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EFFICIENCY OF METHYL JASMONATE AND OLIGOSACCHARIDE FRACTION EXTRACTED FROM FUSARIUM OXYSPORUM F. SP. VASINFECTUM ON POLYPHENOLS ACCUMULATION IN COTTON (GOSSYPIUM HIRSUTUM L.)

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ABSTRACT

This paper aims to stimulate the polyphenols accumulation in cotton by MeJA and oligossacharide fraction from FOV to increase protection to Fusarium wilt. To do this, cotton plants were treated with different concentrations of fungal fractions and different concentrations of MeJA. Leaves were removed and the polyphenols content was determinate. The concentrations of elicitors which stimulated more the polyphenols production were retained. Their effectiveness time on polyphenols production in cotton leaves after application was evaluated. The results revealed that the oligosaccharide fraction 11 (10 %) allowed a large polyphenols accumulation as MeJA. It has a shelf life of 45 days after application while that of 5 mM MeJA is 30 days. The results suggest that a natural molecule such as oligosaccharides could be considered as a treatment to increase the resistance of cotton FOV. However, it would be necessary to apply a second application 45 days after the first to maintain polyphenols production at a high level. This article mainly highlights the use of elicitors such as fungal oligosaccharide aimed at limiting chemical control in cotton culture.

Keywords: Fusarium oxysporum, MeJA, oligosaccharides fraction, polyphenol

Abbreviations: OSF, oligosaccharide fraction; FOV, Fusarium oxysporum f. sp. vasinfectum, MeJA, methyl jasmonate

1. INTRODUCTION

The growing of *Gossypium hirsutum* L. accounts for nearly 95 % of global cotton production (Kouadio *et al*., 2004, Konan and Mergai, 2007). It is grown for its fibers which constitute the main raw material in the textile industry. Cotton production contributes more than 60 % to the Gross Domestic Product (GDP) of African countries (N'Djafa *et al*., 2010). This socio-economic importance is compromised by the fact that the cotton crop is facing heavy pest attacks. Fusarium wilt caused by *Fusarium oxysporum* f.sp. vasinfectum (W. C. Snyder & H. N. Hansen) is the disease that causes more damage in cotton. In addition, most varieties of cotton grown are highly susceptible to Fusarium wilt. Cotton growing requires the use of plant protection products

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to fight the enemies of cotton, especially microorganisms, this in order to ensure a good profitability of cotton and seed production.

Unfortunately, pesticides, especially fungicides, are increasingly indexed for issues of toxicity, environmental pollution, health and even biodiversity (Calvet *et al*., 2005; Faurie *et al*., 2009a). In fact, pesticides can have serious consequences for male infertility, cancers and fetal malformations (Toé *et al*., 2004; Damalas and Eleftherohorino, 2011; Toé *et al*, 2013). To reduce the risks of pesticides using on the environment, it appears necessary to look for more effective alternatives for the development of a sustainable agriculture. One of the alternatives is to give plants the means to defend themselves, or to strengthen their own defenses, rather than directly fighting the aggressor (Amari, 2012, Konan *et al*., 2014; N'goran, 2015). Moreover, a plant falls ill due to lack of defense compounds and / or lack of a good level of synthesis of defense compounds. Elicitors are usually molecules secreted by microorganisms, fungal cell wall derivatives, bacteria and / or host plants (Korsangruang *et al*., 2010). SDNs (stimulators of natural defenses) are most often analogues or derivatives of natural molecules among which salicylic acid, ethylene and methyl jasmonate (MeJA) are the most used (Faurie *et al*., 2009b; Dufour *et al.,* 2013). Other molecules of oligosaccharides released by pathogens (exogenous elicitors) or plant cells (endogenous elicitors) are also capable of initiating a defense reaction in the host plant (Li *et al*., 2003). Authors like Hahn (1996) and Belhadj *et al*. (2006) showed, in the case of the plant / fungus interaction, that fungal-wall-derived oligosaccharides activate defense systems in higher plants such as peas, soybeans and parsley. Also, N'goran *et al*. (2014) have recently shown that the oligosaccharide fraction of fungal origin rich in reducing sugars is capable of inducing defense reactions. Thus, the accumulation of phenolic compounds in cotton is stimulated to increase the natural resistance of cotton against *Fusarium oxysporum* f. sp. Vasinfectum (FOV), responsible for Fusarium wilt. For this purpose, the duration of effectiveness of Methyl Jasmonate (MeJA) and of the FOV culture filtrate, both, as oligosaccharide elicitors after application to cotton is evaluated on the production of phenolic compounds in the leaves of cotton

