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Evaluation of banana hybrids for tolerance to black leaf streak (*Mycosphaerella fijiensis* Morelet) in Puerto Rico

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ABSTRACT

In Puerto Rico, bananas (including plantains) are important agricultural commodities; their combined production totaled over 158,000 tons in 2011. Black leaf streak (BLS) and Sigatoka leaf spot diseases, caused by *Mycosphaerella fijiensis* and *Mycosphaerella musicola*, respectively, are responsible for significant losses of this crop, due to the high susceptibility of the most important cultivars. Diploid, triploid and tetraploid hybrids were introduced from international breeding programs for evaluation in Isabela, Puerto Rico. Accessions were established in the field in a randomized complete block design and were evaluated over two cropping cycles (2007–2010) for response to BLS and agronomic traits. Significant differences ($P = 0.05$) in BLS severity were observed among accessions throughout both crop cycles and were most pronounced at harvest. When averaged across production cycles, severity indices at harvest ranged from very resistant (20% of the leaf surface affected) for 'FHIA 02' to extremely susceptible (97%) for 'Grand Nain'. Yield attributes varied widely among the accessions, including mean bunch weights (6.9–41.0 kg), numbers of hands per bunch (6.6–13.4), and the numbers of fruit per bunch (57.0–239.2). Several accessions, mainly from the Fundación Hondureña de Investigación Agrícola (FHIA), were BLS resistant and had short pseudostems, and large bunches. They could potentially replace susceptible cultivars in commercial production or play roles in a nascent organic market.

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1. Introduction

Bananas (including plantains) (*Musa* spp.) are large tropical monocots that originated in Southeast Asia (Perrier et al., 2011). World production, which was estimated in 2010 at 138 million metric tons, sustains over 400 million people (FAOSTAT, 2010). Bananas are an important nutrient source, especially in Africa and Asia, and are associated with a large export industry that provides important revenue and jobs in many producing countries. Bananas are one of the few agricultural products that are not imported into Puerto Rico (production meets local demand); during the 2009/2010 growing season they were responsible for approximately $80 million in gross income (PRAD, 2012).

Most cultivated edible bananas are natural hybrids among and between *Musa acuminata* (A genome) and *Mycosphaerella balbisiana* (B genome) (Perrier et al., 2011; Ploetz et al., 2007; Stover and Simmonds, 1987). Centers of high genetic diversity (Heslop-Harrison and Shwarzacher, 2007) are confined to the Indo-Malaysian, Asian, and Pacific island tropics and it is from these regions that germplasm has been collected for evaluation and use by banana breeding programs (Rosales et al., 1999; Sharrock, 1990; Simmonds, 1962; Yan et al., 2012). Banana cultivars set fruit by vegetative parthenocarpy (Dodds and Simmonds, 1948; Okoro et al., 2011), and do not set seed. Identifying or recreating commercially acceptable cultivars which also resist important diseases and pests has been most difficult.

In Puerto Rico, pests and diseases limit commercial banana production and integrated pest management practices are essential. Some of the more important fungal diseases include the Sigatoka leaf spots [Sigatoka leaf spot or yellow Sigatoka, caused by *Mycosphaerella musicola* R. Leach ex J.L. Mulder, and black leaf streak (BLS) or black Sigatoka caused by *Mycosphaerella fijiensis*

Abbreviations: CARBAP, Centre Africain de Recherches sur Bananiers et Plantains; EMBRAPA, Empresa Brasiliera de Pesquisa Agropecuaria; FHIA, Fundación Hondureña de Investigación Agrícola; IITA, International Institute for Tropical Agriculture; masl, meters above sea level; TARS, Tropical Agriculture Research Station.

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Table 1
Name, source, genome, type and pedigree of *Musa* spp. synthetic hybrids and standard cultivar controls evaluated for Black leaf streak (*Mycosphaerella fijiensis* Morelet) resistance and agronomic traits over two cropping cycles in Isabela, PR.

Accession	ITC code[a]	Origin		Genome	Type/Use	Pedigree/Derivation
		Country	Institute[b]			
CRBP 39	1344	Cameroon	CARBAP	AAAB	Plantain hybrid	French Clair (AAB) × M-53 (AA)
FHIA-02	–	Honduras	FHIA	AAAA	Dessert	Williams (AAA) × SH-3393 (AA)
FHIA-17	–	Honduras	FHIA	AAAA	Dessert banana	Gross Michel (AAA) × SH-3362 (AA)
FHIA-18	–	Honduras	FHIA	AAAB	Dessert/Pome	Prata Aña (AAB) × SH-3142 (AA)
FHIA-23	–	Honduras	FHIA	AAAA	Dessert banana	Highgate (AAA) × SH-3362 (AA)
PA 03-22	1261	Brazil	EMBRAPA	AAAB	Dessert/Pome	Prata Aña (AAB) × Calcuta 4 (AA)
PA 12-03	1302	Brazil	EMBRAPA	AAAB	Dessert/Pome	Prata Aña (AAB) × Lidi (AA)
PITA 16	1417	Nigeria	IITA	AAB	Plantain hybrid	TMPx 4479-1 (AAAB) × SH 3362 (AA)
PV 03-44	1262	Brazil	EMBRAPA	AAAB	Dessert/Pome	Pacovan (AAB) × Calcuta 4 (AA)
PV 42-320	1313	Brazil	EMBRAPA	AAAB	Dessert/Pome	Pacovan (AAB) × M-53 (AA)
PV 42-53	1310	Brazil	EMBRAPA	AAAB	Dessert/Pome	Pacovan (AAB) × M-53 (AA)
PV 42-81	1312	Brazil	EMBRAPA	AAAB	Dessert/Pome	Pacovan (AAB) × M-53 (AA)
SH 3640	1307	Honduras	FHIA	AAAB	Dessert banana	Prata Aña (AAB) × SH-3393 (AA)
Grand Nain[c]	–	Puerto Rico	Local selection	AAA	Dessert/Cavendish	Selection
Maricongo[c]	–	Puerto Rico	Local selection	AAB	True Plantain	Dwarf mutant of Congo
Yangambi km 5[c]	–	D.R. Congo	Local selection	AAA	Dessert/Ibota	Unknown
FHIA-01[d]	0504	Honduras	FHIA	AAAB	Dessert/Pome	Prata Aña (AAB) × SH-3142 (AA)
FHIA-21[d]	1332	Honduras	FHIA	AAAB	Plantain hybrid	AVP-67 (AAB) × SH-3142 (AA)
FHIA-25[d,e]	1418	Honduras	FHIA	AAB	Cooking banana	SH-3648 (4×) × SH-3142 (AA)
TMB2x 9128-3[d]	1437	Nigeria	IITA	AA	Diploid hybrid	Tjau lagada (AA) × Pisang lilin (AA)

[a] Bioversity International Musa International Transit Center, Leuven, Belgium.
[b] Abbreviations: Centre Africain de Recherches sur Bananiers et Plantains (CARBAP); Fundación Hondureña de Investigación Agrícola (FHIA); Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA); International Institute of Tropical Agriculture (IITA).
[c] Internal controls: Grand Nain – susceptible Cavendish type, Maricongo – susceptible plantain, and Yangambi km 5 – resistant.
[d] Only 7 replicate plants of FHIA-01, FHIA-21, FHIA-25, and TMB2x 9128-3 were established, evaluated and not included in experimental design or in statistical analysis of data.
[e] Somaclonal variants with off-type phenotypes having distorted leaf laminas with only one bunch reaching maturity.

