the computer. The accuracy of probits calculated in this program was verified by comparing them with the table published by Bliss (2).

RESULTS

The equation solved was of the form:

$$\text{probit } Y = bX + a$$

in which Y = proportion of uredinia erupted, X = number of days after infection, b = slope of the line, and a = the y-intercept. Of the 33 regression lines calculated, 32 had coefficients of determination greater than 0.91 (Table 1). For each set of data, the regression equation was used to calculate T_{50}, the time required for 50% of the uredinia to erupt. These T_{50}'s were approximately one-half day shorter than LP's for the same sets of data (Table 1).

Within each experiment the homogeneity of regression coefficients (coefficient b in the above equation) was tested (page 319 in reference 12). Within an experiment and cultivar, coefficients associated with various inoculum levels were homogeneous. However, within all nine experiment-inoculum level comparisons, F-values for heterogeneity of regressions were highly significant. Based upon Duncan's New Multiple Range Test, coefficients for Monon and Suwon 92 were consistently greater than those for Suwon 85 and P6028.

Finney (page 51 in reference 3) points out that in calculating a regression line from probit data, the probits should be weighted. This was not done in the calculations reported above because the coefficients of determination for the unweighted regression equations were so high that weighting was deemed unnecessary. However, weighted regression lines were calculated for the data pooled from all experiments (Fig. 1). The slope and intercept for the weighted regression line was nearly identical to these statistics from unweighted regression for each cultivar. Moreover, the values of T_{50} calculated from the two regression equations were identical when rounded off to one-tenth of a day.

In a mathematical model for slow-rusting resistance, P_j-values are used to characterize the sequence of eruption of uredinia (9). P_j is the probability that an infection that occurred on the $(i-j)$th day will erupt into a uredinium on the ith day. Values for P_j are obtained by calculating the daily increment in percent uredinia erupted from the day the first uredinia erupt until the day all of them have erupted. Solution of the probit regression equation can also generate values for P_j. Probit Y is calculated as a function of X, for X = p (the minimum latent period) to m (the day at which Y = 1). Y_j is calculated as the "antiprobit" of probit Y by using the rational approximation of the normal integral (1). Then,

$$P_j = Y_j - Y_{j-1} \text{ for } j = p \text{ to } m.$$

I have incorporated these operations into the computer program that models rust epidemics by using the equations of Shaner and Hess (9). Instead of entering P_j-values into the computer, one enters the slope and y-intercept of the probit regression line and the

TABLE 1. Comparison of T_{50} and LP[a] for estimating latent period of *Puccinia recondita* infections on *Triticum aestivum*

Cultivar	Mean LP (days)	Mean T_{50} (days)	Range of LP-T_{50} (days)	Samples (no.)
Monon	7.83	7.27	.52-.67	6
Suwon 92	8.05	7.51	.48-.62	9
Suwon 85	10.49	9.94	.44-.75	9
P6028	10.69	10.15	.39-.77	9

[a]T_{50} is the number of days required for 50% of the uredinia to erupt, calculated from probit analysis. LP is the weighted mean latent period (see text for details).

computer calculates P_j-values before simulation of the epidemic begins.

P_j-values derived from probit analysis are similar to P_j-values calculated directly from percent uredinia erupted (Table 2). Disease progress curves (per unit severity plotted against time) from epidemics modeled with either the original P_j-values or P_j-values calculated from probit analysis were almost identical. For each cultivar, the disease progress curves for the two simulations were almost congruent and the areas under the curves differed by no more than 3%.

Because probits transform a sigmoid latent period curve into a straight line, it follows that numbers of uredinia per square centimeter of leaf would not need to be counted daily. Using the probit transformation, counts on alternate days should yield the same slope and y-intercept as daily counts, and hence estimate the same P_j-values and same T_{50}. However, there is a small residual mean square associated with the regression of probit percent uredinia on time, so a line derived from alternate day counts would not necessarily be identical to a line derived from daily counts. To determine how different the regression lines based on daily or alternate-day counts would be, the probit of percent uredinia erupted on alternate days, beginning with the first day uredinia erupted, and including the day on which the maximum number had appeared, was regressed on time. Values of T_{50} estimated from these regression equations were within 0.1 day of values of T_{50} estimated from the original probit lines reported in Table 1. P_j-values were also estimated from the regression equation as described above. These values were likewise similar to the original, directly determined P_j-values. Disease progress curves generated from these P_j-values closely matched those generated from the original P_j-values.

