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Estimation of area under the disease progress curves in a rice-blast pathosystem from two data points

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Abstract Attempts were made to estimate the area under the disease progress curve (AUDPC) of rice blast disease caused by *Pyricularia grisea* from two data points on the disease progress curve. Forty-two rice genotypes were exposed to high disease pressure in a nursery over nine seasons. A conducive condition was created for maximum disease development through high nitrogen application, close spacing and maintenance of high relative humidity. Disease severity was recorded on alternate days beginning from disease initiation until the end of the epidemic. The estimation of AUDPC, and logistic and Gompertz apparent infection rates using either all-points (AP) or two-point (TP) methods revealed significant correlations among them. This was also confirmed through regression analysis and factor analysis. Hence, the estimation of AUDPC from two data points i.e. initial and final disease scores of the disease progress curves is recommended as providing information similar to that from all the data points; this should save valuable time, labour and economic resources.

Keywords Apparent infection rate · Area under disease progress curve · Factor analysis · *Pyricularia grisea* · Rice blast disease

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Abbreviations

AUDPC	Area Under Disease Progress Curve
RAUDPC	Relative Area Under Disease Progress Curve
r	Logistic apparent infection rate
k	Gompertz apparent infection rate
AP	All points
TDS	Terminal Disease Severity scores
TP	Two points

Introduction

Disease severity in any plant-pathosystem can be assessed either once at the peak of the epidemic or several times at some intervals starting from disease initiation until the end of the epidemic. The former method of assessment measures the cumulative effects of all the factors operating during the course of epidemic *viz*. the terminal disease severity scores (TDS), while the latter can be used to estimate different parameters like the area under the disease progress curves (AUDPC), relative area under the disease progress curve (RAUDPC), logistic (r) and Gompertz (k) apparent infection rates, the time required for the disease to reach a specific level of severity in logistic and Gompertz models, and the genotype-score on PC-1 and PC-2 estimated through principal component analysis (PCA). Among these parameters, AUDPC has been widely used in two areas of plant pathological research *viz*. crop loss assessment (Ferrandino and

Elmer 1992) and assessment of partial or quantitative resistance. The estimation of AUDPC is based on simple formulae developed by Wilcoxson et al. (1975), Shaner and Finney (1977), Bjarko and Line (1988), Das et al. (1992), Chen and Line (1995), Miedaner and Sperling (1995) and Broers et al. (1996), following the trapezoidal rule for calculation.

Recently Mohapatra (2002) and Mukherjee et al. (2005) recognized AUDPC, RAUDPC, r, k and genotype-score on PC-1 as top ranking parameters for identification of slow-blasting resistance in rice. The assessment of rate reducing resistance in polycyclic diseases covers a range of sampling frequencies at regular intervals, which is time consuming, labour intensive and costly. Van der Plank (1963) proposed estimating the logistic apparent infection rate (r) using a two-point method *i.e.* initial disease severity and final disease severity data, which was adopted by Berger (1981) through Gompertz transformations. Recently, Jeger and Viljanen-Rollinson (2001) reported that estimation of AUDPC from two data points on the disease progress curves for stripe rust resistance in wheat provided an equivalent amount of information as those from all points. This was followed by estimation of AUDPC from two data points for assessment of late blight resistance in potato by Haynes and Weingartner (2004), who suggested that the estimation from two data points provide the information similar to those from all points, thereby saving time and economic resources. The objective of the present investigation was to illustrate the estimation of AUDPC from two data points on the initial and final day of epidemic progress, which might be as reliable as that from all data points.

Materials and methods

Plant material and growing conditions

Seeds of 42 rice genotypes were collected from the International Germplasm collections, IRRI, Philippines through the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India and the National Germplasm collections maintained at Central Rice Research Institute, Cuttack, India. The seeds were sown in a uniform blast nursery with the susceptible check variety Karuna sown in alternate rows as well as all around the nursery, as spreader rows. Thus each test variety was raised within one meter long single-row plot, surrounded by susceptible spreader rows of Karuna. Seeds were sown with a spacing of 10 cm between rows and 5 cm between plants. The experiment was conducted in a randomized complete block design with three replications. High nitrogen fertilizer (100 kg N ha^{-1}) in the form of ammonium sulfate was applied in split doses. High relative humidity was maintained throughout the period of experimentation, by running the sprinkler irrigation system during hotter periods of the day (10 am to 3.30 pm) with an intermittent stoppage of half an hour after each hour of irrigation. The experiment was repeated over a period of nine seasons (5 dry+4 wet seasons), from dry season 1997 to dry season 2001.

