RESPONSE TO JUGLONE TOXIC EFFECT IN VARIOUS GENOTYPES OF BANANA (MUSA AA, AAA, AAB, AAAA, AAAB)

E. G. D. L. AMARI[1], E. DICK[1], D. KONE[1], K. ABO[2] et K. KOBENAN[3]

[1]Université de Cocody-Abidjan, UFR Biosciences, Laboratoire de Physiologie Végétale, 22 BP 582 Abidjan 22, Côte d'Ivoire. E mail : amariler@yahoo.fr

[2]Laboratoire de Phytopathologie et de Biologie Végétale, INP-HB, BP 1313 Yamoussoukro, Côte d'Ivoire

[3]Centre National de Recherche Agronomique (CNRA), Station de Recherche de Bimbresso, Laboratoire de Phytopathologie, 01 BP 1536 Abidjan 01 Côte d'Ivoire.

ABSTRACT

In Côte d'Ivoire, Black Sigatoka, caused by *Mycosphaerella fijiensis* Morelet, is a major constraint in banana plantain production. The use of juglone, a purified toxin of this pathogen, could constitute an alternative in the early selection of new banana genotypes resistant to Black Leaf Streak Disease (BLSD). Local cultivars and hybrids to be vulgarized, were evaluated in order to elucidate the variability of banana varieties and to assess the possibility of using juglone to breed for resistance to BLSD. Necrosis induction bioassays, electrolyte leakage, combined with phenolic compound quantifications were performed on both diploid and triploid reference cultivars, as well as tetraploid hybrids following juglone infiltration. Results show, based on minimal concentrations and intensities of necrosis, that genotypes sensitivity to juglone was generally related to susceptibility to the disease in the field. The tetraploid genotypes exhibited the lowest necrosis intensities when juglone concentration was less than 100 ppm. Forty eight hours after treatment, phenols content of FHIA23 and PITA14 hybrids was at a basal level. These tetraploid banana probably produced antioxidants with high affinity for the active oxygen species (AOS) inducing their tolerance to the disease. This study confirms the use of juglone for simple and rapid screening of banana varieties with resistance to BLSD. Pathological and physiological parameters related to biochemical evaluation, constitute the best strategy for identifying genotype with the greatest resistance to *Mycosphaerella fijiensis*. However, this great resistance is not systematically related to a higher content in total phenols.

Key words : Differential reaction, juglone, black leaf streak disease, phenolic compounds.

RESUME

REPONSE A L'EFFET TOXIQUE DE LA JUGLONE DE DIVERS GENOTYPES DE BANANIERS (MUSA AA, AAA, AAB, AAAA, AAAB)

Mycosphaerella fijiensis *est un parasite nécrotique occasionnant la cercosporiose noire, qui constitue l'une des contraintes majeures à la production de bananes desserts et de plantains en Côte d'Ivoire. L'utilisation de la juglone, un métabolite toxique purifié du pathogène a été envisagée comme une voie de sélection précoce de nouveaux génotypes de bananiers résistants à la maladie des raies noires (MRN). Des cultivars locaux et des hybrides à vulgariser ont été évalués en vue d'élucider la variabilité de réaction entre génotypes et de confirmer la possibilité d'utiliser la juglone pour le criblage de la résistance contre la MRN. Les tests d'induction de nécroses et de perte d'électrolytes associés au dosage quantitatif des composés phénoliques ont été réalisés sur des cultivars diploïdes et triploïdes, ainsi que des hybrides tétraploïdes infiltrés avec la juglone. Les intensités et les concentrations minimales d'induction des nécroses évaluées ont montré que la sensibilité à la juglone des génotypes de bananiers a été globalement identique à leur niveau d'infestation au champ. Pour les génotypes tétraploïdes, les intensités de nécroses ont été plus faibles (< 40 %) lorsque la concentration de juglone a été inférieure à 100 ppm. En outre, 48 h après traitement, les hybrides FHIA23 et PITA14, ont présenté un retour à la teneur initiale en phénols. La forte tolérance de ces hybrides à la maladie serait due à une synthèse d'antioxydants, à forte affinité pour les formes actives d'oxygène (FAO). Ces résultats confirment l'utilisation de la juglone pour le criblage simple et rapide de génotypes de bananiers résistants à la MRN. Une combinaison des paramètres pathologique, physiologique et biochimique constitue une stratégie idéale pour le criblage fiable du génotype le plus résistant à Mycosphaerella fijiensis. Cependant, cette grande résistance n'est pas systématiquement liée à une teneur plus élevée en phénols totaux.*

Mots Clés : *Réaction différentielle, juglone, maladie des raies noires, génotypes de bananiers, composés phénoliques.*

INTRODUCTION

In many areas of banana and plantain production, Black Leaf streak disease (BLSD), caused by *Mycosphaerella fijiensis*, is the most important constraint to bananas growth in West Africa (Vuylsteke *and al*., 1993). Symptoms caused by BLSD included an important drying of the leaves with disturbance of the photosynthetic assimilation process, thereby inducing a substantial reduction in plant growth and an early fruits ripening (El Hadrami, 2000).

Among various techniques available, genetic resistance is the most suitable for small and resource-poor farmers to fight against the disease (Ortiz *and al*., 1995). Thus, the sensitivity of banana hybrids provided by genetic improvement programs is evaluated under natural conditions of infestation. But, in addition to the slowness and the variability of results obtained, when high numbers of plants were tested, space become a major limiting factor, since each plant requires a large surface area. Therefore, various artificial inoculation methods have been developed, using young tissue-culture plants under greenhouse conditions (Pasberg-Gauhl, 1989). However, these methods are laborious and expensive, because they require sophisticated laboratory equipment and greenhouse facilities. Moreover, *M. fijiensis* is a very slow growing fungus under culture conditions and it is time-consuming to produce enough inoculum (Pasberg-Gauhl, 1989).