Morelet, and Fusarium wilt, caused by *Fusarium oxysporum* Schltdl.:Fr. f. sp. *cubense* (E. F. Sm.) W. C. Snyder & H. N. Hansen (FOC). Tropical race 4 of FOC causes serious losses and threatens Cavendish (AAA) local and export industries worldwide (Dita et al., 2010). Moko disease, caused by the bacterium *Ralstonia solanacearum* (Smith) Smith (race 2), can be highly destructive and requires strict sanitation measures. Several viruses can be important, especially *Banana bunchy top virus* (BBTV), *Cucumber mosaic virus* (CMV), *Banana bract mosaic virus* (BBrMV), and the banana streak viruses (BSVs) (Geering, 2009; Krikorian et al., 1998). And nematodes (*Radopholus similis* and *Pratylenchus* spp.) and the banana weevil borer (*Cosmopolites sordidus* (Germar)) are serious pests.

Since its description in 1963, BLS has spread to most banana-growing regions. The disease's status and outlook are discussed in recent reviews (Chillet et al., 2009; Churchill, 2011; Etebu and Young-Harry, 2011; Jones, 2000; Marin et al., 2003). BLS first appeared in the Western Hemisphere in Honduras in 1972 and subsequently spread throughout the Americas (Carlier et al., 2002; Stover, 1976). Although the Caribbean islands were some of the last locations to which the disease spread, it is now found in most of the chain (summarized by Churchill (2011)). In Puerto Rico, BLS was first noted in 2004 by agricultural extension personnel from the University of Puerto Rico, and Irish et al. (2006) confirmed the presence of *M. fijiensis* with molecular markers.

M. fijiensis spreads within and among fields mostly by wind-borne ascospores that are produced on senescent infected leaf tissues (Gauhl et al., 2000). With high humidity, ascospores and conidia germinate readily and penetrate the leaf surface through stomates. Small black lesions (streaks) form on the leaf lamina surface and as the disease progresses lesions enlarge, turn tan and coalesce. Losses can easily exceed 50% if cultural practices and fungicide applications are not utilized (Gauhl and Pasberg-Gauhl, 1994).

BLS has reduced yields and significantly raised farmer production costs since its arrival in Puerto Rico. For example, BLS has increased the number of fungicide applications that are needed to maintain yields (Alamo et al., 2007). Although fungicides can be effective, their use is constrained by the loss and reduced efficacy of labeled products, as well as associated risks to human health and the environment (Churchill, 2011; Marin et al., 2003). As cultural practices are not entirely effective and fungicides have drawbacks, genetic resistance to the disease is desirable. Several international breeding programs have released BLS-resistant hybrids, most of which are parthenocarpic tetraploids generated by crossing triploid banana and plantain cultivars with wild or improved diploid parents (Ortiz and Vuylsteke, 1998; Rowe and Rosales, 1993; Swennen and Vuylsteke, 1993; Vuylsteke and Ortiz, 1995).

The USDA-ARS, Tropical Agriculture Research Station (TARS) in Mayaguez, Puerto Rico is the official repository for *Musa* spp. germplasm for the National Plant Germplasm System. In this capacity, TARS has introduced hybrids from Musa breeding programs for evaluation. We describe below efforts to identify BLS-resistant genotypes that would satisfy local and export markets and which could be used to replace susceptible cultivars.

2. Materials and methods

2.1. Plant material

Banana hybrids originated from international breeding programs in Brazil (Empresa Brasiliera de Pesquisa Agropecuaria – EMBRAPA), Cameroon (Centre Africain de Recherches sur Bananiers et Plantains – CARBAP), Honduras (Fundación Hondureña de Investigación Agrícola – FHIA), and Nigeria (International Institute for Tropical Agriculture – IITA) (Table 1). Plant material was obtained as virus-indexed *in vitro* tissue cultured plantlets or adventitious sword sucker meristems for *in vitro* induction, either from Bioversity International's *Musa* International Transit Center at the

Table 2
Disease index, days to flowering and harvest, and number of functional leaves, plant height, plant diameter and number of suckers at flowering for banana synthetic hybrids and standard cultivar controls evaluated for black leaf streak (*Mycosphaerella fijiensis* Morelet) tolerance and agronomic traits for a **first cycle (mother)** in Isabela, PR.

Accession	Disease severity index (%)		Days to		At flowering			
	Flowering	Harvest	Flowering	Harvest	Functional leaves	Plant height (m)	Pseudostem diam. (cm)[a]	Suckers[b]
CRBP 39	22 c[c]	79 bc	240.5 cd	360.8 b	12.0 fg	3.46 bc	19.6 d	8.8 a
FHIA-02	02 f	25 i	184.0 fg	310.6 def	14.4 bc	2.54 fg	16.7 f	6.3 cd
FHIA-17	18 cd	41 h	292.0 ab	413.9 a	11.2 gh	3.29 cd	23.6 ab	7.5 abc
FHIA-18	04 f	47 gh	237.3 cde	375.3 b	13.4 bcde	3.08 dce	21.1 c	6.2 cd
FHIA-23	12 de	37 hi	301.1 a	423.9 a	10.1 h	3.41 bc	24.4 a	8.0 ab
PA 03-22	46 a	64 def	255.7 c	314.5 de	7.9 i	3.29 cd	16.3 f	8.1 ab
PA 12-03	05 ef	73 cd	164.6 g	274.5 g	12.7 def	2.11 h	15.2 g	6.0 cd
PITA 16	32 b	98 a	292.2 ab	410.4 a	10.1	3.29 cd	22.9 b	7.0 abcd
PV 03-44	02 f	56 fg	183.0 fg	322.4 cde	13.0 cdef	2.96 de	16.0 gf	5.6 d
PV 42-320	07 ef	91 ab	213.4 def	306.7 ef	14.0 bcd	4.01 a	18.2 e	6.0 cd
PV 42-53	07 ef	78 c	205.4 ef	326.6 cd	14.8 b	3.20 cde	18.3 e	6.5 bcd
PV 42-81	07 ef	98 a	207.6 def	335.5 c	14.4 bc	3.75 ab	18.9 de	5.7 d
SH 3640	16 cd	91 ab	205.3 ef	303.9 ef	16.7 a	2.83 ef	18.2 e	5.8 cd
Grand Nain	21 c	99 a	192.7 fe	295.3 f	16.6 a	2.29 gh	16.1 f	8.0 ab
Maricongo	34 b	68 cde	260.9 cb	366.3 b	11.7 fg	3.72 ab	19.4 d	7.5 abc
Yangambi km 5	38 b	60 ef	255.0 c	379.0 b	12.3 efg	2.97 de	16.5 f	6.8 bcd
FHIA-01[d]	06	23	311.7	431.3	14.3	3.02	18.9	5.0
FHIA-21[d]	05	15	294.8	388.0	12.0	3.35	18.1	7.3
TMB2x 9128-3[d]	24	88	137.8	257.0	10.3	3.34	17.0	5.8

[a] Measured at 1 m above the ground.
[b] Although cultural practices included desuckering, the total number of visible suckers and scars were counted.
[c] Means followed by the same letter within a column are not significantly different according to the least significant difference test at $P = 0.05$.
[d] FHIA-01, FHIA-21, and TMB2x 9128-3 not included in statistical analysis of data.

Catholic University in Leuven, Belgium, or directly from FHIA. Plant material was micro-propagated *in vitro* following conventional protocols (Vuylsteke, 1989) and acclimatized in a greenhouse for 3 months. Plants were regularly watered and fertilized with water soluble Evergreen (20-20-20 + micro elements) and Osmocote®[1] slow release (19-6-12) fertilizers.

2.2. Field site and experimental design

On 20 February 2008, acclimatized material was planted at the USDA-ARS Tropical Agriculture Research Station (TARS) research farm in Isabela, PR (18°28′18.38″N, 67° 3′4.25″W; elevation 118 masl). The experiment was established on a well-drained Oxisol (clayey, kaolinitic isohyperthermic Typic Hapludox) with average pH of about 6.2. Average annual precipitation over the past 6 years (calendar years 2007–2012) was 1520 mm with peaks in precipitation around the months of May and August (U.S. Department of Agriculture, Natural Resources Conservation Service, Soil Climate Analysis Network (online), http://www.wcc.nrcs.usda.gov/scan/, Referenced April 19, 2013).

