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Effect of glyphosate on antibiosis in the coevolution of the pathosystem *Manihot esculenta-Colletotrichum gloeosporioides*

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1 ABSTRACT

The high use of glyphosate for weed and pest control of cassava crops in Africa, particularly in Côte d'Ivoire, is correlated with the increasing extent of cassava anthracnose in the cassava producing regions. This health situation raises the question of the existence of a combined effect of glyphosate and anthracnose infection on the defence response of the cassava plant. The study was on the effect of glyphosate on antibiosis in *M. esculenta* in the pathosystem with *C. gloeosporioides*. To achieve this objective, a classical fully randomized 3-repeat device was used and antibiosis was the factor studied with 3 modalities. Treatments carried out on the plots, 4 months after planting, were inoculation of test plots of cassava with *C. gloeosporoides* before they were treated with 4JAI glyphosate. The assessment of phenolic and flavonoid antibiosis levels was carried out on leaf and stem organs harvested from 1JHA to 45 JHA and from the day of glyphosate treatment to 41 JHA. Comparative flavonoid antibiosis profiles were determined by CCM at the stages of maximum inhibition and maximum accumulation. Results showed that the phenolic and flavonoid antibiosis content of plants in glyphosate-treated plots decreased progressively in all varieties studied from JATr 1 to JATr 7. After the seventh day, the respective amounts of phenolic and flavonoid antibiosis gradually increased from the 8th JATr to the 180th JATr. This study showed a negative but partial interference of glyphosate in the defense mechanism of cassava cultivars (*Manihot esculenta*).

RESUME

L'importante utilisation du glyphosate pour le désherbage et la lutte contre les adventices des cultures de manioc en Afrique et notamment en Côte d'Ivoire, et l'étendue croissante des cas d'anthracnose du manioc dans les régions de production de cette culture, soulève la question d'un possible effet conjugué du glyphosate et de l'infection à l'anthracnose. Les auteurs ont étudié l'effet du glyphosate sur les antibioses chez *M. esculenta* dans le pathosystème avec *C. gloeosporioides*. Pour atteindre cet objectif, un dispositif classique à trois répétions complètement randomisées a été utilisé et les antibioses sont le facteur étudié avec 3 modalités. Les traitements réalisés sur les parcelles, 4 mois après plant plantation, ont été l'inoculation des parcelles tests de manioc par *C. gloeosporoides* avant que celles-ci ne soient traitées par le glyphosate 4JAI. L'évaluation des teneurs en antibioses phénoliques et flavonoïdiques a été réalisée sur les organes feuilles et tiges récoltées de 1JAI à 45 JAI et du jour du traitement au glyphosate à 41 JAT. Les profils comparatifs en antibioses flavonoïdiques ont été déterminés par CCM aux stades de l'inhibition maximale et de l'accumulation maximale. Les résultats ont montré que la teneur en antibioses phénoliques et flavonoïdiques des plantes des parcelles traitées avec le glyphosate ont diminué progressivement chez toutes les variétés étudiées du 1er JATr au 7ème JATr. Après le septième jour, les quantités respectives des antibioses phénoliques et de flavonoïdes ont augmenté progressivement du 8ème JATr au 180ème JATr. Il ressort de cette étude, une interférence négative mais partielle du glyphosate dans le mécanisme de défense des cultivars de manioc (*Manihot esculenta*).