2. MATERIALS AND METHODS

1.1Plant material

Plant material consists of cotton plants obtained from cotton seeds (*Gossypium hirsutum* L.), cultivar Y331B. The seeds, originating from Côte d'Ivoire, were supplied with fiber by CNRA (Centre National de Recherche Agronomique, Côte d'Ivoire, West Africa). This cultivar has a very high sensitivity to *Fusarium oxysporum* f. sp. vasinfectum (W. C. Snyder & H. N. Hansen), causal agent of Fusarium wilt.

1.2. Delinting and seeds selection

In a small vase, a small amount of concentrated sulfuric acid (H_2SO_4) (96 %) was added to the fiber seed provided by the CNRA. These seeds were immediately kneaded with a spatula to get rid of their fibers. These seeds thus delinted were placed in a beaker filled with tap water for 1

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min. Those that have been totally immersed have been said to be viable. These viable seeds were selected, dried in the open area and then selected for further experimentation.

1.3. *In vivo* production of cotton plants

In vivo, two-month-old cotton plants were used. The plants were produced from cotton seeds provided by the CNRA, after their delinting. These seeds were soaked in sterile distilled water at two seeds per 30 mL in a test tube and placed in the dark to facilitate germination. After 48 hours of imbibition in the dark, the seeds from which the embryo radicle points after imbibition were sown on the ridges at the rate of three seeds per hole at 5 cm depth. Each mound contains a row of 10 cotton plants. Plant growth was monitored for 28 days. For water nutrition, 100 mL of water was added to each plant every two days.

2. FUNGAL MATERIAL

Fungal material is the fungus Fusarium oxysporum f. sp. vasinfectum (FOV). FOV strains were provided by the Phytopathology Laboratory of the Superior School of Agronomy of Félix Houphouët-Boigny National Polytechnic Institute, Yamoussoukro-Côte d'Ivoire. Two FOV strains with different virulence were used. These are COT 11 and COT 6, respectively less virulent and very virulent (Abo *et al*., 2005).

2.1. Inoculation of seven-day-old cotton plants

The pathogenicity of the two strains of *Fusarium oxysporum* f. sp. vasinfectum (COT6 and COT11) was demonstrated by inoculating 30 seven-day-old cotton seedlings with 15 seedlings per strain. These seedlings were produced from cotton seeds provided by CNRA, after their delinting. These delinted seeds were sterilized under a laminar flow hood by rapid dipping for 1 min in 70 % (v/v) ethanol. This sterilization was followed by immersion for 20 min in sodium hypochlorite (2.5 % active chlorine). These seeds were then rinsed three times with sterile distilled water. Seeds were then soaked in sterile distilled water at two seeds per 30 mL in a test tube and placed in the dark to facilitate germination. After 48 hours of dark imbibition, seeds whose embryo radicle points after imbibition were placed in 500 mL pots containing soil autoclaved at 121 °C for 30 minutes under a pressure of 1 bar. Inoculation was done using agar explants bearing the pure mushroom contained in Petri dishes. These explants were mixed with soil containing the seedling at the two cotyledonary leaf stage at the rate of one Petri dish per plant. The symptoms of fusarium wilt caused by FOV were followed with the naked eye. The end of the experiment was marked by the death of all the seedlings.