A total of 20 accessions were planted, 16 of which were included in a randomized complete block design (RCBD) experiment with four blocks and six single plant experimental units per block. Since ‘FHIA-01’, ‘FHIA-21’, ‘FHIA-25’ and the diploid breeding line ‘TMB2x 9128-3’ were not received in time for micro-propagation, only seven plants of each of these accessions were established in single plot, non-replicated border rows (Table 1). Three internal control cultivars were included in the evaluations: ‘Grand Nain,’ a susceptible Cavendish cultivar that is a local standard; ‘Maricongo,’ a susceptible plantain cultivar that is a local standard; and ‘Yangambi km 5,’ a resistant control. Observations and data were collected for 19 of the 20 accessions, as ‘FHIA-25’ appeared to be an ‘off-type’/somaclonal mutant that produced inconsistent flowers and bunches.

Treatment blocks were spaced 1.5 m between plants and 3.0 m between rows and consisted of two rows of three replicate plants each. Border rows of ‘Grand Nain’ were established around each treatment and block to ensure uniform disease pressure. With the exception of the false-horn plantain ‘Maricongo,’ male flower buds and false hands were removed from evaluated accessions as soon as they were evident. Commercial practices for irrigation, fertilization and insect control were followed throughout the study (Goenaga and Irizarry, 2000; Irizarry et al., 2001a,b, 1992; PRAES, 1995). Cultural practices and fungicide applications recommended for BLS control were not used.

2.3. Evaluation

Gauhl's modification of Stover's Sigatoka severity scoring system, adapted from Orjeda (1998), was utilized. The scoring system follows a 0–6 scale where, 0 = no symptoms; 1 = 1%; 2 = 2–5%; 3 = 5–15%; 4 = 16–33%; 5 = 34–50%; and 6 = >50% of the leaf area affected. Beginning 6 months after field establishment, which corresponded to the first appearance of BLS symptoms, every leaf on individual plants was scored monthly for two full crop cycles (mother and first ratoon). Following harvest of the mother crop, rating switched to the sword sucker that had been chosen for the second cycle (first ratoon). Disease severity was also recorded at flowering and harvest during both production cycles. A disease severity index was calculated for individual plants using the following formula (Craenen, 1998):

$$\text{Disease severity index} = \left[\left(\sum nb\right)\Big/(N-1)T\right] \times 100,$$

where n = number of leaves with a given rating, b = rating, N = seven total ratings/grades in the scale and T = total number of leaves scored.

Mean severity index for monthly ratings and for ratings at flowering and at harvest were calculated by averaging across individual plants for each accession. During both cropping cycles, the following agronomic and bunch characteristics were

[1] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Table 3
Disease index, days to flowering and harvest, and number of functional leaves, plant height, plant diameter and number of suckers at flowering for banana synthetic hybrids and standard cultivar controls evaluated for black leaf streak (*Mycosphaerella fijiensis* Morelet) tolerance and agronomic traits for a **second cycle (first ratoon)** in Isabela, PR.

Accession	Disease severity index (%)		Days to		At flowering			
	Flowering	Harvest	Flowering	Harvest	Functional leaves	Plant height (m)	Pseudostem diam. (cm)[a]	Suckers[b]
CRBP 39	09 def[c]	52 ef	535.4 bc	635.3 bc	10.3 fgh	4.04 c	20.6 efg	4.6 a
FHIA-02	07 def	16 j	415.1 hg	533.1 ef	12.2 abc	3.26 gh	19.1 g	3.6 ab
FHIA-17	10 bcde	32 ghi	644.8 a	754.5 a	10.4 efgh	3.79 de	27.7 a	2.4 b
FHIA-18	03 f	26 ij	533.1 bcd	659.9 b	12.9 ab	3.78 de	24.5 b	5.3 a
FHIA-23	10 bcde	29 hij	649.7 a	755.4 a	10.5 defgh	3.77 de	27.8 a	2.4 b
PA 03-22	24 a	42 fgh	575.4 b	624.1 bc	9.1 h	3.47 fg	15.6 h	4.2 a
PA 12-03	17 abc	49 ef	379.2 h	499.8 f	9.9 gh	3.10 h	19.7 fg	3.6 ab
PITA 16	12 bcde	89 ab	486.9 def	596.8 cd	11.3 cdefg	3.45 fg	21.3 def	4.5 a
PV 03-44	06 ef	29 hij	397.1 h	532.1 ef	11.8 bcde	4.18 c	19.9 fg	4.7 a
PV 42-320	10 bcde	59 de	461.0 fg	563.3 de	11.2 cdefg	4.52 b	22.4 cd	3.9 ab
PV 42-53	07 def	37 fghi	459.6 fg	569.1 de	13.4 a	4.15 c	22.0 cde	4.1 ab
PV 42-81	10 bcde	68 dc	479.5 ef	593.5 cd	12.6 abc	5.02 a	24.2 b	3.9 ab
SH 3640	14 bcd	77 bc	486.0 ef	574.8 de	12.7 abc	3.58 ef	23.4 bc	3.8 ab
Grand Nain	18 ab	95 a	468.1 f	557.7 de	10.6 defgh	2.58 i	19.2 g	4.3 a
Maricongo	23 a	63 cde	535.8 bc	626.3 bc	11.5 bcdef	3.94 cd	19.0 g	3.6 ab
Yangambi km 5	17 abc	47 efg	516.8 cde	631.1 bc	11.9 bcd	3.99 cd	19.9 fg	3.7 ab
FHIA-01[d]	03	22	511.8	625.4	12.8	3.66	25.5	3.5
FHIA-21[d]	09	25	580.7	681.3	11.3	3.87	21.2	1.9
TMB2x 9128-3[d]	18	88	350.3	456.8	11.0	4.14	18.1	3.3

[a] Measured at 1 m above the ground.
[b] Although cultural practices included desuckering, the total number of visible suckers and scars were counted.
[c] Means followed by the same letter within a column are not significantly different according to the least significant difference test at $P = 0.05$.
[d] FHIA-01, FHIA-21, and TMB2x 9128-3 not included in statistical analysis of data.

recorded: length of crop cycle, number of functional leaves (i.e., mature, fully expanded and photosynthetically active) at flowering and harvest, plant height and pseudostem diameter at flowering, bunch weight, and number of hands and fruit per bunch. In addition, the weight of third hand fruit, as well as, the length and diameter for the middle six fruits in the third hand were recorded. Fruit for the accessions being evaluated were assessed for their flavor and overall appearance in an informal sensory evaluation by personnel at TARS. Detailed postharvest and sensory evaluations, conducted in collaboration with the Food Science Department of the University of Puerto Rico as part of a separate research project, are not reported in this manuscript. Environmental data was downloaded from a USDA Natural Resources Conservation Service (NRCS) weather station on the USDA-ARS TARS research farm in Isabela, PR.

2.4. Statistical analysis

Analysis of variance was carried out using the GLM procedure of SAS for Windows Ver. 9.1 (SAS Institute, Cary, NC). After significant F test at $P = 0.05$, mean separation was performed with the least significant difference test.

2.5. M. fijiensis confirmation

Randomly selected symptomatic leaf samples from ‘Grand Nain’ were collected from the experimental plot. Single ascospores were recovered using the discharge technique (Johanson and Jeger, 1993), transferred to PDA, and later sub-cultured in PDA broth for mycelium production. DNA was extracted from lyophilized and ground mycelium using a FastDNA Spin Kit (MP Biomedicals, Solon, OH) following the manufacturer’s protocols. Isolates were then amplified with the polymerase chain reaction and Internal Transcribed Spacer (ITS) primers that are specific for *M. fijiensis* and *M. musicola* (Irish et al., 2006; Johanson and Jeger, 1993). Amplicons were separated and visualized via electrophoresis in a 1.5% agarose gel.

