African Journal of Plant Science

Volume 11 Number 8, August 2017
ISSN 1996-0824

Academic Journals

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Vol. 11(8), pp. 331-340, August 2017
DOI: 10.5897/AJPS2016.1487
Article Number: 03673C665313
ISSN 1996-0824
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African Journal of Plant Science

Full Length Research Paper

Effects of chitosan and snail shell powder on cocoa (*Theobroma cacao* L.) growth and resistance against black pod disease caused by *Phytophthora megakarya*

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Received 19 October, 2016; Accepted 22 January, 2017

Chitosan and chitin sources have emerged as promising groups of biological substances that can induce plant growth and resistance to diseases. This study is aimed at investigating the potential effect of chitosan and snail shell to promote cocoa growth and induce resistance against *Phytophthora megakarya*. The results showed that treatment of cocoa seeds with chitosan at 1.5 to 2.0% and snail shell at 2.0% increases the percentage of germination and also reduces the time of germination. 12 weeks after planting, a series of morphological changes was observed on the plants treated with chitosan and snail shell. Plant height (34 to 39.5 cm), leaf number (11 to 13 leaves/plant), leaf area (88 to 130 cm^2) and fresh weight of roots and shoots (25 to 44 g/plant) increased significantly in the chitosan and snail shell treated soils. Pods inoculated with chitosan and/or snail shell treated soil suspensions presented very slight necrotic lesions. This could be linked to a decrease in the *P. megakarya* load of the soil suspension. Leaf inoculation showed variation among the treatments with the lowest index (highest level of resistance) recorded in plants treated either with chitosan or snail shell. The treatment of plants with chitosan and snail shell powder before and after inoculation showed higher level of phenolic compounds than in the control plants. Furthermore, the inoculation induced a significant accumulation of proteins in the cocoa plants treated with chitosan and snail shell. The level of proteins accumulation after inoculation was higher in plants treated with snail shell than those treated with chitosan. In conclusion, the effect of snail shell on cocoa growth and resistance showed that it is possible for snail shell powder to be a biofungicide and biofertilizer used in the control of cacao Black Pod Disease in nurseries.

Key words: *Theobroma cacao*, *Phytophthora megakarya*, black pod disease, chitosan, snail shell, biocontrol agents.

INTRODUCTION

In Cameroon and other African countries, cocoa (*Theobroma cacao*) is one of the most important economical cash crops. However, its cultivation is faced with numerous problems such as parasitic attack and the insufficiency of selected genotypes. Among the parasitic constraints, Black Pod Disease (BPD) caused by several

Phytophthora species has been the worst threat of cacao in West and Central Africa (Opoku et al., 2000). Reports have shown that in Cameroon, the BPD caused by *Phytophthora megakarya* is the highest threat; and losses of up to 50 - 80% of cocoa beans have been reported (Ndoumbe-Nkeng et al., 2004). This disease reduces the yield and quality of the product, and also increases the cost of production. The cocoa fruits, stems or roots can be infected by chlamydospores or sporangia, which may germinate to produce swimming zoospores that can be spread by drops of rain, wind-blown rain, soil and soil water. In order to prevent these losses due to *P. megakarya* infection, different strategies have been developed. Chemical control using metalaxyl and copper-based fungicides has been reported to be the most effective strategy to reduce the impact of BPD in nurseries and in farms (Ndoumbe-Nkeng et al., 2004; Sonwa et al., 2008). However, application of fungicides can have drastic effects on the consumer and the environment (Naseby et al., 2000). A promising and safer method for controlling cocoa BPD has been the development of resistant cultivars and the use of appropriate cultural practices (Nyasse et al., 2007; Tchameni et al., 2011). However, disease resistant cultivars are not yet available.

A biocontrol approach using chitosan or chitin sources (snail shell, crab skeleton, shrimp skeleton) is an eco-friendly alternative. Chitosan is a carbohydrate biopolymer derived from deacetylation of chitin, which is found in the shells of Crustaceans, cuticules of insects and cell walls of fungi. The positive charge of chitosan confers to it numerous and unique physiological and biological properties with great potential in a wide range of agricultural practices (Bautista-Banos et al., 2004; Tang et al., 2010). Many studies have reported the capacity of chitosan to stimulate the immune system for plant resistance to pathogen infection, to induce the accumulation of phytoalexins resulting in antifungal responses in order to enhance protection against further infection (Coqueiro et al., 2011) and also to change the soil microorganisms content (Roy et al., 2010). Moreover, chitosan has been widely used as a growth stimulator, germination accelerator and yield enhancer in many crop species such as in orchid (Uthairatanakij et al., 2007), faba bean (El-Sawy et al., 2010), cucumber (Shehata et al., 2012) and corn (Boonlertnirun et al., 2011; Lizárraga-Paulín et al., 2011).

This study is therefore aimed at investigating the ability of chitosan and snail shell powder to promote cocoa growth and induce resistance against *P. megakarya* in nurseries by evaluation of the rate of germination of seeds, plant agro-morphological characteristics, suppressive potential, total phenolic compounds and peroxidase activities. Our findings will contribute to the evaluation of the role of chitosan and snail shell powder as a biofungicide alternative in the control of cocoa BPD.

MATERIALS AND METHODS

Soil

The soil used in this experiment was collected from Yaoundé (Centre region, Cameroon) and are often used by farmers to prepare young cocoa seedlings. The soil was air-dried and passed through a 4 mm sieve before mixing (3:1; v/v) with river sand. Chemical analysis (organic matter, nitrogen, calcium, magnesium, phosphorus contents and pH) of dry samples was carried out before the cultivation period. The contents of available nutrients in the soil were: organic matter, 3.40%; nitrogen, 1.23%; calcium, 6.48×10^{-3} meq.g^{-1} of soil; magnesium, 23.20×10^{-3} meq.g^{-1} of soil; phosphorus, 3.54 mg.g^{-1} of soil; and pH was 6.1. The soil-sand mix was then autoclaved three times at 121°C for 30 min before being transferred into pots.

Fungal strains and snail shell powder production

Zoospore suspensions of *P. megakarya* isolate EL from the core collection of IRAD (Institut de Recherche Agricole pour le Développement, Yaounde, Cameroon) were obtained according to Tondje et al. (2006). Sterilized and unsterilized soils were then inoculated with 10^6 zoospore.kg^{-1} of soil.

Snail shell was obtained following the method of Jideowno et al. (2007). The shells (from Buea, in the South west region of Cameroon) were thoroughly washed using tap water and air-dried for two days. They were then dried in an oven and at 105°C, then pounded in a mortar using a pestle to form the snail shell powder. Sterilized and unsterilized soils were inoculated with zoospores of *P. megakarya* at 10^6 zoospore.kg^{-1} of soil for 48 h before, being treated with 1% chitosan and snail shell powder. Chitosan was kindly provided by Professor Carole Beaulieu, Centre SEVE University of Sherbrooke (Quebec, Canada).

Evaluation of germination rate

For evaluation of germination rate and plant agro-morphological characters, mature cocoa pods (♀MA12 × ♂PA150) hybrids produced by manual pollination were collected from the SODECAO (Société de Développement du Cacao) gene banks of Mengang Station (South Region, Cameroon; Latitude 2°90'N, Longitude 11°20'E). Cocoa seeds were extracted from the pods, washed with distilled water and coated as shown in Table 1. For the germination test, 8 groups of 30 seeds each were sown per germination tray. A 2% chitosan stock solution was prepared as described by Lizárraga-Paulín et al. (2011), and the 1.5% solution was obtained by appropriate dilution of the stock solution. Experimental seeds were sown at a depth of 5 cm in sterilized soils and the trays incubated at 25 ± 1°C. Distilled water was sprinkled on the trays every two days to avoid dryness. Germination was confirmed by a shooting of the radicule and the germination rate evaluated as in Zeng et al. (2012).

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Table 1. Experimental design of seeds coating.

Groups	1st	2nd	3rd	4th	5th	6th	7th
Number of seeds	30	30	30	30	30	30	30
State	Untreated	Treated	Treated	Treated	Treated	Treated	Treated
Chitosan (v/w)	Control	2%	1.5%	-	-	-	-
Snail shell (w/w)	Control	-	-	2%	10%	20%	30%
Soaked for		12 h	12 h	-	-		
Oven-dried at 29°C for		1 h	1 h	-	-	-	-

Evaluation of agro-morphological characters

The agro-morphological characters that were assessed include the weight of the plant root and shoot weight, height, leaf number, length, width and area of the leaf. These parameters were assessed every 4 weeks for a period of 12 weeks. To produce the plants, a single pregerminated cocoa seedling was transplanted into each plastic pot, which contained treated and untreated soil. Each treatment was in duplicates of ten pots. All the pots were kept in the greenhouse and watered with distilled water every two days for a period of 12 weeks. During assessment at the 4 weeks interval, roots of harvested plants were washed to remove soil particles and plant height measured with a caliper tip. Length and width of leaves measured with a graduated ruler and the weight of shoots and roots of freshly harvested plants then measured separately.

The experiment was a completely randomized design with two treatments chitosan and/or snails shells at 1% w/w, and the control (treatments without chitosan and/or snail shells). The treatments without chitosan and/or snail shells are also designed: sterilize Soil (sS), sterilize Soil + *P. megakarya* (sS+Pm), and none sterilize Soil (nsS). The treatments with chitosan and/or snails shells are designed as: sterilize Soil (sS) + Chitosan (sS+Ch), sterilize Soil + *P. megakarya* + Chitosan (sS+Pm+Ch), none sterilize Soil (nsS) + Chitosan (nsS+Ch), sterilize Soil + Snail Shell (sS+SS), sterilize Soil + *P. megakarya* + Snail Shell (sS+Pm+SS), and none sterilize Soil + Snail Shell (nsS+SS). Each treatment consisting of three replicates were repeated twice.

