Author's post-print of the paper published in the Proceedings of the International ISHS-ProMusa Symposium on Recent Advances in Banana Crop Protection for Sustainable Production and Improved Livelihoods, Acta Horticulturae 828, ISHS 2009. Available from www.musalit.org

New Approaches to Select Cultivars of Banana with Durable Resistance to Mycosphaerella Leaf Spot Diseases

C. Abadie[1], Y. Chilin-Charles[1], J. Huat[2], F.Salmon[3], L. Pignolet[4], J. Carlier[4], T.Lescot[4], F.Côte[4] and C. Jenny[1]

[1]CIRAD, Station de Neufchâteau, Sainte-Marie, Capesterre Belle-Eau, 97130, Guadeloupe, France
[2]CIRAD, Station de Dembeni, BP 1304 - 97600 Mamoudzou - Mayotte
[3]CIRAD, PRAM, Le Lamentin, F-97232, Martinique, France
[4]CIRAD, TA B-26 / PS4, Boulevard de la Lironde, F-34398, Montpellier Cedex 5, France

Key words: Banana hybrids, natural and laboratory conditions, resistance durability, screening.

Abstract

Mycosphaerella leaf spot diseases of banana caused by *Mycosphaerella musicola* (Sigatoka leaf spot, SLS) and *M. fijiensis* (black leaf streak, BLS) are very destructive. The cultivation of resistant banana clones appears to be the most appropriate means to combat disease because genetic resistance is usually more durable than chemical control. Since pathogen populations maintain a high level of genetic diversity and single gene resistances, such as is suspected in total or hypersensitive resistance situations, are easily overcome, the phenomenon of 'partial resistance', which may be multigenic and hence more durable, is used in CIRAD breeding programmes. New hybrids of banana developed by CIRAD are routinely selected for resistance against Mycosphaerella leaf spot diseases. There are four steps in the evaluation process. Firstly, the level of resistance to *M. musicola* is assessed under field conditions in Guadeloupe. Secondly, the most resistant hybrids are selected for detached-leaf resistance tests to *M. musicola* and *M. fijiensis* under controlled conditions. Thirdly, the efficacy of partial resistance of selected hybrids against *M. musicola* is confirmed in the field in the French West Indies and against *M. fijiensis* in the field in Mayotte. Finally, the durability of the hybrids' partial resistance is assessed by measuring disease severity in experimental plots for several years. This complete 4-steps process allows, through experimental approaches, to select banana cultivars with durable resistance against Mycosphaerella leaf spot diseases.

INTRODUCTION

Mycosphaerella leaf spot diseases are the most damaging and costly in commercial banana production (Jones, 2000) as they reduce yields and fruit quality. Only a few types of dessert cultivars are grown in monoculture for export production, and most belong to the Cavendish subgroup, which is very susceptible to leaf spot. Crops in the major banana-producing countries in the Latin American-Caribbean region and Africa are affected by *Mycosphaerella fijiensis*, which causes black leaf streak (BLS). Few banana-producing countries remain free of BLS, and these are usually affected by Sigatoka leaf spot disease (SLS), caused by *M. musicola*. BLS and SLS are caused by ascomycete fungi that are spread naturally by wind. The pathogen populations are known to be genetically diverse due to the occurrence of sexual reproduction and high population densities. Studies using neutral molecular markers have shown that genetic differentiation occurs at global, continental and local population levels for *M. fijiensis* (Carlier et al., 1996 ; Rivas et al. 2004) and *M. musicola* (Hayden et al., 2003). Pathogenic variability also occurs in these fungal populations (Abadie et al., unpublished).