3. Results

3.1. Disease index

As cropping cycles showed significant effects, these were analyzed separately (Tables 2–5). For all accessions, the mean disease severity index for the mother crop at flowering and at harvest was higher than that observed for the ratoon crop (*data not shown*).

For the mother crop, mean disease severity at flowering was lowest for ‘FHIA-18’, ‘PA 12-03’, ‘PV 42-320’, ‘PV 42-53’, ‘PV 42-81’, and ‘PV 03-44’ ($P = 0.05$). The internal controls ‘Grand Nain’ and ‘Maricongo’ showed intermediate levels of BLS (21 and 34%, respectively) whereas ‘PA 03-22’ was most susceptible (46%) (Table 2). At harvest, ‘Grand Nain’, ‘SH 3640’, ‘PV 42-81’; ‘PV 42-320’ and ‘PITA 16’ had significantly higher mean disease severity indices (91–98%) whereas all FHIA clones had significantly lower indices (25–47%) (Fig. 1, Table 2).

For the ratoon crop at flowering, ‘Maricongo’ and ‘PA 03-22’ had significantly higher mean disease severity indices whereas ‘FHIA-18’ had the lowest index. At harvest, the ratoon crop of ‘Grand Nain’ and ‘FHIA-02’ had the highest and lowest indices, respectively (Table 3).

From flowering to harvest, disease severity increased considerably for all accessions, but rankings for accessions were similar in both crop cycles (Tables 2 and 3). The FHIA clones, particularly in the ratoon crop, showed significantly lower disease severity at harvest. At flowering, ‘PA 03-22’ had the highest rating in both cycles (46% and 24%). However, disease did not progress much from flowering to harvest for this accession which had intermediate disease indices. The ‘FHIA-21’ (AAAB) plantain hybrid had the lowest disease severity at harvest for the mother crop; however, this accession was not part of the replicated experiment and could not be analyzed statistically (Table 2).

3.2. Monthly ratings

Monthly disease ratings began 6 months after field establishment and continued through harvest for each cycle. Average

Table 4
Number of functional leaves at harvest, bunch weight, number of hands, number of fruit and third hand metrics for banana synthetic hybrids and standard cultivar controls evaluated black leaf streak (*Mycosphaerella fijiensis* Morelet) tolerance and agronomic traits for a **first cycle (mother)** in Isabela, PR.

Accession	At harvest				Third hand fruit		
	Functional leaves	Bunch weight (kg)[a]	# Hands	# Fruit	Weight (kg)	Length (cm)[b]	Width (mm)[c]
CRBP 39	2.1 fg[c]	15.0 f	6.9 g	97.6 f	2.3 efg	20.7 de	35.5 cdef
FHIA-02	10.5 a	23.6 bc	10.1 b	147.6 c	2.7 cd	20.7 de	38.9 bc
FHIA-17	4.6 de	43.1 a	12.4 a	221.3 a	4.3 a	22.4 bc	40.7 ab
FHIA-18	7.8 b	24.1 b	9.5 bc	157.0 c	2.9 c	18.6 f	37.6 bcd
FHIA-23	4.2 de	44.2 a	12.9 a	233.1 a	4.3 a	22.5 bc	40.6 b
PA 03-22	5.5 cd	6.9 h	8.0 ef	120.7 d	1.0 j	12.3 i	28.9 h
PA 12-03	3.7 e	11.2 g	8.0 ef	114.7 de	1.3 i	15.8 g	30.2 gh
PITA 16	0.2 h	25.0 b	8.7 cde	187.8 b	3.2 b	20.0 e	35.3 def
PV 03-44	6.4 c	10.7 g	6.4 g	90.4 f	1.8 h	16.8 g	34.5 def
PV 42-320	1.3 gh	15.6 ef	7.1 fg	101.2 ef	2.3 efg	20.4 e	35.8 cdef
PV 42-53	3.4 ef	17.5 ef	7.2 fg	103.0 ef	2.6 cde	20.8 de	37.1 cd
PV 42-81	0.2 h	15.2 f	7.1 fg	105.8 def	2.3 gf	21.0 de	32.6 fg
SH 3640	1.9 g	18.3 de	8.2 de	115.7 de	2.5 def	21.7 cd	36.3 cde
Grand Nain	0.1 h	20.7 cd	9.1 cd	150.6 c	2.8 cd	23.3 b	36.7 cd
Maricongo	3.4 ef	16.5 ef	8.2 de	57.0 g	2.7 cd	28.0 a	44.1 a
Yangambi km 5	3.6 e	17.1 ef	9.2 bc	198.9 b	2.0 gh	14.3 h	32.9 efg
FHIA-01[d]	8.0	30.6	9.3	136.5	3.5	23.6	39.7
FHIA-21[d]	8.2	28.4	7.8	115.8	4.0	27.0	41.4
TMB2x 9128-3[d]	0.3	13.3	18.0	318.3	0.9	13.8	22.2

[a] Net weight determined by removing hands from rachis and subtracting rachis weight from the gross weight of bunch.
[b] Average of the top and bottom three middle fruit of the third hand.
[c] Means followed by the same letter within a column are not significantly different according to the least significant difference test at $P = 0.05$.
[d] FHIA-01, FHIA-21, and TMB2x 9128-3 not included in statistical analysis of data.

monthly ratings were calculated for each accession and the sum of these monthly ratings was plotted over time for the two crop cycles (Figs. 2 and 3). The sum of monthly ratings was highest for 'Grand Nain' in both cycles, reaching close to 3.5 in the mother crop and over 7.0 in the first ratoon, and was lowest for 'FHIA-02' in both production cycles.

3.3. Agronomic traits

Time from planting to flowering and from planting to harvest was significantly different among accessions within each of the cropping cycles (Tables 2 and 3). In the mother crop, 'PA 12-03' had the shortest time from planting to flower (164.6 days); however two other accessions 'FHIA-02' and 'PV 03-44', which flowered in 184.0 and 183.0 days, respectively, were not significantly different. In contrast, 'FHIA-23' took over 300 days to flower and was not significantly different than 'FHIA-17' and 'PITA 16' which took over 292 days to flower (Table 2). The ratoon crops of 'PV 03-44' and 'PV 12-03' flowered 388 days after planting, whereas 'FHIA-17' and 'FHIA-23' averaged 647 days. In the ratoon crop, 'PA 12-03', 'PV 03-44' and 'FHIA-02' were harvested in significantly less time than 'FHIA-17' and 'FHIA-23' which took significantly longer (Table 3).

Table 5
Number of functional leaves at harvest, bunch weight, number of hands, number of fruit and third hand metrics for banana synthetic hybrids and standard cultivar controls evaluated black leaf streak (*Mycosphaerella fijiensis* Morelet) tolerance and agronomic traits for a **second cycle (first ratoon)** in Isabela, PR.