To over-come these constraints, it is necessary to develop simple and rapid methods of evaluating plant resistance to BLSD. Indeed, elongated necrotic lesions surrounded by chlorosis, suggests a possible involvement of the pathogen phytotoxic-compound (Molina *et al.*, 1989, Upadhyay *et al.*, 1990). Some of these toxins, already identified, could constitute an alternative technique for a rapid screening of resistant genotypes in banana allowing an early selection of young plants (Harelimana *et al*., 1997 ; Etamé, 2003). One of these secondary metabolites is 5-hydroxy-1,4-naphtoquinone ($C_{10}H_5O_3$), commonly called juglone. This toxin has been previously isolated from several plants including walnuts (*juglans sp.*) and species of *Penicillium* and *Verticilium* (Steirle *and al.,* 1991). Although its role in plant disease remains unclear, juglone is generally considered not to be required for pathogenicity, but probably functions as an aggressive factor because its production induces an increase in disease severity (Lepoivre, 2000).

Moreover, the variability in the resistance to oxidative stress induced by juglone in different genotypes of banana might reflect differences in the composition of their respective antioxidant systems. In their time-course, it can induce active oxygen species (AOS) production and scavenging (Busogoro *and al.,* 2004 a et b ; El Hadrami *and al*., 2005). So, before using toxin to breed for tolerant genotypes to BLSD, it is important to elucidate banana resistance mechanisms to the disease.

This study aim to : (i) show if, with various genotypes of banana exhibiting differential behaviour toward BLSD, the use of juglone could allow to establish sensitivity similar to that observed in the field ; (ii) assess if the variability of banana genotypes reaction to juglone is determined by the quantitative metabolism of phenolic compounds.

MATERIAL AND METHODS

PLANT MATERIAL

Six bananas varieties, showing distinct BLSD-resistance in the field were used (N'Guessan, 2000). Highly BLSD-susceptible cultivars Grande Naine, AAA ; Orishele, AAB ; Poyo, AAA ; BLSD-sensitive cultivar Figue Sucreé, AA and BLSD-partially resistant hybrids FHIA23 AAAA ; PITA14, AAAB were grown in the greenhouse, under natural conditions of photoperiod and temperature. Plants, at the stage of 5 to 6 fully expanded leaves, were used for juglone treatments after acclimation, for 2 days, under greenhouse, with a relative humidity of 90 ± 2 %.

PREPARATION OF JUGLONE SOLUTIONS

A commercial juglone product (Sigma-Aldrich Company), with the same properties as the compound isolated in toxins of *M. fijiensis*, was used. Juglone formula was $C_{10}H_6O_3$ (5-hydroxy-1,4-naphtoquinone). Solutions of 0, 12.5, 25, 50, 100, 250 and 500 ppm concentrations were prepared in 10 % methanol (MeOH 10 %). Ten percent methanol (= 0 ppm of juglone) and distilled water were used as the control solutions.

SOLUTION INJECTION INTO BANANA LEAVES

Twenty microliters of each solution (juglone at different concentrations, MeOH 10 %, distilled water) were injected into the fully expanded second leaf of the plants previously acclimated, using a microsyringue, with a rubber stopper covering its needle. Four sites per solution and per leaf were achieved on 3 plants of each variety of bananas and the whole experiment was repeated 3 times independently. Plants were incubated after the injections at 25 ± 2 °C, under ambient conditions of photoperiod and temperature (Harelimana, 1997).

DETERMINATION OF JUGLONE MINIMAL CONCENTRATION INDUCING NECROSIS

For each genotype, the minimum concentration of juglone inducing necrosis was determined 48 h after infiltration of solutions into banana leaves. Necrotic lesions were recognizable by brown or black spot observed at the site of injection.

NECROSIS INTENSITY MEASUREMENT

The severity of necrosis, observed at the injection site, 48 h after treatment, was evaluated using a visual scale adapted from standard values established by Stierle *and al*. (1991). According to the necrosis surface (Sn), as compared to the infiltrated surface (Si), using values ranging from 0 to 4, a necrosis index (i) was assigned to each site injected with the toxin. In this scale : 0 = no necrosis ; 1 = Sn lower than ¼ of Si ; 2 = Sn between ¼ and ½ of Si ; 3 = Sn between ½ and ¾ of Si and 4 = Sn higher than ¾ of Si.

Using the formula infection index defined by Townsend and Herberg (Perez *and al*., 2002), the necrosis intensity (IN) (%) was calculated for each genotype per solution injected in the leave :

$IN\% = (\sum in / 4N) \times 100$

Where, i is the index of the necrosis observed for each infiltrated site per solution ; n the number of the injected sites with the same necrosis index for each solution ; N the total number of infiltrated sites for each solution, N = 4.

DETERMINATION OF CI_{50}

According to necrosis intensity, juglone concentrations were transformed to log of concentrations. The CI_{50}, inducing 50 % of necrosis on infiltrated surface, for each banana genotype, was then calculated using linearization a scheme of the necrosis intensity evolution curves.

ELECTROLYTES LEAKAGE

Young banana leaves were used for each genotypes. Leafs discs of 0,5 cm diameter were cut using a cork bower, then abundantly washed and placed in distilled water. Twelve foliar discs were chosen at random and placed in tubes containing 1 ml of juglone solution at different concentrations (0, 25, 50, 100, 250 ppm). The tubes were transfered on an incubator-shaker, at 100 rpm, under continuous light for 48 h. After this incubation period, the leaf discs were discarded and the mixture of electrolytes was adjusted to 10 ml with distilled water. Electrolytes leakage from the leaf discs, for each solution conductivity (C) was determinated using a conductivitimeter (Selecta Precitherm, electrode : WTW/ Tetracom 325/pH/Cond 340i), at 25 °C.

