Minimum Number of Assessment Times to Compare Chemical Control Treatments for Papaya Fruit Anthracnose

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ABSTRACT

The chemical treatment evaluation in the field to control post-harvest fruit anthracnose (*Colletotrichum gloeosporioides*) requires a suitable disease incidence assessment on harvested papaya (*Carica papaya*) fruits. The minimum number of papaya fruit harvests was determined for valid treatment comparison in field trials for anthracnose chemical control. Repeatability analysis was done using previously published data. The coefficient determination (R^2) estimate range, using four methods, and based on means of 12 assessment times, was $92.58 \leq R^2 \leq 94.45\%$. The number of assessment times required for R^2=90% varied from seven to nine. The R^2 values of $85.1 \leq R^2 \leq 91.3\%$ estimated by ANOVA suggested that any seven successive assessment times were sufficient for treatment comparison.

Additional keywords: repeatability analysis, *Carica papaya*, field control.

RESUMO

Número mínimo de épocas de avaliações para comparar tratamentos de controle químico da antracnose do mamoeiro

Estudos de controle químico da antracnose (*Colletotrichum gloeosporioides*) em mamoeiro (*Carica papaya*), no campo, requerem avaliação da intensidade da doença em frutos após a colheita. Determinou-se o número mínimo de épocas de avaliação (colheitas) necessárias para comparação adequada de tratamentos. A análise de repetibilidade foi aplicada a dados previamente publicados. Estimativas usando quatro métodos resultaram em coeficientes de determinação (R^2), baseados na média de 12 épocas de avaliações, de $92,58 \leq R^2 \leq 94,45\%$ e o número de épocas de avaliações requerido para obter R^2 = 90%, variou de sete a nove. O R^2 estimado por ANOVA indicou que quaisquer sete épocas de avaliação sucessivas foram suficientes para comparação entre tratamentos, apresentando $85,1 \leq R^2 \leq 91,3\%$.

In Brazil, papaya (*Carica papaya* L.) is cultivated in about 40,800 ha (FAO, 2003) mostly with cultivar Sunrise Solo. Harvesting begins about ten months after seeding and the average productive life is about two years with yields up to three fruits per week. Post-harvest anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is the major fungal disease leading to fruit losses of up to 90% in a disease-favoring season (Tatagiba *et al*., 2002). During the development period of about five months (Calegario, 1997), the fungus infects immature fruit and remains quiescent until harvest maturity (Dickman & Alvarez, 1983). Anthracnose symptoms begin to appear during transport and marketing.

Field spraying with fungicides during fruit formation is one of the most effective ways of controlling this disease (Liberato & Zambolim, 2002). Field trials are resource intensive; therefore, it is imperative to determine the minimum number of assessment times (fruit harvests) necessary to assess anthracnose incidence on the harvested fruit during the production period for a reliable comparison among treatments. In a system where the initial ranking of the treatments remains consistent over the successive evaluations, fewer assessments may suffice to provide reliable data for their comparison. The consistency of treatment ranking during successive assessment times is called repeatability (Turner & Young, 1969).

According to Falconer & Mackay (1996), when disease incidence is measured repeatedly over time in each plot, the phenotypic variance (V_P) can be analyzed into a variance component within plots, measuring the differences between the incidences of the same plot, and a variance component between plots, measuring the permanent differences between plots. The within-plot component (V_{Et}) is entirely due to environment as it is caused by temporary environmental differences between successive evaluations. The inter-plot component is partly environmental (V_{Ep}) and partly genetic (V_G, genotypic variance), where the environmental part is the result of circumstances permanently affecting the plot. Thus $V_P = V_G + V_{Ep} + V_{Et}$. The partitioning of the V_P, expressed by the repeatability is thus divided into two components, V_{Et} and ($V_G + V_{Ep}$). The repeatability coefficient is: $r = (V_G + V_{Ep}) / V_P$, which represents the correlation between repeated measurements in the same plot. It expresses the proportion of the variance of single measurements that is due to permanent, or non-localized, differences between plots, both genetic and environmental.

