CHAPTER 2

EVALUATION OF THE CAVENDISH BANANA GCTCV-218 FOR TOLERANCE TO *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* 'SUBTROPICAL' RACE 4 (VCG 0120)

ABSTRACT

Fusarium wilt, caused by the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is one of the most destructive diseases of bananas in the world. In South Africa, all Cavendish banana varieties are highly susceptible to *Foc* 'subtropical' race 4 (VCG 0120). Since no replacement variety is acceptable to the local market, the only way to control Fusarium wilt is by reducing the spread of the pathogen by means of cultural practices such as the use of micro-propagated planting material. A Fusarium wilt-tolerant Cavendish plant known as GCTCV-218 was recently selected in a field severely affected by the disease in Taiwan. In this study, GCTCV-218 is evaluated for tolerance to Fusarium wilt of bananas in South Africa, both under greenhouse and field conditions. The production of phenolics in GCTCV-218 was also compared to that in the susceptible Cavendish variety, Williams, after challenging it with the pathogen. Greenhouse and field results showed that GCTCV-218 is significantly more tolerant to *Foc* than Williams. Significantly more ester-bound and cell wall-bound phenolics were also produced in GCTCV-218, 24 hrs after pathogen attack. GCTCV-218 was tolerant to *Foc* 'subtropical' race 4 (VCG 0120) and the tolerance appeared to be linked to the ability to produce greater amounts of phenolic compounds at an early stage after infection.

INTRODUCTION

Bananas (*Musa* spp.) are regarded as one of the most important agricultural crops produced in tropical and subtropical countries of the world. However, the continued production of bananas is threatened by highly virulent fungal pathogens, such as *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *cubense* (E.F. Smith) Snyder & Hansen (*Foc*). *Foc* is responsible for an important vascular disease that was first reported from Australia in 1876, known as Fusarium wilt or Panama disease (Stover, 1962). Today, Fusarium wilt has been reported from all banana-growing countries except those bordering the Mediterranean (Ploetz, 1990).

Three pathogenic races of *Foc* have been identified. Strains that infect the varieties Gros Michel (AAA), Apple (AAB), Silk (AAB), Taiwan Latundan (AAB) and IC2 (AAAA) belong to race 1. Those that cause disease to Bluggoe bananas (ABB) and close relatives and some Jamaican tetraploids (AAAA) are referred to as race 2, while race 4 causes disease to Cavendish bananas (AAA) as well as race 1 and 2 susceptible varieties (Stover and Buddenhagen, 1986; Hwang, 1999).

Fusarium wilt became known as one of the most important diseases in agricultural history following the demise of more than 40 000 ha of Gros Michel bananas in Central and South America over a period of 50 years (Stover, 1962). In the early 1960's, the international banana export trade in Central America was rescued by the timely replacement of *Foc* race 1-susceptible Gros Michel bananas with resistant Cavendish varieties. Losses of Cavendish bananas to *Foc* race 4, first in the subtropics (Ploetz, 1990), and more recently in the tropics (Pegg *et al*., 1993; Ploetz, 1994), have raised fears that the world trade in banana might once again be threatened. In South Africa, where bananas are planted in the sub-tropics, Fusarium wilt has already been reported from four of the six banana production areas (Viljoen, 2002). Likewise, the disease has destroyed many thousands of hectares of Cavendish bananas in tropical countries such as Indonesia and Malaysia (Hwang and Ko, 2004) and no replacement variety with resistance to *Foc* race 4 exists for the seedless Cavendish banana.

No sustainable control strategy exists for Fusarium wilt of banana, other than replacing susceptible varieties with those resistant to the disease. Conventional breeding efforts to find a resistant replacement for Cavendish bananas have had limited success, often because of the reluctance by local markets to accept the new hybrids (Stover and Buddenhagen, 1986; Rowe and Rosales, 1993; Daniells *et al*., 1995). Hwang and Tang (1996), therefore, initiated a program using unconventional improvement methods for Cavendish bananas in Taiwan for Fusarium wilt resistance by generating somaclonal variants. Two clones, GCTCV-215-1 and 217, with good resistance to *Foc* 'tropical' race 4 (VCG 0121) were found (Hwang, 1999). However, a field selection from Giant Cavendish, known as GCTCV-218, eventually rescued the banana industry in Taiwan from destruction (Hwang and Ko, 2004).

Resistance to Fusarium wilt of banana is dependant on cell wall strengthening and occlusion to prevent fungal colonization of the vascular tissue (Beckman, 1990). This involves the synthesis and deposition of lignin in the cell walls, and the accumulation of cell-wall appositions in the xylem vessels, respectively (Shiraishi *et al*., 1989). Cell wall strengthening and xylem occlusion depend on phenolic-based defence responses by the plant (Matern and Kneusel, 1988). Phenolics are substances that are constitutively expressed and widely distributed in plants. Mace (1963) established that healthy banana roots have specialised cells that contain high concentrations of phenols. Upon challenge by pathogens and pests, a substantial increase in phenolic synthesis takes place as part of the active defence response (Mace, 1963; Mace and Solit, 1966; Beckman and Mueller, 1970; Nicholson and Hammerschmidt, 1992; Matern *et al*., 1995). A successful resistance response depends on the rate and extent of recognition and activation of the defence mechanisms (Beckman, 1987; Beckman, 1990).

In a recent investigation, Groenewald *et al*. (unpublished data, FABI, South Africa) showed that *Foc* strains in Taiwan (VCG 0121) are genetically only distantly related to those causing disease to Cavendish bananas in South Africa (*Foc* 'subtropical' race 4, VCG 0120). The aim of this study, therefore, was to determine whether the Cavendish selection from Taiwan (GCTCV-218) also has tolerance to *Foc* 'subtropical' race 4 (VCG 0120). The role of phenolics production in the resistance response was also considered.