DISCUSSION

The high coefficients of determination for the regression of probit percent uredinia erupted on days after inoculation indicate that probit analysis is a valid and useful technique for dealing with latent period data. The latent period can be expressed as T_{50}, the
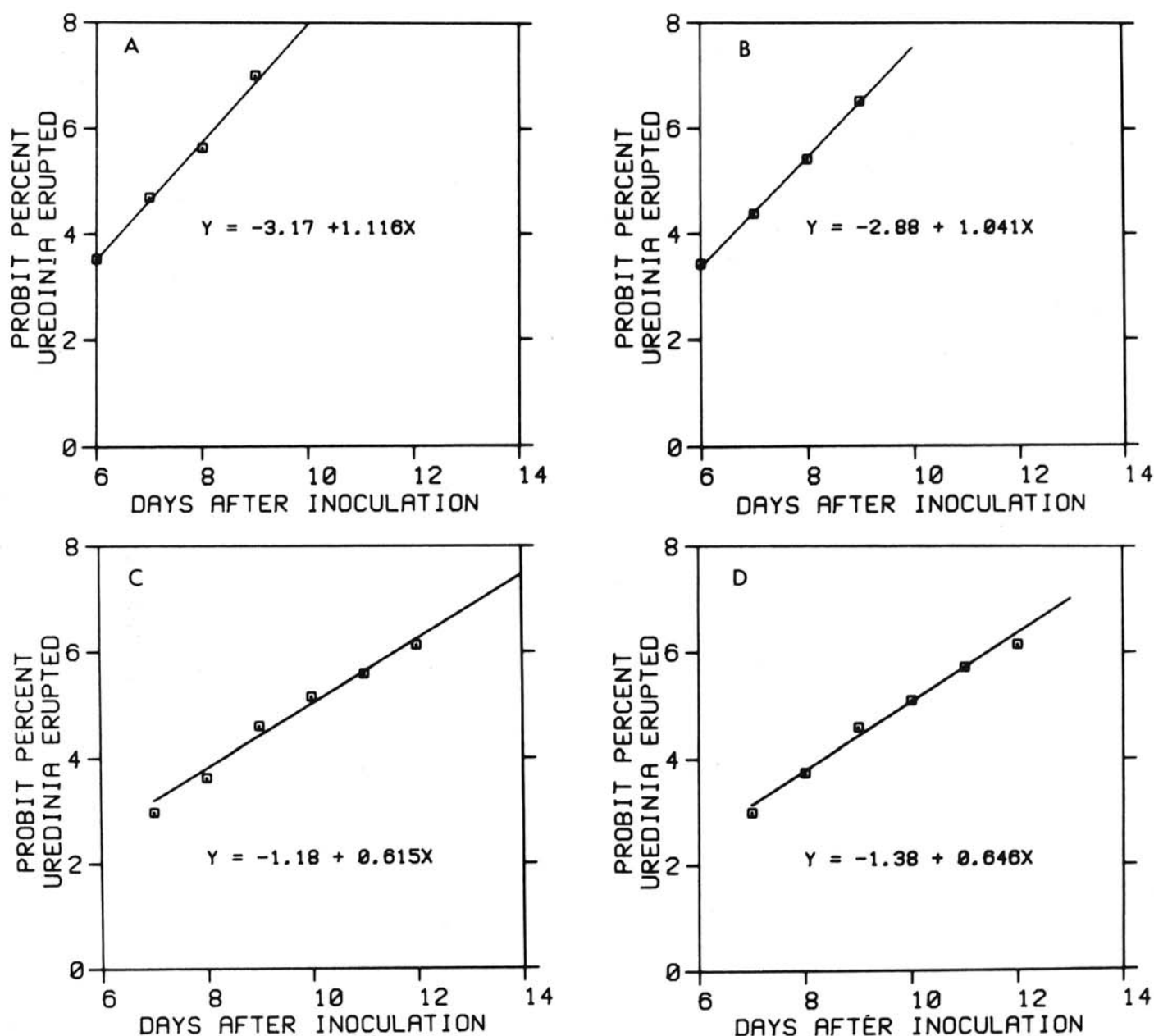

Fig. 1. Weighted regression of probit percent *Puccinia recondita* uredinia erupted on days after inoculation (see text and pages 51–55 in reference 3 for details). Points are based on data pooled from three experiments each involving three inoculum levels: **A**, Wheat cultivar Monon; **B**, Wheat cultivar Suwon 92; **C**, Purdue wheat breeding line P6028 A2-5-9-6-1; and **D**, Wheat cultivar Suwon 85.

