

Full Length Research Paper

Appraisal of methods for assessing black Sigatoka resistance in diploid banana populations

A. Barekye^{1,2*}, P. Tongoona¹, J. Derera¹, M. D. Laing¹ and W. K. Tushemereirwe²

¹African Centre for Crop Improvement (ACCI), School of Agricultural Sciences and Agribusiness, University of KwaZulu-Natal, P/Bag X01, Pietermaritzburg, 3209, South Africa.

²National Banana Research Programme, National Agricultural Research Organisation, P.O. Box 7065, Kampala, Uganda.

Accepted 31 October, 2011

Three black Sigatoka assessment protocols, (i) assessing disease severity 6 months after planting, (ii) estimating disease development over time in the different accessions, and (iii) assessing the youngest leaf spotted (YLS) at flowering were appraised. The assessment was implemented on 18 diploid accessions together with susceptible and resistant checks, planted in a 4 × 5 rectangular lattice design with two replicates at Kawanda Agricultural Research Institute in Uganda during 2005 to 2007. Natural disease inoculum was used with experimental plots planted in locations between the rows of a susceptible local cultivar that acted as a spreader. All the three assessment techniques were able to classify the *Musa* accessions into resistant and susceptible classes. However, the rankings of the clones according to their resistance were not consistent. The rankings of YLS correlated positively with those of area under disease progress curve (AUDPC) ($P < 0.05$). The AUDPC rankings correlated strongly with the rankings of disease development time ($P < 0.001$). The AUDPC and YLS significantly predicted bunch weight although the coefficient of determination was low. Overall AUDPC resulted in the highest coefficient of determination ($R^2 = 0.84$) in detecting black Sigatoka response among the diploid *Musa* clones. Considering the time taken for the banana plants from planting to flowering, it is recommended that the disease resistance be assessed six months after planting and the disease severity data be converted into AUDPC data.

Key words: Youngest leaf spotted, disease development, severity.

INTRODUCTION

Black Sigatoka is one of the major diseases reducing banana yields in Uganda. The disease can cause yield loss of 30 to 50% on bananas and plantains (Mobambo et al., 1993; Tushemereirwe, 1996). This yield loss exposes subsistence farmers who entirely depend on the crop to food insecurity. The most viable and sustainable approach of controlling black Sigatoka for resource limited farmers is by use of host plant resistance (Stover and Buddenhagen, 1986; Swennen and Vulysteke, 1993). Host plant resistance involves evaluating materials in order to select sources of resistance for use in

generating new hybrids. Reliable methods of assessing disease resistance of banana genotypes are essential in order to identify black Sigatoka resistant materials to use as parents in the banana breeding programme in Uganda.

Banana breeding has relied on diploids because they are male fertile and are resistant to pests and diseases (Swennen and Vulysteke, 1993; Vuylsteke, 2001; Tushemereirwe et al., 2005). The initial wild diploids used transmitted the poor agronomic traits to their progenies (Rowe and Rosales, 1996). It therefore became important to improve banana diploids for both agronomic and disease resistance traits. The best way to improve multiple traits (agronomic and disease traits) at the same time would be to use recurrent selection procedures. The use of recurrent selection procedures will help

*Corresponding author. E-mail: a.barekye@kari.go.ug. Tel: +256 712 844097, +256 414 566381.

accumulate alleles for disease resistance as well as other important agronomic traits in the population. Black Sigatoka resistance has been reported to have quantitative resistance (Ortiz and Vuylsteke, 1994) hence need for an assessment technique which can detect small differences among the accessions within the population.

One of the methods, the youngest leaf spotted (YLS) Vakili (1968), can be used to differentiate response of *Musa* genotypes to black Sigatoka. Youngest leaf spotted was also reported to predict yield loss in terms of bunch weights in kilogrammes (Craenen and Ortiz, 1998). Because YLS has been reliable in black Sigatoka assessment and determining reduced banana yields, it has been widely used to assess disease damage in *Musa* species. Youngest leaf spotted, normally done at flowering, involves recording the youngest leaf with at least 10 necrotic lesions by counting from the top-most leaf. It takes bananas about 9 to 12 months from planting to flowering, hence making it expensive to maintain and manage banana plants whose disease response is unknown. Besides, breeding materials need to be selected early so that they participate in crosses at flowering. Youngest leaf spotted is a good method of assessing the disease but it delays the selection process and makes conventional breeding an expensive process.