In commercial plantations, chemical disease control is the only available control method against these diseases. In one year, between 10 and 60 aerial fungicide sprays are applied depending on climatic conditions, spraying strategy (calendar applications or use of forecasting system) and levels of susceptibility of fungal populations. However, fungicide resistance develops rapidly. Moreover, such high frequencies of fungicide applications are unsustainable in an environmental, economic and legislative context, particularly in isolated areas like the French West Indies (de Lapeyre et al., 2009). The use of resistant banana cultivars is considered to be the most appropriate and durable control method. Conventional breeding techniques, based on a sound knowledge of *Musa* diversity and genetics, have been utilised in Guadeloupe for the last decade to develop banana hybrids with resistance against Mycosphaerella leaf spot diseases (Jenny et al., 2003). Hybrids are selected according to their resistance to SLS and BLS, fruit quality and agronomic performance. The breeding program aims at developing triploid hybrids by crossing diploids with resistant tetraploids derived from colchicine treatments (Bakry et al., 2007). Two kinds of resistance -high and partial- have been recognised in interactions between *Musa* spp. and Mycosphaerella leaf spot diseases (Fouré et al., 2000). In many host-pathogen interactions, partial resistance is believed to be the most durable. Thus, partially resistant germplasm is used in the CIRAD banana breeding program. The process (divided in four successive steps) undertaken to select banana hybrids with durable resistance to BLS and SLS is described here.

MATERIALS AND METHODS

Step 1: Selection of Hybrids with Resistance to SLS

Hybrids were selected for their resistance to SLS by measuring disease reaction under conditions of high natural inoculum pressure in the field at Neufchâteau (CIRAD experimental centre) in Guadeloupe. Hybrids were planted in a randomised design in quincunx, with each hybrid surrounded by four plants of SLS-susceptible ‘Grande Naine’ (AAA genome; Cavendish subgroup), at a density of 2000 plants/ha (2 x 2.5 m) in five replications. No fungicides were applied to the plots.

Suckers of 61 hybrids, produced from 20 different crossings using 15 different parents, were planted on 22/07/2005. Disease levels were evaluated during three to five crop cycles from 02/02/2006 to 26/06/2007. The youngest leaf spotted (YLS) and the total number of leaves per plant (TNL) were measured twice a month (Carlier et al, 2003). This allowed an index of non-spotted leaves (INSL) to be calculated as follows: INSL = (YLS-1)/TNL. This parameter is the proportion of standing leaves without any typical late-stage symptoms (necrotic spots). The average of each parameter was calculated with the data of the first three cycles. A statistical analysis (ANOVA) was calculated for data collected on 22/05/2006 (representative of the second cycle) using the software StatBox 6.6, and the accessions were ranked with a Bonferroni test at 5%.

Step 2: Characterisation of the Resistance of Five Selected Hybrids to SLS and BLS

The evaluation of resistance against BLS and SLS of some hybrids and their parents was conducted in controlled conditions by inoculation of banana leaf pieces. Three strains of *M. fijiensis* and *M. musicola*, with different levels of aggressiveness, were inoculated separately onto leaf fragments (25 cm²) cut from 5-7 month old plants of five hybrids ('FlhorBan 916', 'Flhorban 917', 'Flhorban 918', 'Fhlorban 919' and 'FlhorBan 920') derived from tissue culture. Two inoculations were undertaken, with three replicates/strain/hybrid combination. The banana leaf pieces were maintained on a culture medium amended with 50 ppm of the antioxidant benzimidazole (El Hadrami et al., 1998; Abadie et al., 2005). The number of lesions on each leaf fragment and the size of these lesions (five replicates) were scored from the first lesion appearance (after about 18 days) to lesion coalescence or leaf death (after about 70 days). The maximum value for these two parameters for each strain/hybrid combination was used to compare the interactions.