The repeatability analysis has three main uses; one of them shows the quantitative accuracy improvement resulting from the temporal repetition of measurements. When the repeatability is high, thus low V_{Et}, multiple measurements do not significantly improve accuracy. Contrarily, if the repeatability is low, multiple measurements can substantially increase accuracy (Falconer & Mackay, 1996).

The repeatability coefficient has been used in plant or animal breeding to determine the necessary number of assessment times to compare treatments with a certain accuracy and minimum resources (Dias & Kageyama, 1998; Silveira *et al*., 1998; Ferreira *et al*., 1999; Di Renzo *et al*., 2000). This study was done to determine the minimum number of successive assessments (successive harvests) required to reliably evaluate the effect of field fungicide application to control the post-harvest anthracnose of papaya fruits.

The data used in this study were obtained by Tatagiba *et al.* (2002), who evaluated the efficacy of field applications with 11 treatments (ten fungicides and one unsprayed control) to control papaya post-harvest anthracnose. In summary, the trial was done in a completely randomized block design with four replications of eight plants (cv. Improved Sunrise Solo Line 72/12). The fungicides were sprayed bi-weekly or monthly, from March 1997 (beginning of flowering) to March 1998. Harvest was initiated in September, by harvesting nine fruits/plot (color-break stage), at two-week intervals, to give a total of 12 assessments times (harvests). The disease incidence was evaluated ten to 12 days after harvesting on fruits stored at room temperature.

To evaluate for estimation consistency, the repeatability coefficient was estimated by four methods: ANOVA, principal components based on covariance or correlation matrix, and correlation matrix based structural analysis, as described by Mansour *et al.* (1981) and Cruz & Regazzi (1997), using the software packages "GENES" (Cruz, 2001; http://www.ufv.br/dbg/genes/genes.htm). The minimum number of assessment times (n_0) necessary for predicting the true treatment value, based on pre-established determination coefficients (R^2) was obtained according to Cruz & Regazzi (1997):

$$R^2 = \frac{n\,r}{1 + r\,(n - 1)}$$

Where, r is the estimated repeatability coefficient and n is the number of successive assessment times.

Writing the equation above in terms of n, the numbers of harvests (renamed n_0) needed to assess the anthracnose incidence with a specified determination value can be calculated through the following equation:

$$n_0 = \frac{R^2\,(1 - r)}{(1 - R^2)\,r}$$

The repeatability coefficient estimates ranged from 0.51 to 0.59 (Table 1). The treatment comparison based on the mean of 12 assessment times had an accuracy of $92.6 \leq R^2 \leq 94.5$ depending upon the estimation method (Table 1).

Based on fruit anthracnose incidence, the minimum number of assessment times for treatment comparison varied from seven to nine for $R^2 = 90\%$, depending upon the estimation method (Table 2). After calculating the minimum number of assessment times, the sequence of successive assessment time (initial, mid or end of fruit set) was determined, by repeatability analysis of all possible sets of two to 12 successive assessments. The R^2 estimated by ANOVA showed that any seven successive assessments are equally good for an accuracy of $85.1 \leq R^2 \leq 91.3\%$ (Figure 1).

The similarity among the repeatability coefficients obtained by different analysis methods suggests consistency among estimations. High repeatability coefficients indicate low chance of significant interaction between treatments and assessment times; therefore, the real value of treatment performance can be predicted using less assessment times (harvests). There was no significant interaction between treatments and assessment times as estimated by repeated measures ANOVA (Tatagiba *et al*., 2002).

The R^2 shows the accuracy of analysis predicting real value of treatment performance (obtained with infinite assessment times) based on n assessment times. The R^2 is the square of correlation between the average of n successive assessment times and the real value of treatment performance (Cruz & Regazzi, 1997). In this study, a high accuracy level was obtained with n=12, but this number could be reduced to any successive seven to nine assessment times, without affecting accuracy of treatment comparison, thus saving labor and costs. However, when only two successive assessment times were considered, R^2 ranged from 21.9 to 87.3% (Figure 1), which indicates that further reduction of assessment times can reduce the accuracy of treatment performance estimates.