Recording observation and statistical analysis

Disease severity scores of the five most severely infected plants (selected based on initial visual assessment) in each test variety were recorded on alternate days. Disease severity data recorded on alternate days were used to estimate AUDPC for the AP and TP methods. Severity was recorded beginning from the first day of disease initiation until the end of the epidemic in the spreader rows, following the score chart developed by Padmanabhan and Ganguly (1959). The scoring was based on both number and type of spots, each assigned with a numerical value which is summarized below:

Type of spot	Numerical value
Type-A: Just brown specks	1
Type-B: Reddish brown circular discoloration without zonal differentiation	2
Type-C: Circular spots, 2–3 mm. diameters	4
Type-D: Broadly spindle shaped spots, slightly longer than broader with central ashy zone of 4–5 mm. diameters	8
Type-E: Elongated spindle shaped spots with central ashy zone of 3–5 mm. broad and up to 20–30 mm. long	16

The numbers of spots were also assigned numerical values as:

Number of spots	Score	Numerical value
Up to 5 spots	Score-1	1
6–15 spots	Score-2	5
>15 spots	Score-3	10

The products of the respective numerical values for the type and number of spots were considered as the numerical score value for a particular unit of observation (= a plant). The average of five such highest infected plants was considered as the disease score for a particular host genotype. These numerical score values were converted into severity scores of $0<x\leq 1$ by dividing each score by the highest score recorded in the susceptible check Karuna at the end of the epidemic and used for analysis of disease progress curves.

AUDPC was calculated using the AP method ($AUDPC_{AP}$) as suggested by Shaner and Finney (1977):

$$AUDPC_{AP} = \sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2][t_{i+1} - t_i]$$

where

x_i = the proportion of host tissue damaged at the i^{th} day

t_i = the time in days after appearance of the disease at the i^{th} day

n = the total number of observations.

AUDPC was also calculated from two data points i.e. the initial and final assessments, following the methods suggested by Jeger and Viljanen-Rollinson (2001) based on the integration of disease progress models. The details of this method are as follows. For any real continuous function $y=f(t)$ (with $y>0$), the area under the function is simply the definite integral evaluated between the limit of integration t_o and T, where $T>t_o$.

$$\text{Suppose}\quad y = f(t) = 1/(1 + Ae^{-rt}) \tag{1}$$

i.e. f(t) is the logistic function with $A=(1-y_o)/y_o$, where y is the amount of disease in the numerical scale of 0–1, y_o is the value of y at $t_o=0$, and r is the logistic rate parameter. The area under the disease progress described by Eq. 1 is then

$$AUDPC = \int \{dt/(1 + Ae^{-rt})\}$$

which is evaluated between the limits t=0 and T, and substituting in y

$$= T + [\ln\{(y_o/y_T)\}]/[\ln\{y_T/(1 - y_T)\} - \ln\{y_o/(1 - y_o)\}]/T \tag{2}$$

where, y_T is the value of f(t) at t=T.

Thus, only two assessments of disease are necessary, one at the start of an epidemic and the other at the end of epidemic or at a critical growth stage.

The apparent infection rates in the logistic (r) model (Van der Plank 1963) as well as the Gompertz (k) model (Berger 1981) were estimated as the regression coefficients (*b*) of the logit x or gompit x on time (days)

The logistic as well as Gompertz apparent infection rates were also calculated following the TP methods suggested by Van der Plank (1963) and Berger (1981), respectively as:

$$r_{TP} = [\log_e\{x_2/(1 - x_2)\} - \log_e\{x_1/(1 - x_1)\}]/(t_2 - t_1) \text{ and}$$
$$k_{TP} = [\text{gompit}\{x_2/(1 - x_2)\} - \text{gompit}\{x_1/(1 - x_1)\}]/(t_2 - t_1)$$

where, x_1 and x_2 are disease severity scores on the first and last day of observation, and t_1 and t_2 are the initial and final day of observation.

The association among the estimates of AUDPC, the apparent infection rates in the logistic as well as the Gompertz models calculated using both the AP and TP methods were assessed through correlation analysis. The usefulness of each parameter was determined through the estimation of the degree of variability and inter-correlations among them through factor analysis (Kendall and Stuart 1968), carried out by considering the six parameters as entities and the 42 rice genotypes as the variates. The parameters showing higher inter-correlations and explaining the major part of the variations in the communality were considered as the best for use in characterization of the disease progress curves. The factor analysis was carried out with the help of statistical package developed by M/s. Indostat Services, Hyderabad, India.