For each essay, 12 foliar discs whose cells were previously dried for 20 min, at 120 °C, in an oven, were subject to the same treatment. The conductivity of solution after incubation was used as total conductivity (C_T). The cellular membranes percentage of integrity (I) (%) was calculated using the formula :

$I\% = (1 - (C / C_T)) \times 100$

All experiments were repeated 3 times.

DETERMINATION OF CL_{50} AND TL_{50}

According to percentage of integrity, the juglone concentrations and incubation times (foliar discs incubated for 48 h in juglone solution at 100 ppm), were transformed respectively to log of concentrations and log of times. The CL_{50} and TL_{50} inducing 50 % of loss of the cellular integrity for each banana genotype was then calculated using linearization of the integrity percentage evolution curves.

EXTRACTION AND TOTAL SOLUBLE PHENOLIC COMPOUNDS ANALYSIS

Before extraction of total phenolic compound, the survived leaves were infiltrated with 20 µl of juglone solution, at 50 ppm under the same conditions as previously described. After this treatment, the genotypes were kept under a saturated atmosphere. Leaf samples were

harvested following 0, 12, 24 and 48 h after the injection.

For each incubated period, 0.5 g of leaf sample treated and non-treated with juglone solution (50 ppm) were crushed in a mortar in 5 ml of 80 % methanol, 0.5 ml of 0.5 % Na-metabisulfite with a sund pinch of Fontainebleau. The suspension was centrifuged (5 000 trs/min) for 5 min and supernatant was adjusted to 10 ml with the 80 % methanol solution. The extract was immediately kept in ice (at 0 °C) until used to determine phenolic compounds. The extracts obtained from untreated leaf samples were used as controls. Total phenols were determined by the Swann and Hillis method (1959), and tyrosine solution (200 µg ml^{-1}) was used as the standard.

For phenols measurement, the reaction mixture contained 0.25 ml of extract, 3.75 ml of distilled water, 0.25 ml of Folin reagent (1N) and 0.75 ml of sodium carbonate (17 %). The absorbance was recorded at 725 nm, using a spectrophotometer (Model Baush § Lamb). All measurements were done in triplicates

DATA ANALYSIS

Data of necrosis intensity, conductivity and percentage of integrity were analyzed using the STATISTICA 6.0 software. Significant differences between all a homogenous groups were detected using the Newman-Keuls test, at $P < 0.05$. Quantitative results obtained from total soluble phenolic compounds were submitted to analysis of variance using SAS v. 9.1 software. Treatment means were compared using the Fisher test ($P < 0.05$).

RESULTS

JUGLONE TOXICITY AND NECROSIS EXPRESSION

Distilled water and 10 % methanol solution treatments did not induce any necrosis on banana leaves 48 h after injection. The minimal concentrations of juglone inducing necrosis, ranged from 12.5 - 100 ppm (Table 1). Three groups of banana varieties were observed with these concentrations. In the range from 12.5 - 50 ppm of juglone, the first group of genotypes was composed of highly susceptible banana cultivars (Orishele and Grande Naine). The second group was concerned with Poyo and Figue Sucrée, with minimal concentrations, varying from 25 - 100 ppm. Banana hybrids (PITA14 and FHIA23) constituted the group with minimal concentrations, ranging from 50 - 100 ppm of juglone. The common minimal concentration of juglone-inducing necrosis of hybrids and the cultivars, was 50 ppm (Table 1).

The cultivars exhibited the highest susceptibility with the lowest minimal concentration of the toxin inducing necrosis and an important development of necrotic lesions. At 100 ppm, the necrosis intensities were higher than 50 %, with Grande Naine and Poyo. These were 48.64 and 44.4 %, respectively, with Orishele and Figue Sucrée and lower than 40 % with PITA14 and FHIA23 (Table 2). The lowest necrosis intensities were observed with the banana hybrids (partially resistant to *M. fijiensis* infection) when the concentration of juglone was particulary lower or equal to 100 ppm. Except for the control solutions (distilled water and 10 % methanol) and both juglone solutions at 12.5 and 500 ppm, significant ($P < 0.05$, Newman-Keul test) increase in necrosis intensities was observed 48 h after injection between treated genotypes in concentrations of juglone, ranging from 25 - 250 ppm (Table 2).

The CI_{50} was weak and lower than 169 ppm with all the cultivars (Orishele, Grande Naine, Poyo, Figue Sucrée) sensitive to BLSD but higher than 260 ppm with the tetraploïd hybrids (PITA14 and FIHA23) partially resistant to BLSD (Table 3).

RESPONSES OF BANANA GENOTYPES LEAF DISCS TO JUGLONE TOXICITY

Electrolytes leakage from leaf discs of each banana genotype, increased with increasing juglone concentration (Figure 1). Differences between banana genotypes were significant ($P < 0.05$, Newman-Keul test) for conductivity (C) and membrane percentage integrity.

At 0 and 25 ppm, the increase in conductivity and the decrease in percentage integrity were similar between the different genotypes (Figures 1 and 2). Conductivity values were lower than 25 µs/cm and higher than 50 % for the integrities.

Dramatic decreases in membrane percentage integrities were observed after the 48 h-treatment, with the concentrations of juglone higher than 50 ppm (Figure 2). Except Orishele, the other cultivars (Figue Sucrée, Poyo, Grande Naine) exhibited the lowest membrane percentage integrity, as compare to the PITA14 and FHIA23 hybrids. At 250 ppm, with very low percentage integrity, membrane permeability was almost total, in particular for Poyo (I % = 4.02 %). The cultivar Orishele very susceptible to BLSD, exhibited the lowest electrolytes loss as its conductivities were the lowest (Figure 1). This observation might be complex for the banana genotypes classification according to their sensitivity to BLSD. But, the hybrid PITA14, partially resistant to the pathogen infection, exhibited the highest percentage integrities (Figure 2).