Investigations have been made on identifying early assessment techniques to distinguish response of *Musa* genotypes to black Sigatoka. Mobambo et al. (1997) investigated host response of different ages of *Musa* germplasm to black Sigatoka under natural infestation. Disease incubation time, disease development time, youngest leaf spotted and life time of leaf of different banana genotypes of young and old plants were recorded and the disease responses correlated. There were significant correlations for disease development time, youngest leaf spotted and life time of leaf between young and mature plants. However, it was noted that early evaluation for disease response using disease development time could not predict agronomic traits like yield performance (Mobambo et al., 1997). Craenen and Ortiz (1998) investigated influence of black Sigatoka on the growth and yield of diploid and tetraploid hybrid plantains. In diploids, disease incubation time correlated significantly with days to fruit filling. The relationship was not significant for tetraploid hybrids. These findings suggested that disease development is unable to predict yield loss due to black Sigatoka in bananas, although it can detect disease response among different *Musa* accessions.

Recently Twizeyimana et al. (2007) investigated a rapid screening technique of *Musa* species resistant to black Sigatoka using *in-vitro* plantlets, and detached leaves. The plantlets and detached leaves were evaluated *in-vitro* for their response to black Sigatoka. The investigation concluded that the two assays were rapid and effective in space utilization and screened *Musa*

genotypes resistant to black Sigatoka. However, Liu et al. (2007) doubted whether detached leaf pieces support development of disease symptoms and plant responses that would compare to those that would be observed using intact plants with attached leaves.

The deficiencies in all these early assessment techniques suggest that there is need to identify a method of quantifying black Sigatoka resistance that is stable, reliable and able to predict yield. Disease severity using area under disease progress curve (AUDPC) has been used to quantify rusts (Holland and Munkvold, 2001; Kushwaha et al., 2007), mildews (Lipps et al., 1989; Danielsen and Munk, 2004) and leaf spots (Jeger and Viljanen-Rollinson, 2001; Asea et al., 2002) in cereals, legumes and potatoes. In potatoes, AUDPC based on three-leaf method showed the highest negative correlation with yield and is regarded as the best method to predict yield loss caused by downy mildew (Danielsen and Munk, 2004). In bananas and plantains AUDPC was used to differentiate banana and plantain genotypes' response to black Sigatoka (Vera, 2008). However, usefulness of AUDPC in yield prediction in bananas and plantains has not been reported. Unlike YLS, AUDPC is not limited to a standard stage of growth of a banana plant. From information available, it appeared possible to differentiate banana genotypes response to black Sigatoka using youngest leaf spotted, area under disease development curve and disease development over time (DDT) but the efficiency and reliability of AUDPC and DDT have not been established. The objectives of the study were to compare the efficiency of youngest leaf spotted, disease development time and area under disease progress curve for black Sigatoka assessment in a diploid population, and to investigate the relationship between AUDPC and disease development over time with bunch weight in diploid bananas.

MATERIALS AND METHODS

Germplasm

The materials evaluated for black Sigatoka resistance were obtained from the International Institute of Tropical Agriculture (IITA), Fundación Hondureña de Investigación Agrícola (FHIA) and International Network for the Improvement of Bananas and Plantains (INIBAP). Others were developed from Kawanda Agricultural Research Institute (KARI) in Uganda through inter-diploid crosses. Germplasm characteristics are shown in Table 1.

Experimental design and management

The diploid accessions were planted at KARI on 2nd December 2005. Kawanda Agricultural Research Institute is located at 00° 25'N, 00° 32'E at an altitude of 1210 m above sea level and experiences a bimodal type of rainfall with "short" rains starting in March/April to June and the "long" rains starting in August to November/December.

The land was ploughed using a tractor, thereafter it was marked, holes of 45 cm diameter by 45 cm in depth were dug. Soil was

Table 1. Sources and characteristics of diploid materials used in black Sigatoka resistance assessment.