Accession	At harvest				Third hand fruit		
	Functional leaves	Bunch weight (kg)[a]	# Hands	# Fruit	Weight (kg)	Length (cm)[b]	Width (cm)[b]
CRBP 39	3.1 fg[c]	17.4 fg	7.7 ghi	112.0 f	2.5 efg	21.5 d	35.1 cde
FHIA-02	7.6 a	33.6 b	10.9 cd	173.5 e	3.5 abc	21.9 cd	41.3 ab
FHIA-17	5.0 cde	39.7 a	14.0 a	257.1 ab	3.8 a	21.2 d	38.7 bc
FHIA-18	6.7 ab	28.5 c	11.9 bc	207.6 cd	2.8 defg	18.8 f	35.1 cde
FHIA-23	4.7 cdef	36.4 ab	12.9 ab	234.2 bc	3.6 ab	21.2 d	36.8 cde
PA 03-22	6.8 ab	7.0 h	7.5 hi	108.0 f	1.0 h	12.6 h	29.6 g
PA 12-03	4.0 cdefg	19.8 efg	9.0 fg	132.5 f	2.3 g	19.3 ef	35.6 cde
PITA 16	0.5 i	27.4 c	9.3 ef	202.9 de	3.3 abcd	20.6 de	35.1 cde
PV 03-44	5.5 bc	19.7 efg	6.9 i	115.4 f	2.8 defg	19.1 ef	38.5 bcd
PV 42-320	3.8 defg	22.0 def	8.0 fghi	121.5 f	3.0 cde	21.5 d	36.3 cde
PV 42-53	6.8 ab	26.5 cd	8.1 fghi	126.7 f	3.6 abc	22.0 bcd	39.0 bc
PV 42-81	2.3 gh	22.4 de	7.7 ghi	119.2 f	3.1 bcd	23.5 b	34.4 e
SH 3640	2.7 gh	25.7 cd	9.1 ef	135.4 f	3.1 bcd	23.2 bc	36.7 cde
Grand Nain	1.2 hi	24.3 cde	10.3 de	178.9 de	3.1 bcd	23.3 bc	33.6 ef
Maricongo	3.4 efg	15.5 g	8.3 fgh	56.7 g	2.4 fg	26.2 a	45.2 a
Yangambi km 5	5.3 bcd	28.5 c	11.8 bc	269.0 a	2.9 def	16.0 g	34.6
FHIA-01[d]	8.0	41.7	10.7	168.3	4.0	24.3	38.8
FHIA-21[d]	5.7	27.2	8.3	121.0	3.9	25.5	40.4
TMB2x 9128-3[d]	0.5	13.5	8.0	162.3	1.9	16.5	31.1

[a] Net weight determined by removing hands from rachis and subtracting rachis weight from the gross weight of bunch.
[b] Average of the top and bottom three middle fruit of the third hand.
[c] Means followed by the same letter within a column are not significantly different according to the least significant difference test at $P = 0.05$.
[d] FHIA-01, FHIA-21, and TMB2x 9128-3 not included in statistical analysis of data.

Fig. 1. Example of a) resistant 'FHIA-18' and b) susceptible 'Grand Nain' accessions at about the same developmental stage assessed against black leaf streak (BLS) (*Mycosphaerella fijiensis* Morelet) in Isabela, PR.

Significant differences in the number of functional leaves were seen for both mother and first ratoon crops at flowering and at harvest with rather consistent rankings among the accessions in both crop cycles (Tables 2 and 3). 'Grand Nain' and 'SH 3640' had significantly higher numbers of functional leaves (>16) at flowering in the mother crop. In the ratoon, 'SH 3640' 'PV 42-53'; 'PV 42-81', 'FHIA-18', and 'FHIA-02' had higher numbers of functional leaves at flowering (>12). In both crop cycles, 'PA 03-22' had a significantly lower number of functional leaves.

Plant height and pseudostem diameter data were recorded at flowering and were significantly different among accessions in both cropping cycles (Tables 2 and 3). Although plants with taller

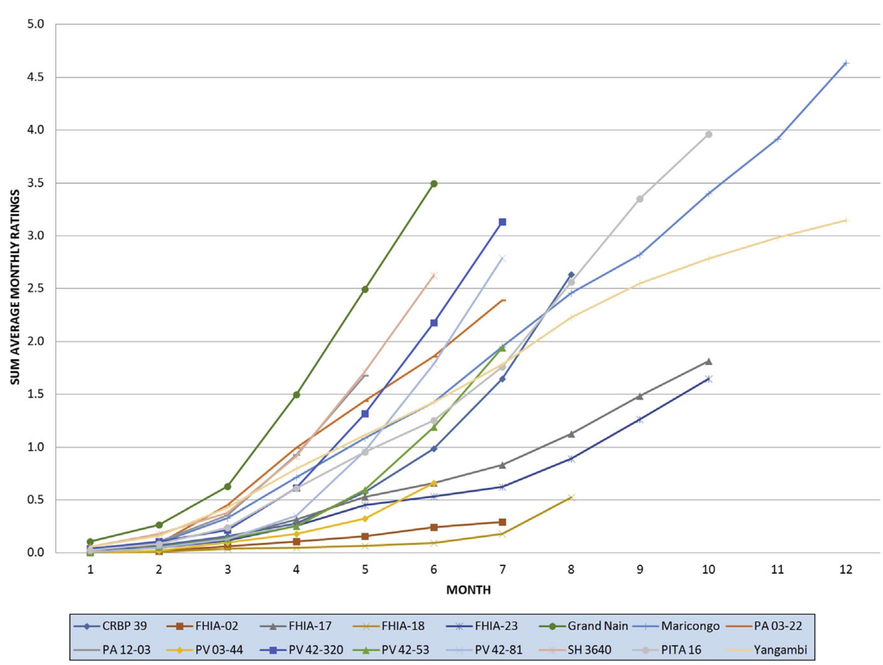

Fig. 2. Mother crop sum of the weighted average monthly disease index ratings for banana hybrids and standard cultivars being evaluated for tolerance to black leaf streak (*Mycosphaerella fijiensis* Morelet) in Isabela, PR. Ratings began 6 months after planting.

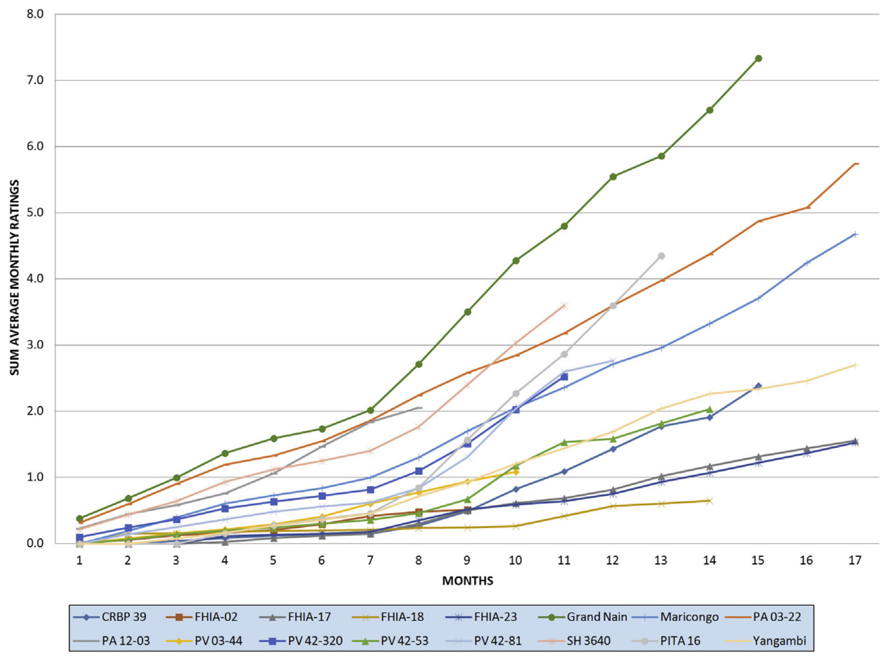

Fig. 3. First ratoon crop sum of the weighted average monthly disease index ratings for banana hybrids and standard cultivars being evaluated for tolerance to black leaf streak (*Mycosphaerella fijiensis* Morelet) in Isabela, PR.

pseudostems generally had wider pseudostems, this was not always the case. All accessions were taller in the second cycle with a similar pattern observed for stem diameter. In the mother crop, 'PV 42-320' was the tallest plant (4.01 m), but was not significantly different from 'PV 42-81' (3.75 m) and 'Maricongo' (3.72 m). The shortest accession was 'PA 12-03' (2.11 m). In the first ratoon, the tallest accession was 'PV 42-81' (5.02 m) and the shortest was 'Grand Nain' (2.58 m). In general, 'FHIA-17' and 'FHIA-23' had the widest pseudostems in the mother and ratoon cycle. 'PA 12-03' and 'PV 03-44' had significantly narrower pseudostems in the mother crop, whereas 'Maricongo', 'FHIA-02', 'Grand Nain', 'PA 12-03', 'PV 03-44', 'Yangambi' and 'CRBP 39' had narrower pseudostems (19.0–20.6 cm) in the ratoon cycle.

