TROPICULTURA, 1997, **15**, 4, 186-189

Field Plot Techniques for Black Sigatoka Evaluation in East African Highland Bananas

Josephine U. Okoro, R. Ortiz[1] & D. Vuylsteke

Keywords: *Mycosphaerella fijiensis* – *Musa* – Optimum plot size.

Summary

Number of plants per experimental unit and number of replications for the efficient and precise assessment of black sigatoka leaf spot disease caused by Mycosphaerella fijiensis *in East African Highland bananas were determined. Two representative cultivars were used. Host response to black sigatoka infection was measured by recording the youngest leaf with necrotic spots. The number of plants per experimental unit was determined, using the methods of maximum curvature and comparison of variances, while the number of replications was estimated by Hatheway's method. The optimum experimental plot size was 3 plants (18 m²) for the beer banana cultivar 'Igitsiri', and 30 plants (180 m²) for the cooking banana cultivar 'Igisahira Gisanzwe', using the comparison of variances method. However, the optimum plot size was 15 plants (90 m²) for both cultivars using the method of maximum curvature. The latter statistical method was preferred because of the low precision of the estimates in the former method. Unreplicated trials with plots of 15 plants could be adequate to assess black sigatoka response in East African bananas if uniform disease pressure exists.*

Résumé

Le nombre de pieds par unité expérimentale et celui des répétitions utilisés en vue d'une évaluation efficace et précise de l'incidence de la cercosporiose causée par Mycosphaerella fijiensis *chez les bananes d'altitude en Afrique orientale, ont été déterminés. Deux cultivars représentatifs ont été utilisés. La réponse de la plante hôte à l'infection de la cercosporiose a été mesurée grâce à la notation de la plus jeune feuille présentant des taches nécrotiques. Le nombre de pieds par unité expérimentale a été déterminé selon la méthode de la courbure maximum et de comparaison des variances, tandis que le nombre des répétitions a été évalué suivant la méthode de Hatheway. La taille optimum des parcelles expérimentales était de 3 pieds (18 m²) pour la banane à bière "Igitsiri", 30 pieds (180 m²) pour le cultivar de banane à cuire "Igisahira Gisanzwe", conformément à la méthode des variances. Cependant, la taille optimum des parcelles était de 15 pieds (90 m²) pour tous les cultivars utilisant la méthode de la courbure maximum. Cette méthode statistique s'est avérée préférable à cause de la faiblesse de la précision des évaluations obtenues par la première méthode. Les essais avec 15 pieds et sans répétition pourraient être indiqués pour l'évaluation de la réponse à la cercosporiose chez les bananes d'altitude en Afrique orientale à condition qu'il existe une pression uniforme de cette maladie.*

Introduction

Plantains and bananas (*Musa* spp.), important food crops and sources of revenue for smallholder farmers, differ according to their ecoregional distribution and specific characteristics (15, 18). The highest per capita consumption of *Musa* fruit worldwide occurs in East Africa where cooking and beer bananas predominate, Black sigatoka, a leaf spot disease caused by the fungus *Mycosphaerella fijiensis* Morelet, is a major constraint to *Musa* production worldwide (8, 19).

To increase efficiency in evaluating germplasm for resistance to black sigatoka disease, an optimum number of plants must be sampled. In field trials, undesirable effects (experimental error) can be reduced by using either large plots (7) or greater number of replications (11). In plantain and banana trials, a high number of replications and large plots (i.e., many plants per experimental unit) are major constraints as each plant requires 6 m² in the field (14).

The main objective of this study was to determine the optimum number of plants per experimental unit and the adequate number of replications to efficiently assess black sigatoka in East African banana *(Musa* spp. AAA group) trials.