Induced resistance assessment

Young cocoa leaves from two-month-old plants were collected from the nursery in the greenhouse washed thoroughly with tap water and sterilized with 70% ethanol for 30 s. Sixteen leaf discs of 1.5 cm diameter from each treatment were made with a cork borer and replicated three times. These leaf discs were placed with their abaxial surface upwards in side trays. On the other hand, six whole leaves of cocoa were set apart in a single tray and arranged according to the protocol described by Djocgoue et al. (2007). These discs and whole leaves were all simultaneously inoculated with 10 µl of 10^6 zoospore ml^{-1} suspension of *P. megakarya*. In each treatment, one control made up of six whole leaves and leaf discs inoculated with 10 µl of sterilized distilled water in a separate tray was included. Inoculation was performed on the underside of each leaf, and the trays were incubated in a dark room at 25 ±1°C. Disease expression was rated six days after, using the rating scale developed by Nyasse et al. (1995). This experiment was repeated twice, and the severity of disease was determined for each treatment by calculating the ratio of the sum of individual scores to the total number of discs leaves used. The disease severity index used to express the resistance level (Paulin et al., 2008) was as follows: Highly Resistant (HR: 0 < index ≤ 1); Resistant (R: 1 < index ≤ 2); Moderately Resistant (MR: 2 < index ≤ 2.5); Susceptible (S: 2.5 < index ≤ 3.5); and Highly Susceptible (HS: 3.5 < index ≤ 5).

Biochemical analyses

Biochemical analyses were carried out following the assessment of infection on the whole leaves. The samples involved were cut at 1 cm beyond the necrosis point or beyond the marked scar (sections with no symptoms). Samples from the same treatments were combined. The parts of the leaves from sterilized Soil (sS) treatment were combined. For biochemical analyses, each treatment was repeated twice.

Determination of the content of total phenolic compounds

The extraction and quantitative measurement of the content of total phenolic compounds were performed as described by Djocgoue et al. (2007) with modification. Total phenolic compounds were extracted twice using 80% methanol. 1 g of fresh tissue of inoculated and healthy leaves plant was ground separately in 10 ml of 80% methanol at 4°C. After 5 min of agitation, the ground material was centrifuged at 10000 g for 5 min at 4°C. The supernatant was collected and the pellet was re-suspended in 5 ml of 80% methanol followed by agitation for 5 min. After the second centrifugation at 4°C, the supernatant was collected and mixed with the previously collected supernatant to constitute the phenolic extract. The concentration of phenolic compounds was determined spectrophotometrically at 725 nm according to the method of Marigo (1973), using the Folin-Ciocalteu reagent. Total phenolic compound contents were expressed in mg equivalent of catechin per g of fresh weight.

Determination of the content of total native protein

For the determination of total native protein content, extraction was performed as described by Priminho et al. (2008) with modification. 1 g of fresh tissue of inoculated and healthy leaves plant was ground separately in 10 ml of extraction buffer (Tris-HCl 10 mM pH 7.5, Triton X-100 1%) at 4°C, stirred for 10 min and kept on ice. The samples were sonicated (8 pulses of 3 s each with 10 s intervals) with the setting at 70% output on an Ultrasonic processor (Gex 130, 130 W), and then centrifuged at 10000 *g* for 25 min at 4°C. The pellet was submitted to a second extraction. Both supernatants were mixed with 0.4 volume of *n*-butanol and 1/10 of 3 M NaAc pH 4.5. The samples were kept on ice for 30 min with agitation every 10 min, and then centrifuged at 10000 *g* for 15 min at 4°C. The supernatant containing total proteins was stored at 4°C. The proteins were quantified using the Bradford (1976) method. 1 ml of Bradford reagent was added to each ml of extract. The absorbance was measured at 595 nm using a UV-VIS 1605 Shimadzu

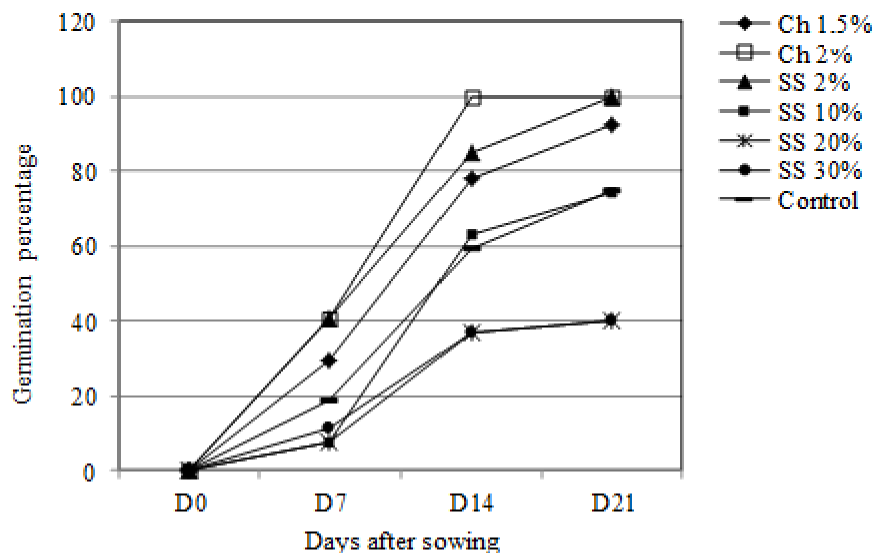

Figure 1. Germination percentage of seed coated and uncoated with chitosan (Ch, 1.5 and 2%) and snail shell (SS) at 2, 10, 20 and 30%. Each treatment consisting of three replicates were repeated twice.

spectrophotometer. BSA was used as the standard.

Peroxidases activity

Peroxidase activity was determined by spectrophotometry at 470 nm in the total native protein extracts according to the method of Boudjeko et al. (2005) using Tris-maleate buffer and oxygenated water (H_2O_2). The enzyme activity was expressed in enzyme unit per g of fresh weight.

Evaluation of the suppressive effect of snail shell

For evaluation of the suppressive effect of snail shell and chitosan in the soil, namely, *P. megakarya*, 3 month-old healthy pods (SNK10, susceptible clone) were harvested, washed with tap water, sterilized with 70% ethanol (for 1 min), 10% (v/v) commercial sodium hypochlorite (for 5 min) and rinsed 3 times with sterilized distilled water. The inoculation was carried out by the deposition of 500 µl suspensions of untreated and treated soils collected after 12 weeks of experiment on the scar obtained with hand utensils. The scars are then closed with cotton that has been immersed in sterilized water. The soil suspension was obtained by mixing soil with sterilized distilled water. That is, 2 g of soil sample was mixed with 10 ml of sterilized distilled water, shaken and allowed to stand for 10 min. A control constituted of pods inoculated with only sterilized distilled water was realized. The entire inoculated pods were incubated in a dark room at 25 ± 1°C in a humid chamber. The rate of necrosis was qualitatively evaluated on daily basis for 6 days using the sign + or -.

Statistical analyses

Data analysis was performed using the SPSS software version 17.0. All the results were expressed as means ± standard deviation and subjected to Analysis of Variance (ANOVA). Where significant differences were found, pairs of samples were compared by Tukey's test at $p \leq 0.05$.

RESULTS

Effect of chitosan and snail shell on seed germination

The treatment of cocoa seeds with chitosan at 1.5 to 20% and snail shell at 2% increases the percentage of germination and also reduces the time of germination compared to the control seeds (Figure 1). Treatment of seeds with chitosan at 2% increased the germination percentage after sowing from 0 to 40% within 7 days (D7) and to 100% within 14 days (D14). Treatment of seeds with snail shell (SS) coating at 2% also increased the germination percentage from 0 to 40% within 7 days (D7) but reaches 100% germination within 21 days (D21). The treatment with snail shell at 10 and 20% did not increase the germination percentage at the same rate. Hence, with these concentrations, the germination percentage increased from 0 to 60% within 14 days (D14) and reaches 75% after 21 days (D21). The effect of treatment with snail shell at 30% concentration was at the same level with the control seeds, that is, 40% of germination within 21 days (D21).

Effect of chitosan and snail shell on agro-morphological characteristics

The agro-morphological characteristics of cocoa

Table 2. Effect of chitosan and snail shell soil treatments on agro-morphological characteristics of cocoa seedling after twelve weeks of growing.