Although cultural practices included the removal of suckers every 3 months, the numbers of suckers produced per plant (as well as scars left from the removal of suckers) at flowering were counted. The number of suckers at flowering was significantly different among accessions for both cropping cycles, but inconsistent in order/rank (Tables 2 and 3).

At harvest, accessions differed in numbers of functional leaves, net bunch weight, numbers of hands per bunch and third hand traits (Tables 4 and 5). In the mother crop, 'FHIA-02' had a significantly higher number of leaves (10.5) than the rest of the accessions. In the first ratoon, 'FHIA-02' also had a high number of functional leaves (7.6), which was not significantly different from 'FHIA-18', 'PA 03-22' and 'PV 42-53'. Accessions with the fewest functional leaves in the mother crop included 'Grand Nain' (0.1), 'PITA 16' (0.2), 'PV 42-81' (0.2) and PV 42-320 (1.3). In the ratoon crop, 'PITA 16' and 'Grand Nain' had significantly fewer functional leaves, averaging only 0.9.

The cultural practice of removing false hands and male inflorescences as soon as possible influences bunch weight, number of hands, and third hand metrics. Net bunch weight, determined by subtracting the weight of the rachis from the total bunch weight, varied significantly among accessions and generally increased during the second cycle. In the mother crop, 'FHIA-23' and 'FHIA-17' had significantly higher bunch weights whereas 'PA 12-03' had the lowest bunch weight (Table 4). Similar results were obtained in the ratoon crop except that 'PA 03-22' had the lowest bunch weight. The lowest numbers of hands were found in the 'PV' accession series and ranged from 6.4 to 7.2 hands in the mother crop to 6.9–8.1 hands in the first ratoon. 'FHIA-23' had the highest number of fingers in the mother crop with 233.1, which was not significantly different from 'FHIA-17' with 221.3. In the first ratoon crop, 'FHIA-17' produced significantly more fingers (257.1) than all other accessions. For both production cycles, the 'Maricongo' plantain control produced significantly fewer fingers with 57 in both cycles.

Both 'FHIA-17' and 'FHIA-23' had significantly heavier third hands in the mother and ratoon cropping cycles (Tables 4 and 5). In both cycles, 'PA 03-22' had significantly lower third hand weight. In both cycles, 'Maricongo' had significantly longer fingers than other

Fig. 4. Field of view (40×) of *Mycosphaerella fijiensis* Morelet ascospores on water agar Petri dishes used in single ascospore isolations. Note characteristic one-septate ascospore with bipolar germination.

accessions whereas ‘PA 03-22’ had significantly shorter finger length values. ‘Maricongo’ had the widest fingers in both production cycles, but did not differ significantly from ‘FHIA-17’. In both cycles, ‘PA 03-22’ had the narrowest fingers, which were not significantly different from ‘PA 12-03’ in the mother crop. In sensory evaluations of fresh fruit, the accessions with pure *M. acuminata* genomes generally rated higher in flavor and quality.

3.4. *M. fijiensis* confirmation

A total of 40 randomly recovered *Mycosphaerella* single-ascospore isolates (Fig. 4) were tested with ITS species-specific primers (Johanson and Jeger, 1993). All 40 samples tested, as well as the internal standard positive control DNA sample, clearly amplified the diagnostic ~700 bp fragment for *M. fijiensis* (Fig. 5). Except for the internal positive DNA control, none of the 40 DNA samples from randomly recovered isolates produced an amplicon with the *M. musicola* specific primers.

4. Discussion

In Puerto Rico, bananas (*Musa* spp.) are among the few agricultural commodities that do not rely on imports to meet consumer demand. Since its arrival on the island, black leaf streak has seriously affected plantain and banana production. Integrated disease management practices are needed in Puerto Rico to sustain production of these important commodities. Unfortunately, cultural practices are only partially effective, and fungicides are expensive, can lose effectiveness due to resistance in pathogen populations, and may be harmful to the environment and human health. BLS-resistant hybrids would be a most useful addition to the limited number of measures that are available to manage this disease. To this end, several accessions that were evaluated in this study compared favorably to susceptible commercial standards. Their superior BLS tolerance and yields indicate that they could replace susceptible cultivars that are now used in Puerto Rico.

In general, the number of functional leaves at flowering and harvest reflected BLS rankings in that the most resistant (i.e., lowest disease index) had the highest number of functional leaves. Generally, the FHIA hybrids were consistently more resistant, and developed less disease between flowering and harvest than other accessions (i.e. had more functional leaves at harvest). These results agree with those of Romero and Sutton (1997) who reported that time for BLS symptom development (incubation period) was greater for ‘FHIA-02’ and ‘FHIA-01’ than for ‘Grand Nain.’ In addition to their superior performance against BLS, these accessions tolerate Fusarium wilt and the burrowing nematode, *Radopholus similis*, two important constraints that were not evaluated in the present study.

Although many accessions were more resistant to BLS than ‘Grand Nain’ and ‘Maricongo,’ some had intermediate reactions. Interestingly, the “resistant” control, ‘Yangambi km 5’, developed moderate levels of disease in both cycles (ca. 50% at harvest). This is similar to findings reported by Mouliom-Pefoura (1999), who indicated that this cultivar became susceptible in Cameroon 8 years after it was first determined to be resistant. Similar responses of ‘Yangambi km 5’ have been reported in other field studies (Alvarado-Capo et al., 2003; Fullerton and Olsen, 1995).

An unexpected result in this study was the decrease in disease indices from the mother to ratoon cycle. Environmental factors may have played a role in these differences, in that a period of high temperatures, relative humidity and precipitation immediately preceded the average flowering date for the mother crop. Average flowering date for the mother crop was 14 October, 2008 and 13 July, 2009 for the ratoon crop. Mean daily temperature (24.0 °C) and relative humidity (95.0%) were high for the three months preceding flowering of the mother crop. Average precipitation ($n = 30$ years) for the Isabela site is 153 mm (http://www.noaa.gov/), but was 314 mm for the month of September, 2008. In contrast, the three months preceding the average flowering date for the ratoon crop were cooler (23.6 °C) with a lower average humidity (84.2%). In addition, the precipitation recorded for the month preceding flowering in the ratoon crop was about half (153.4 mm) of what had been observed in the mother crop (*data not shown*). Previously, Gauhl (1994) and Meredith et al. (1973) had associated increased ascospore production for *M. fijiensis* with high relative humidity and rainfall.

Several agronomic traits are important in commercial banana and plantain production. Short cycling intervals between harvests are very desirable, but only one accession, ‘PA 12-03’, had shorter cycle times than ‘Grand Nain’ and ‘Maricongo.’ In contrast, many accessions had longer cycles, which appeared to be related to plant height and pseudostem diameter. For example, two of the largest accessions (‘FHIA-17’ and ‘FHIA-23’) took over 755 days to produce a mother and a ratoon crop. ‘FHIA-17’ and ‘FHIA-23’ produced the largest bunches, but were not affected by toppling due to their large pseudostems and rhizomes; however, their height and bunch weights made them difficult to harvest. Despite their BLS tolerance and high yields, the long cycle times and harvesting difficulties for ‘FHIA-17’ and ‘FHIA-23’ may impact their adoption in Puerto Rico.