Material and methods

Two common cooking and beer banana cultivars of the highlands of East Africa ('Igisahira Gisanzwe' and

Plantain and Banana Improvement Program, International Institute of Tropical Agriculture, Oyo Road, P.M.B. 5320, Ibadan, Nigeria, West Africa.
[1] Corresponding author; current address: KVL, Dept. of Agricultural Sciences, 40 Thorvaldsensvej, DK-1871, Frederiksberg C, Copenhagen, Denmark.
Received on 06.11.95 and accepted for publication on 03.04.97.

'Igitsiri', respectively) were planted in August 1992 at the High Rainfall Station of the International Institute of Tropical Agriculture (IITA) at Onne (4°51'N, 7°3'E), which is in the humid forest zone of southeastern Nigeria. Annual rainfall at Onne averages 2400 mm with a monomodal distribution from February through December.

Cultivars were evaluated in separate uniformity trials consisting of 10 rows of 12 plants each with a distance of 3 m between rows and 2 m within rows giving a land requirement of 6 m^2 $plant^{-1}$.

The assessment of host plant response to black sigatoka was done at flowering in July 1993 (mid rainy season). Methods for characterization of host response for black sigatoka were explained by Foure (3). The youngest leaf spotted (YLS) with a necrotic center was recorded on each plant (12), before flowering after which leaf production ceases. This method of assessment was used because YLS is highly correlated with the timing of disease development and other methods to assess host plant resistance to black sigatoka (1, 2). Also, YLS is highly heritable (9). Recording YLS also has the advantage of being a simple trait to score, requiring the surveyor to merely record the number of the youngest leaf, counting down from the first (top) unfurled leaf, to the first leaf that shows mature spots caused by black sigatoka. Black sigatoka became ubiquitous at Onne since late 1980s. Results obtained by Ortiz & Vuylsteke (10) confirmed this uniform widespread of black sigatoka at Onne in 1991-1992. Artificial inoculation was, therefore, not considered necessary for field screening.

The data were statistically analyzed following a hierarchical (i.e., nested) system of classification (13). Accordingly, this trial was divided into two blocks of 60 plants each. Each block was subdivided into two plots, which consisted of five rows with six plants each. The plot was further split into two subplots, comprising five rows with three plants each. Each subplot was then split into five sub-subplots of three plants each, and each sub-subplot was divided in three units of one plant each. This desing resulted in experimental units of 60, 30, 15, 3, and 1.

The objective of our research was to evaluate the variability for assessement of host response to black sigatoka in relation to the number of plants per plot. Optimum number of plants per experimental unit was determined using the methods of maximum curvature (5) and comparison of variances (17), while number of replications was determined as explained by Hatheway (4). In field trials the interaction between plots should be considered. Plot size recommended by our experiment are for trials where experimental plots are surrounded by susceptible border rows to ensure uniform disease pressure.

Method of maximum curvature

The coefficients of variation (CV) were estimated from the data collected for experimental units, consisting of different number of plants. The number of plants per experimental unit was plotted against the CV obtained in each experimental unit. The optimum number of plants per experimental unit, which corresponds to the point of maximum inflection, was visually located on the curve (6).

Comparison of variances method

The variance of YLS per experimental unit, consisting of different number of plants (1, 3, 15, 30, and 60 plants per plot), was estimated. Tests of homogeneity of variances were performed, excluding in each test the smaller experimental unit whose variance was significantly different from the largest experimental unit. The test was continued until the experimental units were homogeneous or had statistically similar variances. The optimum number of plants per experimental unit was then considered to be that of the smallest experimental unit among those experimental units with similar variances (17).

Number of replications and index of environmental variability

True mean differences (d), were detected using the following equation : $d = \{[2\{t_1 + t_2\}^2 C_x^2]/rx^{b)(1/2)}$ (4), where t_1 is the critical Student's value for a significance level of 0.05 (type I error); t_2 is the tabulated value of *t* corresponding to $2(1\text{-}P) = 0.20$ where *P* is the probability of obtaining a significant result at the 90% level (power with 1-P being the type II error); C_x is the plot size coefficient of variation used in the estimation; r is the number of replications; x is the plot size (in number of plants): and b is the index of environmental variability. The number of replications to detect true mean differences was calculated assuming experiments in which 25 entries were tested with a CV equal to 0.045 (= 4.5%). This was the average CV for experimental units of 15 plants.