BLSD-partially resistant genotypes (PITA14 and FHIA23) had the most important CL50 (> 45 ppm of juglone), whereas they were lower than 34 ppm with Poyo and Gande Naine, highly BLSD-susceptible cultivars (Table 4). CL_{50} was respectively 42.11 and 42.4 ppm of juglone, with Orishele and Figue Sucrée cultivars (Table 4). Moreover, tetraploid hybrids exhibited the most important TL_{50} (> 13 h of incubation), while it was only 5 h with Poyo and 10 h with Figue Sucrée, Orishele and Grande Naine (Table 4).

Table 1 : Range of the minimal concentrations of juglone inducing necrosis for the different banana genotypes.

Gammes de concentrations minimales de juglone ayant induit des nécroses chez les différents génotypes de bananiers.

Genotypes	Level of reaction to the infection in conditions field	Range of minimal concentrations of juglone-inducing necrosis (ppm)
Orishele (AAB)	HS	12.5 – 50
Grande Naine (AAA)	HS	12.5 – 50
Poyo (AAA)	HS	25 – 100
Figue Sucrée (AA)	S	25 – 100
PITA (AAAB)	PR	50 – 100
FHIA23 (AAAA)	PR	50 – 100

HS = highly BLSD-susceptible, S = BLSD-sensitive, PR = BLSD-partially resistant. For the control a 10 % methanol solution, or distilled water was injected. The concentration of juglone is expressed in mg/l (ppm).

Table 2 : Necrosis intensities of the different concentrations of juglone 48 h after infiltration into leaves of the different banana genotypes.

Intensités de nécroses des différents génotypes de bananiers en fonction des concentrations de juglone, 48 h après infiltration dans les feuilles.

Genotypes	Necrosis intensities (%)						
	Juglone concentration (ppm)						
	0	12.5	25	50	100	250	500
Orishele (AAB)	0 a	2.77 a	13.88 b	33.33 b	48.61 c	68.75 b	74.30 a
Grande Naine (AAA)	0 a	0.69 a	13.19 b	29.16 b	50.69 c	60.41 ab	67.36 a
Poyo (AAA)	0 a	0 a	0.69 a	9.72 a	57.63 c	65.97 ab	69.44 a
Figue Sucrée (AA)	0 a	0 a	2.08 a	19.44 ab	44.44 bc	59.72 ab	72.22 a
PITA (AAAB)	0 a	0 a	0 a	5.55 a	32.63 ab	53.47 ab	63.88 a
FHIA23 (AAAA)	0 a	0 a	0 a	6.94 a	28.47 a	45.83 a	63.19 a

The concentration of juglone is expressed in mg/l (ppm). For the control at 10 % methanol solution, or distilled water was injected. In the same column, means followed by different letters are significantly different, according to Newman-Keuls test (p = 0.05).

Table 3 : Juglone concentration (CI_{50}) inducing 50 % of necrosis on infiltrated surface of different banana genotype.

Concentrations de juglone (CI_{50}) induisant 50 % de nécroses de la surface totale infiltrée chez les différents génotypes de bananiers.

Genotypes	Behaviour to the infection in field	CI_{50} (ppm)
Orishele (AAB)	HS	122.06
Grande Naine (AAA)	HS	151.84
Poyo (AAA)	HS	155.00
Figue Sucrée (AA)	S	168.27
PITA 14 (AAAB)	PR	260.20
FHIA 23 (AAAA)	PR	312.88

TS = très sensible, S = sensible, PR = partiellement résistant à la MRN.

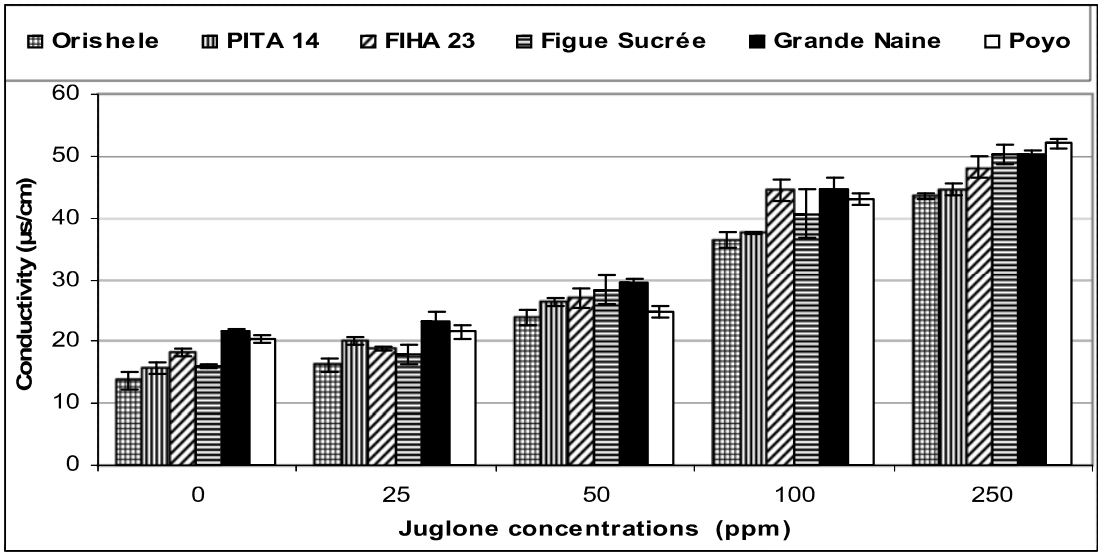

Figure 1 : Conductivities of different banana genotypes leaf discs during 48 h exposure to various concentrations of juglone.