MATERIALS AND METHODS

Greenhouse Trials

Plant material

Cavendish banana varieties GCTCV-218 (tolerant to *Foc*) and Williams (susceptible to *Foc*) were micro propagated at Du Roi Laboratories in Letsitele, South Africa. Once the plantlets were grown to 10-cm plants, they were removed from seedling trays and transplanted into 250-ml polystyrene cups containing water. Plants were maintained in a greenhouse at 18/25ºC with a 16 hrs natural sunlight/8 hrs dark photoperiod, and fertilized once a week with a nutrient solution (0.6 g/l $Ca(NO_3)_2.H_20$, 0.9 g/l Agrasol-0® (Scotts, Scotts-Europe B.V., The Netherlands), 1 ml/l Micromax® Stock solution (3 g/l) (Fleuron, P.O. Box 31245, Braamfontein 2017). Inoculation with *Foc* was carried out after 21 days to allow the young banana plants to recover from stress related to the planting procedure, and to develop a strong root system.

Inoculation

Single-spore cultures of the highly virulent *Foc* isolates (CAV 045, 092, 105), belonging to 'subtropical' race 4, vegetative compatibility group (VCG) 0120, were used as starter inoculum. The isolates were first grown on 90-cm-diameter Petri dishes with half-strength Potato Dextrose Agar (PDA) (Biolab, Merck Laboratories, Wadeville, Gauteng, South Africa) for 7-10 days. The inoculum was prepared by pouring sterile distilled water onto the surface of Petri dishes containing the isolates for inoculation, and loosening the fungal spores with a sterile glass rod. The spore suspensions were then filtered through sterile cheese cloth, combined, and the spore concentration was adjusted to 2.5×10^5 conidia.ml^{-1} using a haemocytometer.

The roots of all the banana plantlets were slightly wounded by crushing the entire root system, prior to inoculation. Each plantlet was inoculated by adding 2.5 ml of the spore suspension to each cup containing 250 ml water in order to achieve a final inoculum concentration of 2.5×10^3 conidia.ml^{-1}. Sterile distilled water was added to cups containing the control plants. Plants were kept in the greenhouse for a further 6 weeks. They were watered only when approximately 50 ml water was left in the

cups. Five replicates with six plants in each replicate were inoculated for both GCTCV-218 and Williams, and the entire experiment was repeated.

Disease rating

Disease development was evaluated 5-6 weeks after inoculation using a disease modified version of the severity rating scale for Fusarium wilt of banana (Carlier *et al*., 2002). The rating scale ranged from 0-5, with plants showing no internal symptoms scoring a 0 and plants showing 100% vascular discoloration scoring 5 (Fig. 1). Disease severity was calculated using the formula of Sherwood and Hagedorn (1958):

Disease severity (%) = [∑ (No. plants in a disease scale category)*(Specific disease scale category)/ (Total no. of plants in the trial)*(Maximum disease scale category)] *100

Statistical analysis for the data was conducted using the General Linear Models (GLM) procedure of *STATISTICA*, version 7 (STATSOFT Inc. 2004). Experiments were analysed using One-way analysis of variance (ANOVA) and the Tukey Honest Significant Difference (HSD) test. Significance was evaluated at $P<0.05$.

Field Trial

Plant material

Three separate field trials were conducted over a period of 2 years in the Kiepersol area, Mpumalanga, South Africa. Tissue-cultured banana plantlets approximately 40 cm high were planted in three different fields infested with *Foc* 'subtropical' race 4 (VCG 0120) in January 2002. Experimental plots consisted of a completely randomised block design, with 15 or 20 plants of either GCTCV-218 or Williams per block, dependent on the trial site, and five replicate blocks randomised in the plantation. Standard banana cultivation methods were applied to the trial.

Disease rating

Disease development was rated according to the presence or absence of external disease symptoms. Healthy plants were awarded a value of 0, while diseased plants were scored as 1. Disease severity was calculated using the formula of Sherwood and Hagedorn (1958). Statistical analysis for the data was conducted as described above.

Phenolic Assays

Plant material

GCTCV-218 and Williams tissue cultured banana plantlets were grown in the polystyrene cup-system described above. Plants were inoculated with a *Foc* spore suspension with a final concentration of 2.5 x 10^3 conidia.ml^{-1} and maintained in the greenhouse until sampling. Control plants were treated with sterile distilled water. Roots were harvested at 0, 6, 24 and 48 hrs after inoculation, and immediately placed in liquid N_2. The roots were then ground with an electric homogeniser (IKA A11 Basic analytical Mill, United Scientific (Pty) Ltd., San Diego, USA), freeze dried and stored at -80°C until the phenol assays were performed. Each assay was performed in triplicate.

Extraction of phenolics

Phenolics were extracted using a modification to the method described by De Ascensao and Dubery (2003). Phenolics from the root material (0.05 g) of the control and treated plants (GCTCV-218 and Williams) were extracted with 1 ml of a solution containing MeOH/AC/H_2O (7:7:1 (v/v/v)). The suspension was homogenised for 1 min before being shaken for 1 h at 200 rpm and centrifuged for 5 min at 12 000 x g. After centrifugation, the supernatant was saved. The remaining precipitate was re-homogenised and centrifuged as above. The second supernatant was combined with the first and the procedure was repeated two more times. The four combined supernatants were concentrated to 1 ml. Aliquots were made in order to determine total soluble phenolic acids, free phenolic acids, MeOH soluble ester-bound phenolic acids and MeOH soluble glycoside-bound phenolic acids. The remaining precipitate was dried at 70°C for 24 hrs. The resulting alcohol insoluble residue (AIR) yielded the cell wall material that was used to extract the ester-bound cell wall phenolic acids.

Total soluble phenolic acids

The method used to determine the total soluble phenolic content is based on the reduction of the phospho-molybdene/phosphor-tungstate present in the Folin-Ciocalteau reagent (Swain and Hillis, 1959). The aliquoted supernatant was concentrated in a speedy vac (SPD111V vacuum centrifuge) (Savant, Holbrook, New York, USA) to 5 µl and diluted to 175 µl with water and added to 25 µl of 20% (v/v) Folin-Ciocalteau reagent and mixed. After 3 min, 50 µl of saturated aqueous sodium carbonate ($NaCO_3$) was added, mixed and incubated at 30°C for 30 min. A blank of water was used as control. Gallic acid was used as a phenolic standard to construct a standard curve ranging from 0 - 400 $\mu g.ml^2$ ($y = 1.3527x – 0.0109$, $R^2 = 0.9986$). The concentration of phenols in the various extracts was calculated from the standard curve and expressed as µg gallic acid g^{-1} dry weight.