2 INTRODUCTION

Manihot esculenta Crantz, known as manioc or cassava, is a plant cultivated for its tuberous roots and leaves. Native to Latin America (Kenneth and Barbara, 1999; Lenis *et al.,* 2006), it is an important source of income for the people who grow it (Ekou, 2003; Manso, 2005), and serves as a staple food (N'Zué *et al.,* 2005; Fokounang and Dixon, 2006) for more than 500 million people worldwide (Ambang *et al.,* 2007). It is highly prized by the African population because of the high use of its leaves and roots in the diet and the richness of its leaves in nutrients (iron) (Akpingny *et al.,* 2017). Thus, cassava contributes in its various forms to food and nutrition security (Patricio M. *et al.,* 2018). In Côte d'Ivoire, cassava cultivation covers about 80% of the national territory (Akpingny *et al.,* 2017). Its annual production has increased from about 2.4 million tons (FAO, 2013) to about 5 million tons while experiencing an 11% decline in 2016 (Patricio M. *et al.,* 2018). Despite the figures and the discovery of new varieties such as Bocou 1, Bocou 2, and Bocou 3, the cassava sector is struggling to grow. This situation is largely explained by the fact that cultivars are subject to diseases caused by pathogenic microorganisms and various pests in a growing environment where *Manihot esculenta* farms are increasingly maintained by chemical weeding with the glyphosate. This product has been shown to be effective in controlling weeds. However, pesticides are responsible for polluting the environment and the entire food chain through the accumulation of their toxic residues (Buhot, 2003; Thakore, 2006). Moreover, their continuous (or abusive) use is correlated to the increase in pest pressure and nuisance (Johal & Hubert, 2009). This phenomenon is due to the appearance of pathogenic strains (plant enemies) resistant to the products used (He *et al.,* 2005; De Lapeyre *et al.,* 2010). Moreover, information obtained from the literature indicates that glyphosate inhibits the synthesis of amino acids in plants (Hoffmann, 2003), some of which are precursors to the biosynthesis of phenolic defence markers such as flavonoids (Hopkins & Evrard, 2003). To this end, *Manihot esculenta* has been the subject

of several studies. However, there is not much information on the natural defence of *Manihot esculenta* in field culture with glyphosate under stress conditions has not been reported in the literature. The general objective is to elucidate the role of phenolic constituents and glyphosate under stress conditions in the natural defence of *Manihot esculenta* against *Colletotrichum gloeosporioides*. The objective of this study is to evaluate the effect of glyphosate on the involvement of phenolic and flavonoid antibiosis in the pathosystem *M. esculenta-C. gloeosporioides*. The objective of this study is to evaluate the effect of glyphosate on the involvement of phenolic and flavonoid antibiosis in the pathosystem *M. esculenta-C. gloeosporioides*.

3 MATERIAL

3.1 Plant material: The plant material used consists of powders (Figure 1) of leaves (F) and stems (T) of 3 cultivars of *Manihot esculenta* including 1 traditional cultivars (Figure 2), Yace (Y) and 2 improved cultivars (Figures 3) which are 9620A (A) and TMS30572 (S). All these varieties were provided by the CNRA.

Figure 1: Powder of leaves (F) and stems (T) of cassava cultivars

Figure 1: Improved (A and S) and traditional (Y) cassava cultivars

3.2 Fungal material: The fungal agent used is *Colletotrichum gloeosporioides* f. sp manihotis. It is a necrotrophic fungus (Perfect *et al.,* 1999). It was isolated from *Manihot esculenta* plants affected by anthracnose, on an experimental: The chemical material used for the experiments consists of glyphosate (commercially purchased herbicide), solvents for extractions (methanol, chloroform, hexane) and for the development of chromatograms (n-butanol, acetic acid, water), and reagents for the revelation of flavonoid antibiosis (Neu, Follin-Ciocalteu, DPPH (2, 2-diphenyl-1-picrylhydrazyl)). All these chemicals

(solvents and reagents) were purchased from CARLO ERBA and are of analytical purity.

3.4 Method

3.4.1 Parcel setting device: The experimental site consists of two (2) blocks (A and B) of plots. Each block included a replication of 3 plots that were totally randomized according to Fisher's design (Dagnelie, 2003). Each plot consisted of 6 elementary plots arranged according to N'Zué et al. (2005).

3.4.2 -Preparation and application of glyphosate slurry: The glyphosate slurry was prepared according to the protocol of Lienhard (2003), Kébé *et al.* (2009) and Brou *et al.* (2012). This spray (2 doses of glyphosate solution solubilized in 15 L of water) was applied at a rate of 150 L/ha after sprayer calibration (OSATU Star 2016). The directed treatment approach consisted in directing the sprayer nozzles between the stems of *Manihot esculenta* plants during spray application (Cirad, 2000; Brou *et al.,* 2012).