Evolution des conductivités des disques foliaires chez les différents génotypes de bananiers 48 h après incubations dans les solutions de juglone.

Bars represent standard deviations / *Les barres représentent les déviations standards.*

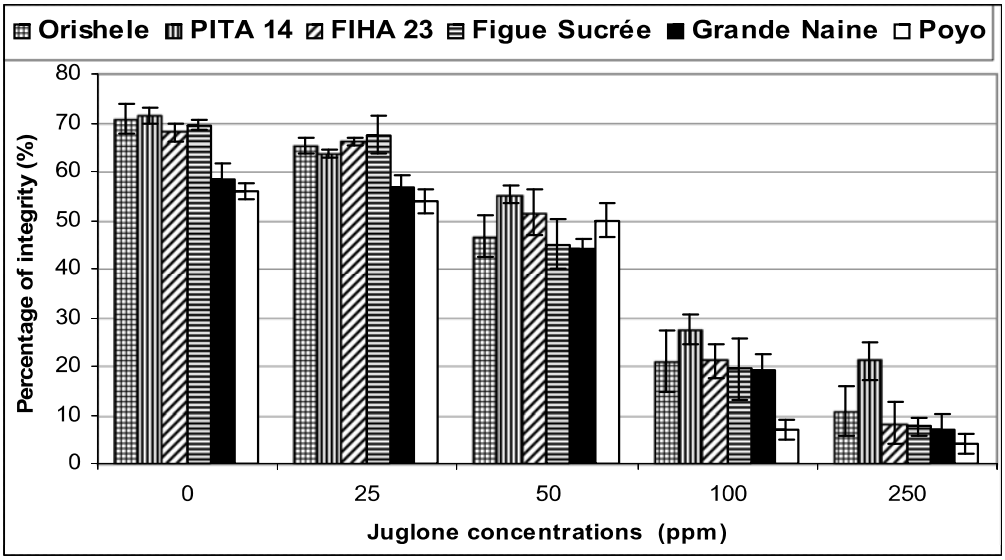

Figure 2 : Percentage of integrity of the leaf disc membranes after a 48 h-incubation in juglone solutions at different concentrations.

Evolution du taux d'intégrité cellulaire des rondelles foliaires en fonction de la concentration de juglone chez les différents génotypes de bananiers 48 h après incubations.

Bars represent standard deviations / *Les barres représentent les déviations standards.*

Table 4 : Juglone concentration (CL_{50}) and incubation time (TL_{50}) inducing 50 % loss of the cellular integrity in different banana genotypes.

Concentration de juglone (CI_{50}) et durée (DP_{50}) de perte de 50 % de l'intégrité cellulaire des tissus foliaires chez les différents génotypes de bananiers.

Genotypes	Behaviour to the infection in field	CL_{50} (ppm)	TL_{50} (h)
Poyo (AAA)	HS	31.84	5.37
Grande Naine (AAA)	HS	33.36	10.65
Orishele (AAB)	HS	42.11	10.48
Figue Sucrée (AA)	S	42.40	10.27
FHIA23 (AAAA)	PR	45.40	13.46
PITA14 (AAAB)	PR	49.95	18.56

HS = highly BLSD-susceptible, S = BLSD-sensitive, PR = BLSD-partially resistant

FREE TOTAL PHENOLIC COMPOUNDS PRODUCTION

Banana resistance to oxidative stress induced by juglone toxic effect was recorded. Two groups of banana genotypes were distinguished according to time-course appearance peak of phenols content (Figure 3). In the first group, banana varieties exhibited a late accumulation of the phenolic compounds (48 h after toxin infiltration) while in the second group, it was a quick stimulation of phenols (24 h after treatment). The first group, showing susceptibility to the toxin was composed only by the BLSD-highly sensitive cultivars (Grande Naine and Orishele). The second group, resistant-juglone was composed of sensitive cultivars (Figue Sucrée and Poyo) as well as tolerant hybrids (FHIA23 and PITA14) according to their respective behaviour following *M. fijiensis* infection (Figure 3). In this group, the high phenols content recorded with susceptible cultivars (11320.4 and 9838.5 µg/gFW, respectively for Poyo and Figue Sucrée at 24 h), constitute a more complex indicator of such quantitative evaluation. Nevertheless, at 48 h when necrosis appeared in all the treated varieties, only PITA14 and FHIA23 hybrids exhibited levels of phenolic compounds similar to those observed at 0 h (initial time). In addition, before increasing phenolic contents, in a first reaction after juglone infiltration in the leaves, the hybrid FHIA23 exhibited a decrease in its phenolic content after 12 h (Figure 3). Very highly significant differences ($P < 0,001$, Fisher test) were observed between banana genotypes for their total phenolic content after treating with 50 ppm of juglone solution.