Clone	Source	Principal selection criteria
Yangambi Km 5	INIBAP	Resistant to black Sigatoka
SH 3142	FHIA	Test material
<i>Morong Princessa</i>	INIBAP/IITA	Resistant to black Sigatoka
Wambo	INIBAP/IITA	Test material
Opp 861	IITA	Test material
9719	IITA	Resistant to black Sigatoka
Pagtou	INIBAP	Test material
8615-1	KARI	Test material
8075	IITA	Resistant to black Sigatoka
Calcutta 4	FHIA	Resistant to black Sigatoka
1535K-1	KARI	Test material
5365	KARI	Test material
202SH	KARI	Susceptible to black Sigatoka
Pisang lilin	INIBAP	Resistant to black Sigatoka
8532	KARI	Test material
Pitu	IITA	Test material
3202	KARI	Test material
Galeo	INIBAP	Test material
<i>Musa acuminata</i> subsp <i>malaccencis</i>	FHIA/IITA	Resistant to black Sigatoka
Grand Naine	INIBAP	Susceptible to black Sigatoka

IITA, International Institute of Tropical Agriculture; FHIA, Fundación Hondureña de Investigación Agrícola; INIBAP, International Network for the Improvement of Bananas and Plantains; KARI, Kawanda Agricultural Research Institute.

mixed with about 5 kg of kraal manure in the hole before planting. The planting materials were obtained from the banana fields at KARI. Before planting, the banana corms were pared and dipped in chlorpyrifos, 20% EC (15 ml per 20 L of water) for about one hour to disinfect the corms against nematodes and banana weevils.

Apart from 18 diploids (AA), two other clones (one black Sigatoka susceptible check, Grand Naine, AAA and the resistant check Yangambi Km 5, AAA) were included to make a total of 20 accessions which were planted in a 4 × 5 rectangular lattice design. Each plot had six plants per block replicated two times. The natural disease pressure which is high at KARI was the source of inoculum. However, to ensure that each plant had high exposure to the disease, the experimental plots were planted between rows of a local cultivar 'Mbwazirume', AAA-EA that was used as a spreader for black Sigatoka.

Weed control was implemented regularly by spraying with glyphosate applied at least four times in a year. Detrushing was practiced minimally because there was need to maintain the disease inoculum around the test plants. Desuckering, removal of excess plants to maintain at most three plants per mat was also implemented two times a year.

Black Sigatoka was assessed and compared using three disease assessment protocols. These were assessing disease severity six months after planting, estimating disease development over time in the different accessions and assessing the youngest leaf spotted (YLS).

Six months after planting, black Sigatoka was assessed using the modified Stover (1971) scale. The proportion of the diseased leaf was estimated out of 100%. Each leaf was assessed individually and the overall disease damage per plant was computed. This assessment was repeated four times at intervals of 14 days.

Disease damage was converted into the AUDPC using the formula given below as suggested by Shaner and Finney (1977):

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

Where, X_i = proportion of the host tissue damaged at i^{th} day, t_i = the time in days after appearance of the disease at i^{th} day, and n = the total number of observations.

Assessing disease development over time started six months after planting. It is presumed that an emerging leaf (cigar leaf), picks the disease spores from the atmosphere. The date of leaf emergence was recorded. This leaf was observed continuously through when the disease symptoms appeared, when the leaf was 12% dead, 25% dead, 50% dead up to when the leaf was completely dead. This data was used to compute days from leaf emergence to symptom appearance, days from symptom appearance to 12% leaf damage, days from symptom appearance to 25% leaf damage, days from symptom appearance to 50% leaf death and days from symptom appearance to complete leaf death. Disease development was followed on three successive leaves per plant in order to capture variations in disease pressure over time. At flowering the youngest leaf spotted (the first leaf showing at least 10 necrotic spots) was recorded. Also, the total number of leaves both at flowering and harvest were recorded. Youngest leaf spotted was assessed for the plant crop and the first ratoon.

Data analysis

The generalized linear model was used to perform the analysis of

Table 2. Means of Youngest Leaf spotted (YLS), Area Under Disease Progress Curve (AUDPC) and days from symptom appearance to 25% leaf damage (DTQ) of *Musa* accessions at KARI.