3.4.3 Inoculum and inoculation: The nutrient medium Potato Dextrose Agar (PDA, MERCK) was used for the preparation of culture medium for the fungus *Colletotrichum gloeosporioides* f. sp manihotis. The inoculum was prepared from an aggressive strain of *Colletotrichum gloeosporioides* isolated from field anthracnosed cassava plants. The strain was then purified 7 times at intervals of 8 days in preparation for inoculation of cassava plants. The inoculation of the plants was done according to the methods of Terry et al. (1983) and, Ambang *et al.* (2007). It was carried out in 3 steps: wounding of the stems, introduction of the inoculum in pellet form at the wound site and finally covering the inoculated stem portion. This inoculation was carried out on plants 4 months after planting

3. 4.4 Sampling of organs for extraction: The leaves and stems of the used plants were collected between the 5th and 20th eye protuberance from the apex of the plant (Brou *et al.,* 2012). Organs were harvested at different periods ranging from 1 to 180 days. For the same period, the leaves and stems of a cultivar that had undergone identical treatment were harvested from the elementary plots (3 replicates) and homogenized for the preparation of extractions (Brou *et al.,* 2012).

3.4.5 -Extraction and quantification of phenolic and flavonoid antibiosis: Fifteen grams (15g) of leaf and stem powder were macerated for 24 h in 65 mL of 70% (v/v) MeOH at room temperature, respectively (Brou *et al.,* 2010). This is repeated twice. After filtration, the hydro-methanol extracts of all cultivars (Y, B2, M, S, I and A) were stored in the freezer for subsequent use in phytochemical screening, polyphenol and total flavonoid assays. Extracts of cultivars TMS30572 (S), 9620A (A) and Yacé (Y) were used for selective flavonoid extraction. Their selected hydro-methanol extracts were treated with (3 × 20 mL) hexane, chloroform, ethyl acetate and n-butanol, respectively. Phenolic antibiosis was determined by the method of Singleton and Rossi (1965). Flavonoids were measured according to the formula of Hariri et al. (1991) and Brou et al. (2012).

3.5 -Statistical analysis of the data: All the experiments conducted in this work were repeated at least three times. The data obtained were processed using the statistical software version 7.0 stat soft. They were subjected to a one-factor ANOVA analysis of variance after prior verification of the homogeneity of the variances using a Leven test. When the p values (level of probability) of the one-factor ANOVA are significant ($p \leq 0.05$), the analysis is completed by classifying the means into homogeneous groups using the Duncan's test at the 5% threshold. This method is based on the comparison of all pairs of means. A level of probability $p \leq 0.05$ (risk α) is said to be significant, highly significant when $p \leq 0.01$ and very highly significant when $p \leq 0.001$.

4 RESULTS

4.1 Comparative evaluation of the levels of antibiosis in plants from inoculated plots and those inoculated and treated with glyphosate:

The effect of glyphosate on the content of phenolic defense markers in plants infected with A. gloeosporioides was studied with plant organ extracts collected from the plots of Block B. For their preparation, the harvests of the first 15 days, 30th, 45th and harvest day were used. Thus, 54 extracts were obtained from the leaves of plants inoculated and then treated with glyphosate (18 extracts of AFITr, 18 extracts of SFITr, 18 extracts of YFITr) and 54 extracts from the stems of plants treated with glyphosate (18 extracts of ATITr, 18 extracts of STITr, 18 extracts of YTITr). The phenol and flavonoid compositions of the plant extracts inoculated and then treated with glyphosate were evaluated by TLC and spectrophotometric assay. The results of these analyses were compared with those of plant extracts treated with glyphosate (controls) to show the role of flavonoid markers in the defence against anthracnose caused by C. gloeosporioides in a glyphosate predisposed culture environment.