The coefficient of environmental variability, b, was estimated by solving the relationship $V_x = V_1/X_j^b$, where V_x is the variance, calculated on a per experimental unit basis, of the youngest leaf spotted per unit area among experimental units of X plants, and V_1 is the variance among experimental units consisting of single plants [modified from Smith, (12)]. Thus, $b = [(\log V_1 - \log V_x)/\log X_j]$. In a vegetatively propagated crop such as banana, b is largely a function of the effect of black sigatoka due to fungal dispersal, other pathogens and pests, and soil fertility, in an uniformly designed trial.

Results and discussion

There were significant differences between rows within subplots ($P < 0.05$) for the two cultivars (Table 1) and between plots in the same block for cultivar 'Igitsiri'. The other sources of variation were not significant in the hierarchical analysis of variance. However, the significant variation of a specific source was always relative to the subsequent mean square error (Table 1), which may mask significant variation. Hence, interpretations regarding the variation in uniformity trials of host response to black sigatoka in East African banana

Table 1

Hierarchical analysis of variance of black sigatoka disease caused by *Mycosphaerella fijiensis*, as measured by the youngest leaf spotted, in East African banana uniformity trials.

Source of variation	Degrees of freedom	Expected mean squares	Mean square	
			Igitsiri	Igisahira Gisanzwe
Blocks (b)	1	$\sigma^2+n\sigma^2_R+nr\sigma^2_s+nrs\sigma^2_P+nrsp\sigma^2_B$	2.13	0.41
Plots (p) within blocks	2	$\sigma^2+n\sigma^2_R+nr\sigma^2_s+nrs\sigma^2_P$	5.23*[a]	0.21
Subplots (s) within plots	4	$\sigma^2+n\sigma^2_R+nr\sigma^2_s$	0.38	0.64
Row (r) within subplots	32	$\sigma^2+n\sigma^2_R$	1.38*	0.99*
Plants (n) within rows	80	σ^2	0.79	0.57

[a]* An asterisk indicates significant at the 0.05 probability level.

cultivars, based solely on this hierarchical analysis of variance, should be taken with caution.

Method of maximum curvature

The coefficients of variation, which were estimated for experimental units comprising different number of plants, generally decreased as the number of plants per experimental unit increased (Table 2). The optimum experimental unit consisted of 15 plants (90 m^2),

Table 2

Number of plants, mean, standard error and coefficient of variation for the youngest leaf spotted by *Mycosphaerella fijiensis* in East African banana trials.

Number of plants	Mean		SE ±		CV (%)	
	Igisahira	Igitsiri	Igisahira	Igitsiri	Igisahira	Igitsiri
1	6.34	6.07	0.82	1.01	13.0	16.6
3	6.35	6.06	0.55	0.71	8.7	11.7
15	6.34	6.07	0.18	0.37	2.8	6.1
30	6.34	6.07	0.10	0.38	1.6	6.3
60	6.34	6.07	0.08	0.19	1.3	3.1

as indicated by the point of inflection in Fig. 1. Ortiz & Vuylsteke (10) reported that 4 to 5 plants per experimental unit were enough to determine significant differences in plantains. Perhaps the plants needed to show significant differences in that study were so few because of the difference in the genetic constitution of plantains (*Musa* spp. AAB) and East African Highland bananas (*Musa* spp. AAA) and the difference in their response to black sigatoka with highland bananas considerably less suceptible than plantains.