Accession	YLS		AUDPC		DTQ	
	Mean±se	Rank	Mean±se	Rank	Mean±se	Rank
Yangambi Km 5**	9.5±0.59	1	157±91.5	5	38.2±3.06	8
SH 3142	8.8±0.88	2	531±92.1	12	43.5±4.17	4
<i>Morong Princesa</i>	7.9±0.62	3	77±98.3	3	49.9±3.42	1
Wambo	7.3±0.88	4	621±120.7	15	26.3±3.28	15
Opp 861	7.2±0.67	5	124±94.1	4	39.3±3.17	5
9719	7.2±0.59	6	67±119.1	2	31.3±3.60	14
Pagtou	6.1±0.61	7	924±95.2	16	20.5±3.14	17
8615-1	6.0±0.55	8	596±93.4	14	32.2±2.75	12
8075	5.9±0.59	9	278±91.6	9	45.9±3.17	3
Calcutta 4	5.9±0.59	10	46±110.3	1	38.8±3.25	6
1535K-1	5.7±0.59	11	311±89.9	10	33.2±2.84	11
5365	5.6±0.59	12	576±99.3	13	31.5±2.97	13
202SH	5.5±0.72	13	1789±100.7	20	15.2±3.37	18
Pisang lilin	5.1±0.57	14	344±92.7	11	33.4±2.77	10
8532	5.0±0.57	15	164±91.2	6	38.4±2.97	7
Pitu	4.8±0.58	16	1027±87.5	19	15.1±2.97	19
3202	4.6±0.57	17	230±92.8	8	48.2±3.10	2
Galeo	4.6±0.74	18	1020±103.1	18	23.6±2.98	16
<i>Musa acuminata</i> subsp <i>malaccencis</i>	4.5±0.69	19	202±104.9	7	33.7±3.17	9
Grand Naine*	3.7±0.67	20	924±114.5	17	9.9±3.16	20
LSD(0.05)	1.6		226		7.3	
R ²	0.59		0.84		0.76	
CV (%)	23		44		19	

** Resistant check; * susceptible check; se standard errors of the means.

variance (SAS Inc., 2002) using the model

$$Y_{ij} = \mu + \beta_i + g_j + \epsilon_{ij}$$

where, Y_{ij} = black Sigatoka response, μ = overall mean, β_i = block effect (nested in replication), g_j = genotype response, ϵ_{ij} = experimental error.

For those parameters that were significant, the least significant means (lsmeans) for each accession were computed. To compare how the three assessment protocols ranked the *Musa* accessions, means generated using the different assessment techniques were used to rank the accessions in reference to the susceptible and resistant checks. These were separated using the least significant difference at a probability level of $P=0.05$. Phenotypic correlations were computed in order to compare the relationships between the disease assessment protocols and agronomic parameters and the correlation coefficients were tested at a probability level of $P=0.05$ using Pearson method. Multi-collinearity among the associated data sets was eliminated using variance inflation ratio (VIR). The regression analysis was carried out between bunch weight, and number of functional leaves at flowering, and disease parameters using a stepwise regression approach, in order to find out if the coefficients of the parameters had a genuine effect on the dependent variable (yield and total leaves at flowering). In order to find out the efficiency of the assessment techniques, their

coefficients of variation and determination were compared.

RESULTS

Ranking of genotypes by the three methods

All the three methods ranked genotypes into resistant and susceptible clones (Table 2). According to YLS, Yangambi Km 5 was the most resistant genotype, Grande Naine the most susceptible genotype and Calcutta 4 was ranked as having moderate resistance. On the other hand, AUDPC ranked Calcutta 4 as the most resistant and 202SH as the most susceptible clone. Days from symptom appearance to 25% leaf damage (DTQ) ranked *Morong Princesa* as the most resistant and Grande Naine as the most susceptible. Generally, DTQ and YLS ranked Grande Naine as the most susceptible genotype. On the contrary, YLS ranked 3202 as a susceptible genotype but DTQ ranked it as one of the resistant genotypes.

The AUDPC had the highest R^2 of 0.84 followed by DTQ with R^2 of 0.76. The youngest leaf spotted had the least R^2 of 0.59. However, AUDPC had the highest

Table 3. Spearman rank correlations of different assessment methods for black Sigatoka in banana diploids at Kawanda Agricultural Research Institute.

Variable	Youngest leaf spotted	Area under disease progress curve	Days from leaf emergence to 25% leaf damage	Total leaves at flowering
Youngest leaf spotted	1			
Area under disease progress curve	0.37143*	1		
Days from leaf emergence to 25% leaf damage	0.35789	0.73835**	1	
Total leaves at flowering	0.66466**	0.31729	0.32632	1

*Data significant at $P < 0.05$; ** Data significant at $P < 0.001$.