4.1.1 Determination of total phenols by spectrophotometry: The histograms in Figures 1 and 2 showed a stimulation threshold and an optimum stimulation level. The stimulation threshold, according to Duncan's test ($F = 39.41$; $P < 0.05$), was achieved after 2 JHA (i.e. 48 h after inoculation). At the leaf level, this threshold corresponded to 2391.67 ± 33.55 µg/g DM for cultivar A and 2042.71 ± 31.21 µg/g DM for cultivar S. Finally, the threshold for Y was 1753.84 ± 32.03 µg/g DM. The optimum stimulation level was found after 15 JAI/11 JATr. Thus, at leaf level, the optimum value is 6754.35 ± 31.75 µg/g DM for A, 4352.26 ± 32.28 µg/g DM for S and 3144.68 ± 32.13 µg/g DM for Y (Figure 1)

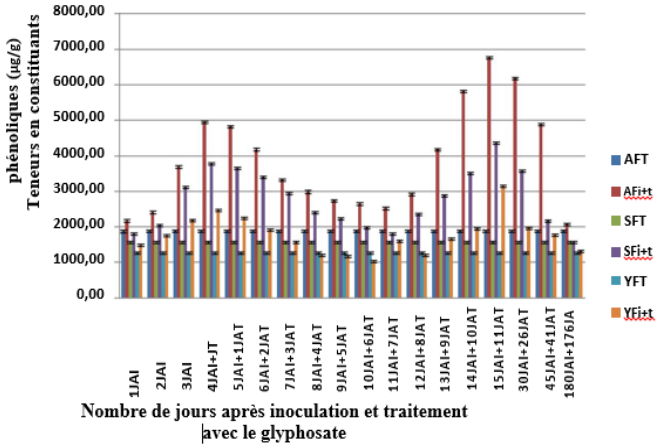

Figure 1 : Phenolic antibiosis levels of control (F) and inoculated and treated (i+Tr) leaves (F) of cultivars (A, S and Y) of *Manihot esculenta*. JAI+JAT: days after inoculation with *Colletotrichum gloeosporioides* and after treatment with glyphosate.

The stimulation threshold at the stems corresponded to 1065.41 ± 29.95 µg/g dry matter (DM) for A, 738.75 ± 29.91 µg/g DM for S and 511.92 ± 31.33 µg/g DM for Y. The optimum stimulation was achieved after 15 JHA (360 h after inoculation) and 11 JAT (264 h after treatment with glyphosate). This optimum value at the stem level was 3506.47 ± 30.15 µg/g DM for A, 2004.78 ± 31.18 µg/g DM for S and 1253.45 ± 30.07 µg/g DM for Y (Figure 2).

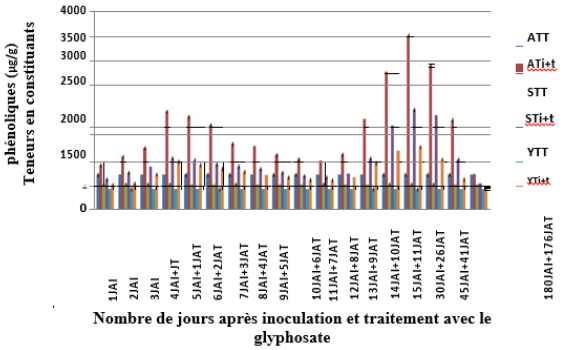

Figure 2: Phenolic antibiosis levels of control (T) and inoculated and treated (i+Tr) stems (T) of cultivars (A, S and Y) of *Manihot esculenta*. JAI+JAT: days after inoculation with *Colletotrichum gloeosporioides* and after treatment with glyphosate.

4.1.2 Evaluation of the content of flavonoid antibiosis: The results of the evaluation of the content of flavonoid constituents (in µg/g quercetol equivalent) in the leaves and stems of plants inoculated with *Colletotrichum gloeosporioides* and treated with glyphosate revealed that the levels of total flavonoid constituents increased over the inoculation time (Figures 3 and 4). These increases reached maximum levels 360 h after infection (15 JHA) before gradually decreasing until harvest date. However, the response of the different infected cultivars was noticeable 48 h after inoculation (i.e. 2 JHA). This response, after a slight increase to 4 JAI, decreased from 4 JAI to 11 JAI (i.e. 7 JATr of glyphosate) before resuming accumulation at 12 JAI and reaching maximum values at 15 JAI.