TABLE 2. Comparison of P_j-values directly observed[a] with P_j-values calculated from probit analysis of wheat leaf rust latent period for four wheat cultivars

Day after infection (i)	Monon			Suwon 92			Suwon 85			P 6028[c]		
	Direct[a]	Probit[b]		Direct	Probit		Direct	Probit		Direct	Probit	
		D	A		D	A		D	A		D	A
6	.071	.068	.064	.058	.042	.046						
7	.311	.296	.287	.209	.239	.256	.022	.019	.017	.021	.023	.024
8	.358	.423	.425	.396	.434	.439	.082	.074	.080	.064	.070	.072
9	.238	.187	.196	.273	.242	.224	.236	.194	.220	.259	.165	.169
10	.022	.025	.028	.064	.041	.034	.201	.291	.318	.218	.250	.255
11		.001	.001		.002	.001	.223	.253	.243	.161	.247	.247
12							.110	.127	.098	.148	.158	.154
13							.123	.037	.021	.085	.065	.062
14								.006	.002	.044	.018	.016
15								.001			.003	.003

[a] From Shaner and Hess (9).
[b] P_j-values under column D were derived from the regression equation relating probit percent uredinia erupted to days after inoculation. P_j-values under column A were derived from the regression equation relating probit percent uredinia erupted on alternate days to days after inoculation. See text for details. P_j is the probability that an infection that occurred on the (i-j)th day will erupt into a uredinium on the ith day.
[c] P6028 is Purdue breeding line P6028-5-9-6-1.

time required for 50% of the uredinia to erupt, analogous to the LD_{50} of toxicology. Latent period expressed as T_{50} is nearly the same as the weighted mean latent period (LP) we have used in previous studies (6,7,10) but consistently about one-half day shorter. The advantage of the probit method is that it can be used without having data on percent uredinia erupted for each day. This eliminates the need to examine every inoculated plant every day from the 6th day after inoculation until all uredinia have erupted. Alternate-day observations would suffice. This doubles the amount of experimental material that can be handled because the time required to estimate percent uredinia erupted limits population size in slow-rusting studies.

The fact that the probit transformation of latent period data gives a close fit to a straight line suggests that the theoretical basis for probits applies to the outbreak of uredinia following infection. Probits were used by Bliss (2) for the analysis of dosage-mortality data. Finney (3) provided a thorough analysis of the method of probits. Briefly, probits were developed for analysis of quantal data, such as the ability or inability of a fungal spore to germinate. They are based on the assumption that the quantal response of a population of organisms to a stimulus is normally distributed. Probits express the cumulative percentage response to various levels of stimulus in units of standard deviation. Thus, we may reason that data which lie close to a straight line when the probit transformation is used, fit the assumption of a population whose response to a stimulus is normally distributed. In the present case the experimental population consists of infection sites on a leaf of a wheat cultivar, rather than of individual organisms. The "stimulus" is time after inoculation. If a population of individual organisms is genetically heterogeneous for several genes that additively affect the response to a stimulus and if the alleles assort at random, the phenotypes will be normally distributed. The normal distribution of latent periods of infections on an individual leaf is not a consequence of genetic assortment because the population of host infection sites on a leaf are genetically identical. Likewise, gross environmental conditions such as soil conditions or temperature cannot be invoked to explain the variation in latent periods of infection sites that may be within 1 or 2 mm of each other. Rather, there is a randomness in the response of an infection site due either to developmental events prior to infection or to the cellular environment during infection. Possibly some subtle interaction between host cell, fungal mycelium, and environment at the infection site determines how quickly and to what degree the host retards the development of the pathogen. The fact that the regression coefficients of the probit equations were significantly greater for Monon and Suwon 92 than for Suwon 85 and P6028 indicates that the population of infections on these latter two cultivars had not only a greater mean latent period, indicated by the greater T_{50}, but also a greater variance of response. The standard deviation of the normal distribution of latent periods that has been

fit by probit analysis is the reciprocal of the regression coefficient (3).

Probits were developed as an analytic method for experiments in which different subsets of a population of organisms are each exposed to different doses of toxicant or other material. Thus, the response at each dose is an independent estimate of the population response. When probits are applied to latent period data, the subsets of infections examined each day are not independent because the same infected leaves are examined repeatedly. There is no reason to believe that this invalidates the use of probits for analysis of latent period data. It should improve the precision of estimation of T_{50} for the actual experimental plants used and, with sufficient replication, the general conclusions that can be drawn about various wheat genotypes under a given environment.

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