Table 4. Pearson correlation coefficients of different assessment protocols in *Musa* accessions with bunch weight (kg) and total leaves at flowering at KARI.

Variable	YLS	TLF	AUDPC	DAA	DQQ	DTQ	DH	DFF
Youngest leaf spotted (YLS)								
Total leaves at flowering (TLF)	0.7061***							
Area under disease (AUDPC)	-0.3139**	-0.4096**						
Dys symptom appearance (DAA)	0.1447ns	0.1692ns	-0.4109***					
Dys leaf infection 12% (DQQ)	0.2142*	0.2922**	-0.6560***	0.5742***				
Dys 25% leaf damage (DTQ)	0.1958*	0.3219**	-0.7074***	0.5195***	0.8680***			
Dys 50% leaf damage (DH)	0.1371ns	0.2994*	-0.6645***	0.4294***	0.7731***	0.9009***		
DFF (100% damage-DAA)	0.0872ns	0.1685ns	-0.5257***	0.2395*	0.6398***	0.7789***	0.8829***	
Bunch weight (BWT)	0.287*	0.1749ns	-0.3958***	0.3958***	0.3828***	0.3370**	0.3646**	0.3093*

ns = non significant, *, **, *** significant at $P=0.05$, $P=0.01$ and $P=0.0001$, respectively.

coefficient of variation of 44%. When the AUDPC data were transformed ($\log(x+1)$), the coefficient of variation reduced to 17%. Although the R^2 of transformed AUDPC slightly reduced to 80%, it was still higher than the R^2 of YLS. The YLS and DTQ had relatively low coefficients of variation of 19 and 23%, respectively (Table 2).

Relationships among disease rating methods

The different assessment protocols ranked the diploid accessions (1 most resistant and 20 most susceptible). The rankings were correlated and results are shown in Table 3. The ranks of genotypes using AUDPC and days from leaf emergence to 25% leaf damage were positively correlated ($P < 0.001$). The rank correlations of AUDPC and YLS were positively correlated and significant ($P < 0.05$). The correlation coefficients of ranks of DTQ and YLS were not significant although they had a positive relationship. The ranks of total leaves at flowering correlated positively ($P < 0.001$) with the ranks of YLS.

Youngest leaf spotted had a strong but negative correlation ($P < 0.01$) with AUDPC. The YLS had a weak and positive correlation ($P < 0.05$) with days from leaf emergence to 25% leaf damage (DTQ). The area under

disease progress curve had a negative but strong correlation ($P < 0.001$) with total leaves at flowering (Table 3).

In addition to disease assessment protocols, YLS had a positive and strong correlation with total leaves at flowering ($P < 0.001$) and a weak but significant positive correlation with bunch weight ($P < 0.05$). Also AUDPC had a negative and strong correlation ($P < 0.01$) with total leaves at flowering and a negative strong correlation ($P < 0.001$) with bunch weight. The DTQ had a strong positive correlation ($P < 0.01$) with total leaves at flowering and a positive correlation with bunch weight ($P < 0.01$) (Table 4).

When a stepwise regression was carried out between total leaves at flowering (TLF) and disease parameters, AUDPC, days from leaf infection to symptom appearance (DAA) and YLS were involved in the regression equation ($TLF = 5.97 - 0.0008AUDPC + 0.005DAA + 0.82YLS$; $R^2 = 53.8$). The AUDPC was significant ($P = 0.039$) and YLS was highly significant ($P < 0.001$). Although DAA was included in the equation, it was not significant ($P = 0.894$) (Table 5).

To find out which of the disease parameters could be used to predict yield in the *Musa* accessions, a stepwise regression was carried out between bunch weight and

Table 5. Stepwise regression of total leaves at flowering (TLF) on disease assessment parameters.

Variable	Parameter estimate	Standard error	t-value	P-value
Constant	5.97	1.37	4.35	<0.001
Area under disease progress curve	-0.000979	0.00047	-2.09	0.039
Days to symptom appearance	0.0047	0.0349	0.13	0.894
Youngest leaf spotted	0.8166	0.0939	8.7	<0.001

$$\text{TLF} = 5.97 - 0.0008\text{AUDPC} + 0.005\text{DAA} + 0.82\text{YLS}; R^2 = 53.8.$$

Table 6. Stepwise regression of bunch weight (BWT) on disease parameters and days from flowering to harvest.