Treatment	Number of leaves	Leaf area (cm^2)	Plant height (cm)	Shoots (g)	Roots (g)
nsS	7.33 ± 0.57^{a}	44.16 ± 1.4^{a}	24 ± 0.57^{a}	13.77 ± 0.05^{b}	3.6 ± 0.25^{a}
sS	7.33 ± 0.57^{a}	55.96 ± 2.7^{a}	28.5 ± 0.57^{a}	12.57 ± 0.05^{a}	3.3 ± 0.05^{a}
nsS+Ch	11.5 ± 0.57^{b}	130.02 ± 1.1^{c}	38 ± 0.57^{c}	44.16 ± 0.05^{g}	9.55 ± 0.05^{d}
nsS+SS	12 ± 0.57^{b}	95.15 ± 1^{b}	36.25 ± 057^{c}	23.33 ± 0.57^{d}	10.2 ± 0.05^{e}
sS+Ch	$13 .16 \pm 0.57^{b}$	114.66 ± 5.2^{c}	39.5 ± 0.57^{c}	38.86 ± 0.05^{f}	12.46 ± 0.005^{f}
sS+SS	12.5 ± 0.57^{b}	104.89 ± 5.1^{bc}	38 ± 0.57^{c}	27.33 ± 0.88^{e}	11.63 ± 0.01^{f}
sS+Pm	7 ± 0.57^{a}	37.87 ± 6.4^{a}	25 ± 0.57^{a}	16.7 ± 0.57^{c}	4.2 ± 0.05^{b}
sS+Pm+Ch	10.5 ± 0.57^{b}	87.93 ± 9.7^{b}	34.6 ± 0.57^{b}	26.9 ± 0.5^{e}	8.82 ± 0.05^{c}
sS+Pm+SS	12.5 ± 0.57^{b}	88.41 ± 1.1^{b}	33.25 ± 0.57^{b}	25.33 ± 0.88^{d}	8.8 ± 0.05^{c}

Each treatment consisting of ten replicates was repeated twice. Means with the same letter within a column are not significantly different at $P<0.05$. nsS: none sterilize Soil; sS: sterilize Soil; nsS+Ch: none sterilize Soil + Chitosan; nsS+SS: none sterilize Soil + Snail Shell; sS+Ch: sterilize soil + Chitosan; sS+SS: sterilize soil + Snail Shell; sS+Pm: sterilize Soil + *P. megakarya*; sS+Pm+Ch: sterilize Soil + *P. megakarya* + Chitosan; sS+Pm+SS: sterilize Soil + *P. megakarya* + Snail Shell.

seedlings (height of the plant, number of leaves, area of leaf, weight of shoots and roots) grown in soils treated with chitosan and snail shell were variably affected in different stages after 12 weeks of growth (Table 2). The heights of cocoa seedlings grown in soil treated with chitosan and snail shell powder were both significantly different from those of the control plants. The control plants (nsS, sS and sS+Pm) belonged to the same group with an average height of 26 cm. The plants grown in sterilized soil (sS) and unsterilized soil (nsS), treated either with chitosan (sS+Ch and nsS+Ch) or with snail shell (sS+SS and nsS+SS) had an average height of 38 cm and belonged to the same group. The presence of *P. megakarya* in the sterilized soil (sS) slowed down this growth in height as shown on Table 2, with an average height of 34 cm for the treatments sS+Pm+Ch and sS+Pm+SS. Furthermore, the number of leaves and the area of leaves of cocoa plants grown in the soils (nsS, sS and sS+Pm) treated either with chitosan or snail shell powder varied according to the treatment after 12 weeks of growth (Table 2). The average number of leaves in the control plants (nsS, sS and sS+Pm) was 7 leaves and was significantly different from those of the treated plants which had an average of 12 leaves, with no significant difference between the treatments. The area of leaves showed 2 different groups: the control plants and treated plants. The treatments without chitosan and/or snail shells (sS, sS+Pm, nsS) showed level of leaf areas significantly lower than those the treatments with chitosan and/or snails shells (sS+Ch, sS+Pm+Ch, nsS+Ch,sS+SS, sS+Pm+SS, nsS+SS).

The weights of fresh shoots and roots of the cocoa plants grown in soils treated either with chitosan or snail shell powder were significantly different between the treated plants and the control (Table 2). The weight of the shoots had 3 different groups: the first group (nsS, sS and sS+Pm) had a mean of 14 g, the second group (sS+Ch, sS+Pm+Ch, nsS+Ch,sS+SS, sS+Pm+SS, nsS+SS) had a mean of 33 g and the third group (sS+Ch and nsS+Ch) had the highest. The weight of the roots had 2 different groups: the first group (nsS, sS and sS+Pm) had a mean of 4 g, the second group (sS+Ch, sS+Pm+Ch, nsS+Ch,sS+SS, sS+Pm+SS ,nsS+SS) had a mean of 10 g. Chitosan treatments consistently improved the weight of the cocoa shoots and roots more than that of snail shell treatment.

Evaluation of disease severity

The discs of cocoa leaves inoculated with 10 µl of 10^6 zoospore.ml^{-1} suspension of *P. megakarya* developed a clear lesion six days after, while no symptom was seen on discs of leaves inoculated with sterilized distilled water. The disease severity was significantly ($p < 0.05$) different among the treatments. The highest level of disease severity was observed with the control treatments (sS, nsS and sS+Pm). While, the lowest level of disease severity was recorded in plants treated with either chitosan or snail shell, showing a disease severity index of 0.83 to 1.15 for chitosan treatments and 1 to 1.4 for snail shell treatments (Figure 2).

Biochemical analyses

The amount of total phenolic compounds in non-inoculated plants was lower than the inoculated ones. The inoculations of leaves had a significant effect on total phenolic contents in all the treatments. The treatment of plants with chitosan and snail shell powder before and after inoculation showed higher level of phenolic compounds than in the control plants. Chitosan treatment had a more significant effect as compared to snail shell

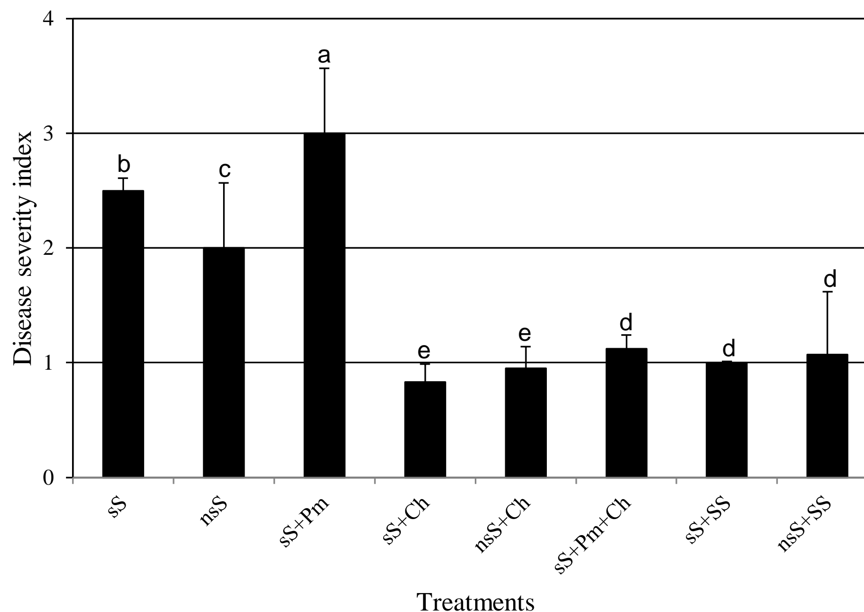

Figure 2. Disease severity of plants treated and untreated with chitosan and snail shell six days after inoculation. Each treatment consisting in three replicates were repeated twice. Means with the same letters are not significantly different at *P*< 0.05. sS: sterilize Soil; nsS: none sterilize Soil; sS + Pm: sterilize Soil + *P. megakarya*; sS + Ch: sterilize Soil + Chitosan; nsS + Ch: none sterilize Soil + Chitosan; sS + Pm + Ch: sterilize Soil + *P. megakarya* + Chitosan; sS + SS: sterilize Soil + Snail Shell; sS + SS: sterilize Soil+ Snail Shell; nsS + SS: none sterilize Soil + Snail Shell.

treatment (Figure 3). The presence of *P. megakarya* in the soil seemed to slow down the increase of the amount of phenolic compounds except for the chitosan treatment. Chitosan treatment showed an increase of the amount of phenolic compounds after inoculation, with an increase of 14, 23 and 44% for the treatments nsS+Ch, sS+Ch and sS+Pm+Ch, respectively. This was not the case with the snail shell treatment which showed an increased level of phenolic compounds after inoculation that was significantly lower, regardless of the nature of treatment.

The amount of proteins was much lower in the plants grown in control soils (nsS, sS and sS+Pm) before and after inoculation. The treatment with snail shell and chitosan increased the protein level in healthy and inoculated plants (Figure 4). The presence of *P. megakarya* in the soil had no significant effect on the protein in the plants compared to the other treatments. The inoculation induced a significant accumulation of proteins in the cocoa plants treated with chitosan and snail shell. The proteins accumulation after inoculation was higher in plants treated with snail shell than those treated with chitosan (Figure 4).

The peroxidase activity of plant leaves grown in control soils (nsS, sS and sS+Pm) before and after inoculation was not significantly different from that of the treatments nsS+SS before and after inoculation (Figure 5). In plants treated with chitosan, the peroxidase accumulation was higher in the non-inoculated plants with an average of 0.92 UE/min/g fresh weight, and this amount significantly rose to 76% in all the plant leaves after inoculation (sS+Ch, sS+Pm+Ch and nsS+Ch). Furthermore, there was a significant difference in peroxidase accumulation between the leaves of plants treated with chitosan and those treated with snail shell.

Effect of *P. megakarya* soil inoculum load on cocoa pods

The cocoa pods inoculated with sterile soil containing *P. megakarya* and non-sterile soil treated with chitosan and snail shell, respectively showed very low levels of necrotic lesions that appeared only 6 days (D6) after inoculation. For the treatments without chitosan and snail shell (nsS and sS+Pm), necrotic lesions appeared and spread on the inoculated cocoa pods from D4 and D2 after inoculation till D6. The pods inoculated with sterilized soil and with zoospores of *P. megakarya* (sS+Pm) got completely rotten (Table 3).