Non-conjugated phenolic acids (Free acids)

Twenty-five µl of triflouroacetic acid (TFA) was added to 500 µl of aliquoted supernatant to acidify the solution prior to extraction with 1 ml anhydrous diethyl ether (Saarchem, Merck Laboratories) (Cvikrová *et al*., 1993). The ether extract was dried and the resulting precipitate was resuspended in 250 µl 50% aqueous MeOH. This solution was used to determine the free phenolic content with Folin-Ciocalteau reagent.

Glycoside-bound phenolics

The aliquoted supernatant (500 µl) for MeOH soluble glycoside-bound phenolic content determination was hydrolysed in 50 µl concentrated pure HCl for 1 h at 96°C, directly placed on ice for 15 min and then extracted three times with 1 ml anhydrous diethyl ether (Saarchem, Merck Laboratories). The ether extract was dried in a speedy vac and the remaining precipitate was resuspended in 250 µl 50% aqueous MeOH. This solution was used to determine the glycoside phenolic content with Folin-Ciocalteau reagent in the same way as described for total phenolic acids.

Ester-bound phenolics

Soluble ester-bound phenolic acids were extracted after alkaline hydrolysis under mild conditions (Cvikrová *et al.,* 1993). One hundred and twenty five µl of 2 M NaOH was added to the aliquot of supernatant and the tubes were left to stand at room

temperature for 3 hrs and then placed on ice for 15 min. After hydrolysis, 150 µl 1 M HCl was added and the phenolics extracted with 1 ml anhydrous diethyl ether (Saarchem, Merck Laboratories) as described above. The final solution was used to determine the phenolic ester content using the Folin-Ciocalteau reagent.

Cell wall-bound phenolics

The ester-bound phenols incorporated into the cell wall were extracted following alkaline hydrolysis (Campbell and Ellis, 1992). Dry cell wall material (AIR) was weighed (0.01 g) and resuspended in 0.5 M NaOH (1 ml for 10 mg) for 1 h at 96°C. Cell wall-esterified hydroxycinnamic acid derivatives were selectively released under these mild saponification conditions. The supernatant was acidified to pH 2 with HCl, centrifuged at 12 000 x g for 10 min and then extracted with 1 ml diethyl ether (Saarchem, Merck Laboratories). The extract was dried in a speedy vac and the precipitate was resuspended in 250 µl 50% aqueous MeOH. This solution was used to determine the cell wall-esterified phenolic acids content with Folin-Ciocalteau reagent.

Statistical analysis for the phenolic assay data was conducted using the General Linear Models (GLM) procedure of *STATISTICA*, version 7 (STATSOFT Inc. 2004). Experiments were analysed using One-way analysis of variance (ANOVA) and the Tukey Honest Significant Difference (HSD) test. Significance was evaluated at $P<0.05$.

RESULTS

Greenhouse Trials

Yellowing of banana leaves and wilting appeared 4-5 weeks after inoculation with *Foc*. After 6 weeks, the Williams plants developed more severe internal symptoms than GCTCV-218. Many of the Williams plants scored 5 compared to 1 (few) or 0 (no) internal symptoms in GCTCV-218. Disease severity values for Williams were 65 and 57% for the two trials, compared to disease severity values of 34 and 38% in

GCTCV-218 (Fig. 2). No symptoms developed in the control plants of either GCTCV-218 or Williams.

Field Trials

GCTCV-218 consistently showed better tolerance to Fusarium wilt in the field than Williams. In the three sites, GCTCV-218 plants had a disease severity of 10, 34 and 14%, compared to the disease severity rating for Williams of 52, 76 and 72% (Fig. 3).

Phenolic Assays

Total soluble phenolics

GCTCV-218 produced a significantly higher total phenolic content when compared to Williams at 0 and 6 hrs after inoculation with *Foc* (Fig. 4). Although there were no significant differences between the treatments, an induction in total phenolics was observed in GCTCV-218 after 6 hrs, while induction in Williams occurred only after 24 hrs. No significant differences in total phenolic production in GCTCV-218 and Williams were obvious at 24 and 48 hrs after inoculation.

Non-conjugated phenolic acids (Free acids)

Free acids were significantly higher in GCTCV-218 when compared to Williams before inoculation with *Foc*, but decreased after inoculation in both varieties. GCTCV-218 also had a significantly higher level of free acids at 24 hrs after inoculation (Fig. 5).

Glycoside-bound phenolics

A significant decrease in glycoside phenolics occurred in GCTCV-218 6 hrs after inoculation with *Foc*, with an increased induction taking place after 24 and 48 hrs (Fig. 6). GCTCV-218 showed a significant induction of phenolics after 24 hrs, and Williams only at 48 hrs. Glycoside-bound phenolics were significantly higher in GCTCV-218, 24 and 48 hrs after inoculation when compared to Williams.

Ester-bound phenolics

Ester-bound phenolics were expressed at similar levels in GCTCV-218 at 0 and 6 hrs after inoculation, but a significant increase in content was observed at 24 hrs and reached a plateau at 48 hrs after inoculation with *Foc* (Fig. 7). The ester-bound phenolics in Williams, however, only increased at 48 hrs. At 24 hrs, GCTCV-218 further had a slightly higher ester-bound content when compared with the susceptible cultivar Williams.

Cell wall-bound phenolics

Williams showed a significantly higher basal content of cell-wall bound phenolics at 0 and 6 hrs after inoculation with *Foc* when compared to GCTCV-218. After *Foc* infection however, there was a significant decrease in the content (Fig. 8). GCTCV-218 responded to pathogen infection at 24 and 48 hrs after inoculation with a significant increase in cell-wall bound phenolics as well as a significantly higher phenolic content when compared to Williams.