Step 3: Evaluation of the Efficacy of Resistance of Three Selected Hybrids to SLS and BLS

The efficacy of the resistance of hybrids to SLS was evaluated under field conditions in the French West Indies and to BLS in field trials in Mayotte from July 2006 onwards as follows: (1) a plot of 110 plants of 'FlhorBan 918' in Guadeloupe (Neufchâteau), (2) a plot of 130 plants of 'FlhorBan 916' in Martinique (Lamentin-CIRAD experimental centre) and (3) two plots of 27 plants of 'FlhorBan 918' and 36 plants of 'FlhorBan 920' in Mayotte. A plot of the susceptible Cavendish cultivar 'Grande Naine' was cultivated close to each hybrid plot in each location. No fungicide was applied on the hybrids plots. Because the trials in the French West Indies were maintained under commercial conditions, the susceptible 'Grande Naine' plot was treated with fungicide to protect against SLS. However, no fungicides were used in Mayotte. The resistance efficacy was evaluated through the measurements of YLS and TNL described above and also the score of the disease severity using a scale of 7 grades as described by Carlier et al. (2003). The INSL was calculated as described above. The parameters were measured during two to three cycles, and the values for each hybrid were compared with the values for the control 'Grande Naine'.

To complete the characterisation of the hybrids' partial resistance, the sexual sporulation capacities (amount of perithecia per cm²) were evaluated on necrotic banana samples collected in hybrids plots.

Step 4: Evaluation of the Durability of the Resistance of 'Flhorban 920' to SLS and BLS

The evaluation of the durability of resistance of one selected hybrid ('Flhorban 920') to SLS was undertaken by measuring the disease levels of 200 plants from 2004 onwards in a trial plot in Guadeloupe (Neufchâteau) and on two hectares of a commercial banana plantation in Martinique (Bellevue).

RESULTS AND DISCUSSION

Step 1: Selection of Hybrids for Resistance to SLS

The inoculum pressure was high during the observation period with an average YLS of 6 and INSL of 55% for the susceptible control 'Grande Naine' over three cycles. The average YLS of the 61 hybrids over three cycles varied from 6.2 to 16 and the INSL from 55 % to 100%. Fifty-nine hybrids expressed at least once a necrosis, which means a compatible reaction as defined by Fouré et al. (2000). The reaction of the hybrids was characterised as follows: susceptible for INSL <70 % and YLS <8; partial resistance for INSL 70-90% and YLS 9-10; strong partial resistance for INSL >90% and YLS >11, and high resistance when no necrosis was observed during the three cycles. The boundaries of each characterisation were not strict and were determined according to knowledge of the disease in this location and comparison with the disease level on the susceptible control 'Grande Naine'. Only eight hybrids (13%) were susceptible having a reaction similar to 'Grande Naine'. Other hybrids expressed partial resistance varying from low partial resistance (21%) to strong partial resistance (62%) (Table 1). Necrotic spots were never observed on two hybrids (3%), and these were classified as highly resistant. Because of the occurrence of some variability in the data set, the classification of the hybrids showing partial resistance is not definitive.

The statistical analysis demonstrated that the INSL (measured on 22/05/2006) was significantly different between the two extreme classes of hybrid reaction (one very susceptible and similar to the control and the other very resistant). It also showed that the reaction of intermediate classes corresponded to low partial resistance (Fig. 1). Of the 61 hybrids tested, 40 had strong partial resistance. However, most of these hybrids had poor agronomic and fruit quality parameters, and only five were selected for further investigation.

Step 2: Characterisation of the Resistance of Five Selected Hybrids to SLS and BLS

The artificial inoculation of leaf squares cut from five selected hybrids allowed their partial resistance to *M. musicola* and *M. fijiensis* to be evaluated under laboratory conditions. For the susceptible Cavendish control, the maximum number of lesions counted on the leaf squares (mean of lesions caused by three strains of *M. fijiensis*) varied from 75 to 150 with an average of 117 (Fig. 2). The maximum number of lesions was scored on 'Pisang Madu' (PMA). Due to high variability in lesions measurements, no significant differences were found for lesion numbers.