2.2. Isolation, characterization and identification of fungal strains

To confirm the presence and virulence of the strain used, seedlings inoculated and exhibiting symptoms of the disease, including the control (uninfected), were recovered to effect re-isolation of the pathogen. Re-isolation was made from hypocotyl fragments because FOV caused by FOV being a vascular disease, the fungus is much more present in the stem than in other parts of the plant (Delattre, 1973). Transplantation of the hypocotyl fragments of about 0.5 cm was performed in Petri dishes on 8 % (w/v) PDA medium, previously sterilized by autoclaving at 120

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°C for 30 min. Then the Petri dishes were incubated for seven days in a culture room at 25 ± 2 °C under a 12h photoperiod. The fungal cultures obtained are compared to their parent culture. After one week of culture on PDA medium, the different fungal strains isolated and then transplanted were identified using the key of Botton *et al*. (1990).

2.3. Preparation and production of fungal filtrates

Two isolated fungus strains were cultured on solid sterilized PDA medium, as previously, for 10 days in a culture room at 25 ± 2 °C to obtain spores. Then, the spore colonies obtained were wetted with 5 ml of sterile distilled water containing one drop of Tween 20. The surface of the cultures was gently scraped using a curved sterile Pasteur pipette to obtain two suspensions of spores. The concentration of spore suspensions was determined using a Malassez cell. Aliquots of each spore suspension were added to the modified Czapek-Dox liquid medium to optimize the production of fungal elicitors previously autoclaved under the same conditions as before. The culture is large volume. The final concentration is approximately 2.5 10^4 spores / mL of medium. The cultures were placed at 21 ° C in the dark and stirred for 28 days (Fanizza *et al*., 1995). The two suspensions of spores obtained were stirred and then filtered on sterile Wattman paper. The filtrates obtained constituted exocellular oligosaccharide fractions (OSF). These two fractions are recovered in labeled Erlenmeyer flasks, sterilized as before and then stored at -4 °C.

3. DETERMINATION OF THE GLUCOSE EQUIVALENT OF FUNGAL FRACTIONS

The amount of sugars in the "eliciting" solutions obtained from the fungal fractions was determined according to the method described by Dubois *et al*. (1951) modified and adapted to our plant material. In this method, sulfuric acid (H_2SO_4) breaks the saccharide bonds between D-glucose and D-fructose bringing all the present sugars into solution, which will be revealed by phenol. For the dosage, 0.2 mL of phenol (5 %) is added to 0.2 mL of fungal extract. This mixture is supplemented to 1 mL with distilled water, followed by an addition of 1 mL of concentrated sulfuric acid (H_2SO_4) (96 %). After incubation for 5 min in a boiling bath, the reaction medium is cooled in the dark for 30 min. The yellow-orange color intensity that is proportional to the amount of sugar is measured spectrophotometer at the wavelength of 480 nm against a control containing no extract. The quantity of sugar was determined using a standard curve made with different concentrations of a stock glucose solution (200 µg/mL) and was expressed in mg glucose equivalent per liter of oligosaccharide fraction (mg EqGlu/L of OSF).

4. TREATMENTS OF COTTON PLANTS BY DIFFERENT CONCENTRATIONS OF FUNGAL FRACTIONS

Exocellular oligosaccharide fractions were dissolved in distilled water to obtain solutions at different concentrations (2; 5; 10; 20; 50 and 100 %). To each fungal solution, an aqueous solution containing Triton X-100 (0.1 %) was added. The purpose of this Triton is to act as an adjuvant and to allow a longer retention of the fungal fractions on the leaves while conferring penetrating power in the leaves. Thus, after four weeks of culture, 21 plants (at a rate of three plants per treatment) each having about 12 to 17 spread leaves were sprayed with the solutions resulting from mixing the different fungal solutions with Triton. During the treatments, plastic

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bags were used to separate the treatments of the controls, in order to avoid the contact of these controls with the different fungal solutions. The treatments were carried out by spraying with jars of perfume in a wash bottle, previously washed with sodium hypochlorite (3.6 % active chlorine) and cleaned with 80 % ethanol. Each plant was sprayed with 10 mL of the fungal solution. Cotton plants treated with culture medium (Modified Czapek-Dox) containing Triton X-100 (0.1 %) were used as a control for each condition tested.