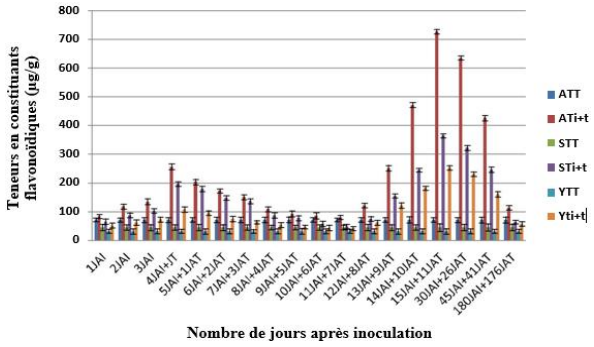

Figure 3: Flavonoid antibiosis levels in treated or control (F) and inoculated and treated (I+Tr) leaves (F) of cultivars Y, S and Y. JAI+JAT: days after inoculation and treatment

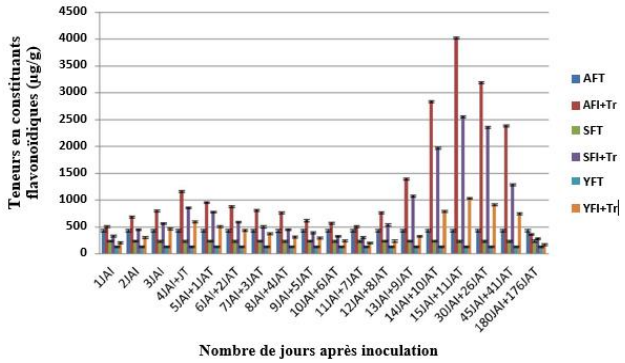

Figure 4: Flavonoid antibiosis levels of treated or control (T) and inoculated and treated (i+t) stems (T) of cultivars Y, S and Y. JAI+JAT: days after inoculation and after treatment.

Thus, these figures presented two parts. These two portions were blurred by a curvilinear part for each of the three cultivars. The characteristics of these curves showed a threshold of stimulation and an optimum of stimulation. The stimulation threshold, determined according to Duncan's test ($F = 37.41$; $P < 0.05$), was found after 2 JAI (i.e. 48 hours after inoculation) for all cultivars studied. For cultivar A, 118.18 ± 7.21 µg/g DM at the stem and 689.72 ± 18.36 µg/g DM at the leaves, 88.75 ± 8.06 µg/g DM at the stem and 454.72 ± 14.25 µg/g DM at the leaves for cultivar S, respectively. Finally, the thresholds are 62.89 ± 9.25 µg/g DM on stems and 306.01 ± 18.15 µg/g DM on leaves for cultivar Y. The optimum stimulation was 726.13 ± 7.65 µg/g DM at the stem and 4023.86 ± 18.65 µg/g DM at the leaf for cultivar A, 364.26 ± 7.01 µg/g DM at the stem and 2556.31 ± 17.01 µg/g DM at the leaf for cultivar S. Finally, 252.35 ± 8.26 µg/g DM on stems and 1033.16 ± 16.26 µg/g DM for cultivar Y. This maximum accumulation stage was reached after 15 JAI (360 h after inoculation) for all three cultivars. At the maximum accumulation stage, cultivar A accumulated 10.21 times its initial content of flavonoid constituents on the stems and 9.34 times its basic content of flavonoid constituents on the leaves. In contrast, cultivar S has accumulated an intermediate content of 8.16 times its initial content of flavonoid constituents on the leaves and 10.83 times its initial content of flavonoids on the stems. At this level, the proportions of accumulation of flavonoid constituents of the improved cultivars were similar to each other and better than those of cultivar Y. However, all three cultivars reacted rapidly and significantly ($F = 37.41$; $P < 0.05$) 48 hours after infection (2 JAI) by C. gloeosporioides and showed a decrease in levels after treatment with glyphosate. Thus, maximum decreases in flavonoid constituents were observed 11 JHA + 7 JATr. At the stage of this maximum decrease, the levels of flavonoid constituents in the leaves of cultivars A, S and Y were 505.96 ± 17.35, 301.63 ± 17.65 and 201.92 ± 14.28 µg/g DM, respectively. Stems at this stage showed the following minimum values 79.65 ± 7.35; 48.44 ± 7.65 and 41.02 ± 7.28 µg/g DM for cultivars A, S and Y, respectively. Therefore, a glyphosate inhibitory effect was observed in cassava plants attacked by C. gloeosporioides (Figures 3 and 4).