DISCUSSION

Greenhouse and field evaluation in this study showed that GCTCV-218 developed significantly less Fusarium wilt than Williams bananas in South Africa, and could be considered tolerant to *Foc* 'subtropical' race 4 (VCG 0120). This result has major implications for the continued cultivation of Cavendish bananas in countries affected by *Foc* race 4. In Taiwan, GCTCV-218 was highly resistant against race 4 and also showed high yields of high quality fruit (Hwang and Ko, 2004). Hybrids with good resistance to the pathogen, such as FHIA-01 ('Goldfinger') (Moore *et al.*, 1995; Jones, 2000) and SH-3640/10 ('High Noon') (Eckstein *et al.*, 1996; De Beer, 1997), are not always acceptable to the Cavendish-dominated markets. In these situations GCTCV-218 could be considered a good replacement for susceptible Cavendish varieties in countries affected by *Foc* race 4.

Planting of a tolerant Cavendish selection such as GCTCV-218 would need to be supported by an integrated disease management strategy. This might also include treatment of plants with chemicals that induce systemically acquired resistance,

biological control organisms, and fertilisers that reduce pathogen growth and enhance plant vigour (Nel, 2004).

Evaluation and confirmation of disease tolerance in GCTCV-218 provides an opportunity to study resistance mechanisms in Cavendish bananas against *Foc*. The plant could be considered an isogenic line of Giant Cavendish, the susceptible Cavendish variety from which it was selected. Techniques such as Suppression Subtractive Hybridisation (SSH) (Diatchenko *et al*., 1996 and 1999) provide an opportunity for isolating genes differentially expressed in response to *Foc* race 4. If these genes are linked to resistance, they could be used as markers for the early identification of tolerant somaclonal variants, or they could be re-introduced into high-yielding susceptible plants to improve disease tolerance.

Higher levels of total soluble phenolics in GCTCV-218 compared to Williams in this study suggests that constitutive defence compounds are present in higher concentrations in tolerant bananas, even before contact with the pathogen. This increased presence of phenolics could contribute to an enhanced plant response after infection. The role of phenolics in defence responses in banana has been well illustrated previously. De Ascensao and Dubery (2000) reported a prominent increase in total soluble phenolics in FHIA banana roots, 8 hrs after treatment with elicitors from *Foc* race 4. Williams, however, only responded after 12 hrs and did not show the same prominent increase as the tolerant hybrid. Histochemical observations of root sections of banana varieties susceptible (Poyo) and partially resistant (Yangambi Km5) to nematodes also revealed striking differences in phenolic content (Valette *et al.*, 1998). Resistant roots had high levels of lignin, flavanoids, dopamine, cafeic esters and ferulic esters that were associated with a very low rate of nematode root penetration in the resistant cultivar.

Early induction of phenolic compounds could contribute to tolerance in GCTCV-218 against *Foc* race 4 compared to Williams, where no phenolics were induced after 48 hrs. This early response may be a key factor in preventing pathogen spread into and throughout the vascular system of the tolerant plant. Phenolics are precursors of several secondary metabolites and proteins involved in disease resistance, such as phytoalexins and lignin (Matern *et al.*, 1995). In this study, ester-bound phenolics

were expressed at increased levels in GCTCV-218, 24 hrs after pathogen attack. Evidence strongly suggests that esterification of phenols to cell wall materials, is a common theme in the expression of disease resistance (Fry, 1987). The early increase of glycoside-bound phenolics in GCTCV-218 may further contribute to disease tolerance by being toxic to the pathogen and thereby preventing pathogen spread. Cell wall-bound phenolics were significantly induced at 24 and 48 hrs after pathogen attack. De Ascensao and Dubery (2003) reported a 3-fold increase of glycoside-bound phenolic, a 4.2-fold increase of ester-bound phenolics and a 6.3-fold increase in cell wall-bound phenolics in the resistant Goldfinger 36 hrs after *Foc* – elicitor treatment.

In this study, GCTCV-218 had a greater capacity for phenolic metabolism in response to *Foc* and it also displayed a high basal level of total soluble phenolics, free acids and glycoside-bound phenolics before pathogen infection. Strengthening of cell walls is generally accomplished by infusion of phenolics into, or the apposition of phenolic-containing material against the cell wall (Beckman *et al*., 1974; Ride, 1975). The constitutive presence of phenolics suggests that GCTCV-218 has pre-existing defence mechanisms that protect the plant. GCTCV-218 responded strongly to pathogen attack by producing high levels of ester-bound and cell wall-bound esterified phenolics. This suggests that cell wall strengthening and subsequent lignin deposition is taking place. The high level of phenolics found in GCTCV-218 may also possibly contribute to the effective and timeous production of papillae and gels in response to *Foc*. GCTCV-218, therefore, is able to actively induce a structural and biochemical defence response against *Foc*. Apart from simply inducing strong defence responses, GCTCV-218 appears to be able to induce them early enough to contain *Foc* and prevent further spread.