5. TREATMENTS OF COTTON PLANTS BY DIFFERENT CONCENTRATIONS OF METHYL JASMONATE

After four weeks of culture, 18 cotton plants (3 plants / concentration) each having about 12 to 17 spread leaves were treated with 2.5; 5; 10; 15 and 20 mM of MeJA. These different concentrations of MeJA were previously dissolved in ethanol (1 %). To each of these MeJA solutions was added an aqueous solution containing Triton X-100 (0.1 %). These solutions thus treated were then sprayed on the leaves using a hand sprayer. Triton X-100 acts as adjuvant and allows a longer retention of the product on the leaves while imparting a penetrating power in the leaves. In order to promote the penetration of MeJA into the plants through the opening of the stomata, watering of the plants was done according to the humidity of the substrate. Thus, each plant received approximately one 10 mL application of the MeJA solution. MeJA contact times were 24; 48 and 72h. Cotton plants sprayed with a solution of ethanol (1 %) and Triton X-100 (0.1 %) served as controls. During the treatments, plastic bags were used to separate the treated plants from the controls, in order to avoid contact of these controls with the solutions.

6. EVALUATION OF THE EFFICIENCY TIME OF ELICITORS ON POLYPHENOLS PRODUCTION IN COTTON LEAVES

For this study the concentration of 5 mM for MeJA and 10 % for OSF 11 which strongly stimulated the production of phenolic compounds were used.

6.1. Preparation of elicitors and treatments of cotton plants

Methyl jasmonate (MeJA) was dissolved beforehand in ethanol (80 %) in the presence of 0.5 ml of Triton X-100 (0.1 %) and then distilled water was added. As for each oligosaccharide fraction FOS 11 (10 %), an aqueous solution containing Triton X-100 (0.1 %) was added. After four weeks of culture, six plants each having about 12 to 17 spread leaves were separately sprayed with 5 mM MeJA solution and FOS 11 (10 %).

6.2. Extraction and determination of polyphenols in cotton leaves

On each cotton plant treated at various concentrations of fungal fractions and methyl jasmonate, three leaves of the third leaf stage were taken and then lyophilized for the determination of polyphenols. As for the evaluation of the duration of effectiveness of the elicitors on the production of phenolic compounds in cotton leaves after treatments, the leaves were taken, after 72h of contact with the stimulators, every three days for two months. These leaves were then frozen and lyophilized for the determination of total phenols.

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The extraction of polyphenols was carried out according to the method of Kouakou *et al*. (2008; 2009). A sample of 500 mg of leaves of each treatment was immersed in 10 ml of pure methanol and placed in the dark at 4 °C., with stirring, for 24 hours corresponding to the time necessary for the extraction of the phenolic compounds. The leaves were subsequently removed. The mixture is then centrifuged at 2000 rpm / min for 10 min. The methanolic supernatant obtained is filtered through a Millipore membrane (0.45 µm) and the filtrate constitutes the polyphenols extract. The determination of the total phenols is done according to the method described by Singh (2000) modified and adapted to our plant material. Thus, 0.5 mL of the Folin-Ciocalteu reagent and 0.9 mL of water are added to 0.1 mL of polyphenols extract. After stirring at room temperature, 1.5 mL of a 17 % (w/v) sodium carbonate solution was added. After 20 min incubation at 25 °C in the dark, the intensity of the blue color of the reaction mixture which is proportional to the concentration of phenolic compounds was monitored spectrophotometer at 765 nm. During the assay, a control was carried out by replacing the phenolic extract with distilled water. Polyphenols content was determined using a calibration line ($y = 0.032x + 0.078$, $R^2 = 0.99$, where y is absorbance and x is the concentration of gallic acid) made with different concentrations of a stock solution of gallic acid (200 µg/mL). These contents were expressed in milligrams per gram of dry matter (mg/g of DM). Each measurement was repeated three times.