Variable	Parameter estimate	Standard error	t-value	P-value
Constant	-5	2.75	-1.82	0.073
Area under disease progress curve	0.004317	0.00099	4.35	<0.001
Days to harvest (DTH)	0.00562	0.00855	0.66	0.512
Total leaves at flowering (TLF)	0.286	0.242	1.18	0.24
Youngest leaf spotted (YLS)	0.713	0.294	2.43	0.017

$$\text{BWT} = -5 + 0.004\text{AUDPC} + 0.006\text{DTH} + 0.29\text{TLF} + 0.713\text{YLS}; R^2 = 0.24.$$

disease parameters. AUDPC, and YLS were significant in yield prediction with probabilities of ($P < 0.001$ and $P = 0.017$, respectively). Total leaves at flowering, and days from flowering to harvest were not significant ($P > 0.05$) although they were included in the regression equation (Table 6).

DISCUSSION

Ranking of genotypes by the three methods

Generally, the assessment protocols classified the *Musa* accessions into resistant and susceptible clones. However, the rankings of the diploid genotypes were not consistent across the three assessment methods. For example YLS ranked Yangambi Km 5 as the most resistant while AUDPC ranked *Morong Princesa* as the most resistant. Also genotype, 3202 was ranked by YLS among the susceptible genotypes but DTQ indicated the same genotype was resistant to black Sigatoka. The ranking of Yangambi Km 5 as the most resistant genotype by YLS was expected because this genotype was used as a resistant check and it was chosen based on its YLS (IITA, 1989; Orjeda, 1998).

The high variation of black Sigatoka resistance among the diploid accessions evaluated using AUDPC could have caused a high coefficient of variation of AUDPC. However transforming AUDPC data with $\log(x+1)$ reduced the coefficient of variation. Youngest leaf spotted and days from leaf infection to 25% leaf damage had reasonably low coefficients of variation. The AUDPC had the highest coefficient of determination R^2 (0.84) among all the assessment techniques. This indicated that AUDPC accounted for most of the variation in disease

resistance among the diploid clones. AUDPC also correlated significantly ($r^2 = -0.3139$) with YLS which has been used to assess black Sigatoka resistance among *Musa* species. In the bunch weight prediction model, AUDPC was also significant implying that it might also be used to predict yield losses in banana diploids due to black Sigatoka. Days from leaf infection up to 25% leaf damage due to black Sigatoka also had a higher R^2 (0.76) than YLS (0.59), lower coefficient of variation (19%) than AUDPC (44%). Yet the days it took a leaf up to 25% leaf damage to black Sigatoka could not be used either to predict bunch weight or total leaves at flowering. Also a lot of time had to be committed in the field to follow up a leaf from emergence up to 25% leaf damage. Therefore, AUDPC seemed to be able to assess black Sigatoka damage before flowering, and appeared reliable in discriminating diploid *Musa* clones into resistant and susceptible ones. Days from leaf infection to symptom appearance (incubation time) was not significantly correlated with youngest leaf spotted and did not significantly affect total leaves at flowering. In previous studies, Jones (2000) reported that incubation time did not correlate with black Sigatoka resistance. Results from this study also suggest that it might not be feasible to use incubation time to assess black Sigatoka resistance among *Musa* genotypes because it had a low $R^2 = 0.34$ (results not shown).

Relationship among disease rating methods

The number of functional leaves at flowering is an indication of disease resistance. The number of total leaves at flowering is also important in fruit filling. The negative correlation between total leaves at flowering

with AUDPC suggested that in this diploid population plants with high disease severity assessed by AUDPC were expected to have reduced number of leaves at flowering. This is true since the disease causes leaf death through necrosis.