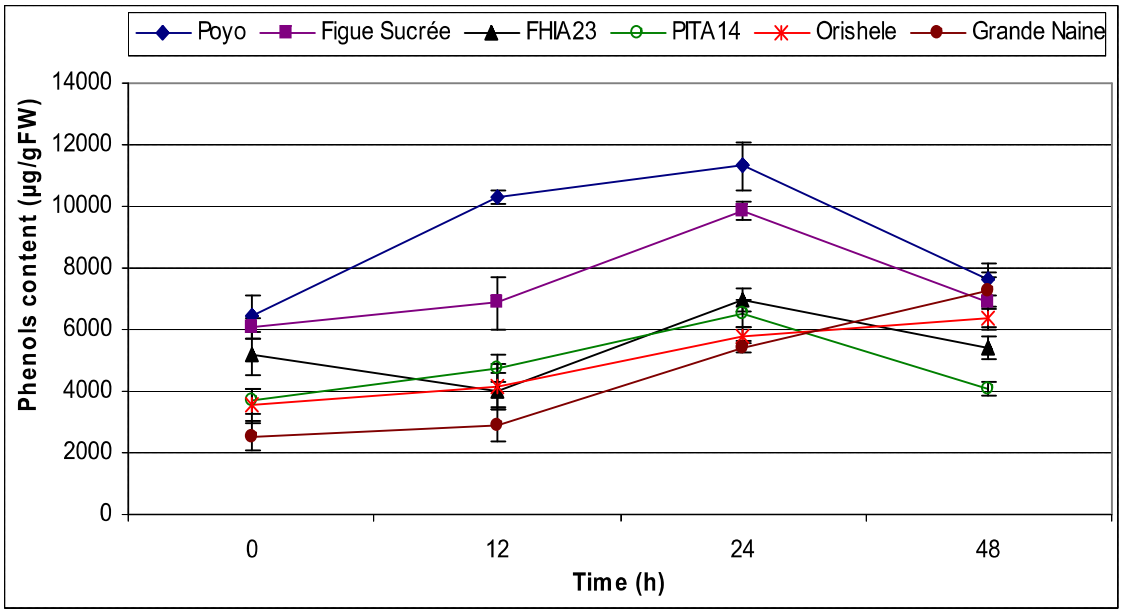

Figure 3 : Total phenolic compounds content of leaves of six genotypes of banana injected with 50 ppm of juglone as a function of times after injection.

Evolution de la teneur en composes phénoliques libres dans les feuilles des bananiers infiltrés de 50 ppm de juglone en fonction du temps.

Bars represent standard deviations / *Les barres représentent les déviations standards.*

DISCUSSION

Necrosis induction, disturbance of membranes permeability and phenolic compounds accumulation caused by juglone toxic effect were evaluated in banana genotypes. Juglone, at various concentrations, induced necrosis on the treated leaves, as opposed to distilled water and 10 % methanol. There was a constant correlation between the minimal concentration inducing necrosis, the necrosis intensity and CI_{50} in the different genotypes of banana for the sensitivity to juglone. The reaction to juglone of the banana cultivars (Grande Naine, Orishele, Poyo and Figue Sucrée) and the hybrids (FHIA23 and PITA14) confirmed their differential behaviour to BLSD in the field (N'Guessan *and al.*, 2000). Sensitivity related to necrosis induction by juglone was similar to genotypes susceptibility infection by *M. fijiensis*. Tetraploid hybrids (FHIA23 and PITA14) tolerant to BLSD, were most resistant to juglone toxic effect. Juglone injection into survived leaves proved more sensitive than the injection of pathogen toxic crude extract. Indeed, with juglone, small concentrations (12,5 ppm) were sufficient for necrosis development. But, with toxic crude extracts, high concentrations (500 ppm) were required to induce necrosis (Harelimana, 1997). Minimal concentration of juglone commonly inducing necrotic lesions was 50 ppm for all tested banana varieties. The appearance of necrotic lesions on banana leaves, after juglone infiltration, reveals active oxygen species (AOS) production. Indeed, AOS are able to produce necrosis (Desikan *and al.*, 1998 ; Busogoro *and al.*, 2002).

Disturbance of membranes permeability caused by juglone toxicity allowed to classify the different banana genotypes according to their conductivity, percentage of integrity, CL_{50} and TL_{50}. Genotype sensitivity to juglone due to electrolyte leakage through cellular tissues, was partially in conformity with the level of field infection by *M. fijiensi*s (N'Guessan *and al.*, 2000). Except, for the Orishele cultivar, banana hybrids partially tolerant to the infection, by the pathogen, were the least sensitive to juglone toxic effect. The importance of electrolytes leakage could be related to a disturbance of membrane transporters, involving an ionic imbalance of the cytoplasm (Johal and Briggs, 1992). However, for Orishele (BLSD-susceptible cultivar), the low quantity of released electrolytes could be independent to its resistance ability to disturbance of membrane permeability due to

toxin. Indeed, in the release of electrolyte from leaves infiltrated with juglone solutions, chlorophyll loss was not only dependent on membrane integrity (Amari *and al.*, 2008). Chlorophyll loss is a more complex indicator of toxicity related to the mode of action of the toxin (Abbas a*nd al*., 1998). The electrolytes content of foliar tissues of Orishele could be low. Juglone like other toxins, can increase conductivity in the incubated solutions if there is an important electrolyte leakage from foliar tissues. Electrolyte leakage constitutes a very sensitive measure to an early evaluation of the physiological phytotoxicity of juglone before morphological symptoms can be detected visually or microscopically (Abbas *and al*., 1998).

In preliminary biochemical study on the resistance to juglone toxic effect, our investigations were focused on the assessment of phenolic compound content.This quantitative evaluation revealed an apparent contrast between levels of phenolic compounds in the leaves of BLSD-susceptible cultivars (Figue Sucrée and Poyo) and BLSD-partially resistant hybrids (FHIA23 and PITA14). For Figue Sucrée and Poyo showing high phenolic contents, the toxin concentration (50 ppm) used in this biotest, could be the required level for optimal reaction to disease development. Indeed, juglone solution, at 50 ppm, constituted the minimal concentration from which the necrosis were initiated into leaves of banana hybrids. Whereas, 12.5 ppm were sufficient for banana cultivars. In these conditions, the mode of action of the toxin may be influenced by banana genotypes resistance, with a possible modification of their classification. In addition, like other toxins of the pathogen, juglone could not be the only one determinin pathogenicity (Etamé, 2003). The correlation between sensitivity in the field and tolerance to the toxin can be possible, if this toxin is the pathogenicity limitant factor.