7. STATISTICAL ANALYSIS

The statistical analyses were performed with STATISTICA version 7.1 software. An analysis of variance (ANOVA) with one or two classification criteria was performed on all treatments applied. When this analysis shows a difference between the means, the Newman-Keuls test was performed in order to determine the significant differences between treatments at the 5% threshold. All the experiments were performed three times

8. RESULTS

1. Estimate of total soluble sugars from FOS from FOV

The analysis in Table 1 shows that the OSF 11 expressed a glucose equivalent (56.39 mg Eq Glu/L of OSF) content significantly higher than that generated by the FOS 6 (27.27 mg Eq Glu/L of OSF). In fact, the glucose content displayed by COT 11 is approximately three times higher than that presented by COT 6.

Table 1. Sugar content of two oligosaccharide fractions of FOV

Fractions of (OSF)	Sugar content (mg Eq Glu/L of OSF)
FOS 6	27,27 ± 1,24 [b]
FOS 11	56,39 ± 0,17 [a]

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In the same column, the values followed by the same letter are not significantly different (Newman-Keuls test at 5%). EqGlu: equivalent Glucose; OSF 6: Oligosaccharide fraction of strain COT 6; OSF 11: Oligosaccharide fraction of the COT 11 strain.

2. Effect of concentration of fungal fractions on polyphenols content in cotton leaves

The analysis in Table 2 shows that the oligosaccharide fractions (OSF) of COT 6 and those of COT 11 induce an increase in the production of total phenols in cotton leaves when their concentrations vary from 0 to 10 %. Above 10 %, this production of total phenols decreases inversely with the concentration of the fungal fractions. With the exception of the control treatment where the total phenol contents were statistically of the same order of magnitude in both strains, the strain COT 11 generated total phenols contents much higher than those expressed by the strain COT 6, whatever the concentration of these two strains. The comparison of the total phenol content expressed by the FOV strain COT 11 at the concentration of 10 % (430.95 ± 0.07 mg/g DM) to that displayed by the control (40.46 ± 0.00 mg/g DM) showed a multiplication factor of about 10. On the other hand, in FOV strain COT 6, this comparison, 10% (248.55 ± 0.02 mg/g DM) and control (41 13 ± 0.78 mg/g DM) showed a multiplication factor of only about 6-fold. In cotton leaves, the phenolic production induced by the less virulent FOV strain COT 11 was greater than that produced by the more virulent FOV strain COT 6.

Table 2. Polyphenol content of cotton leaves treated with different concentrations of fungal fractions of two strains of FOV

Fraction of FOV	Concentration (%)	Polyphenol content (mg/g of DM)
FOS 6	0	41,13 ± 0,78 ^{m}
	2	108,37 ± 0,04 ^{l}
	5	188,18 ± 0,16 ^{h}
	10	248,55 ± 0,02 ^{e}
	20	196,70 ± 0,12 ^{g}
	50	142,42 ± 0,15 ^{i}
	100	116,25 ± 0,35 ^{k}
FOS 11	0	40,46 ± 0,00 ^{m}
	2	207,58 ± 0,19 ^{f}
	5	283,72 ± 0,04 ^{c}
	10	430,95 ± 0,07 ^{a}
	20	345,73 ± 0,07 ^{b}
	50	267,36 ± 0,05 ^{d}
	100	135,35 ± 0,20 ^{j}