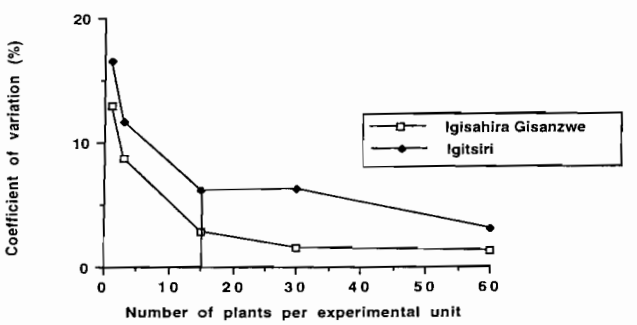

Figure 1. Coefficients of variation for the youngest leaf spotted due to black sigatoka caused by *Mycosphaerella fijiensis* in two East African banana trials plotted against number of plants per experimental unit. Line perpendicular to X axis indicates optimum plot size for both cultivars.

Comparison of variance method

The variance estimates for the youngest leaf spotted for experimental units of different sizes and for each clone also indicate a decrease in variance with increase in number of plants per experimental unit, i.e., the larger the number of plants per experimental unit the smaller the variance. The variance for experimental units of 30 and 60 plants were not significantly different for the cultivar 'Igisahira Gisanzwe' while the variances for experimental units of 3, 15, 30, and 60 plants were statistically similar for the cultivar 'Igitsiri' using Bartlett's test of homogeneity of variances (Table 3).

Table 3

Variance estimates of black sigatoka response (*Mycosphaerella fijiensis*), as measured by the youngest leaf spotted, in 'Igisahira Gisanzwe' and 'Igitsiri' of plots with different number of plants.

Plot size	Estimates of Variance	
	'Igisahira Gisanzwe'	'Igitsiri'
1	0.681 a	1.021 a
3	0.304 b	0.486 b
15	0.032 c	0.134 b
30	0.009 d	0.142 b
60	0.007 d	0.036 b

Variances followed by the same letter are not statistically different at the 5% probability level according to Bartlett's test.

Determination of number of plants per experimental unit using this method, however, must be taken with caution due to the poor precision of the variance estimates for subplots and blocks in cultivar 'Igitsiri', and for subplots, plots, and blocks in cultivar 'Igisahira Gisanzwe', as shown by the respective analysis of variances (Table 1).

Number of replications and index of environmental variability

The number of replications depends on the level of precision that is required in an experiment. Estimates of true differences between treatment means, expressed in percentage, were calculated for experi-

mental units of 15 plants using 2, 3, 4, 5, 6, and 7 replications in a randomized complete block design including 25 simulated treatments. For example, two replications are required to detect a significant difference of 4% between two genotypes for youngest leaf spotted, i.e., 0.33 or 1/3 of a leaf. This suggests that unreplicated trials with plots of 15 plants may be useful for a preliminary assessment of black sigatoka response in East African bananas in fields under uniform disease pressure as in Onne.

The value of the coefficient of environmental variability was about 1, which suggests that no serial correlation of errors occurred when black sigatoka disease was scored in both uniformity trials because of the uniform spread of black sigatoka at Onne. This also indicates that in the determination of black sigatoka response in East African bananas, big experimental units (15 plants or more) instead of small experimental units should be used. This finding was confirmed by an independent study with the black sigatoka susceptible Cavendish banana cv. 'Valery' carried out at Onne from 1994 to 1996 (S. Nokoe and R. Ortiz, IITA, unp. results). The recommended optimum plot size consisted on average of 13 ± 1 plants for the assessment of host response to black sigatoka as measured by incubation time, evolution time, disease development time, youngest leaf with symptoms, and youngest leaf spotted.

Conclusion

The results from the statistical analyses used for this study suggest that 1) assessment of black sigatoka response in East African bananas with experimental units comprising less than 15 plants (90 m^2) should be avoided or taken with caution, and 2) replications may not be required to assess black sigatoka in East African bananas if an optimum plot size of 15 plants is used and uniform disease pressure is assured. Experimental plots should be surrounded by border plants of a black sigatoka susceptible cultivar to ensure uniform natural inoculum pressure of the pathogen throughout the field.