4.2 Comparative chromatographic profiles of antibiosis of plants in inoculated plots and those inoculated and treated with glyphosate: The chromatographic profiles (Figures 5 and 6) of flavonoid antibiosis of the stems and leaves of inoculated plants (Block A) differ from those of the stems and leaves of plants inoculated with C. gloeosporioides and treated with glyphosate (Block B). This difference mainly concerned the absence of the yellow-orange component (Rf 0.88) on the chromatogram of the inoculated/treated plants in cultivars S and Y. In addition, the absence of the fluorescent yellow component (Rf 0.83) is

noted only in treated inoculated plants of cultivar Y. Like the flavonoid profiles of the leaves of inoculated and treated inoculated plants, those of the stems of inoculated plants (A^A, S^A et Y^A) also differ from those of the stems of inoculated/treated plants (A^B, S^B et Y^B). However, the constituent of Rf 0.88 is present only on inoculated/treated stems in cultivar Y and absent in cultivars A and S. These differences were observed at the stages of maximum decrease (11 JAI + 7 JATr) in flavonoid levels (Figures 5 and 6).

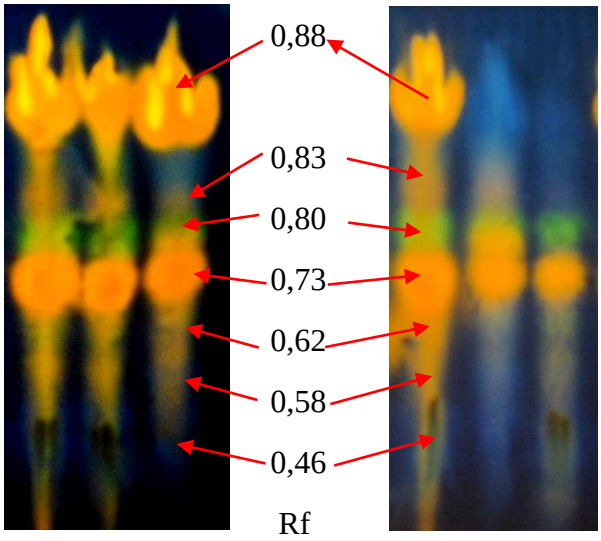

Figure 5 : Chromatographic profiles of flavonoid leaf (F) antibiosis of inoculated cultivars (**A^A**, **S^A** and **Y^A**) and inoculated/treated (**A^B**, **S^B** and **Y^B**) at the maximum inhibition stage

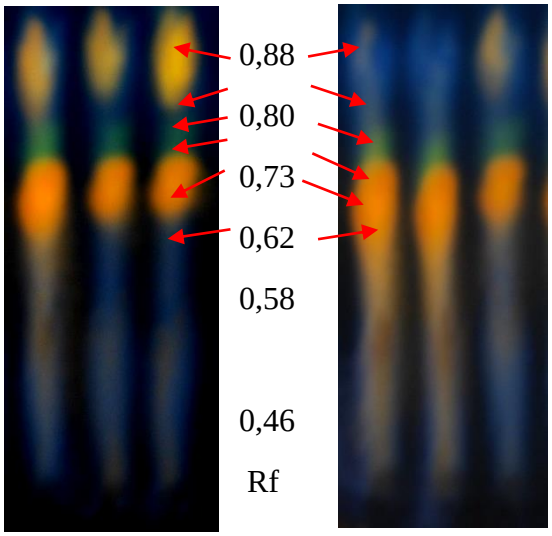

Figure 6: Chromatographic profiles of flavonoid stem antibiosis (**T**) inoculated cultivars (**A^A**, **S^A** and **Y^A**) and inoculated/treated (**A^B**, **S^B** and **Y^B**) at the maximum inhibition stage