The early reversal of the phenolic compounds, at their initial content in the hybrids (FHIA23 and PITA14), might explain the low intensities of necrosis on the leaves of these genotypes, as compared to the cultivars (Figue Sucrée and Poyo). The FHIA23 and PITA14 hybrids probably produced antioxidants, with high affinity for the active oxygen species (AOS) inducing weak necrotic lesions. These antioxidants scavenged in time the AOS leading to an early detoxification and less tissue injuries. The time course of AOS-production, AOS-scavenging and the antioxidant systems accumulation may involve the general level of the resistance to juglone (EL Hadrami *and al.,* 2005).

The complexity in interpretating the phenols content between susceptible and tolerant banana genotypes seems to demonstrate that these compounds are not only involved in plants defence. Indeed, plants have produced many antioxidant systems to scavenge AOS and to keep stress conditions under control (Baker and Orlandi, 1995). Nwaga *and al*. (2002) observed that inoculations with mycorrhiza fungi, are able to significantly modify the metabolism of polyphenol biosynthesis in cowpea leaves. This treatment may reduce the quantity of phenolic compounds in cowpea, but a qualitative increase in the number of phenolic compounds was noticed. The same authors reported that these observations were also related to ecological factors such as biotic stresses. These results show the qualitative role of phenolic compounds in the resistance mechanisms of different banana varieties. Moreover, according to Gire *and al.* (1994), there are more flavanols (80 %) primarily derived from quercitin than other phenolic compounds in the mesophyl cells of banana varieties infected by BLSD. However, the global level of resistance to *M. fijiensis* of banana cultivar could be determined by the low contents of proanthocyanidins (Gire *and al.*, 1994). The variable phenols content observed here between sensitive and tolerant banana varieties is probably related to different mechanisms of action involving the antioxidants system.

In light of the present findings, it could be concluded that the composition of antioxidant systems and/or their time-course of production determine the differences between the various banana genotypes reaction to oxidative stress caused by juglone :

- In the hybrids (FHIA23 and PITA14) partially resistant to BLSD, strong antioxidants, with high affinity to AOS such as phenolic compounds of the family of proanthocyanidins, may be preformed and/or have their synthesis stimulated.

- In the cultivars Figue Sucrée and Poyo sensitive to BLSD, antioxidants with low affinity to AOS such as flavonols derived form quercitin may be preformed and/or accumulated quickly in high quantity than proanthocyanidins.

- In the cultivars Grande Naine and Orishele, very susceptible to BLSD, it may have low and

late synthesis of the phenolic compounds without proanthocyanidins production.

CONCLUSION

The level of resistance established after juglone infiltration in banana leaves, was broadly the same with that observed in the field with infection by *M. fijiensis.* The hybrids (FHIA23 and PITA14) partially resistant to BLSD were generally more resistant to juglone toxic effect than the cultivars. Among these cultivars, Figue Sucrée and Poyo were most resistant to juglone than Grande Naine and Orishele. Genotypes sensitivity to juglone established only with phenolic compounds content was partially in conformity with that observed in field. A combination of the recorded parameters in different bioassays after infiltration of toxin might constitute the best strategy for identifying genotypes exhibiting greatest resistance to the pathogen.

The results also show that juglone is selective and can allow early and reliable classification of banana genotypes-resistance to BLSD. Juglone might have a determinant role in *M. fijiensis* pathogenesis mechanisms, but others biochemical analysis are required for confirmation. After juglone infiltration into leaves, there were modifications of phenols biosyn-thesis. However the quantitative changes could be related to the qualitative composition of phenols. Banana plants evolved various protective mechanisms to reduce or to completely eliminated AOS occurred by juglone toxic effect. One of the protective mechanisms is the control of the oxidative stress, which operated with the amount and the time-course of phenols production and that might depend, for a large part, on the qualitative mobilization of these antioxidants. The results of this work suggest that more basic studies are needed to improve our knowledge on the nature and the role of the phenolic compounds in plants defence strategies and to help to further understanding of the mechanisms involved in resistance to *M. fijiensis* toxins.

REFERENCES

Abbas H. K., Paul R. N., Riley R. T., Tanaka T. and W. T. Shier. 1998. Ultrastructural effects of AAL Toxin TA from the fungus *Alternaria alternata* on black nightshade (*Solanum nigrum* L.) leaf discs and correlation with biochemical measures of toxicity. Toxicon 36 (12) : 1821 - 183.

Amari L. D. G. E., Koné D., Dick E., Traoré S., Kobenan K. et P. A. Anno. 2008. Etude comparée de paramètres photosynthétiques chez différentes variétés de bananiers après infiltration de la juglone, un métabolite toxique de *Mycosphaerella fijiensis,* agent causal de la cercosporiose noire. Journal of Applied Biosciences 10 (2) : 523 - 531.

Baker C. J. and E. W. Orlandi. 1995. Active oxygen in plant pathogenesis. Annual Review of Phytopathology 33 : 289 - 321.

Busogoro J. P., Panis B., Messiaen J., Van Custen P., Swennen R. et P. Lepoivre. 2002. Evènements oxydatifs induits par les métabolites de *Mycosphaerella fijiensis* chez la cercosporiose noire du bananier (*Musa* spp.) et analyse de la faisabilité d'une sélection précoce de la résistance à cette maladie. Promusa 9 : XXIV.

Busogoro J. P., Etame J. J., Harelimana G., Lognay G., Messian J., Lepoivre P. and P. Van Custem. 2004a. Experimental evidence for the action of *Mycosphaerella fijiensis* toxins on banana photosynthetic apparatus. *In :* Banana improvement : Cellular, Molecular, Biology and Induced Mutations. SM, Jain and R. Sweennen (Eds.). Science Publishers, Enfield, USA. pp 161 - 170.