The maximum area of a lesion was significantly lower on the hybrids and parents, varying from 3 to 8 cm^2, than on the Cavendish control, with an average area/lesion of 14 cm^2. The five hybrids expressed partial resistance to BLS, which was mainly due to a slow lesion development. Similar results were obtained for SLS (data not shown). For 'FlhorBan 918', a higher number of lesions was scored, but the average surface of a lesion (2.7 mm^2) was three times smaller than on the Cavendish control (8 mm^2) (Fig. 3). Its parent had the same behaviour, which suggests a good inheritance of this trait. As in the field, the disease intensity was lower for SLS than for BLS.

Previous studies have shown the occurrence of different types of resistance in cultivars or wild bananas (Abadie et al., 2003). The presence of different resistance components in a hybrid could have a strong impact on resistance durability. In this study, two different measurements of resistance –number of lesions and lesion area– were evaluated for five hybrids. Results showed that the partial resistance to BLS and SLS mainly occurred through a slow lesion development. However, the data showed high variability due to visual scoring. New tools (such as a quantitative molecular approach) are being developed to improve the resistance characterisation, especially in the context of strong partial resistance.

The sexual sporulation capacities could not be scored on the five hybrids under field conditions because no leaf necrosis appeared on any hybrid. Disease development was so slow that the infection cycle was not complete.

Step 3: Evaluation of the Efficacy of Resistance of Three Selected Hybrids to SLS and BLS

Results showed a high inoculum pressure at the three locations used for screening with an average disease severity on the susceptible 'Grande Naine' of 7, 12 and 43% in Martinique (SLS), Guadeloupe (SLS) and Mayotte (BLS) respectively. The higher disease severity in Mayotte could be explained by a shorter infection cycle for BLS than for SLS and because the control plot in Mayotte was not treated with fungicide.

The three hybrids were resistant to SLS and BLS. No necrosis was observed on the hybrids. The average number of leaves/plant was 11 for 'FlhorBan 916', 9-10 for 'FlhorBan 918' (in Mayotte and Guadeloupe) and 10.3 for 'FlhorBan 920'. The INSL reached 100% for the three hybrids during the 4^{th} cycle in Martinique, and the three first cycles in Mayotte and Guadeloupe (Fig. 4). The mean INSL of the susceptible 'Grande Naine' plot varied from 65 and 70% in Martinique and Guadeloupe to 28% in Mayotte.

The similar reaction of the hybrids to SLS and BLS is explained by the choice of parents resistant to BLS and the early selection of hybrids for resistance to SLS. The resistance of the hybrids characterised by a very slow lesion development did not allow the occurrence of a complete infection cycle. In Guadeloupe, the average youngest leaf with young lesions was 8.1 on 'FlhorBan 918' (data not shown) with a total leaf number of 10. This did not allow lesions to develop into necrotic lesions until leaves were senescing. As a result, there was a very low production of inoculum on the hybrids. This could reduce the inoculum pressure in a banana production zone. Further selection will be based on agronomic traits.

Step 4: Evaluation of the Durability of the Resistance of 'Flhorban 920' to SLS

The durability of the resistance of 'FlhorBan 920' to SLS was evaluated over several years. Results showed a high disease pressure in Guadeloupe with an average severity varying from 25 to 32% on the susceptible 'Grande Naine' control during four cycles (Table 2). Little necrosis was observed on 'FlhorBan 920' during the first cycle, and the disease severity was nil (no necrosis) during the other cycles. On the commercial plantation in Martinique, the disease severity has been zero since 2004 without any fungicide treatment (data not shown).

The resistance of 'FlhorBan 920' to SLS seems to be durable in the short term. However, the experimental conditions (small size of the trial plot) did not allow creating a selection pressure high enough for an evolution (through a higher level of aggressiveness) of *M. musicola* populations. The cultivation of 'T8' and its diploid parent 'Paka' in the Cook Islands over 8 years allowed the pathogen to overcome the resistance of these cultivars (Fullerton and Olsen, 1995).