Youngest leaf spotted and AUDPC had a significant linear relationship with TLF and bunch weight. This suggested that YLS at flowering and AUDPC six months after planting could be used to predict TLF and BWT. Prediction of Bunch weight (BWT) using AUDPC will help in selection of materials that combine high yield with disease resistance. In a study by Craenen and Ortiz (1998) disease resistance based on YLS helped in selection of *Musa* genotypes that combined higher yields with disease resistance. The positive correlation in rankings of YLS and AUDPC also suggest that AUDPC might be as efficient as YLS in discriminating the clones according to their disease resistance. However, YLS is carried out at flowering when a lot of resources could have been spent to maintain materials whose disease response is unknown. Besides, it might also be late to design/plan crosses to use to improve susceptible banana plants especially in a recurrent selection improvement programme. At the moment, there are no molecular markers that have been applied in selection of black Sigatoka resistant *Musa* genotypes. There is need to select an appropriate early and reliable assessment technique that can predict yield in the selected genotypes. The AUDPC might be used to select resistant banana materials earlier than the YLS method.

Table 2 shows Calcutta 4 as the most resistant accession to black Sigatoka disease using AUDPC. On the other hand, YLS indicated that Calcutta 4 has moderate resistance. The high level of resistance identified by AUDPC could imply a hypersensitive disease reaction of Calcutta 4. In other studies Calcutta 4 was reported to exhibit a hypersensitive disease reaction to black Sigatoka (Craenen and Ortiz, 1998). This type of resistance is not good for quantitative resistance breeding since it can easily break down. The breakdown of resistance in Calcutta 4 has been reported elsewhere (Jones, 2000). It is therefore necessary to use an assessment technique that can detect hypersensitive reaction so that we avoid selecting such materials in a population improvement programme. It appears that AUDPC can identify such type of disease reaction.

Normally, banana plants do not flower at the same time due to genotype and environmental differences. This implies that using YLS method, plants will be assessed over a period of time. The fluctuations in environmental conditions may introduce differences even among the same genotypes. Similarly, following disease development in a leaf from emergence up to when leaf is 25% damaged takes a minimum of 50 days in resistant genotypes (Table 2). Therefore environmental changes may also influence disease expression even among the same genotype thus introducing errors in the data. On

the contrary, AUDPC is not restricted to a stage of either plant growth or leaf damage. Therefore AUDPC assessment can be implemented at any time. However, at least three assessments are required to compute AUDPC (Shaner and Finney, 1977). Assessing the disease severity at an interval of 14 days all the AUDPC assessments can be conducted within one month. From the present study, because of its high R^2 (0.84), and its ease of use, this investigation recommends using AUDPC to assess black Sigatoka resistance in *Musa* genotypes.

Conclusions

In conclusion, there was a positive relationship between youngest leaf spotted, AUDPC, and disease development time in assessing diploid clones for black Sigatoka resistance. However, youngest leaf spotted and area under disease progress curve were the only methods found in the present study to predict bunch weight and total leaves at flowering. AUDPC was considered to be the best method among the three because it had the best coefficient of determination ($R^2=0.84$) and required less time than disease development time to assess disease damage. Therefore, AUDPC would be recommended to assess disease resistance of *Musa* genotypes to black Sigatoka.

ACKNOWLEDGEMENTS

This work was part of a PhD research that was funded by the Rockefeller Foundation through the African Centre for Crop Improvement. We are grateful to the Rockefeller Foundation for the financial support, the Director of Kawanda Agricultural Research Institute for providing land that hosted the trials. We are grateful to the International Institute of Tropical Agriculture (IITA), the Fundación Hondureña de Investigación Agrícola (FHIA), and the International Network for the Improvement of Bananas and Plantains (INIBAP) (now Bioversity International) for providing germplasm that was used in this investigation.

REFERENCES

- Asea G, Bigirwa G, Adipala E, Pratt SRC, Lipps PE (2002). Effect of *Cercospora zeae-maydis* infested maize residue on progress and spread of grey leaf spot of maize in central Uganda. *Ann. Appl. Biol.*, 140: 177-185.
- Craenen K, Ortiz R (1998). Influence of black Sigatoka disease on the growth and yield of diploid and tetraploid hybrid plantains. *Crop Prot.*, 17: 13-18.
- Danielsen S, Munk L (2004). Evaluation of disease assessment methods in quinoa for their ability to predict yield loss caused by downy mildew. *Crop Prot.*, 23: 219-228.
- Holland JB, Munkvold GP (2001). Genetic relationship of crown rust resistance, grain yield, test weight and seed weight in oat. *Crop Sci.*, 41: 1041-1050.