Several other accessions performed well with moderate to high levels of disease tolerance and acceptable agronomic traits. For example, ‘Yangambi km 5’ showed commercial potential as it had an intermediate response to BLS and produced large bunches with many fruit, especially in the ratoon crop during which yield metrics exceeded previously reported performances for the cultivar (Daniells and Bryde, 1995; Vargas and Sandoval, 2007). Thus, ‘Yangambi km 5’ is a good potential substitute for Sucrier (AA) which is commercially produced and popular on the island.

Although not part of the experiment, ‘FHIA-21’ was highly tolerant, had a high number of functional leaves in both production cycles, and good bunch sizes and fruit numbers. Unfortunately, one of the plants of this accession did not produce a bunch in either cycle, apparently due to Banana streak virus (BSV). BSV has been reported in hybrids with the B genome, including ‘FHIA-21’ (Geering, 2009; Krikorian et al., 1998). ‘FHIA-21’ produces a ‘French-type’ bunch that requires de-handing and has fruit that is generally softer with a shorter shelf life (i.e., ripens quickly). These drawbacks may impede its adoption and/or replacement of BLS-susceptible plantain cultivars such as ‘Maricongo.’

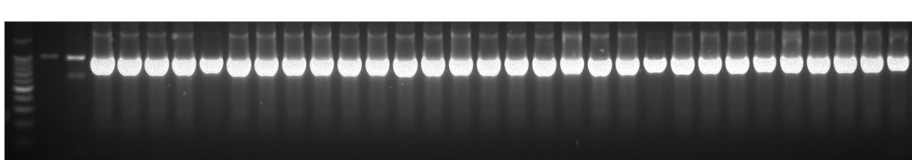

Fig. 5. Amplification of the 700 bp fragment of the Internal Transcribed Spacer (ITS) unit in 40 randomly single-ascospore isolate samples from a field evaluation trial in Isabela, PR. *Mycosphaerella fijiensis* Morelet specific primes utilized.

Lastly, several of the PV and PA hybrids from the EMBRAPA breeding program with Pome backgrounds (AAB) have been reported to resist Fusarium wilt. Although not screened against this disease in the present study, these cultivars may be good substitutes for the 'Manzano' (AAB) Silk group cultivar. The precocious and short 'PA 12-03' might be a good substitute for 'Manzano' and other sub-acid dessert bananas that are susceptible to Fusarium wilt.

Although several of the hybrids evaluated had excellent tolerance to BLS, their adoption could be limited by farmer and customer acceptability. A potential market may exist for these bananas in organic markets. In this regard, the first large scale commercial farm (Bananera Fabre, Sabana Grande, PR) was recently certified organic in Puerto Rico (Jose V. Fabre, *personal communication*). Preliminary data collected on sensory evaluations showed several of the hybrids evaluated in the study meet postharvest and consumer standards and could be incorporated into organic production systems. As many of the hybrids evaluated differ from those that consumers purchase and consume, a detailed postharvest and sensory evaluation was also performed as a separate study at the Food Science Department of the University of Puerto Rico and these data will be published in a subsequent paper.

It is thought that *M. fijiensis* spread to Puerto Rico from the neighboring Dominican Republic as the disease had been reported there previously (Ploetz, 2001). Wind-dispersed ascospores (Brown and Hovmoller, 2002) may have been responsible, as only 100 km separates the islands at their closest points. Another possible scenario would have been the introduction of infested host materials (Alamo et al., 2007).

None of the 40 *Mycosphaerella* spp. isolates randomly recovered from the experimental site were *M. musicola*. This is in contrast to a survey in 2005 in which several isolates of *M. musicola* were recovered from the farm on which this research was conducted. The replacement of *M. musicola* by *M. fijiensis* typically occurs soon after it is introduced to a new location in the lowland tropics (Carlier et al., 2000). A third leaf disease that produces similar symptoms, Eumusae leaf spot disease (caused by *Mycosphaerella eumusae*), is not present in the Western Hemisphere, but could cause significant damage if it is introduced (Carlier et al., 2000). Finally, Cordana leaf spot, caused by *Cordana musae* (A. Zimmerm.) Höhn, was noted on several of the BLS-resistant FHIA clones. However, since other hybrids were significantly more susceptible to BLS, their response to Cordana leaf spot may have been less noticeable.

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References

Alamo, C., Evans, E., Brugeras, A., Nalampang, S., 2007. Economic impact and trade implications of the introduction of black Sigatoka (*Mycosphaerella fijiensis*) into Puerto Rico. J. Agric. Appl. Econ. 39, 5–17.

Alvarado-Capo, Y., Leiva-Mora, M., Dita-Rodriguez, M.A., Acosta, M., Cruz, M., Portal, N., Gomez-Kosky, R., Garcia, L., Bermudez, I., Padron, J., 2003. Early evaluation of black leaf streak resistance by using mycelial suspensions of *Mycosphaerella fijiensis*. In: Jacome, L., Lepoivre, P., Marin, D., Ortiz, R., Romero, R., Escalant, J.V. (Eds.), *Mycosphaerella* Leaf Spot Diseases of Bananas: Present Status and Outlook, Proceedings of the Workshop on *Mycosphaerella* Leaf Spot Diseases, San José, Costa Rica, 20–23 May 2002, pp. 169–175.

Brown, J.K.M., Hovmoller, M.S., 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 297, 537–541.

Carlier, J., Mourichon, X., Jones, D.R., 2000. Sigatoka-like leaf spots: septoria leaf spot. In: Jones, D.R. (Ed.), Diseases of Banana, Abacá and Enset. CABI Publishing, New York, pp. 93–96.

Carlier, J., Waele, D., Escalant, J.V., 2002. Global evaluation of *Musa* germplasm for resistance to Fusarium wilt, Mycosphaerella leaf spot diseases and nematodes. In: Vézina, A., Picq, C. (Eds.), INIBAP Technical Guidelines 6. The International Network for the Improvement of Banana and Plantain, Montpellier, France.

Chillet, M., Abadie, C., Hubert, Q., Chilin-Charles, Y., Lapeyre, D., de Bellaire, L., 2009. Sigatoka disease reduces the greenlife of bananas. Crop Prot. 28, 41–45.

Churchill, A., 2011. *Mycosphaerella fijiensis*, the black leaf streak pathogen of banana: progress towards understanding pathogen biology and detection, disease development, and the challenges of control. Mol. Plant Pathol. 12, 307–328.

Craenen, K., 1998. Technical Manual on Black Sigatoka Disease of Banana and Plantain. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, p. 60.

Daniells, J., Bryde, N., 1995. Semi-dwarf mutant of Yangambi Km 5. InfoMusa 4, 16–17.

Dita, M., Waalwijk, C., Buddenhagen, I.W., Souza Jr., M.T., Kema, G.H.J., 2010. A molecular diagnostic for tropical race 4 of the banana fusarium wilt pathogen. Plant Pathol. 59, 348–357.

Dodds, K.S., Simmonds, N.W., 1948. Sterility and parthenocarpy in hybrids of *Musa*. Heredity 2, 101–117.

Etebu, E., Young-Harry, W., 2011. Control of black Sigatoka disease: challenges and prospects. Afr. J. Agric. Res. 6, 508–514.

FAOSTAT, 2010. http://faostat.fao.org/site/291/default.aspx (verified – Dec. 2012). FAO, Rome.

Fullerton, R.A., Olsen, T.L., 1995. Pathogenic variability in *Mycosphaerella fijiensis* Morelet, cause of black Sigatoka in banana and plantain. N. Z. J. Crop Hort. Sci. 23, 39–48.