CONCLUSION

This paper outlines the four successive steps that have been undertaken to select banana hybrids with durable resistance to SLS and BLS. The first step consisted of the selection of hybrids resistant to SLS in the field. The second step screened hybrids for their resistance to BLS under laboratory conditions and characterised their resistance to BLS and SLS. The third step tested the efficacy of resistance to the two diseases displayed by the hybrids under field conditions and the last step focused on the stability of resistance over several years.

These steps facilitated the selection of three SLS- and BLS-resistant hybrids ('FlhorBan 916', 'FlhorBan 918' and 'FlhorBan 920'). This resistance, which is associated with a slow lesion development, has now been durable for 3.5 years. The selection of disease resistance is based on an experimental approach. A complementary approach based on modelling (disease simulation) is being developed to test the efficacy of many types of disease resistance and to study their durability over several years.

ACKNOWLEDGEMENTS

Thanks to C. Dubois and J.F. Côte for statistical analyses and to A. Pieyre for data calculations from Guadeloupe.

Literature Cited

Abadie, C., Elhadrami, A., Fouré, E. and Carlier, J. 2003. Efficiency and durability of partial resistance against black leaf streak disease. Proc. *Mycosphaerella* Leaf Spot Diseases of Bananas International Workshop, San José, Costa-Rica 20-23 May. p. 161-168.

Abadie, C., Pignolet, L., Elhadrami, A., Habas, R., Zapater, M.F. and Carlier, C. 2005. Inoculation avec *Mycosphaerella* sp., agent de cercosporioses, de fragments de feuilles de bananiers maintenus en survie. Cahier des Techniques de l'INRA ; Numéro spécial "Méthodes d'appréciation du comportement variétal vis-à-vis des bioagresseurs". p. 131-134.

Bakry, F., Paulo de la Reberdière, N., Pichot, S. and Jenny, C. 2007. In liquid medium colchicine treatment induces non chimerical doubled-diploids in a wide range of mono- and interspecific diploid banana clones. Fruits 62:3-12.

Carlier, J., Lebrun, M.H., Zapater, M.F., Dubois C. and Mourichon, X. 1996. Genetic structure of the global population of bananas black leaf streak fungus *Mycosphaerella fijiensis*. Molecular Ecology 5:499-510.

Carlier, J., De Waele, D. and Escalant, J.V. 2003. Global Evaluation of *Musa* Germplasm for Resistance to *Fusarium* Wilt, *Mycosphaerella* Leaf Spot Diseases and Nematodes. Performance Evaluation. INIBAP Technical Guidelines 7. A. Vézina and C. Picq (eds.), Montpellier, France.

de Lapeyre de Bellaire, L., Essoh Ngando, J., Abadie, C., Chabrier, C., Blanco, R., Lescot, T., Carlier, J. and Côte, F. 2009. Is chemical control of Mycosphaerella foliar diseases of banana sustainable? Acta Horticulturae, this volume.

El Hadrami, A., Zapater, M.F., Lapeyre, F., Abadie, C. and Carlier, J. 1998. A leaf disk assay to assess partial resistance of banana germplasm and agressiveness of *Mycosphaerella fijiensis*, the causal agent of black leaf streak disease. Proc. 7th International Congress of Plant Pathology, Edinburgh, Scotland. 9-16 August. BSPP vol. 2, p. 1.1.24.

Fouré E., Mourichon, X. and Jones, D. 2000. Evaluating germplasm for reaction to black leaf streak. p.62-72. In: D.R. Jones (ed.), Diseases of Banana, Abaca and Enset, CABI Publishing, Wallingford, UK.

Fullerton, R.A. and Olsen, T.L. 1995. Pathogenic variability in *Mycosphaerella fijiensis* Morelet cause of black Sigatoka in banana and plantain. New Zealand Journal of Crop and Horticultural Science 23:39-48.

Hayden, H., Carlier, J. and Aitken, E. 2003. Population differentiation in the banana leaf spot pathogen *Mycosphaerella musicola*, examined at a global scale. Plant Pathology 52:713-719.