5 DISCUSSION

In contrast to the levels of antibiosis in inoculated plants (Brou, 2014), the levels of phenolic and flavonoid antibiosis in treated inoculated plants dropped after the stimulation threshold (4JAI) rather than increasing and reaching an accumulation threshold of 9JAI. However, these results are similar to those of Brou et al (2012) who showed a regression, compared to untreated controls, of phenolic and flavonoid antibiosis levels from 4JHA to 11JHA (i.e. 7JATr of glyphosate) before seeing a resumption of 12JHA accumulation and reaching maximum 15JHA values. The decrease in phenolic and flavonoid antibiosis levels in plots that were treated with glyphosate following inoculation of plants with *C. gloeosporioides* contrasts with the levels of this same antibiosis in the case of the *Manihot esculenta-Colletotrichum gloeosporioides* relationship as presented by Brou (2014). This decrease would then be due to the inhibitory action of glyphosate at the stage of synthesis of these secondary metabolites. Glyphosate absorbed from the aerial organs of the plant (leaves and stems) would first be transferred into the phloem, which carries it to the tips of the roots and rhizomes (Gougler & Geiger, 1981; Satchivi et al, 2000; Sharma & Singh, 2001; Wakelin *et al.,* 2004) where it would block aromatic amino acid biosynthesis by binding through phosphoenolpyruvate to the enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) to exert competitive inhibition. Which enzyme is believed to be involved in the metabolic pathway of shikimic acid by catalysing the condensation of shikimate-3-phosphate and phosphoenolpyruvate to 5-enolpyruvylshikimate-3-phosphate (Tomlin, 1995; Roberts, 1998; Hoffman, 2003). This would result in a halt to protein synthesis by inhibiting the synthesis of precursors including phenylalanine and tyrosine. The deficit in tyrosine and phenylalanine, two aromatic amino acid precursors of phenols, would lead to the stopping or at least reducing the flow of synthesis of phenolic and flavonoid antibiosis (Cole, 1985). In addition, phenols synthesized prior to glyphosate application (constituent phenols) would be used by the plant to satisfy its multiple needs such as parietal reinforcement (Kouakou *et al.,* 2004; Hückelhoven, 2007), protection against oxidative stress (Hernandez *et al.,* 2008), defence against pathogens (Treutter, 2006; Kanti & Syed, 2009). Above the maximum inhibition threshold (7JAT/11JAI), the respective levels of phenolic and flavonoid antibiosis gradually increased in all cultivars and reached maximum levels (11JATr/15JAI), whereas in uninoculated but treated cultivars it was close to the levels of their constituents. A halt in the regression of phenolic and flavonoid antibiosis levels was observed when a reasonable and recommended rate of pesticide use for treatment was used selectively (CIRAD, 2000; Lienhard, 2003; Kébé *et al.,* 2009; Brou *et al.,* 2012). In addition, glyphosate is a biodegradable pesticide and degradation may begin around 7 days after application (Motekaitis & Martell, 1985; Agritox, 2004; Mamy, 2004; Mamy & Barriuso, 2005 & 2007). It should be added that there would be physiological selectivity in cassava as in the case of maize (Edwards *et al.,* 2000) and wheat (Cummins & Edwards, 2004) which would result in slow transport of the pesticide in the plant. On the other hand, since cassava is rich in carbohydrates and proteins (Brou *et al.,* 2010), physiological selectivity would be justified by the presence of enzymes such as glutathione-S-Transferases (G-S-T) (Marrs *et al.,* 1995) and glycosyltransferases (O-GT and N-GT) (Gachon *et al.,* 2005), which degrade the glyphosate molecule before it reaches all sites of action, such as EPSPS enzymes whose inhibition induces inhibition of the synthesis of secondary metabolites. In addition, infection with C. gloeosporioides would induce the synthesis of glutathione S-transferase molecules (Doug *et al.,* 2003) which, in addition to their basic content, would undergo an increase in volume. These enzymes would be best known for their ability to conjugate glutathione (GSH) with electrophilic molecules in plants, followed by their sequestration as conjugates in vacuoles where they are further metabolized (Cole & Edwards, 2000). Several functions of GSH in plant