Acknowledgement

IITA/94/JA/61. Plantain and banana research at IITA has benefited from grants by several donors, particularly the Belgian Administration for Development Cooperation (BADC).

Literature

1. Craenen K., 1994. Assessment of black sigatoka resistance in segregating progenies. MusAfrica **4**: 4-5.
2. Craenen K. & Ortiz, R., 1996. Black sigatoka affects growth and yield characteristics in *Musa* germplasm. MusAfrica **10**: 21.
3. Foure, E., 1985. Les cercosporioses du bananier et leurs traitements. Comportement de variétés. Etude de la sensibilité variétale de bananiers et plantains à *Mycosphaerella fijiensis* Morelet au Gabon. Fruits **40**: 393-399.
4. Hatheway W.K., 1961. Convenient plot size. Agronomy J. **53**: 279-280.
5. Immer F.R., 1932. Size and shape of plot in relation to experiments with sugar beets. J. Agr. Res. **44**: 649-668.
6. Le Clerg E.L., 1966. Significance of experimental design in plant breeding. In: "Plant Breeding". K.J. Frey (ed.). Ames, Iowa: University Press. 243-311.
7. Mckenzie H., Holmes N.D., Peterson L.K. & Grant M.N., 1964. A comparison of three plot sizes in studies of host resistance to the wheat stem sawfly. Can. J. Plant Sci. **44**: 485.
8. Mobambo K.N., Gauhl F., Vuylsteke D., Ortiz R., Pasberg-Gauhl C. & Swennen R., 1993. Yield loss in plantain from black sigatoka leaf spot and field performance of resistant hybrids. Field Crops Res. **35**: 35-42.
9. Ortiz R., 1995. *Musa* genetics. In: "Bananas and Plantains" S. Gowen (ed.) UK: Chapman and Hall. 84-109.
10. Ortiz R. & Vuylsteke D., 1994. Inheritance of black sigatoka resistance in plantain-banana (*Musa* spp.) hybrids. Theor. & Appl. Genet. **89**: 146-152.
11. Rampton H.H. & Peterson R.G., 1962. Relative efficiency of plot size and numbers of replications as indicated by yields or orchard grass seed in a uniformity test. Agron. J. **54**: 247-249.
12. Smith H.F., 1938. An empirical law describing heterogeneity in the yields of agricultural crops. J. Agric. Sci. **28**: 1-23.
13. Sokal R.R. & Rohlf F.J., 1981. Biometry. 2nd ed. New York: W. H. Freeman.
14. Swennen R., 1990. Plantain cultivation under West African conditions. A reference manual. Ibadan, Nigeria: International Institute of Tropical Agriculture.
15. Swennen R. 1 Vuylsteke D., 1991. Bananas in Africa: diversity, uses and prospects for improvement. In: "Crop Genetic Resources of Africa". Q.N. Ng, P. Perrino, F. Attere, H. Zedan (eds.). UK: The Trinity Press. **2**: 151-160.
16. Vakili N.G., 1968. Responses of *Musa acuminata* species and edible cultivars to infection by *Mycosphaerella musicola*. Tropical Agriculture (Trinidad). **45**: 13-22.
17. Vallejo R.L. & Mendoza H.A., 1992. Plot technique studies on sweetpotato yield trials. J. Amer. Soc. Hort. Sci. **117**: 508-511.
18. Vuylsteke D., Ortiz R. & Ferris S., 1993a. Genetic and agronomic improvement for sustainable production of plantain and banana in sub-Saharan Africa. African Crop Sci. **1**: 1-8.
19. Vuylsteke D., Swennen R. & Ortiz R., 1993b. Development and performance of black sigatoka resistant tetraploid hybrids of plantain (*Musa* spp AAB group). Euphytica **65**: 33-42.

Josephine Okoro, Nigerian Research associate.
R. Ortiz, Peruvian, Scientist and Program Leader.
D. Vuylsteke, Belgian, Scientist.