DISCUSSION

The aim of this study was to assess the ability of chitosan and snail shell powder to induce growth and resistance of cocoa against *P. megakarya*, the causal agent of BPD in

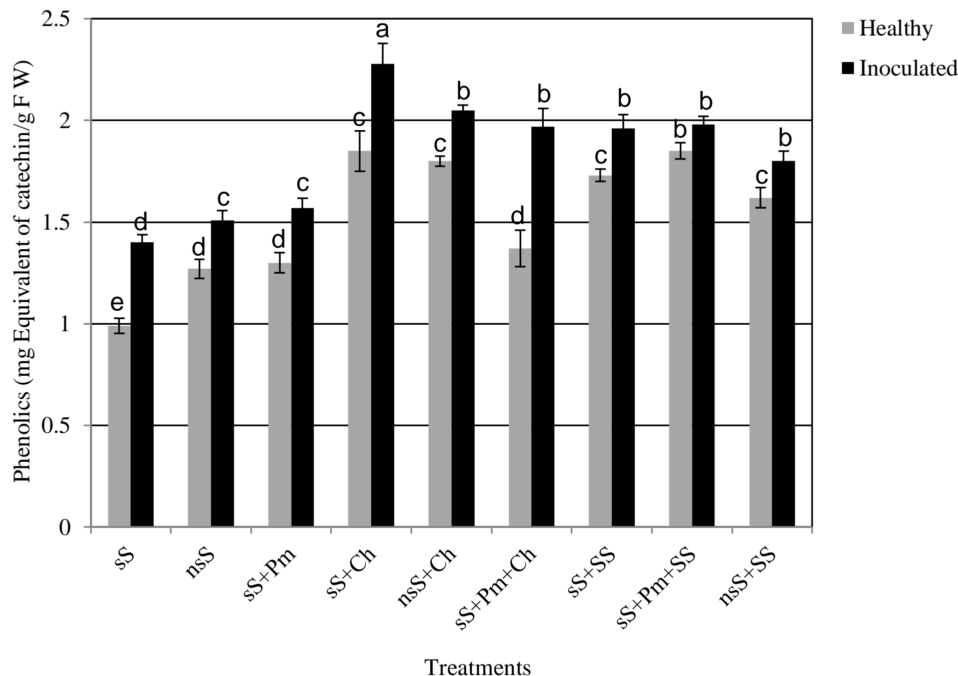

Figure 3. Variation of total phenolic content in treated and untreated plant before and six days after inoculation. Each treatment consisting of three replicates were repeated twice. Means with the same letters are not significantly different at *P*< 0.05. sS: sterilize Soil; nsS: none sterilize Soil; sS + Pm: sterilize Soil + *P. megakarya*; sS + Ch: sterilize Soil + Chitosan; nsS + Ch: none sterilize Soil + Chitosan; sS + Pm + Ch: sterilize Soil + *P. megakarya* + Chitosan; sS + SS: sterilize Soil + Snail Shell; sS + SS: sterilize Soil+ Snail Shell; nsS + SS: none sterilize Soil + Snail Shell.

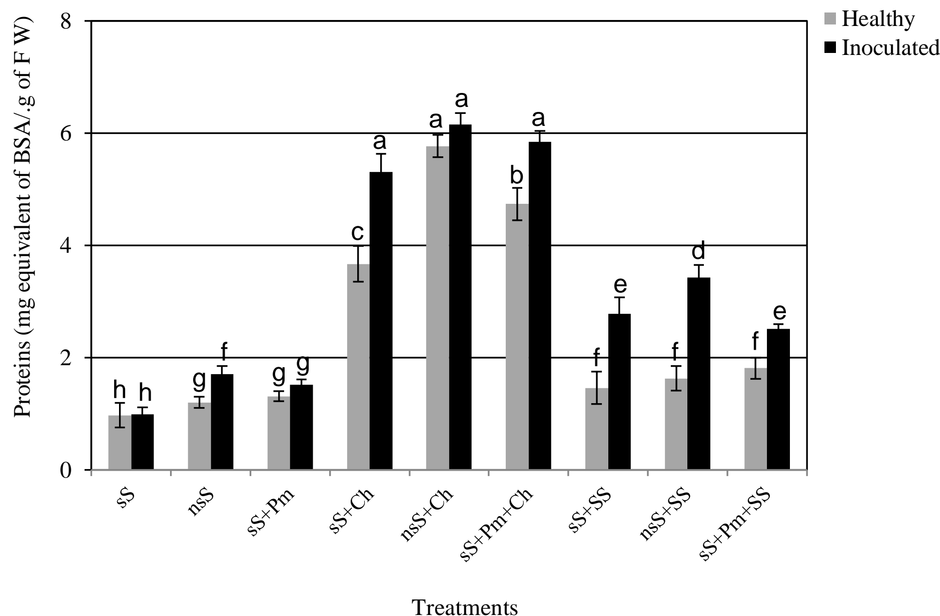

Figure 4. Variation of total proteins content in treated and untreated plant before and six days after inoculation. Each treatment consisting of three replicates were repeated twice. Means with the same letters are not significantly different at *P*< 0.05. sS: sterilize Soil; nsS: none sterilize Soil; sS + Pm: sterilize Soil + *P. megakarya*; sS + Ch: sterilize Soil + Chitosan; nsS + Ch: none sterilize Soil + Chitosan; sS + Pm + Ch: sterilize Soil + *P. megakarya* + Chitosan; sS + SS: sterilize Soil + Snail Shell; sS + SS: sterilize Soil+ Snail Shell; nsS + SS: none sterilize Soil + Snail Shell.

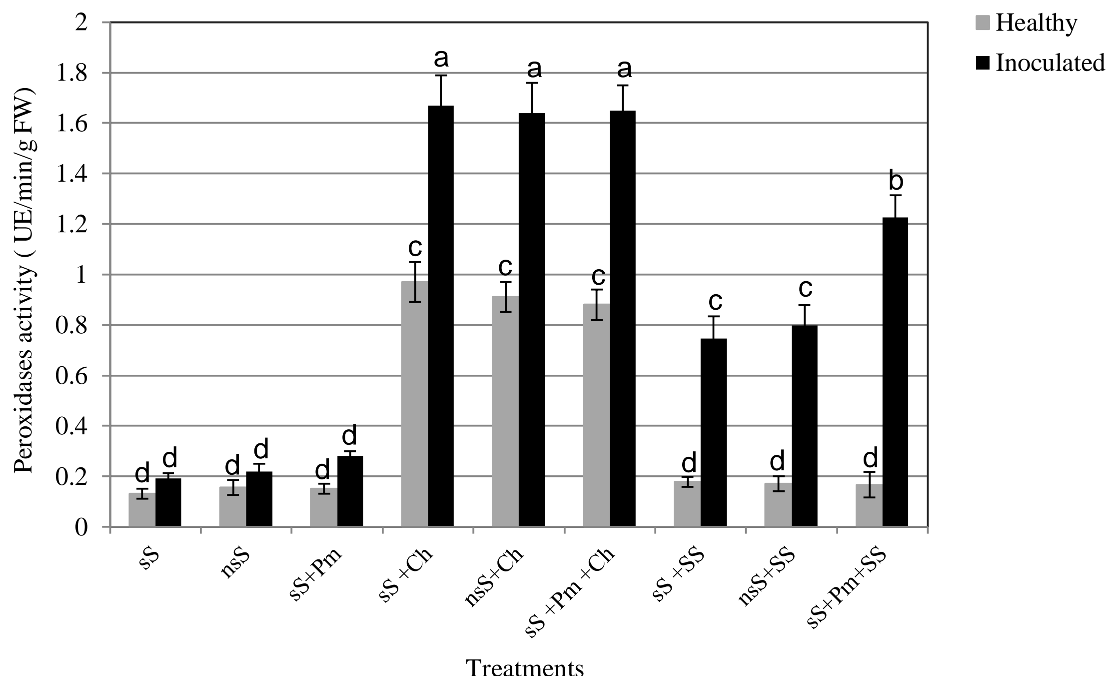

Figure 5. Variation of total peroxidases activities in treated and untreated plant before and six days after inoculation. Each treatment consisting of three replicates were repeated twice. Means with the same letters are not significantly different at *P*< 0.05. sS: sterilize Soil; nsS: none sterilize Soil; sS + Pm: sterilize Soil + *P. megakarya*; sS + Ch: sterilize Soil + Chitosan; nsS + Ch: none sterilize Soil + Chitosan; sS + Pm + Ch: sterilize Soil + *P. megakarya* + Chitosan; sS + SS: sterilize Soil + Snail Shell; sS + SS: sterilize Soil+ Snail Shell; nsS + SS: none sterilize Soil + Snail Shell.

Table 3. *P. megakarya* soil inoculums load effect on cocoa pods.