Jenny, C., Tomekpe, K., Bakry, F. and Escalant, J.V. 2003. Conventional breeding of bananas. Proc. *Mycosphaerella* Leaf Spot Diseases of Bananas International Workshop, San José, Costa-Rica 20-23 May. p. 199-208.

Jones, D. 2000. Diseases of Banana, Abaca and Enset. CABI Publishing, CAB International, Oxon, UK.

Rivas, G., Zapater, M.F., Abadie, C. and Carlier, J. 2004. Founder effects and stochastic dispersal at the continental scale in the fungal pathogen of banana *Mycosphaerella fijiensis*. Molecular Ecology 13:471-482.

Tables

Table 1. Summary of the different reaction phenotypes of 61 banana hybrids challenged by *Mycosphaerella musicola* in the field in Guadeloupe.

Reaction phenotype *	Number of hybrids	% of total
Susceptible	8	13.1
Partial resistance	13	21.3
Strong partial resistance	38	62.3
High resistance	2	3.3

* Reaction phenotype was determined from the index of non-spotted leaves (INSL) and the youngest leaf spotted (YLS) as follows: susceptible phenotype when INSL <70 % and YLS 6-8, partial resistance when INSL between 70 and 90% and YLS 9-10, strong partial resistance when INSL >90% and YLS >11, high resistance without any necrotic leaf spots.

Table 2. Severity of Sigatoka leaf spot disease (% necrotic leaf surface) on the banana hybrid 'FlhorBan 920' compared to susceptible 'Grande Naine' over four crop cycles.

Cultivar	1st cycle	2nd cycle	3rd cycle	4th cycle
'Grande Naine'	32	28.8	25.6	30.1
'FlhorBan 920'	4	0	0	0