- International Institute of Tropical Agriculture (IITA) (1989). Annual Report. Ibadan, Nigeria.
- Jeger MJ, Viljanen-Rollinson SLH (2001). The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. *Theor. Appl. Genet.*, 102: 32-40.
- Jones DR (2000). Diseases of Banana Abaca and Enset. CAB International, Wallingford, UK.
- Kushwaha C, Srivastava CP, Chand R, Singh BD (2007). Identification and evaluation of a critical time for assessment of slow rusting in pea against *Uromyces fabae*. *Field Crops Res.*, 103: 1-4.
- Lipps PE, Madden LV (1989). Assessment of methods of determining powdery mildew severity in relation to grain yield of winter wheat cultivars in Ohio. *Phytopathology*, 79: 462-470.
- Liu G, Kennedy R, Greenshields DL, Peng G, Forseille L, Selvaraj G, Wei Y (2007). Detached and attached *Arabidopsis* leaf assays reveal distinctive defense responses against hemibiotrophic *Colletotrichum* spp. *Mol. Plant-Microbe Interact.*, 20: 1308-1319.
- Mobambo KN, Gauhl F, Vuylsteke D, 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.
- Mobambo KN, Pasberg-Gauhl C, Gauhl F, Zuofa K (1997). Host response to black Sigatoka in *Musa* germplasm of different ages under natural inoculum conditions. *Crop Prot.*, 16: 359-363.
- Orjeda G (1998). Evaluation of *Musa* germplasm for resistance to Sigatoka diseases and Fusarium wilt. INIBAP Technical Guidelines 3. International Plant Genetic Resources Institute, Rome, Italy. International Network for the Improvement of Banana and Plantain, Montpellier France: ACP-EU Technical Centre for Agricultural and Rural Cooperation, Wageningen, The Netherlands.
- Ortiz R, Vuylsteke D (1994). Inheritance of black Sigatoka disease resistance in plantain-banana (*Musa* spp.) hybrids. *Theor. Appl. Genet.*, 89: 146-152.
- Rowe P, Rosales F (1996). Bananas and plantains. In: Janick J, Moore JN (eds) Fruit breeding, tree and tropical fruits. Wiley, New York, pp. 167-211.
- SAS (2002). SAS Institute Inc., Cary, NC, USA.
- Shaner G, Finney R (1977). The effect of nitrogen fertilisation on the expression of slow-mildewing in knox wheat. *Phytopathology*, 67: 1051-1056.
- Stover RH (1971). A proposed international scale for estimating intensity of banana leaf spot. *Trop. Agric. (Trinidad)*, 48: 185-196.
- Stover RH, Buddenhagen IW (1986). Banana breeding: Polyploidy, Disease Resistance and Productivity. *Fruits*, 41: 175-191.
- Swennen R, Vuylsteke D (1993). Breeding black Sigatoka resistant plantain with a wild banana. *Trop. Agric.*, 70: 74-77.
- Tushemereirwe WK (1996). Factors influencing the expression of leaf spot diseases of highland bananas in Uganda. PhD Thesis, University of Reading, U.K.
- Tushemereirwe WK, Gahakwa D, Batte M, Ssali T, Namanya P, Pillay M, Talengera D (2005). Development and promotion of banana genotypes resistant to weevils, black Sigatoka, nematodes and bacterial wilt. In Abstract. Biotechnology, Breeding and Seed systems for African Crops, 24-27 January 2005, Nairobi, Kenya. The Rockefeller Foundation, Nairobi, Kenya, p. 186.
- Twizeyimana M, Ojiambo PS, Tenkouano A, Ikotun T, Bandyopadhyay R (2007). Rapid screening of *Musa* species for resistance to black leaf streak using *in vitro* plantlets in tubes and detached leaves. *Plant Dis.*, 91: 308-314.
- Vakili NG (1968). Responses of *Musa acuminata* species and edible cultivars to infection by *Mycosphaerella musicola*. *Trop. Agric. (Trinidad)*, 45: 13-22.
- Vera (2008). www.ipmcenters.org/IPMSymposiumV/posters/041.pdf. Accessed May 2009.
- Vuylsteke D (2001). Strategies for utilisation of genetic variation in plantain improvement. Kathoelieke, Universiteit Leuven, Belgium. PhD Thesis.