Parameter	sS+Pm	sS+Pm+Ch	sS+Pm+SS	nsS	nsS+Ch	nsS+SS	Control
D1	-	-	-	-	-	-	-
D2	+	-	-	-	-	-	-
D3	++	-	-	-	-	-	-
D4	+++	-	-	+	-	-	-
D5	++++	-	-	+++	-	-	-
D6	+++++++	+	+	+++++	+	+	-

D1 to D6: Days after inoculation. The control is pod inoculated with distilled water. Each treatment was repeated twice. + = Presence of necrotic lesion, - = Absence of necrotic lesion. sS+Pm: sterilize Soil + *P. megakarya;* sS+Pm+Ch: sterilize Soil + *P. megakarya* + Chitosan; sS+Pm+SS: sterilize Soil + *P. megakarya* + Snail Shell; nsS: none sterilize Soil; nsS + Chitosan: none sterilize Soil + Chitosan; nsS + SS: none sterilize Soil + Snail Shell.

nurseries and fields in Cameroon. Differences that emerged from seed germination, measurement of plant growth and quantitative production of selected biochemical molecules, following the infection of the plants with *P. megakarya*, provided a number of underlying evidence to show that soil treatment with organic matter could improve growth of cocoa seedlings and induce resistance to BPD. In our study, chitosan (up to 2%) and snail shell (up to 2%) seed coating increased the percentage of germination compared to control. These results confirm the earlier findings that seeds soaked with chitosan increased the germination percentage (Zeng et al., 2012). This could be due to the excellent film-forming capacity of chitosan, making it easy to form a semi-permeable film on the seed surface which can maintain the seed moist and absorb the moisture from the soil. Furthermore, Tahereh et al. (2012) reported that seeds coated with chitosan increased the lipase and β-1,3 glucanase enzyme activity which caused better germination. However, despite the evidence of its activity from many studies, the mechanism of chitosan effect on seed germination is still unknown. The plant growth

promoting effects of chitosan observed in this study are consistent with the results of many authors who reported the positive effects of chitosan incorporated into soil on early growth stages of soybean, mini-tomato, upland rice and lettuce (Hilal et al., 2006). Like chitosan, the promoting effect of snail shell powder on the growth of the cocoa seedlings, is in agreement with the study of Beauséjour et al. (2003) who showed that soil amendment with chitinous material stimulated gram-positive bacteria which reduced common scab incidence and induced potato growth.

The absence of necrotic lesions on pods inoculated with soil treated with organic matter could be due to a decrease of *P. megakarya* load in the soil. This decrease could be correlated to the healthier condition observed in the treated plants compared to the control plants. Several studies have also reported that chitosan and chitinous soil amendment, contribute to plant protection by modifying the microbial community and stimulating plant defense mechanisms (Benhamou et al., 1994; Roy et al., 2010). Furthermore, Xing et al. (2013) showed that non-treated and heat-treated oyster shell powder exhibited antifungal activities.

This study shows that the incidence of disease was significantly reduced in plants obtained from soils treated with chitosan or snail shell. Under controlled conditions, chitosan and snail shell were efficient elicitors of some defense reactions in cocoa. This effect was higher in plants obtained from soil treated with chitosan. The induction of systemic resistance could be explained by the capacity of chitosan and snail shell to stimulate plant defense mechanisms and higher synthesis of plant defense metabolites like phenolic compounds and pathogen-related proteins (Benhamou and Picard, 2000; Mbouobda et al., 2010; Coqueiro et al., 2011). This induction of systemic resistance by snail shell could be due to its composition. It is well known that snail shell is mainly composed of calcium carbonate. Recently, Arfaoui et al. (2015) demonstrated that pretreatment with calcium base formulation enhanced defense-related genes' expression in soybeans response to *Slerotinia sclerotiorum*. This phenomenon was confirmed in this study by higher production of phenolic compounds, total native protein contents and higher peroxidase activity in the leaves of cocoa seedlings following chitosan and snail shell soil treatment. At the concentration used, chitosan and snail shell does not affect the development of the cocoa plant. This efficacy at low concentrations suggests that this compound was recognized by plant cells and the observed protection was at least partly due to the induction of plant defense responses. In summary, this study contributes to show that chitosan and snail shell soil treatment initiated a series of morphological as well as biochemical changes in the plants which are considered to be part of the plant defense response. The effect of snail shell on cocoa growth and resistance showed that it is possible for snail shell powder to be a biofungicide and biofertilizer used in the control of cocoa Black Pod Disease in nurseries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors acknowledge the institutional and material support from the Biotechnology Centre of the University of Yaounde 1. The study was partially supported by the *Agence Universitaire de la Francophonie* (AUF). The authors are also grateful to R.A. Mouafo Tchinda for his excellent technical assistance.

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Vol. 11(8), pp. 341-350, August 2017
DOI: 10.5897/AJPS2017.1570
Article Number: B37BE1665315
ISSN 1996-0824
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African Journal of Plant Science

Full Length Research Paper

Effect of glyphosate used as a sugarcane chemical ripener in Côte d'Ivoire

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Received 8 May, 2017; Accepted 14 July, 2017

Sugarcane chemical ripening is aimed at accelerating the sucrose accumulation in the stems for harvesting when the climate conditions of crop year are hardly optimal for natural ripening. The study aims at determining the best response of sugarcane varieties to glyphosate ripening effect and the harvest delay after its application in Ferké 2 Sugar Bowl, Northern Côte d'Ivoire, in order to improve the technological qualities of canes at the early harvest season. Twenty plantations (700 ha) hosting two commercial varieties NCo376 and SP711406 were treated with glyphosate (360 emulsion concentrated) at 0.8 L/ha. For each plantation, two sample plots of 1 ha control and treated were determined. Those samples were analyzed to determine the sucrose gradient all along the stalks and monitor their technological qualities after 10, 15 and 20 days. As results, glyphosate improved sucrose content and the recoverable sugar of treated varieties. SP71-1406 was more sensitive than NCo376 with uniform qualities all along stalks after 20 days. Gains of 1.6% sucrose content and 1.5% recoverable sugar were obtained, compared to the control. So, the uppermost parts preservation of harvested stalks is justified and a sugar gain of 0.13 t/ha except those generated by the ripener.

Key words: Glyphosate, ripener, technological quality, gradient, sucrose, recoverable sugar.

INTRODUCTION

Glyphosate (N-phosphonométhyl glycine, $C_3H_8NO_5P$) is a glycine analogue. It is considered to be the most used herbicide worldwide for its biological efficiency as total weed-killer, its affordable cost and low toxicity (Goscinny and Hanot, 2012; Guimaraes et al., 2005).

It is used at low dose for cereal or oleaginous cropping, like desiccant for pre-harvest (Steinmann et al., 2012). The diversified exploitation of the glyphosate properties made it a multiform uses pesticide in agriculture.

In sugarcane cropping, the application of glyphosate at the end of crop cycle before tillage has helped to develop practices of zero tillage and minimum tillage in view of

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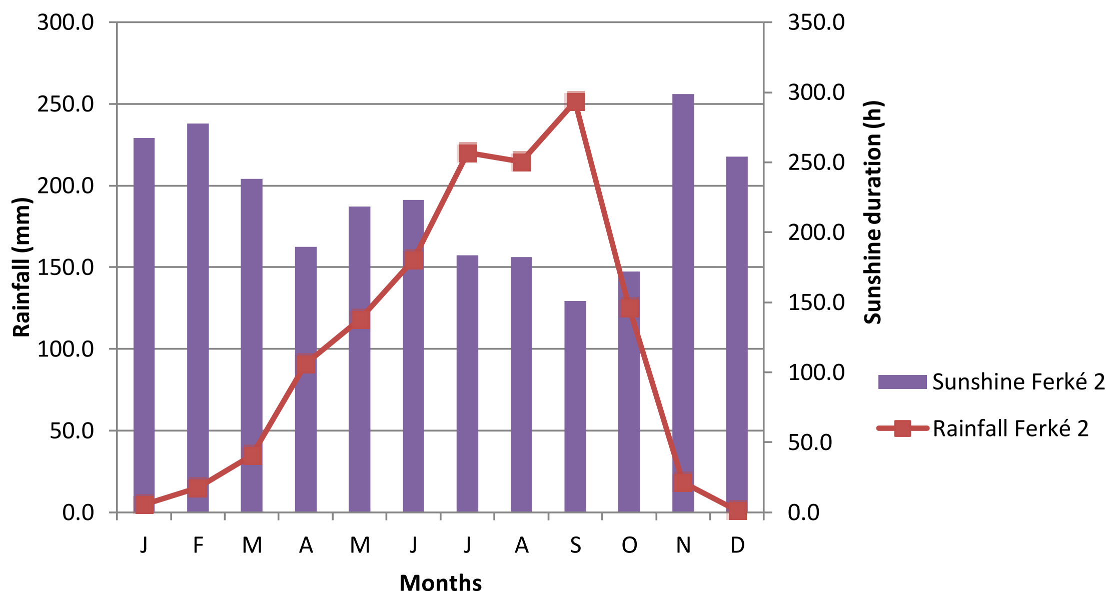


Figure 1. Monthly sunshine and rainfall at Ferké 2 Sugar Bowl for 10 years.

reducing production costs (Almeida et al., 2005). At low dose (0.8 to 1 L/ha), glyphosate has long been used as sugarcane ripener in order to carry out the harvest when climatic conditions are unfavorable to the natural ripening process (low daily thermal difference, soil moisture, high relative humidity of the air) (Meschede et al., 2010). Glyphosate once absorbed by the leaves of the cane, is the only herbicide that can block the activity of the enolpyruvylshikimate-3-Phosphate synthase (EPSPS). This enzyme is located at the beginning of shikimic acid path and that of pentose phosphates involved in the conversion of carbohydrate precursors derived from glycolysis into aromatic amino acids. The enzyme is *a priori* in the chloroplasts where it catalyzes the combination of shikimate-3-phosphate (S3P) with phosphoenol pyruvate to form 5-enolpyruvylshikimate-3-phosphate (ESP). The latter is a precursor of aromatic amino acids (tryptophan, phenylalanine and tyrosine), hormones, vitamins and other essential metabolites in plants. Structural similarities with phosphoenol pyruvate enable glyphosate to be attached to the fixation site of the EPSPS substrate, to inhibit its activity and thereby block its translocation into the chloroplast. By blocking the activity of the EPSPS, glyphosate therefore prevents the degradation of sugars synthesized and stored in the sugarcane stalks into aromatic amino acids. The constant presence of the active site of the EPSPS enzyme in plants enables glyphosate to act on a wide range of weeds. The inhibition of the functioning of the shikimic acid pathway causes a deficiency in aromatic amino acids, and eventually, the death of the plant by nutritional deficiency (Geiger and Fuchs, 2002; Zablotowicz and Reddy, 2004).