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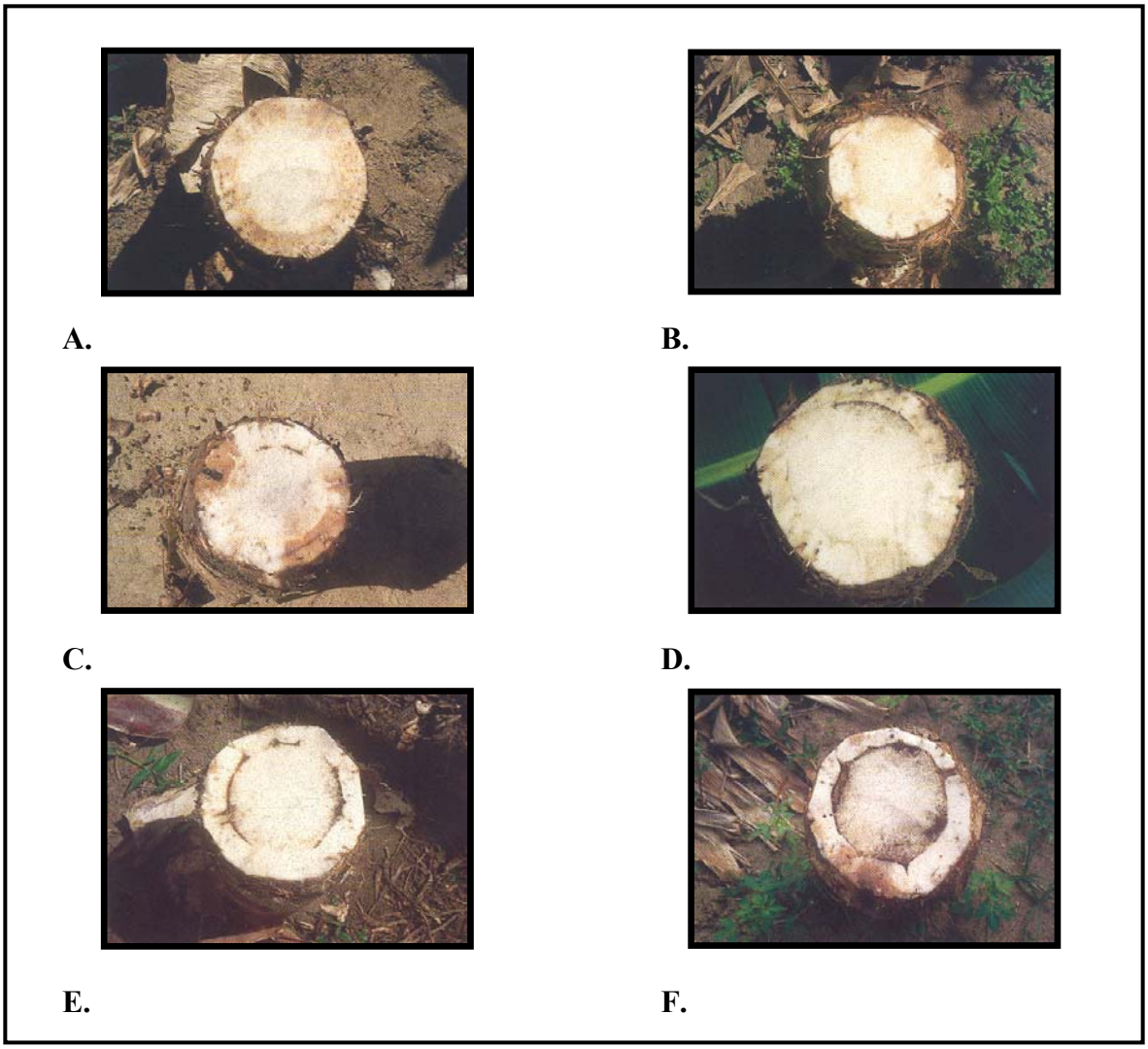

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Figure 1. Visual internal disease rating scale used to evaluate disease severity of *Fusarium oxysporum* f. sp. *cubense* in bananas.

Disease severity	Internal disease symptoms in banana corm
0 (Fig. 1A)	Corm completely clean, no vascular discoloration
1 (Fig. 1B)	Isolated points of discoloration in vascular tissue
2 (Fig. 1C)	Discoloration of up to 1/3 of vascular tissue
3 (Fig. 1D)	Discoloration of between 1/3 and 2/3 of vascular tissue
4 (Fig. 1E)	Discoloration greater than 2/3 of vascular tissue
5 (Fig. 1F)	Total discoloration of vascular tissue (dark purple)

(Carlier *et al.*, 2002)

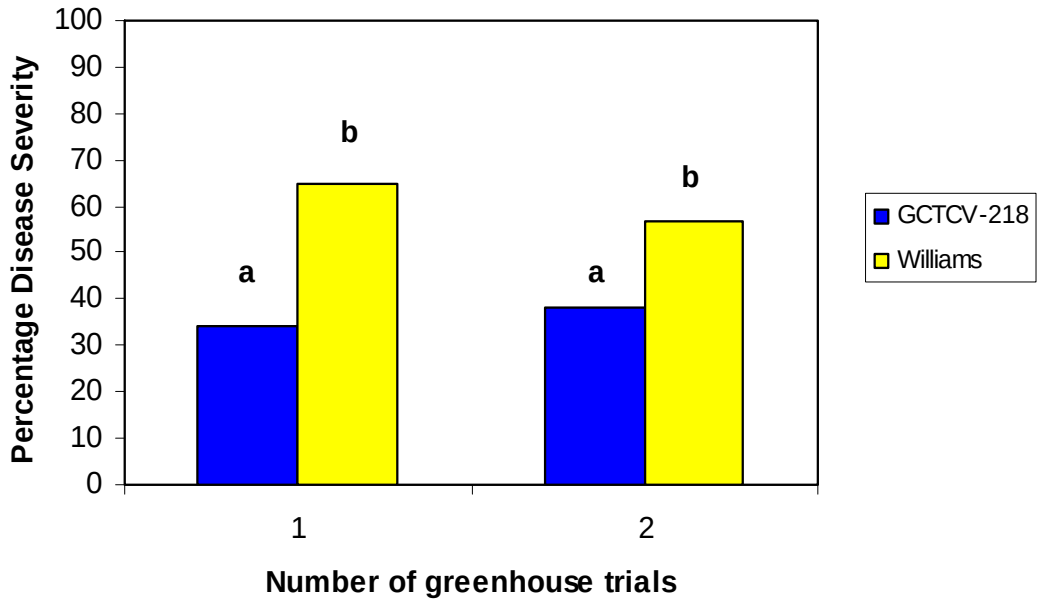

Figure 2. Disease severity index of GCTCV-218 and Williams bananas infected with *Fusarium oxysporum* f. sp. *cubense* during two independent greenhouse trials. Experiments were analysed using One-way analysis of variance (ANOVA) and the Tukey Honest Significant Difference (HSD) test. [a,b] Percentage disease severity values in the same trial with the same letter are not significantly different at $P<0.05$.