Busogoro J. P., Etame J. J., Lognay G., Messian J., Van Custem P. and P. Lepoivre. 2004b. Analysis of the mechanisms of action of *Mycosphaerella fijiensis* toxins during the development of black leaf streak disease. *In* : Banana improvement : Cellular, Molecular, Biology and Induced Mutations. SM, Jain and R, Sweennen (Eds.). Science Publishers, Enfield, USA. pp 171 -181.

Desikan R., Reynolds A., Hancok J. T. and S. J. Nell. 1998. Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on defence gene expression in Arabidopsis suspension cultures. Biochemical Journal, 330 : 115 - 120.

El Hadrami. 2000. Caractérisation de la résistance partielle des bananiers à la maladie des raies noires et évaluation de la variabilité de l'agressivité de l'agent causal *Mycosphaerella fijienis* Morelet. Thèse de Doctorat, Faculté universitaire des Siences Agronomiques de Gembloux (Belgique), 153 p.

El Hadrami A., Koné D. and P. Lepoivre. 2005. Effect of juglone on active oxygen species and antioxidant enzymes in susceptible and partially resistant banana cultivars to Black Leaf Streak Disease. European Journal of Plant Pathology 113 : 241 - 254.

Etamé A. J. J. 2003. Caractérisation des toxines de *Mycosphaerella fijiensis*, l'agent de la cercosporiose noire du bananier pour leur utilisation comme agent de sélection précoce de variétés de bananier résistantes à cette maladie. Thèse de doctorat, Faculté Universitaire des Sciences Agronomiques de Gembloux (Belgique), 171 p.

Gire A., Beveraggi A., Macheix J. J. and X. Mourichon. 1994. Evidence of a constitutive polyphenolic component in resistance of banana the *Mycosphaerella fijiensis*. *In* SEP (Eds.). Société Française de Phytopathologie.

Harelimana G. 1997. Etude de l'implication des toxines de *Mycosphaerella fijiensis*, Morelet dans les mécanismes de résistance partielle des bananiers à la cercosporiose noire. Thèse de doctorat en sciences agronomiques et ingénierie biologique, Unité de phytopathologie de la Faculté Agronomique de Gembloux (Belgique), 153 p.

Harelimana G., Lepoivre P., Jijakli H. and X. Mourichon. 1997. Use of *Mycosphaerella fijiensis* toxins for the selection of banana cultivar resistant to Black Leaf Streak. Euphytica 96 : 125 - 128.

Johal G. S. and G. S. Briggs. 1992. Reductase activity encoded by the HM disease resistance gene in maize. Science, 258 : 985 - 987.

Lepoivre P. 2000. Fungal disease of the foliage. *In* Jones D. R. (Eds.). Disease of Banana, Abaca and Enset. CABI Press. New York, USA. pp 71 - 72

Molina, G. G. and J. P. Kranz. 1989. A phytotoxic activity in extracts of both culture of *Mycosphaerella fijiensis* var difformis and its use to evaluation host resistance to black Sigatoka, Plant Disease 73 : 142 - 144.

N'Guessan A. E. B., Koné D., Kobenan K., Aké S. et A. Tenkouano. 2000. Caractéristiques et évaluation de comportement de quelques hybrides tétraploïdes de bananiers plantain dans le Sud de la Côte d'Ivoire. Bioterre (Revue Internationale des sciences de la Vie et de la Terre), 1 : 6 - 21.

Nwaga D. N. W. L., Fokom R., Oneya S. et A. Ngakon. 2002. Variabilité des composes phenoliques chez *Vigna unguiculata* (L.) Walp. (Légumineuses) et influence de l'inoculation par les rhizobia et les champignons mycorhiziens sur leur biosynthèse. African Journal of Science and Technology. 3 (2) : 17 - 24.

Ortiz R., Ferris R. S. B. and D. R. Vuylsteke. 1995. Banana and plantain breeding. *In* Bananas and plantains, S. Gowen. Chapman & Hall, London, Uk. pp 110 - 146.

Pasberg-Gauhl C. 1989. Untersuchungen zur symptomentwicklung und bekämpfung der schwargen Sigatoka- Krankleit (*Mycosphaerella fijiensis* Morelet) an Bananan (*Musa* sp.) *in vitro* und freiland. Göttinger beiträge zur land und forstwirtochaft in den tropen and subtropen. Heft 40, Goltze, Göttinger, Germany, 142 p.

Pérez L., Hernandez A., Hernandez L. and M. Pérez. 2002. Effect of trifloxystrobin and azoxystrobin on the control of black Sigatoka (M*ycosphaerella fijiensis* Morelet) on banana and plantain. Crop Protection 21 : 17 - 18.

Stierle A. A., Upadhayay R., Hershenhorn J., Strobel G. A. and G. Molina. 1991. The phytotoxins of *Mycosphaerella fijiensis*, the causative agent of Black Sigatoka disease of banana and plantains. Experientia 47 : 853 - 859.

Swain T. and Hillis W.E., 1959. Phenolic constituents of *Prunus domestica*. The quantitative analysis of phenol constituents. J. Sci. Food Agric. 10 : 63 - 68.

Vuylsteke D., Ortiz R. and S. Ferris. 1993. Genetic and agronomic improvement for sustainable production of plantain and banana in sub-Saharan Africa. African Crop Science 1 : 1 - 8.

Upadhyay R. K., Stierle A. A., Hershenhorn J., Strobel G. A. and G. Molina. 1990. Fijiensis, the first phytoxin from *Mycosphaerella fijiensis*, the cauasative agent of Black Sigatoka disease, Experentia 46 : 982 - 984.