In Côte d'Ivoire, the use of glyphosate as ripener is mainly practiced in sugarcane cropping, at the beginning of the harvest season where it has a beneficial effect on sucrose content. In this country, sugarcane harvest season spreads over 5 to 6 months from November to April, with a period of severe drought in December and January, which is very favorable to the accumulation of sucrose in the stalks (ripening). It is especially during the first month of the harvest season that climatic conditions are highly unfavorable to the natural maturation of many sugarcane varieties (usually with abundant flowering) intended to be harvested at that time. This explains the resort to chemical ripening of these varieties with weed-killer like glyphosate so as to accelerate the sucrose accumulation process in the stalks and the time of harvest. The second challenge is to avoid over-ripening of plots treated beyond a certain time which may depend on the variety cultivated (Péné et al., 2016).

The study aims at determining, on the one hand, the sugarcane variety that has the best response to the glyphosate ripening treatment and, on the other hand, the efficiency period of the treatment between the date of application and the date of harvest.

MATERIAL AND METHODS

Experimental site

The sugar bowl of Ferké 2, where the study was conducted, is located at Ferkessédougou in northern Côte d'Ivoire (9°14'- 9°35'N and 5°15'- 5 °24'W and 323 m altitude). The prevailing climate is humid sub-tropical, with a dry season from November to March and a rainy season from April to October (Figure 1). The average annual rainfall is 1200 mm and there is a diversity of soils whose majority is ferralitic and shallow (40 to 60 cm) because of induration (Bigot et al., 2005; Brou, 2005).

Plant material

The effect of glyphosate was assessed on commercial varieties of sugarcane NCo376 originating from South Africa and SP71-1406, Brazilian variety, which were introduced in Côte d'Ivoire, in May 1960 and January 1987 respectively. The first one was the most cultivated in 2007 with 15% of cultivated surface areas at the time of the study and the second one was developing then.

Experimental design

Twenty plots in ripening phase, a total of approximately 700 ha were treated in late September-early October by aerial spraying with glyphosate (Roundup®) applied at a dose of 0.8 L/ha through a slurry dose of 15 L/ha.

Each plot was divided into two experimental sample plots of 1 ha from which 12 canes were sampled 10, 15 and 20 days after treatment and analyzed in the laboratory in order to determine the technological qualities of sugarcane.

Saccharimetric analyses

In the laboratory, each sampled stalk was cut into four pieces or quarters, a base (Q1), two middles (Q2 and Q3), and a top (Q4). Each set of 12 quarters was individually ground using an electric grinder ("Jeffco" food and fodder cutter grinder, model 265B size 10, L1710 series). The pulp resulting from each set of cane was submitted separately to a hydraulic press (Pinette Emidecau Ind.125). Saccharimetry analyses were carried out separately on the collected extract from each pulp. The brix juice (total sugar) was measured using a refractometer (SCHMIDT+HAENSCH, model DURSW, 29129 series) at 20°C. A part of the juice was clarified according to the basic lead acetate method of Horne (lead acetate hydroxide (II) or Horne salt) at 2.5 g per 250 ml of undiluted juice (ICUMSA GS5/7-1, 1994 quoted by Hoareau et al., 2008 and Kouamé et al., 2010). The juice was then filtered through WHATMAN paper 91, and the Pol was read out by polarimeter (SACCHAROMAT Z, 29305 series). The juice Pol was determined from the Brix and Pol read out by Schmidt table for saccharimeter. The juice purity (Pol rate in Brix) was then calculated. The fiber rate was determined using a correspondence table from the weight of the fiber obtained after pressing the ground material.

The sucrose content (SC% or Pol%C) was determined by multiplying the juice Pol by an *n* factor read out on a second table for a weight of 500 g cane pulp cake (Hoarau, 1970). The recoverable sugar (RS%) was determined as follows (Fauconnier, 1991):

$$\textbf{Recoverable Sugar (RS\%)} = [(\mathbf{0.84\ X\ Pol\%C})\ \mathbf{X}\left(\mathbf{1.6} - \frac{\mathbf{60}}{\mathbf{Purity}}\right) - (\mathbf{0.05\ X\ Fiber\%C})]$$

Measurement of cane losses in the top parts of the stalks

The cane losses on farm in the top parts (white tips) were collected in five experimental sample plots of 10 m² each spread across each of the 11 treated plots before harvest and sampled (350 ha). For each sampled plot, the stuffed top parts from each of the five plots were collected separately and weighed in order to determine the average weight of cane per hectare.

Statistical data analysis

An analysis of variance was applied to data collected using the Statistica 7.1 software on Windows 7. The Newman-Keuls post-hoc test was used in case of significant differences between treatments for each agronomic or technological criterion considered (Newman, 1939; Keuls, 1952; Shaffer, 2007).

RESULTS

Effect of glyphosate on the variety NCo376 ripening

The effects of the ripening treatment on variety NCo376 related to the gradient of sucrose content in the cane stems and the period of time after treatment proved significant at 5% threshold (Table 1). However, the lack of interaction between the different treatments of the study for this variety showed that the gradients of sucrose content and recoverable sugar in the stalks, particularly between the top part and the other part of the stalk, were not attenuated despite the application of glyphosate and the period observed after the treatment (Figures 2 and 4).

Effect of glyphosate on the ripening of variety SP71-1406

Unlike NCo376, the effects of the ripening treatment on SP71-1406 related to the gradient of sucrose content in cane stalks was proved not significant (Table 2). The significant interaction effect between the different treatments of the study for SP71-1406 showed that the gradients of sucrose content and recoverable sugar in the stalks, particularly between the top part and the basal and middle parts of the stalk, were mitigated due to the application of glyphosate and time periods of 20 d observed there after (Figures 3 and 4).

Regarding variety SP711406, statistical analyzes showed that the effect of the ripening treatment had canceled the gradients of sucrose content and existing recoverable sugar in cane stalks between the basal and middle parts, on the one hand, and between the basal and the top parts, on the other hand (Table 2).

For variety SP71-1406, the time period of 20 days enabled to obtain a gain of 1.6% of sucrose content and 1.5% of recoverable sugar compared to the control.

These results show that the treatment with glyphosate helps to obtain gains in sucrose content and sugar in both sugarcane varieties considered, but with a shorter treatment response time period for SP71-1406 (more sensitive) compared to NCo376.

Cane weight and sugar losses on-farm

The cane losses on-farm relating to the top parts were estimated to 1 t per ha (Table 3). With 16.7% sucrose content and 12.6% recoverable sugar observed 20 days after treatment on variety SP71-1406, losses could be estimated at about 0.13 t of recoverable sugar per ha, that is, nearly 43 tons of sugar over the 350 ha of

Table 1. Variety NCo376 after treatment by glyphosate in Ferké 2: Averages relating to sucrose content and recoverable sugar rate for three harvesting periods.

Sources of variation		Sucrose content (Pol%C)	Recoverable sugar (RS%)
Quarters or pieces of cane	Q1	14.9[b]	11.1[b]
	Q2	15.1[bc]	11.2[b]
	Q3	15.3[c]	11.4[c]
	Q4	14.7[a]	10.8[a]
Harvesting period after treatment	D1 (10 days)	14.8[a]	10.9[a]
	D2 (15 days)	15.1[b]	11.2[b]
	D3 (20 days)	15.2[b]	11.3[b]
Treatments	TE (Control)	14.8[a]	11.0[a]
	TR (Treated)	15.2[b]	11.3[b]
Average		15.0	11.1
Standard deviation		0.9	0.8
Coefficient of variation (%)		5.9	7.4
Effect quarters		0.00[hs]	0.00[hs]
Effect treatments		0.00[hs]	0.00[hs]
Effect harvesting period		0.00[hs]	0.00[hs]
Effect interaction treatments*quarters		0.89[ns]	0.96[ns]
Effect interaction treatments*harvest period		0.66[ns]	0.58[ns]

The averages followed by the same letters in the same column and for the same variation source are not significantly different at 5% threshold according to the Newman-Keuls test. Hs: High significant; ns: Non-significant.