Results

Disease progress

Conducive conditions facilitated early disease initiation, rapid disease development and highest disease pressure in the susceptible genotypes during all the nine seasons of testing. Delayed disease initiation, slower rates of disease development, and lower final disease severities were recorded for the slow-blasting genotypes. Disease severities in the susceptible genotypes were significantly higher than those on the slow-blasting genotypes on all assessment dates. These genotypes were earlier grouped into four clusters (A, B, C and D), following principal component analysis and cluster analysis by Mohapatra (2002). Among them, clusters A and B were classified as fast-blasting and clusters C and D as slow-blasting genotypes. Disease progress curves for these four clusters of genotypes in one representative season depict differences in the progress of the disease over time (Fig. 1).

Disease severity in these 42 rice genotypes ranged between 0 to 100% over nine seasons. Out of a total of 378 genotypes-season combinations, 70 did not exhibit any disease reaction or exhibited consistently low but similar disease scores over all the assessments. These 70 genotype-season combinations represented 16 genotypes namely DM-27, Tieu-phai, Sam-Houang, Chokoto, Raj bhawalta, Sechi aman, IR-5533-PP-854-1, Madhukar, Milayeng-51, PTB-8, Lien tsan 50B, Salum pikit, PTB-18, DNJ-55, UCP-188 and Goda heenati, showing consistently resistant reactions over all nine seasons. Seven among the rest of the genotypes, namely DZ-192, Sakai, Seritus-malam-B, Laurent-TC, Lien tsan-50A, DJ-88 and CR-570 were consistently resistant over all nine seasons. These 23 genotypes might be possessing stable resistance to blast disease. The disease progress curves for the highly susceptible genotypes reached a level of 100% severity within a period ranging from 10 to 22 days; while the remaining genotypes took 4 to 26 days to reach severity levels ranging from 0.8% to 66.7%.

In total, 308 disease progress curves were analyzed for the purpose of estimation and comparison of parameters. The distribution properties of the six parameters viz AUDPC_{AP}, AUDPC_{TP}, r_{AP}, r_{TP}, k_{AP}, and k_{TP} over nine seasons of testing are presented in Fig. 2, depicting the measures of dispersion in the data. The box position for all the six parameters are shifted towards the lower end, signifying that the distributions are positively skewed. This was further substantiated by noticeable shift of the respective medians towards the lower end of the box; the median values were 0.50, 0.44, 0.13, 0.10, 0.03 and 0.02 for AUDPC_{AP}, AUDPC_{TP}, r_{AP}, r_{TP}, k_{AP}, and k_{TP}, respectively. Small quartile (q1–q3) deviations implied high uniformity, having small variation among the 50% of central observations. The very thin boxes for r_{AP}, r_{TP}, k_{AP} and k_{TP} indicated that a very high number of observations are contained within a very small range, which signified a distribution with a thinner peak compared to the wider boxes for AUDPC_{AP} and AUDPC_{TP}.

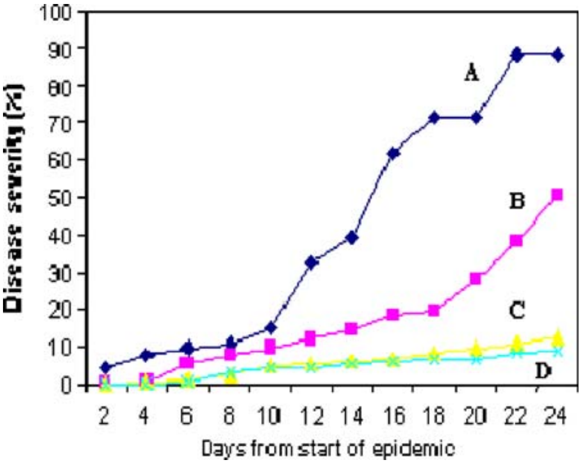


Fig. 1 Blast disease progress curves in respect of four clusters of rice genotypes (**a**) and (**b**) for the fast-blasting; (**c**) and (**d**) for the slow-blasting genotype-clusters)