metabolism have been proposed, including the binding and transport of phytochemicals between cell compartments (Edwards *et al.,* 2000). The complexities of these chemicals are made possible by the fact that herbicides would have certain chemical groups common to those of xenotoxic products, and would therefore be recognized as such (Yuan *et al.,* 2007). Their degradation would occur in three phases (Hatzios & Burgos, 2004) and would start with functional activation reactions (oxidation and hydrolysis) that would introduce or reveal functional groups in the herbicide molecule that are necessary for the conjugation phase. In the second stage, the functional groups of the herbicide molecule would be conjugated with endogenous plant substrates (glutathione and glucose). At the end of this step, the herbicide molecule would lose its phytotoxicity and become more water-soluble and less mobile. The third step would be to store the detoxified molecules in the vacuole and/or in the cell walls (Coleman *et al.,* 1997) where, according to Bartholomew et al. (2002), they would be permanently degraded. This physiological selectivity could finally result in a low glyphosate uptake by the plant, since according to Michitte *et al.* (2007), resistant plants retained 35% less glyphosate spray than sensitive plants, and the penetration of this herbicide would be 40% lower. In parallel with the quantitative evaluation of phenolic and flavonoid antibiosis, a variable chromatographic profile of flavonoid antibiosis was noted during the time of infection of cassava plants by *C. gloeosporioides*. This variation in profile has already been observed in the case of the *M. esculenta-C. gloeosporioides* relationship. Thus, the results of the study, especially those at the stage of maximum accumulation, indicate that no significant difference is observed in the qualitative composition of flavonoid antibiosis between extracts from *M. esculenta* organs treated and untreated with glyphosate. This similarity suggests that glyphosate did not have a significant influence on the chalcone synthase (CHS) and chalcone isomerase (CHI) genes that code for the biosynthesis enzymes of flavonoid antibiotics (Rabbani *et al.,* 2003; Buchanan *et al.,* 2005). However, based on the chromatographic profiles of the maximum inhibition stage, disruption of the biosynthetic mechanism by this herbicide is known to occur. This disruption could be explained by the absence of flavonoid defense markers of Rf 0.88 in cultivars S and Y. This suggests that glyphosate exerted partial inhibition (Brou *et al.,* 2012). Comparison of the chromatographic profiles of extracts at the stages of maximum inhibition and maximum accumulation, and the levels of flavonoid antibiosis revealed their involvement in the defence of *Manihot esculenta* (Brou, 2014). Indeed, the number and diversity of flavonoid antibiosis synthesized, by the appearance of spots of different colours that are characteristic of them (Wagner and Bladt, 1996; Brou *et al.,* 2010), during the time of infection and especially at the dates of maximum accumulation are indicative of the relative but significant influence of glyphosate in the defense action of antibiosis during the pathosystem *Manihot esculenta-C. gloeosporioides* (Behr *et al.,* 2010). Therefore, a positive correlation can be established between the quantitative and qualitative aspects of this antibiosis.

6 CONCLUSION

This study reveals the negative interference of glyphosate on the biosynthesis of phenolic and flavonoid antibiosis during the defence reaction of cultivars in the pathosystem *M. esculenta-C. gloeosporioides*. However, under the influence of glyphosate, inoculated cassava plants have, however, accumulated late high levels of phenolic and flavonoid antibiosis. Thus, the study shows that glyphosate exerts a relative inhibition on the biosynthesis of phenolic and flavonoid antibiosis while delaying the defence reaction of cassava plants against *C. gloeosporioides*.

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