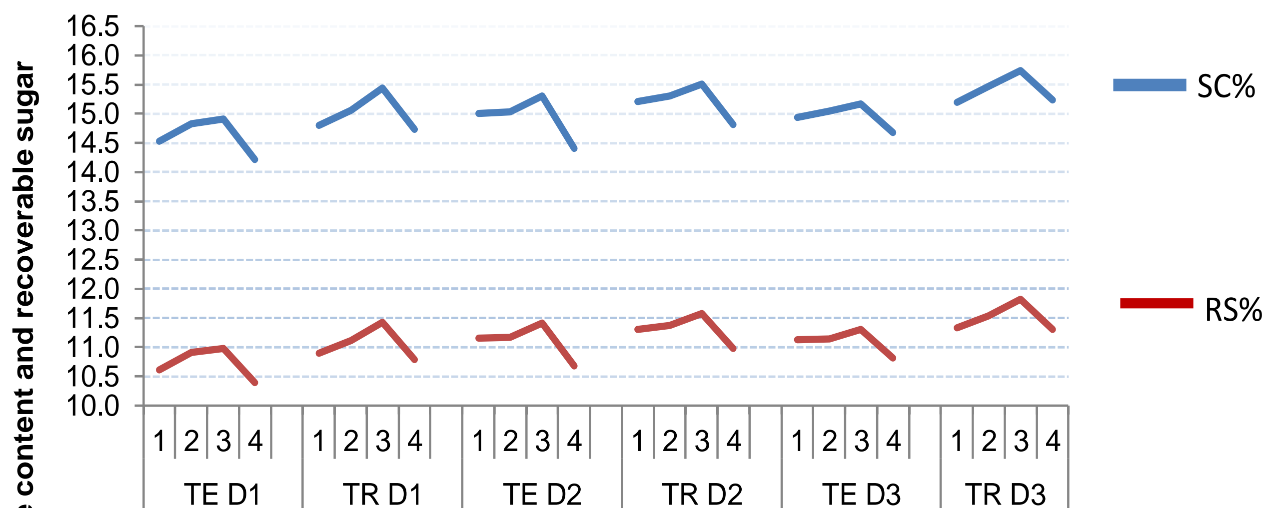
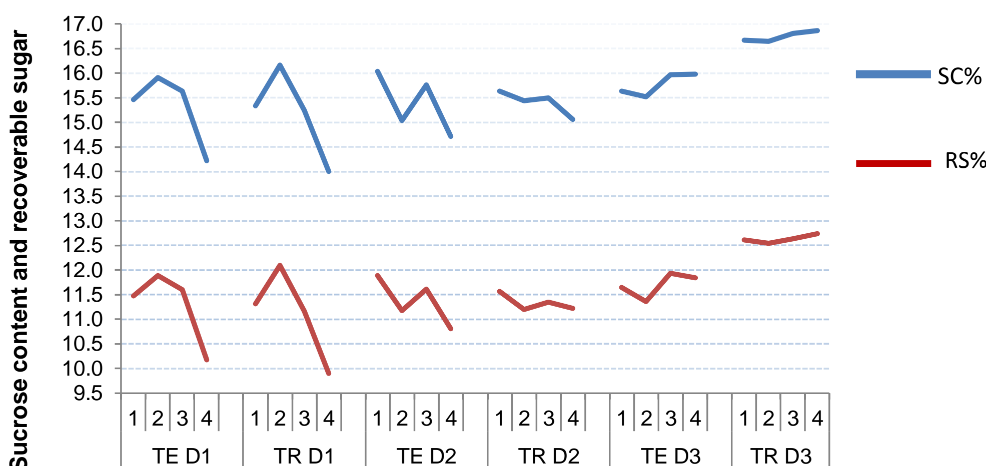


Figure 2. Variety NCo376 after treatment by glyphosate: Gradient of sucrose content or recoverable sugar in stalks on 3 harvesting dates.

Table 2. Variety SP71-1406 after treatment by glyphosate in Ferké 2: Averages relating to sucrose content and recoverable sugar rate for3 harvesting periods.

Sources of variations for SP711406		Sucrose content (Pol%C)	Recoverable sugar (RS%)
Quarters or pieces of cane	Q1	15.7[a]	11.7[a]
	Q2	15.8[a]	11.7[a]
	Q3	15.8[a]	11.7[a]
	Q4	15.0[a]	11.0[a]
\Harvesting period after treatment	D1 (10 days)	15.2[a]	11.2[a]
	D2 (15 days)	15.4[a]	11.3[a]
	D3 (20 days)	16.3[b]	12.2[b]
Treatments	TE (Control)	15.5[a]	11.4[a]
	TR (Treated)	15.7[a]	11.6[a]
Interaction Treatments * Harvesting period	TE*D1 (10 days)	15.3[a]	11.3[a]
	TE*D2 (15 days)	15.4[a]	11.4[a]
	TE*D3 (20 days)	15.8[a]	11.7[a]
	TR*D1 (10 days)	15.2[a]	11.1[a]
	TR*D2 (15 days)	15.4[a]	11.3[a]
	TR*D3 (20 days)	16.7[b]	12.6[b]
Average trial		15.6	11.6
Standard deviation		1.0	1.0
Coefficient of variation (%)		6.4	8.3
Effect quarters		0.11[ns]	0.11[ns]
Effect treatments		0.29[ns]	0.36[ns]
Effect harvesting period		0.01[hs]	0.01[hs]
Effect interaction treatments*quarters		0.90[ns]	0.92[ns]
Effect interaction treatments*harvesting period		0.02[s]	0.03[s]

The averages followed by the same letters in the same column and for the same variation source are not significantly different at 5% threshold according to the Newman-Keuls test. Hs: High significant; ns: Non-significant; s: Significant.



Pieces of cane (1, 2, 3, 4) of controls (TE) and treated (TR) on dates D1=10 days, D2=15 days and D3=20 days

SC%: sucrose content RS%: recoverable sugar

Figure 3. Variety SP71-1406 after treatment by glyphosate: Gradient of sucrose content or recoverable sugar in stalks on 3 harvesting dates.

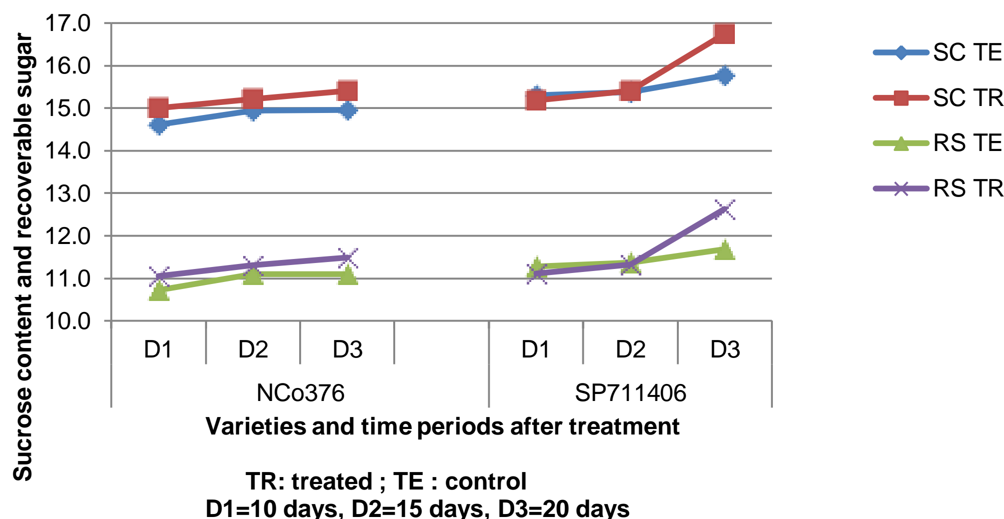


Figure 4. Interaction between harvesting time period and treatments by glyphosate for both varieties NCo376 and SP71-1406 regarding sucrose content (SC) and the recoverable sugar (RS).

Table 3. Cane losses on-farm in top parts of stalks for variety SP71-1406 in Ferké 2 sugar bowl.

N° Plot	Surface area (ha)	Cane losses on-farm	
		Weight (t)	tc/ha
110	40.8	30.0	0.73
118	33.2	21.6	0.65
136	25.2	25.7	1.01
150	19.8	17.8	0.90
239	34.0	23.5	0.69
228	39.4	54.0	1.37
204	33.8	37.9	1.12
409	33.7	47.8	1.42
148	24.1	32.2	1.34
203	42.3	37.6	0.89
303	21.3	9.8	0.46
Total or average	347.6	337.9	0.97

sampled plots.

DISCUSSION

Sugar yield gain related to the treatment

The results of the study showed that the ripening treatment by glyphosate helped significantly improve the sucrose content, the recoverable sugar and therefore sugar yield among the tested varieties. They confirm those obtained in many previous studies (Villegas and Torres, 1993; Bennett and Montes, 2003; Viator et al., 2003).

The treatment of the variety SP71-1406 has significantly improved the technological qualities of top parts so much so as to cancel the gradient of sucrose content in the stalks.

The good response of SP71-1406 to ripening treatment compared to NCo376 using glyphosate reinforces earlier observations made by Silva and Caputo (2012) on varietal differences in sugarcane vis-à-vis this treatment.

It was observed that the harvesting period of time after ripening treatment by glyphosate was generally 25 to 35 days (Silva and Caputo, 2012), while the one observed for SP71-1406 in this study is shorter (15 to 20 days).

Moreover, the varieties respond differently to ripening treatment depending on climatic parameters such as temperature and solar radiation. The particularly upright shape of SP71-1406 (including the active leaf apparatus) enables it to capture more efficiently light energy compared to varieties more sensitive to pouring down or having plagiotropic leaves such as NCo376. This helps explain in part the differences in response to the ripening effect in both varieties (Hopkins, 1995).

Effect of glyphysate on the gradient of sucrose content in cane stalks

In conditions of natural ripening in sugarcane, sucrose accumulation in stalks occurs first in the basal parts before moving progressively towards the top parts. As the ripening goes on, the sucrose content tends to be uniform along the cane stalks (McCormick et al., 2008; Silva and Caputo, 2012). In most cases, the tops are poor in sucrose and very rich in starch unlike to the lower parts of the stalk. This is why in most countries where manual harvesting of sugarcane is practiced, the top parts, immature and very poor in sucrose, are eliminated. These are characterized by an active cell growth that causes quick hydrolysis of part of the sucrose accumulated in the stalks by the vacuolar invertase acid cells. Hexoses stemming from this hydrolysis migrate into the cytoplasm of cells to be used for the benefit of growth (Lingle, 1999). One of the effects of glyphosate is to cause the death of the apical bud of cane stalk or inhibit the synthesis of indole acetic acid (IAA) which is a growth hormone. This results in an increase in ethylene synthesis by the action of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (Liang et al., 1992).