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In the same column, the values followed by the same letter are not significantly different (Newman-Keuls test at 5%), FOS 6: oligosaccharide fraction of the strain COT 6; FOS 11: Oligosaccharide fraction of strain COT 11; DM: dry matter; FOV: *Fusarium oxysporum* f. sp. vasinfectum; Frac of FOV: fraction of FOV; Conc: concentration; ± S: standard error

3. Effect of concentration and incubation time of methyl jasmonate on polyphenol content in cotton leaves

The polyphenols level induced by all cotton leaves treated with methyl jasmonate (MeJA), with the exception of leaves treated with 20 mM MeJA (55.36 ± 0.32 mg/g DM vs. 60 88 ± 0.04 mg/g of control DM) after 72h of incubation were greater than those induced by the controls (Table 3). For the 24h incubation time, the total phenol content increased with the methyl jasmonate concentration until reaching the peak with 15 mM MeJA (248 ± 0.11 mg/g DM). Above 15 mM, the total phenol content decreased from 248 ± 0.11 to 165.23 ± 0.08 mg/g DM (20 mM MeJA), lower total phenols in the incubation time of 24h). In contrast, the production of total phenols in cotton leaves increased when the concentrations of methyl jasmonate for incubation times of 48 and 72h ranged from 0 to 5 % mM. Above 5 % mM, this polyphenols production decreased inversely with MeJA concentration until reaching the lowest values at 20 mM MeJA. Polyphenol levels from treated plants after 72h with MeJA concentrations ranging from 0 to 10 mM were highest, followed in descending order by those from treated plants after 48h and 24h. However, treatment of the leaves by spraying 5 mM MeJA after 72h of incubation yielded the highest polyphenols content (325.46 mg/g DM). On the other hand, high concentrations of MeJA (15 and 20 mM) induced the lowest levels of total phenols, whatever the incubation time.

Table 3. Polyphenol content in MeJA-treated cotton leaves as a function of concentration and incubation time

I.T (h)	Concentration of MeJA (mM)	Total phenol content (mg/g de DM)
24	0	43,42 ± 0,02 ^{r}
	2,5	172,24 ± 0,02 ^{k}
	5	189,37 ± 0,04 ^{h}
	10	206,66 ± 0,57 ^{g}
	15	248 ± 0,11 ^{e}
	20	165,23 ± 0,08 ^{l}
48	0	58,2 ± 0,03 ^{p}
	2,5	186,23 ± 0,08 ^{i}
	5	286,18 ± 0,00 ^{c}
	10	278,67 ± 0,26 ^{d}
	15	178,22 ± 0,08 ^{j}

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	20	110,83 ± 0,46 ^{n}
72	0	60,88 ± 0,04 ^{o}
	2,5	210,54 ± 0,21 ^{f}
	5	325,46 ± 0,29 ^{a}
	10	293,14± 0,18 ^{b}
	15	152,03 ± 0,38 ^{m}
	20	55,36 ± 0,32 ^{q}

In the same column, the values followed by the same letter are not significantly different (Newman-Keuls test at 5%). MeJA: methyl jasmonate; I.T: incubation time; DM: dry matter; ± S: standard error

4. Comparison between the efficiency time of MeJA and fungal fraction FOS 11 (10 %) on polyphenols production

The analysis of figure 1 below shows the variation of the duration of effectiveness of MeJA and that of the fungal fraction (FOS 11 (10 %)) on the production of total phenols as a function of time. Compared with the total phenol levels expressed by untreated (control) leaves, those of leaves treated separately with MeJA and FOS 11 (10 %) increased significantly. Maximum concentrations, either at the level of MeJA or the fungal fraction (FOS 11 (10 %)), were reached after three days of incubation. Indeed, at the third day after the treatment, the activity inducing after treatment of MeJA which was (326.63 mg/g of DM) was 10 times higher than that of the control (41.47 mg/g of DM). This eliciting activity of MeJA on the leaves remained very effective until the first 30 days. However, after a 30-day treatment, polyphenols contents are significantly low and tend to reach the contents of untreated (control) leaves. Beyond 33 days, a considerable drop in polyphenols was noted. On the third day after treatment, with respect to the eliciting activity of the fungal fraction FOS 11, the total phenol content was 432.81 mg/g of DM. This content was practically 8 times higher than that of the control (41.47 mg/g of DM). This eliciting activity of the fungal fraction remained very effective until the first 45 days (one and a half months). However, after a 48-day treatment, a considerable decrease in total phenols was noted.