Figures

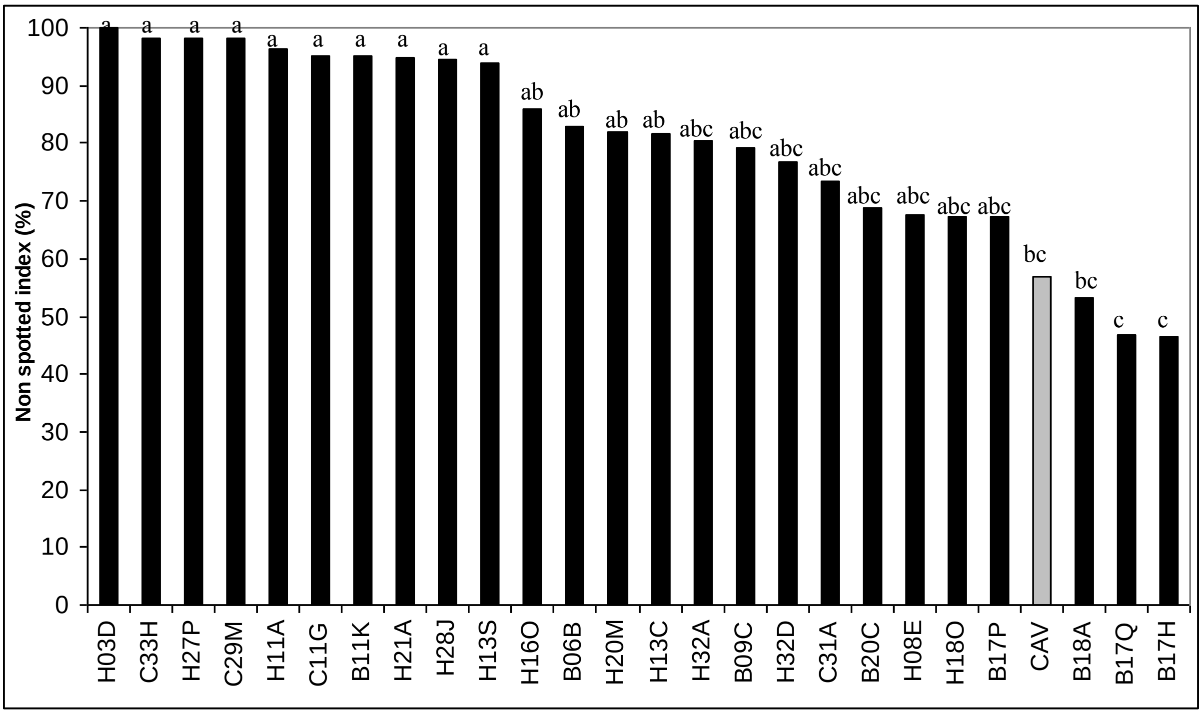

Fig. 1. Index of non-spotted leaves (INSL) for 25 banana hybrids and a susceptible 'Grande Naine' control (CAV) exposed to Sigatoka leaf spot disease under field conditions in Guadeloupe.

Average of INSL measured on five replicates on 22 May 2006. Means followed by the same letter are not statistically different according to Bonferroni's test (p = 0.05)

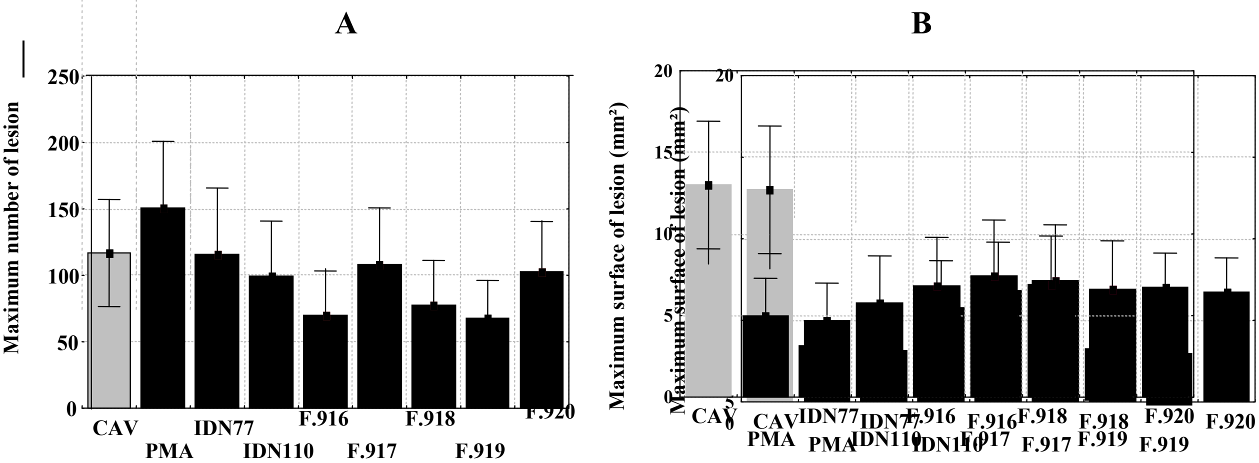

Fig. 2. Evaluation of the resistance of five hybrids ('FlhorBan 916', 'FlhorBan 917', 'Flhorban 918', 'FlhorBan 919' and 'FlhorBan 920') and three parents ('PMA', 'IDN77' and 'IDN110') to Black leaf streak disease compared to the susceptible 'Grande Naine' (CAV) control. (A) Maximum number of lesions on 25 cm² leaf fragment inoculated individually with three strains of *Mycosphaerella fijiensis*; (B) Maximum area of one lesion on 25 cm² leaf fragment inoculated with three strains of *M. fijiensis*.