Since the data regarding post-harvest disease on fruits were collected from distinct harvest times (distinct fruits in each assessment time), they do not characterize a cumulative growth curve. Therefore, Tatagiba *et al.* (2002) did not compare treatments with the growth curve models or area under the disease progress curve (AUDPC) suggested by Campbell & Madden (1990), but instead carried out repeated-measures ANOVA (Madden, 1986; Winer *et al.*, 1991). Nevertheless, the repeatability analysis may be useful for data

TABLE 1 - Repeatability coefficients (r) and determination coefficient (R^2) estimated by different methods for anthracnose incidence on papaya (*Carica papaya*) fruits sprayed with 11 fungicide treatments during 12 biweekly harvests

Method [a]	r	R^2 (%)
Analysis of variance with two factors	0.510	92.58
Covariance matrix based principal components	0.586	94.45
Correlation matrix based principal components	0.545	93.49
Correlation matrix based structural analysis	0.533	93.20

[a] Mansour *et al.* (1981); Cruz & Regazzi (1997).

TABLE 2 - Minimum number of harvests (n_0) associated with determination coefficient (R^2) estimated different methods for anthracnose incidence on papaya (*Carica papaya*) fruits sprayed with 11 fungicide treatments during 12 biweekly harvests

Method [a]	r	R^2				
		0.80	0.85	0.90	0.95	0.99
Analysis of variance with two factors	0.510	4	6	9	19	95
Covariance matrix based principal components	0.586	3	4	7	14	70
Correlation matrix based principal components	0.545	4	5	8	16	83
Correlation matrix based structural analysis	0.533	4	5	8	17	87

[a] Mansour *et al.* (1981) and Cruz & Regazzi (1997).

that characterize a cumulative growth curve, as in foliar disease epidemics.

The pathosystem characteristics, high disease incidence during the trial period, and the fungicide sprays (treatments) some months before and during disease assessments, all appear to have contributed to the similarity of potential for treatment discrimination by all the assessment times. These factors may not occur in foliar diseases of annual crops where, the evaluations at the beginning of the epidemics can not discriminate the treatment effects, and the ranking of efficacy of systemic and protectant fungicides may not be the same at the beginning and at the end of the epidemic. In such pathosystems, it is advisable to investigate the possibility of discarding the initial evaluations for estimating the repeatability coefficient.

The repeatability analysis can be useful to determine the minimum number of assessment times, but the timing and frequency of disease assessments will be determined by the pathosystem and the objectives of the assessment (Campbell & Madden, 1990). Obviously, the set of the successive assessment times should be obtained during the disease favorable period (when the disease intensity is high in control plots). In this study, the weather conditions affected treatment efficacy but not their ranking over time (Tatagiba *et al.*, 2002).

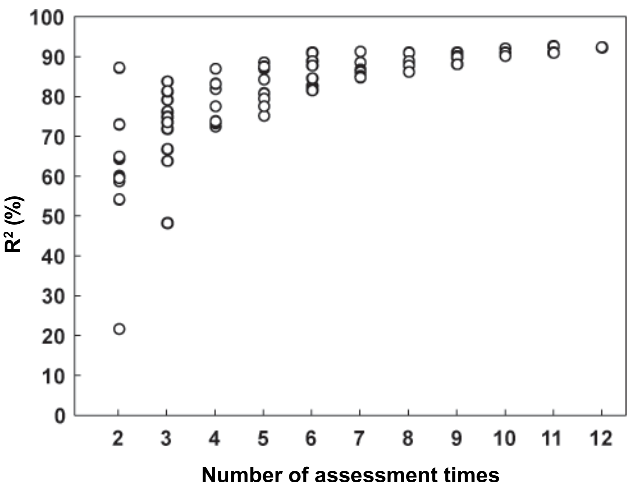

FIG. 1 - Determination coefficient (R^2) related to number of successive harvests of papaya (*Carica papaya*) fruits for evaluation of anthracnose incidence, estimated by analysis of variance with two factors (Mansour *et al.*, 1981; Cruz & Regazzi, 1997).

The repeatability coefficient may differ under other environmental or experimental conditions, but the concept itself is useful to estimate an adequate number of assessment times for papaya fruit anthracnose. Repeatability analysis after each assessment time may also indicate the final disease assessment when R^2 reaches a pre-established value.

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