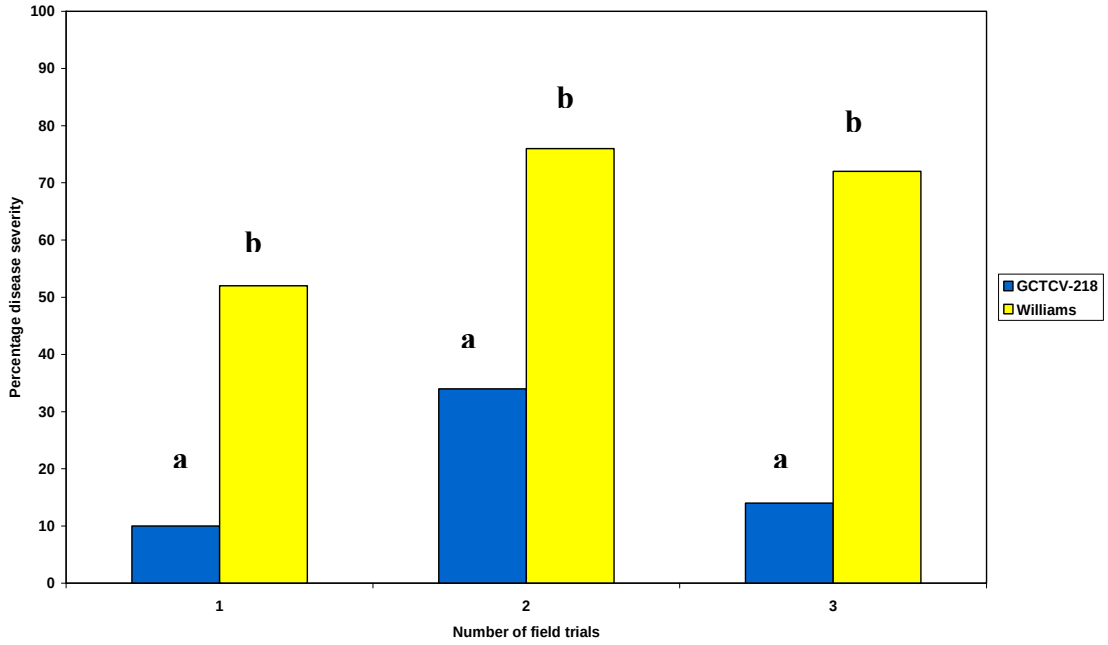

Figure 3. Disease severity index of GCTCV-218 and Williams bananas during three independent field trials in *Fusarium oxysporum* f. sp. *cubense* infected areas in South Africa. Experiments were analysed using One-way analysis of variance (ANOVA) and the Tukey Honest Significant Difference (HSD) test. [a,b] Percentage disease severity values in the same trial with the same letter are not significantly different at $P<0.05$.

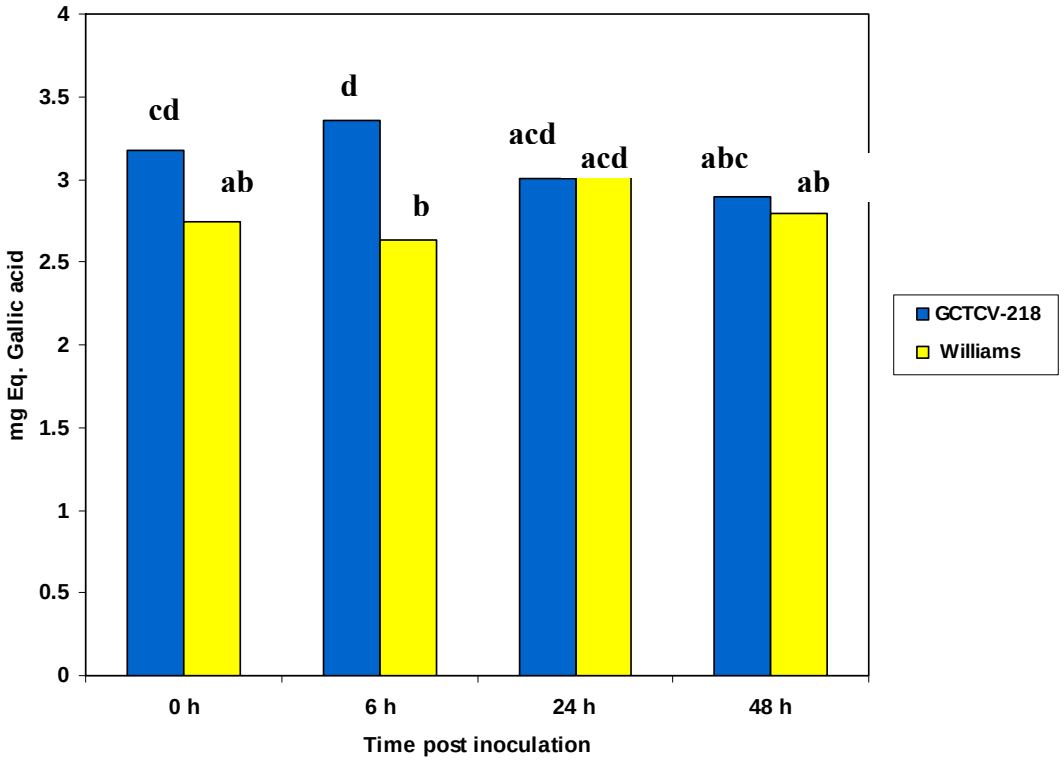

Figure 4. Total phenolic content in GCTCV-218 and Williams banana cultivars at different time points after inoculation with *Fusarium oxysporum* f. sp. *cubense*. Phenolics were determined with the Folin reagent as milligrams of Gallic acid per gram dry weight. Experiments were analysed using One-way analysis of variance (ANOVA) and the Tukey Honest Significant Difference (HSD) test. [a,b,c,d] Bars presented with the same letter are not significantly different at $P<0.05$.