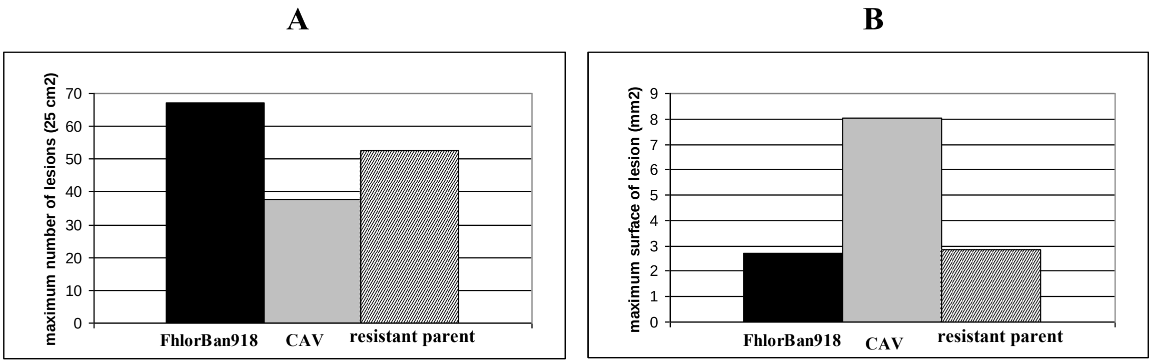

Fig. 3. Evaluation of the resistance of ‘FlhorBan918’ to Sigatoka leaf spot compared to its resistant parent and a susceptible ‘Grande Naine’ control (CAV). (A) Maximum number of lesions on 25 cm² leaf fragment inoculated with three strains of *Mycosphaerella musicola*; (B) Maximum surface area of one lesion on 25 cm² leaf fragment inoculated with 3 strains of *M. musicola*.

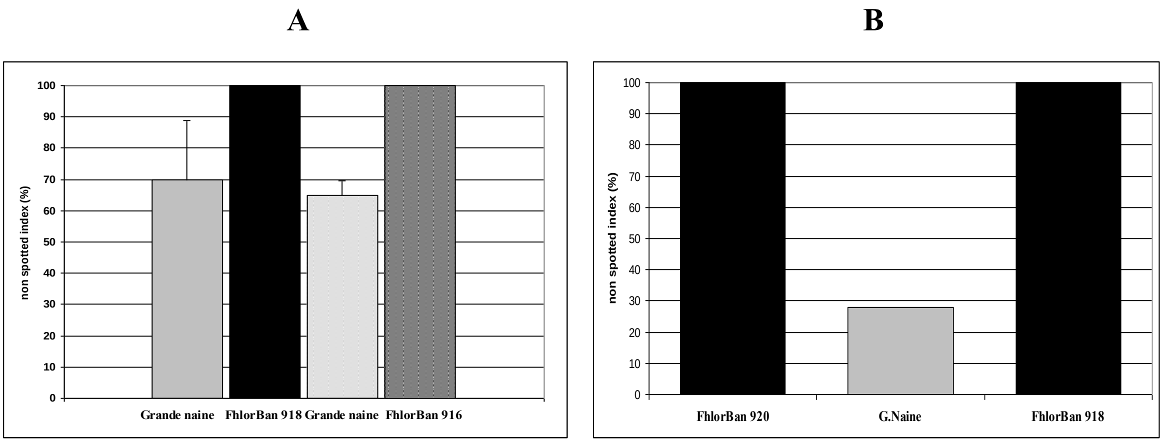

Fig. 4. Efficacy of the resistance of banana hybrids ‘FlhorBan 916’and ‘FhlorBan 918’ to Sigatoka leaf spot (SLS), and ‘FlhorBan 918’ and ‘Fhlorban 920’ to Black leaf streak as demonstrated under field conditions by average index of non spotted leaves (= % green leaves/plant): (A) Germplasm screened against SLS for three crop cycles in Guadeloupe (solid columns) and for four crop cycles in Martinique (hatched columns); (B) Germplasm screened against SLS for two crop cycles in Mayotte. Scores of the Cavendish cultivar ‘Grande Naine’ are shown as a susceptible control.