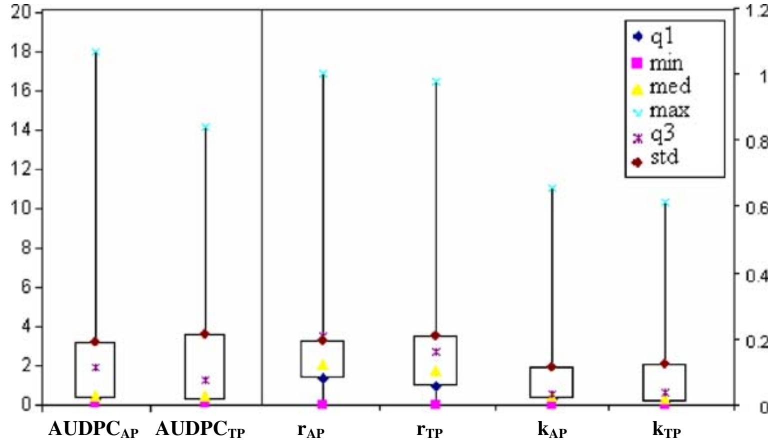
Comparison between the all-points and two-point methods

Six parameters were calculated for the 42 rice genotypes that were tested during all nine seasons of this study: AUDPC, r, and k for both the AP and TP methods. The logistic apparent infection rates were always higher than the Gompertz apparent infection rates in both AP and TP methods. AUDPC ranged from 0.053 to 18.01 in the AP method, while those in the TP method ranged from 0.08 to 14.18, with corresponding median values of 0.50 and 0.44, respectively. Similarly, the r values ranged from 0 to 0.99 in the AP method compared with those from the TP method, ranging from 0 to 0.9; the corresponding median values were 0.13 and 0.10. The Gompertz apparent infection rates ranged from 0 to 0.65 in the AP method and from 0 to 0.61 in the TP method with corresponding median values of 0.03 and 0.02. The correlations among AUDPC, r and k for both the AP and TP methods were highly significant (Table 1).

The relative importance of six parameters

The relative importance of these six parameters in characterization of disease progress curves was determined by estimation of the degree of variability and inter-correlation through factor analysis. The factor analysis extracted two factors explaining 82.30% and 17.70% of variation present in the communality and also

Fig. 2 Box plots depicting the distribution properties of six parameters. The y-scale for $\mathrm{AUDPC}_{\mathrm{AP}}$, $\mathrm{AUDPC}_{\mathrm{TP}}$ presented at *left*, and r_{AP}, r_{TP}, k_{AP}, k_{TP} at the *right* axis



some inter-correlations between parameters (Table 2). The first factor showed a high positive inter-correlation for $\mathrm{AUDPC}_{\mathrm{AP}}$, $\mathrm{AUDPC}_{\mathrm{TP}}$, r_{AP}, r_{TP} and k_{TP}. Hence these five were recognized as the top ranking parameters for evaluation of resistance. The second factor revealed high positive inter-correlation for k_{AP} and hence was recognized as the second ranking parameter. Thus $\mathrm{AUDPC}_{\mathrm{AP}}$ and $\mathrm{AUDPC}_{\mathrm{TP}}$ as well as r_{AP} and r_{TP} were recognized as equally top ranking parameters in characterization of disease progress curves.

Discussion

Several measures for evaluating plant disease resistance have been advocated for use in identification of slow-blasting resistance in rice, including single-point, or multi-point assessments, AUDPC, RAUDPC, r, k and number of lesions (Mohapatra 2002; Mukherjee et al. 2005). However multiple data point assessment methods involve recording observations at regular intervals during the epidemic and thus are time consuming, labour intensive and expensive. Moreover, frequent observations, although providing detailed information on most of the elements of disease progress curves, may inadvertently affect the progress of the epidemic due to human interferences. Recently, Jeger and Viljanen-Rollinson (2001) suggested that estimation of AUDPC for stripe rust resistance in wheat from two data points provided as much information as estimation of AUDPC from repeated assessments, which was successfully adopted by Haynes and Weingartner (2004) for assessment of late blight resistance in potato.