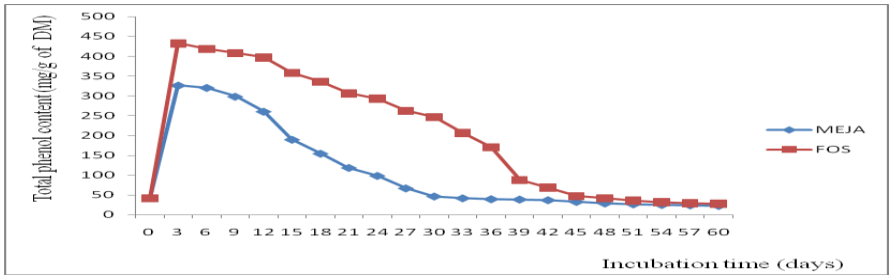

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Figure. Curves of the variation of the total phenols contents as a function of the incubation time after treatments with the elicitors

MeJA: methyl jasmonate; DM: dry matter

9.DISCUSSION

Evaluation of the sugar content of oligosaccharide fractions and mycelial fractions of two strains of Fusarium oxysporum f. sp. vasinfectum (W. C. Snyder & H. N. Hansen) (strain COT6 and strain COT11) showed that the less virulent strain COT 11 excretes oligosaccharides which are higher in glucose than the more virulent strain COT 6. This suggests that the nature of the polysaccharides may differ from one fungal strain to another and, therefore, their degradation would be dependent strain. The culture medium having an acidic pH (5.8), the polysaccharides excreted by the strain COT 11 underwent acid hydrolysis, that is to say a degradation more rapid than those excreted by the strain COT 6. Thus, according to Nita-Lazar *et al*. (2004), FOV strains excreting polysaccharides sensitive to acid hydrolysis have the most bioactive oligosaccharides. Moreover, Côté and Hann (1994) were reported that the degree of polymerization of an oligosaccharide derived from FOV is a test that can determine its ability to induce defense reactions. The evaluation of the effect of FOS concentrations of FOV on the production of total phenols showed a similar evolution of the total phenol content of the two fungal strains. These results suggest the existence of a relationship between the production of total phenols and the concentrations of FOS. In fact, FOS concentrations below 10 % boosted the production of total phenols while that above 10 % inhibited this production of total phenols. This decrease in total phenol production is due to the fact that high FOV oligosaccharide fraction concentrations (> 10 %) induce a hypersensitivity reaction that leads to cell death (Mukundan and Hjorsoto 1990; Roewer *et al*., 1992; Ngoran, 2015): hence a decrease in the accumulation of total phenols. The 10 % FOS concentration was the one that stimulated maximum production of total phenols, regardless of the FOV strains used. However, the FOS of FOV strain COT 11 resulted in the best accumulation of polyphenols compared to that of FOV strain COT6. The COT 11 strain seems to induce an systemic acquired reaction (SAR). Indeed, according to several studies, RSA is induced for a hypersensitivity reaction (HR) caused by an avirulent strain of a pathogen (Pieterse and Van Loon, 1999) or non-pathogenic microorganisms (Van Loon, 1997). This indicates the important role played by the degree of degradation of polysaccharides on the activation of polyphenols biosynthesis pathways. These results are similar to those of several authors (Lattanzio *et al*., 2006, Yamaner *et al*., 2013). These authors reported that the oligosaccharide fraction of fungal origin rich in reducing sugars is capable of stimulating the production of phenolic phytoalexins and also inducing defensive reactions. Also, our results are similar to those of Derckel *et al*. (1999) carried out on two strains of *Botrytis cinerea* Pers. (T4 and T8) of different virulence. The less virulent strain rapidly and strongly induces the production of phytoalexin and PR protein in contrast to the virulent strain. Thus, the pathogenicity or degree of virulence of a strain is therefore negatively correlated to the stimulation of polyphenols production.