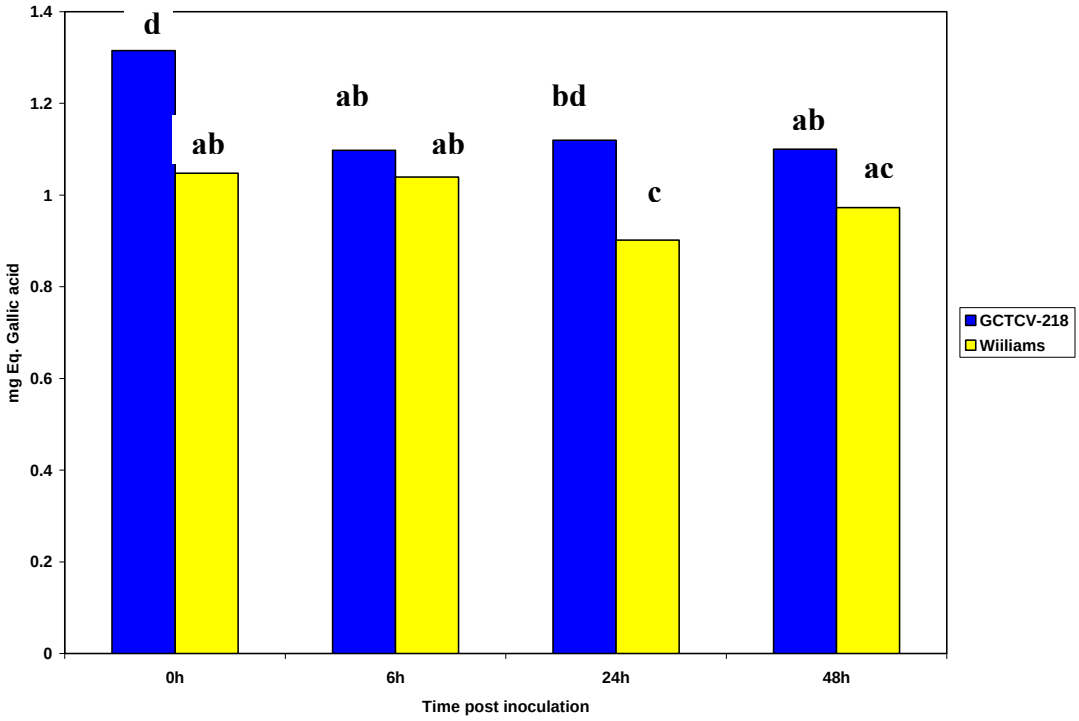

Figure 5. Non-conjugated phenolic acid content in GCTCV-218 and Williams banana cultivars at different time points after inoculation with *Fusarium oxysporum* f. sp. *cubense*. Phenolics were determined with the Folin reagent as milligrams of Gallic acid per gram dry weight. Experiments were analysed using One-way analysis of variance (ANOVA) and the Tukey Honest Significant Difference (HSD) test. [a,b,c,d] Bars presented with the same letter are not significantly different at $P<0.05$.

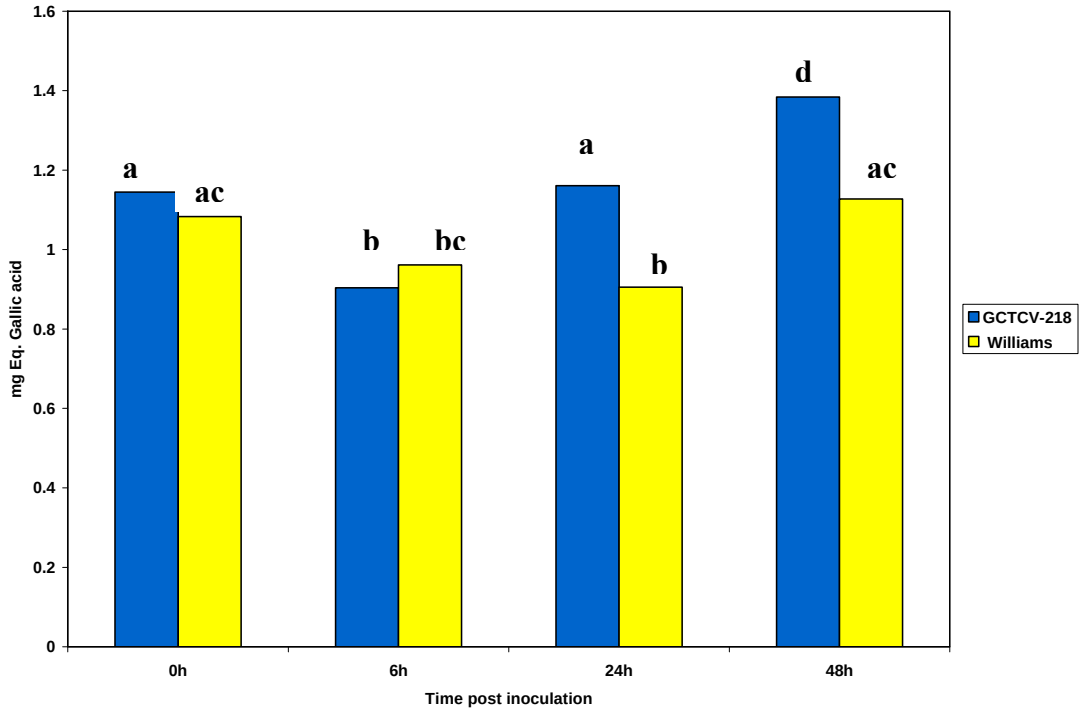

Figure 6. Glycoside-bound phenolic content in GCTCV-218 and Williams banana cultivars at different time points after inoculation with *Fusarium oxysporum* f. sp. *cubense*. Phenolics were determined with the Folin reagent as milligrams of Gallic acid per gram dry weight. Experiments were analysed using One-way analysis of variance (ANOVA) and the Tukey Honest Significant Difference (HSD) test. [a,b,c,d] Bars presented with the same letter are not significantly different at $P<0.05$.