AUDPC estimated from two data points in the present experiment showed good correspondence with AUDPC estimated from all data points during repeated experimentations over a period of nine seasons of testing. Estimated AUDPC values were also highly correlated with the apparent infection rates

Table 1 Correlation among the parameters estimated in all-points (AP) and two-point(TP) methods

	$\mathrm{AUDPC}_{\mathrm{TP}}$	r_{AP}	r_{TP}	k_{AP}	k_{TP}
$\mathrm{AUDPC}_{\mathrm{AP}}$	0.890*	0.947*	0.781*	0.950*	0.841*
$\mathrm{AUDPC}_{\mathrm{TP}}$		0.899*	0.893*	0.923*	0.990*
r_{AP}			0.809*	0.964*	0.884*
r_{TP}				0.820*	0.902*
k_{AP}					0.899*

AUDPC Area under disease progress curve, r and k = Apparent infection rates in logistic and Gompertz models, respectively

* Significant at $P=0.01$ level

Table 2 The factor matrix from the factor analysis to compare parameters describing progress curves of rice blast

Parameters	Vector 1	Vector 2	Factor 1	Factor 2
$\mathrm{AUDPC}_{\mathrm{AP}}$	0.438	−0.041	**0.935**	0.053
$\mathrm{AUDPC}_{\mathrm{TP}}$	0.549	−0.049	**0.981**	0.050
r_{AP}	0.447	−0.012	**0.950**	0.084
r_{TP}	0.431	−0.028	**0.918**	0.064
k_{AP}	0.076	0.997	0.064	**0.998**
k_{TP}	0.454	−0.041	**0.969**	0.056
% variance explained	82.3	17.7	81.7	18.3

Bold figures indicate significance at $P=0.01$ level

AP all points, *TP* two points

from the logistic and Gompertz models for both the AP and TP methods. This was further supported by the relative importance of these parameters determined by the degree of variability and inter-correlations analyzed through factor analysis. AUDPC and logistic apparent infection rates calculated by the AP and TP methods as well as the Gompertz apparent infection rates calculated by the TP method were superior to the Gompertz apparent infection rate calculated by the AP method. Previous reports revealed the superiority of $\mathrm{AUDPC_{AP}}$ and '$\mathrm{r_{AP}}$' over many other parameters for evaluation of slow-blasting resistance in rice (Mohapatra 2002; Mukherjee et al. 2005; Mohapatra et al. 2008). The superiority of $\mathrm{AUDPC_{AP}}$ and $\mathrm{r_{AP}}$ in the present investigation corroborates these previous reports. However, those previous studies did not compare the estimations by the TP method. Evaluating slow-blasting resistance using $\mathrm{AUDPC_{TP}}$ and $\mathrm{r_{TP}}$ in the present investigation is as informative as estimation of $\mathrm{AUDPC_{AP}}$ and $\mathrm{r_{AP}}$ but with minimum labour and cost.

Jeger and Viljanen-Rollinson (2001) delineated three conditions that must be fulfilled for such a conclusion. The first qualification was that resistance must be expressed in terms of a rate parameter rather than in terms of the asymptotic level of disease. This condition was fulfilled in the present study by terminating the disease scoring and AUDPC calculation immediately after severity in the susceptible spreader rows was rated 100% and the plants succumbed to the disease. This made it possible to calculate the logistic and Gompertz apparent infection rates for better comparisons. The second qualification was the period of time over which the disease was present in the crop should be the same for each of the host genotypes. This was achieved in the present investigation with spreader rows of the susceptible check Karuna which was sown in alternating rows with the evaluated genotypes as well as all around the blast nursery. Conducive environmental conditions favored disease development in the spreader rows; disease was established quickly and reached 100% severity within a very short period. This situation ensured that all the tested genotypes were exposed to high disease pressure during the entire period of epidemic development. Third, anomalous results might occur if disease progress is not continuous and cannot be described by a sigmoid curve. In the present investigation, all the disease progress curves were continuous and more or less sigmoid as evidenced from the disease progress curves (Fig. 1). Thus the three qualifications proposed by Jeger and Viljanen-Rollinson (2001) are fulfilled under the present experimental conditions. However, the application of this method in situations of asymptotic level of disease expression like adult plant resistance needs further investigations in the lines adopted by Haynes and Weingartner (2004) by calculating AUDPC for several subsets of the complete data set from beginning to the end of epidemic during the entire period of crop growth.

The highly significant correlations between the AP and TP methods of AUDPC and r over nine seasons of repeated testing, consistent inclusion of these two parameters into factor-1 with high positive inter-correlations and large contributions to the total variability present in the communality suggest that estimation of both of these parameters by the TP method was as effective as the AP method. Thus, assessment of relative resistance to rice blast disease from two data points i.e. initiation of the epidemic and end of the epidemic could provide similar information obtained from all points. This would save valuable time and labor.

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