Gauhl, F., 1994. Epidemiology and Ecology of Black Sigatoka (*Mycosphaerella fijiensis* Morelet) on Plantain and Banana (*Musa* spp.) in Costa Rica, Central America. INIBAP, Montpellier, France.

Gauhl, F., Pasberg-Gauhl, C., 1994. Epidemiology and ecology of black Sigatoka disease in Nigeria. Phytopathology 84, 100.

Gauhl, F., Pasberg-Gauhl, C., Jones, D.R., 2000. Black leaf streak: disease cycle and epidemiology. In: Jones, D.R. (Ed.), Diseases of Banana, Abacá and Enset. CABI Publishing, New York, pp. 56–62.

Geering, A.D.W., 2009. Viral pathogens of banana: outstanding questions and options for control. In: Jones, D., Van den Berg, I. (Eds.), Proceedings International Symposium Crop Protection, Sustainability, Production, and Improved Livelihoods, ActaHortic. 828, pp. 39–50.

Goenaga, R.J., Irizarry, H., 2000. Yield and quality of banana irrigated with fractions of class A pan evaporation on an Oxisol. Agron. J. 92, 1008–1012.

Heslop-Harrison, J.S., Shwarzacher, T., 2007. Domestication, genomics and the future for banana. Ann. Bot. 100, 1073–1084.

Irish, B.M., Goenaga, R., Ploetz, R., 2006. *Mycosphaerella fijiensis*, causal agent of black Sigatoka of *Musa* spp. found in Puerto Rico and identified by polymerase chain reaction. Plant Dis. 90, 684.

Irizarry, H., Goenaga, R., González, O., 2001a. Characterization and grouping of plantain clones on the basis of their genomic constitution and morphological traits of economic importance. J. Agric. Univ. P. R. 85, 105–126.

Irizarry, H., Goenaga, R., Gonzales, O., 2001b. Yield potential of the false-horn "Huamoa" plantain. J. Agric. Univ. P. R. 85, 33–40.

Irizarry, H., Rivera, E., Rodríguez, J.A., 1992. Bunch and ratoon management for profitable production of high quality bananas (*Musa acuminata*). J. Agric. Univ. P. R. 76, 119–130.

Johanson, A., Jeger, M.J., 1993. Use of PCR for detection of *Mycosphaerella fijiensis* and *M. musicola*, the causal agents of Sigatoka leaf spots in banana and plantain. Mycol. Res. 97, 670–674.

Jones, D.R., 2000. Sigatoka. In: Jones, D.R. (Ed.), Diseases of Banana, Abacá and Enset. CABI Publishing, New York, pp. 79–92.

Krikorian, A.D., Irizarry, H., Goenaga, R., Scott, M.E., Lockhart, B.E.L., 1998. Stability in plant and bunch traits of a `French-type' dwarf plantain micropropagated from the floral axis tip and five lateral corm tips of a single mother plant: good news on the tissue culture and bad news on banana streak virus. Sci. Hortic Amsterdam 81, 159–177.

Marin, D.H., Romero, R.A., Guzman, M., Sutton, T.B., 2003. Black Sigatoka: an increasing threat to banana cultivation. Plant Dis. 87, 208–222.

Meredith, D.S., Lawrence, J.S., Firman, I.D., 1973. Ascospore release and dispersal on black leaf streak disease of bananas (*Mycosphaerella fijiensis*). Trans. Brit. Mycol. Soc. 60, 547–554.

Mouliom-Pefoura, A., 1999. First observation of the breakdown of high resistance in Yangambi km 5 (*Musa* sp.) to the Black Leaf Streak Disease in Cameroon. Plant Dis. 83, 78.

Okoro, P., Shaibu, A.A., Ude, G., Olukolu, B.A., Ingelbrecht, I., Tenkouano, A., Ogburia, M.N., Moonan, F., Dimkpa, C., 2011. Genetic evidence of developmental components of parthenocarpy in Apomictic *Musa* species. J. Plant Breed. Crop Sci. 3, 138–145.

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., 1998. PITA-14: a black Sigatoka-resistant tetraploid plantain hybrid with virus tolerance. HortScience 33, 362–365.

Perrier, X., De Langhe, E., Donohue, M., Lentfer, C., Vrydaghs, L., Bakry, F., Careel, F., Hippolyte, I., Horry, J.-P., Jenny, C., Lebo, V., Risterucci, A.-M., Tomekpe, K., Doutrelepnt, H., Ball, T., Manwaring, J., Maret, de P., Denham, T., 2011. Multidisciplinary perspectives on banana (*Musa* spp.) domestication. Proc. Natl. Acad. Sci. U. S. A. 108, 11311–11318.

Ploetz, R.C., 2001. The most important disease of a most important fruit. Plant Health Instr.. http://dx.doi.org/10.1094/PHI-I-2001-0126-01. APSnet Education Center.

Ploetz, R.C., Kepler, A.K., Daniells, J., Nelson, S.C., 2007. Banana and plantain-an overview with emphasis on Pacific island cultivars, ver. 1. In: Elevitch, C.R. (Ed.), Species Profiles for Pacific Island Agroforestry. Permanent Agriculture Resources (PAR), Hōlualoa, Hawai'i. http://www.traditionaltree.org.

PRAD - Puerto Rico Agriculture Department, 2012. Gross Agricultural Income for Puerto Rico 2010/2011. Agricultural Statistics Office, San Juan, Puerto Rico, 2009.

PRAES - Puerto Rico Agricultural Experiment Station, 1995. Technological Package for the Production of Plantains and Bananas. Agric. Exp. Stn. Publ. 97. College of Agricultural Sciences, U. Puerto Rico, Mayaguez.

Romero, R.A., Sutton, T.B., 1997. Reaction of four *Musa* genotypes at three temperatures to isolates of *Mycosphaerella fijiensis* from different regions. Plant Dis. 81, 1139–1142.

Rosales, F., Aranud, E., Coto, J., 1999. A Catalogue of Wild and Cultivated Bananas – a Tribute to the Work of Paul Allen. International Network for the Improvement of Bananas and Plantain, Montpellier, France.

Rowe, P., Rosales, F., 1993. Diploid breeding at FHIA and the development of Goldfinger (FHIA-01). Infomusa 2, 9–11.

Simmonds, N.W., 1962. The Evolution of the Bananas. Longmans, London.

Sharrock, S., 1990. Collecting *Musa* in Papua New Guinea. In: Jarret, R.L. (Ed.), Identification of Genetic Diversity in the Genus *Musa*. International Network for the Improvement of Bananas and Platain, Montpellier, France, pp. 140–157.

Stover, R.H., 1976. Distribution and cultural characteristics of the pathogens causing banana leaf spots. Trop. Agric. 53, 111–114.

Stover, R.H., Simmonds, N.W., 1987. Bananas, third ed. Longman Scientific and Technical, Essex, England.

Swennen, R., Vuylsteke, D., 1993. Breeding black Sigatoka resistant plantains with a wild banana. Trop. Agric. 70, 74–78.

Vargas, A., Sandoval, J.A., 2007. Agronomic evaluation of production and quality of 'Yangambi km 5' (AAA) and 'Datil' (AA). InfoMusa 14, 6–10.

Vuylsteke, D.R., 1989. Shoot-tip Culture for the Propagation, Conservation and Exchange of *Musa* Germplasm. International Institute of Tropical Agriculture, Ibadan, Nigeria, p. 82.

Vuylsteke, D., Ortiz, R., 1995. Plantain-derived diploid hybrids (TMP2x) with black Sigatoka resistance. HortScience 30, 147–149.

Yan, J.Y., Quan, Q.X., Long, X., Peng, H.X., Berg, I.V., Smith, M., Swennen, R., Hermanto, C., 2012. Collecting and conserving Musa germplasm in Guangxi, China. Acta Hortic. 897, 255–257.