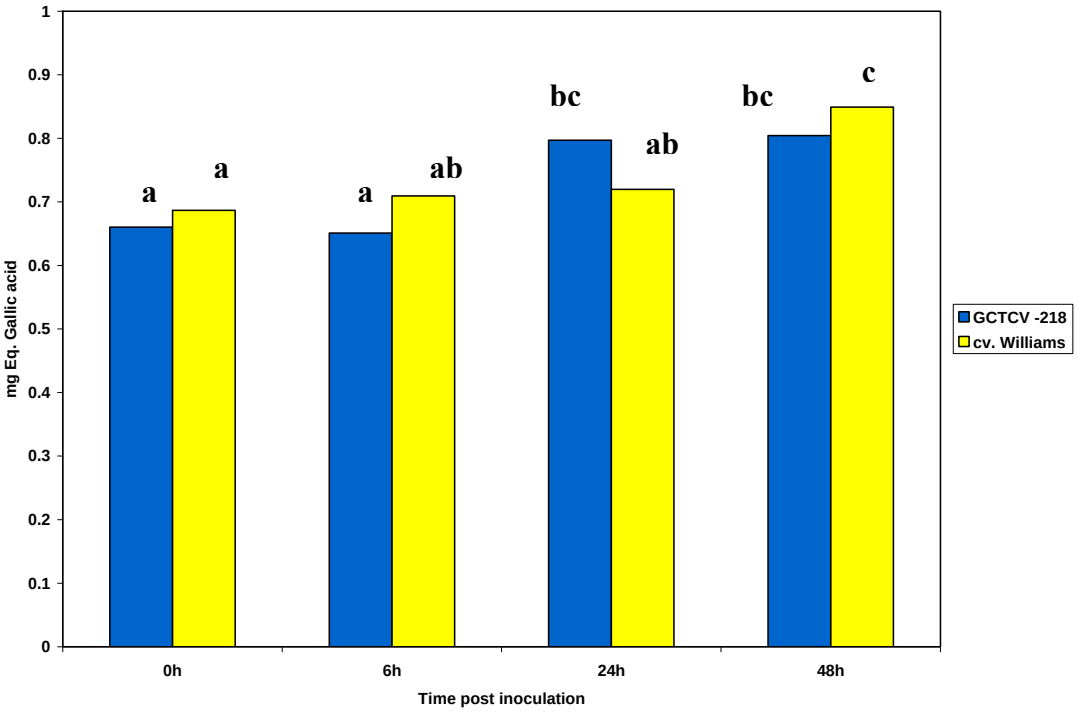

Figure 7. Ester-bound phenolic content in GCTCV-218 and Williams banana cultivars at different time points after inoculation with *Fusarium oxysporum* f. sp. *cubense*. Phenolics were determined with the Folin reagent as milligrams of Gallic acid per gram dry weight**.** Experiments were analysed using One-way analysis of variance (ANOVA) and the Tukey Honest Significant Difference (HSD) test. [a,b,c,d] Bars presented with the same letter are not significantly different at $P<0.05$.

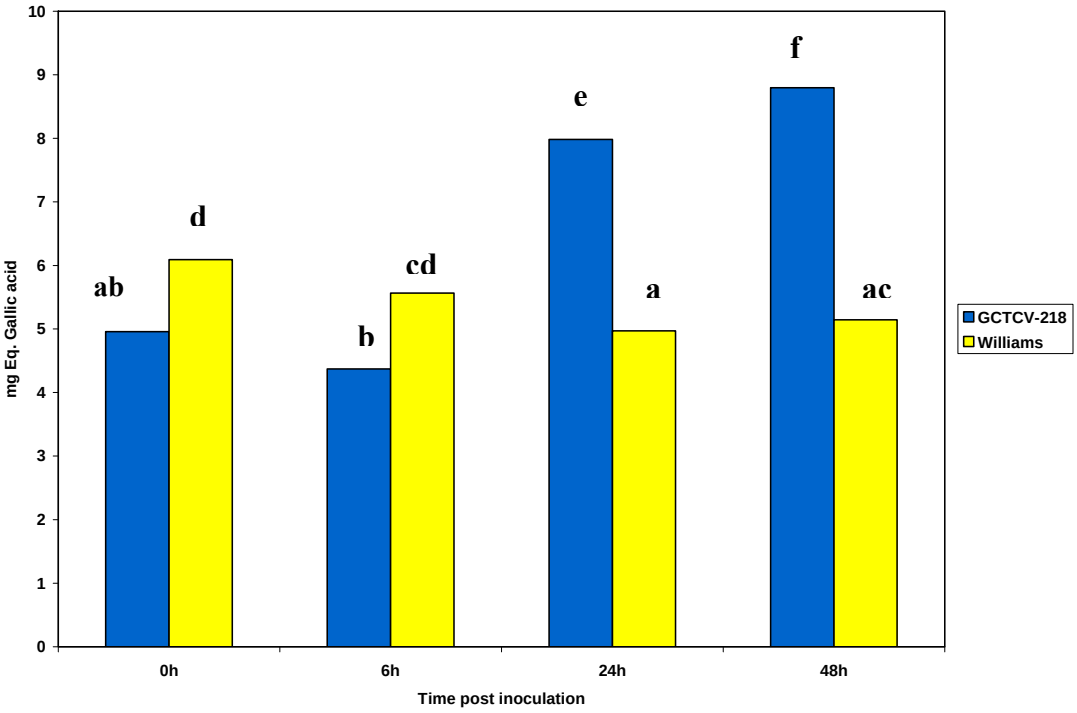

Figure 8. Cell wall-bound phenolic content in GCTCV-218 and Williams banana cultivars at different time points after inoculation with *Fusarium oxysporum* f. sp. *cubense*. Phenolics were determined with the Folin reagent as milligrams of Gallic acid per gram dry weight. Experiments were analysed using One-way analysis of variance (ANOVA) and the Tukey Honest Significant Difference (HSD) test. [a,b,c,d,e,f] Bars presented with the same letter are not significantly different at $P<0.05$.