In the study of the effect of the concentration and incubation time of MeJA on the polyphenols biosynthesis, the results showed that the polyphenols production varies with concentration and

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time incubation of MeJA. Thus, the high polyphenols content observed in leaves treated with 5 mM MeJA for 72 h reveals that this concentration would be optimal for stimulating phenolic metabolism and natural defenses in cotton (Inderjit, 2000). Similar results have been reported by Konan *et al*. (2014). These authors have shown that spraying 5 mM MeJA on plants for 72 h stimulated a high production of total phenols in cotton. In addition, the decrease in total phenol content in plants treated with MeJA at concentrations greater than 5 mM is related to symptoms of stress or toxicity in chlorosis form. This result seems to suggest that in cotton, MeJA induces the production of total phenols to an optimum beyond which it becomes toxic. In fact, the phytotoxicity of MeJA at high concentrations has already been reported in pine (Heijari *et al*., 2008; Moreira *et al*., 2009). Our results also show that the oligosaccharide fraction (FOV 11 (10 %)) produces more phenolic compounds than MeJA. Indeed, several studies on the vine have also revealed an important induction of phenolic compounds by the cells, after addition of oligosaccharide fractions extracted from the *Botrytis* mycelium (Liswidowati *et al*., 1991; Repka *et al*., 2001; Poinssot *et al*., 2003). It was also noted that the accumulation of phenolic compounds in MeJA-treated leaves occurred over a period of 30 days. However, the FOS 11 has had a long time of effectiveness than MeJA, due to a large accumulation of polyphenols contents over a long period (about 45 days). These results could be explained by the fact that the type of treatment has an influence on the quality and the quantity of polyphenols production and would be at the origin of the phenolic variation observed in the treated leaves. Moreover, in vine, the culture filtrate of *Botrytis cinerea* stimulates a large production of stilbenes which, according to Repka *et al*. (2001) and Poinsot *et al*. (2003), are phytoalexins that have an effective action on pathogens. Similarly, in cotton, Konan *et al*. (2014) showed that MeJA strongly stimulates the biosynthesis of phenolic acids, flavonoids and even stilbenes. Their antifungal activity has been demonstrated against FOV, the causal agent of Fusarium wilt in cotton (Konan *et al*., 2014; Ngoran, 2015), and in banana against *Mycosphaerella fijiensis* Morelet, causal agent of black leaf streak disease (Ncho *et al*., 2016). Also, the short time of accumulation of polyphenol compounds observed in the leaves treated with MeJA would be due to the fact that the production of phenolic compounds was not strongly stimulated by the MeJA. According to Sarni-manchado and Cheynier (2006), these compounds participate more in the preformed defense of plants such as the formation of the leaf cuticle than in the induced defense. These results suggest that phenolic compounds accumulate more and in the long term in leaves treated with FOS 11.

Conclusion

This study showed that FOV strain COT 11 excretes oligosaccharides higher in glucose than strain COT 6. The oligosaccharide fraction of strain COT 11 (FOS 11) stimulates more the accumulation of phenolic compounds at the concentration of 10 %. MeJA further stimulates the accumulation of phenolic compounds at a concentration of 5 mM after 72h of incubation. After treatment, FOS 11 (10 %) allowed the accumulation of phenolic compounds until 45 days after incubation. MeJA meanwhile allowed the accumulation of phenolic compounds until the 30th day after incubation.

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