This study showed that after the artificial maturation, it was not necessary to carry out the elimination of the top parts of stalks (except leaf apexes and flower scapes) in varieties like SP71-1406 whose good response to ripening has enabled uniformity of sugar along the stems. So, production losses on-farm through the top parts on treated plots, planted with this variety were assessed at 1 t of cane/ha or 0.13 t of sugar/ha.

The differences in chemical ripening, observed between both varieties could be partly explained by their contrasting skills at flowering. NCo376 is a variety with a very high flowering rate (80 to 100%) in cropping conditions on the perimeter of Ferké unlike SP711406 that flowers very little there. When the treatment does not occur before flowering in varieties highly sensitive to photoperiod, inflorescences intercept a portion of the chemical ripener to the detriment of the leaf apparatus resulting less beneficial effect of the treatment. Previous studies have shown that abundant flowering could cause in the event of over-ripening the phenomenon of pith process (formation of a marrow in the center of the cane stem). This phenomenon, whose magnitude depends on the variety cultivated, causes the reduction of technological qualities. The pith process induces the drying of inner stem from the top parts with weight loss through dehydration, significant drop in cane yield and difficulty in extracting sugar (Caputo et al., 2007; Silva and Caputo, 2012; Leite et al., 2011).

Although artificial ripening by glyphosate is an alternative to natural ripening of sugarcane when climatic conditions are unfavorable (Cardozo and Sentelhas, 2013), some drawbacks associated with its use have been reported. Glyphosate induces senescence of the apical bud and development of lateral buds that are detrimental to the technological qualities of stalks. The growth inhibition by glyphosate causes the reduction in the average number of internodes and the average weight per cane stem. In particular, the use of glyphosate as ripener negatively affects ratoon and number of stalks after harvesting. The height and number of cane stalks per unit surface area is reduced and undulations are observed in the plots (Dalley and Richard-Junior, 2010).

Other cane ripening substances

According to Azania et al. (2013), there are two types of sugarcane ripeners of which one causes lethal stress and the other non-lethal stress for the plant. Glyphosate is considered as lethal stress ripener for sugarcane. It is a growth inhibitor that causes sucrose accumulation in sugarcane while preventing it from being used as an energy source for developing meristems. This ability to reduce the growth rate forces the cane to ripen. Richard et al. (2006) reported a better sugar yield in sugarcane varieties treated with trinexapac-ethyl, imazapyr, or nicosulfuron compared to glyphosate related to the reduction of cane yield caused by the latter. These include non-lethal stress ripeners for sugarcane whose action does not cause permanent growth failure or death of the apical bud as in the case of glyphosate, but which induce the production of ethylene which is responsible for sucrose accumulation in stems (Bueno et al., 2011).

Fluazifop-p-butyl (aryloxyphenoxypropionate group) has a systemic foliar action. Applied at low doses (0.1 to 0.3 l/ha), it is quickly absorbed and migrates into the growth points by inhibiting acetyl coenzyme Acarboxylase (ACCase) which is an enzyme responsible for the biosynthesis of fatty acids (Hugh, 2000). It thus limits the formation of membrane lipids necessary for cell growth. It causes mortality of apical bud and necrosis as in the case of glyphosate, but more slowly by maintaining photosynthesis always active with sucrose accumulation in stems. The treated areas can be harvested between 28 to 35 days with a risk of loss in cane technological qualities beyond that period (Silva and Caputo, 2012). Fluazifop-p-butyl inhibits flowering thus avoiding the risk of pith process. It has no depressive effect on ratoon unlike glyphosate. However, previous studies conducted

in Louisiana showed that fluazifop-p-butyl was less efficient than glyphosate because of its depressive effect on cane yield in the treated areas (Watson and Stefano, 1986; Dalley and Richard-Junior, 2010).

Maleic hydrazide (1.2-dihydro-3.6-pyridazinedione) is a growth regulator which favors suppression of apical dominance in plants. It is considered as potential ripener in sugarcane inducing sucrose accumulation in the stalks with growth reduction (Silva and Caputo, 2012).

The chemical compound Imazapyr (groups of imidazolinones) is absorbed through the leaves and rapidly migrates into the meristematic zones where it accumulates. By inhibiting acetolactate synthase (ALS), it blocks the synthesis of amino acids with branched chains (valine, leucine, isoleucine), thereby stopping protein synthesis (including DNA) and cane growth. Imazapyr does not control flowering in cane according to Lavanholi et al. (2002), but rather favors accumulation of sucrose in stalks.

Ethephon (2-chloro-ethylphosphonic acid) is a growth regulator with systemic action which penetrates the tissues of the plant and decomposes into ethylene, a compound highly soluble in water and stable in aqueous solution at a pH below 3.5 and temperatures above 75°C. It reduces growth but is widely used as a flowering inhibitor, stimulating the emergence and tillering of ratoon until six months after harvesting and as ripener in sugarcane having flowered or not. A differential response of sugarcane varieties to ethephon applications as ripener has been reported (Silva et al., 2007; Castro et al., 2001; Gururaja Rao et al., 1996; Tomlin, 1994). Its inhibitory action on flowering helps avoids the risk of pith process of cane stalks which has the effect of significantly reducing impaired cane and sugar productivity. Furthermore, ethephonhelps anticipate the harvest by at least 21 days and its effect persists for 60 to 90 days after application, which enables to exploit the treated plots for a relatively long period from the beginning until the middle of crop harvest (Caputo et al., 2007; Dalley and Richard-Junior, 2010).

Sulfometuron-methyl (sulfonylurea) is characterized by its systemic action on meristematic zones after foliar uptake inhibiting thus growth and cell division without directly interfering with mitosis and DNA synthesis. It inhibits the synthesis of amino acids with non-cyclic carbon chains such as valine, leucine, and isoleucine by affecting the acetolactate synthase enzyme (ALS) from the precursor alpha-ketobutyrate. Herbicides of this group do not directly block the action of growth activators that are auxin, gibberellins or cytokynins but strongly stimulate the production of ethylene, which is a response of the plant to the phyto-toxicity of the product. This causes paralysis and inhibition of apical meristem development causing in cane the reduction of internodes length formed after application of the herbicide as ripener. Leaf formation is thus inhibited in favor of sucrose accumulation in stems. After application, the treated areas can be harvested after 25 to 45 days according to Silva and Caputo (2012), while Almeida et al. (2005) showed that harvesting could be anticipated by15 days. It is a ripener which does not act on the apical bud so that the stems recover their normal growth even if the treated areas are not harvested or are harvested late (Leite et al., 2011).

Trinexapac-ethyl (cyclohexanedione group) is a chemical compound that induces a large accumulation of sucrose in cane stems. It is preferentially absorbed by the leaves and roots and then passes in meristematic zones where it inhibits the synthesis of gibberellic acid which is involved in cell growth and division, inhibiting thus the development of the plant while favoring sucrose accumulation in cane stems without adversely affecting cane yield as in the case of glyphosate (Van Heerden et al., 2015). The other benefits of trinexapac-ethyl as sugarcane ripener lie in flowering reduction, brix increase and cane juice purity, and the absence of depressive effect on subsequent ratoon. The recommended dose as ripener ranges from 0.8 to 1.2 l/ha and the areas treated with this compound can be harvested after 35 to 55 days (Guimaraes et al., 2005; Rainbolt, 2005; Richard et al., 2006; Dalley and Richard-Junior, 2010; Leite et al., 2011; Silva and Caputo, 2012).

Impact of chemical treatments on biodiversity

The advantages of the application of glyphosate and other ripening products have been proved. However, the impact of these treatments on the status of populations of certain sugarcane pests must be emphasized. Thus, in recent years, increased attacks of *Eldana saccharina,* sugarcane stem borer in the sugar bowl northern Côte d'Ivoire have been reported (Péné et al., 2016). This intensification of stem borer attacks could be explained by the destruction of resources and shelter for natural enemies (parasitoids) of stem borers that are Trichogramma, preventing their maintenance and survival in nature (Goebel et al., 2010). Chemical treatments, especially by air, destroy the natural hosts of parasitoids and thus have the effect of reducing the natural parasitism of *Trichogramma.* The diversity of trichograms is important especially as the host plant diversity is large (Lamy et al., 2013). For this purpose a study is underway in Côte d'Ivoire in order to determine the parasitism rate and identify the natural enemies of the tropical stem borer with a view of biological control of *E. saccharina.*

Conclusion

The study shows that glyphosate, applied as ripener in the early crop harvest season at the dose of 0.8 L/ha, proved efficient on varieties NCo376 and SP71-1406, with a significant improvement of their sucrose content

and recoverable sugar, that is, respectively 1.6 and 1.5%, at 20 days after treatment. SP71-1406 proved particularly sensitive to the treatment with induced ripening in 20 days and a deletion of the gradients of sucrose content and recoverable sugar between the top and basal parts of stalks, unlike NCo376. Taking into account cane losses on-farm across the top parts estimated at 1 t/ha, this corresponds to sugar losses of 0.13 t/ha for variety SP71-1406 when treated. It appears thus relevant, during the manual harvest, to cut the highest possible top parts of the stems so as to limit sugar losses on such varieties responding well to the ripening treatment. The application of glyphosate as chemical ripener causes lethal stress on sugarcane with a depressive effect on ratoons. The study exposes the existence of other substances with non-lethal stress, such as trinexapac-ethyl, ethephon and sulfometuron-methyl. However, all these chemical treatments, whatever their agro-technological benefits and their targets, destroy the habitats of natural enemies of stem borers and therefore result in reduction of their parasitism and change of the status of borers whose attacks and geographic areas have been increasing in recent years.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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