RESEARCH ARTICLE

Pre-harvest chemical elicitor treatment enhances induced resistance in harvested banana fruit cv. 'Embul' and reduces anthracnose caused by *Colletotrichum musae*

U.W.N.P. Wanigasekara, N.K.B. Adikaram* and C.L. Abayasekara
Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya.

Abstract: Developing banana fruits cv. 'Embul' were treated in the field with chemical elicitors, Bion®, salicylic acid (SA) or K_2HPO_4 at two stages, 2 and 4 or 9 and 11 weeks after bunch emergence. Fruits harvested 14 – 15 weeks after bunch emergence were inoculated with *Colletotrichum musae,* and the development of anthracnose was assessed during ripening. Treatment with Bion® or SA at 9 and 11 weeks after bunch emergence while delaying the disease incidence significantly reduced ($p < 0.05$) the severity of the disease by 43 % and 65 %, respectively compared to the control. SA (500 mg/L) treatment was 22 % more effective than Bion® (200 mg/L) while the treatment with K_2HPO_4 (20 mg/L) increased the disease severity by 5 %. Bion® or SA-treated fruits when challenge inoculated with *C. musae* after harvest showed a significantly enhanced chitinase activity ($p < 0.05$). There was also an increased β–1,3–glucanase activity and antifungal activity of the fruit peel compared to the controls. The elicitor treatment did not affect the fruit weight or postharvest quality.

Keywords: β–1,3–glucanase, chemical elicitors, chitinase, induced defense.

INTRODUCTION

Anthracnose caused by *Colletotrichum musae* (Berk. & Curt.) v. Arx is by far the most destructive postharvest disease in all banana producing and marketing countries in the world. The fungus infects the young developing fruit in the field and remains quiescent until the fruit approaches maturity and ripening (Swinburne, 1983). The fungi causing quiescent infections are comparatively more difficult to control than wound invaders.

Quiescence of fungi in fruit has been implicated to constitutive (Karunanayake *et al.,* 2011) or induced

(Adikaram *et al*., 1982) antifungal substances. Fruit losses due to postharvest fungal rots could be reduced if the infections could be kept in their quiescent phase for extended periods during storage and marketing. One possible way to prolong quiescence is to maintain the natural antifungal barrier present in the unripe fruit at an inhibitory level into the post-climacteric phase. Induction of natural resistance has been of considerable interest in the control of postharvest diseases for many years (Adikaram, 1990; Yang *et al*., 2007).

Resistance responses are triggered following the recognition of a range of biological, chemical or physical factors termed 'elicitors' (Terry & Joyce, 2004). The identification of salicylic acid (SA) as a natural inducer of plant defense responses has prompted the characterization of synthetic chemicals that are able to mimic SA (Conrath *et al*., 1995). There is evidence of activation of defense responses due to exogenous application of SA in many fruit crops such as sweet cherry (Yao & Tian, 2005; Bi *et al*. 2007), mango (Zeng *et al*., 2006) and pear (Tian *et al*., 2006). Acibenzolar-S-methyl ester (ASM, BTH, Actigard®, Bion®), a functional analogue of SA, activates disease resistance in crops to a wide variety of pathogens (Hammerschmidt *et al*., 2001). The resistance induced in strawberry (Terry & Joyce, 2000), peach (Liu *et al*., 2005), Yali pear (Cao *et al*., 2005) and pear (Tsiantos *et al*., 2003) has protected fruits from postharvest pathogens. Relatively little information is available on K_2HPO_4 as an elicitor of defenses in fruit crops. However, foliar sprays of phosphates (K_2HPO_4 and KH_2PO_4) have been shown to control powdery mildew in field-grown nectarines, mango trees and grapevine

*Corresponding author (nkba@pdn.ac.lk)

(Reuveni & Reuveni, 1995) and also anthracnose disease in cashew (Lopez & Lucas, 2002).

There is limited information on the resistance responses induced in banana fruit following fungal infection or treatment with chemical elicitors especially applied as preharvest treatments. The present study investigated the possibility of inducing resistance in banana fruit cv. 'Embul' against anthracnose disease, *via* pre-harvest application of chemical elicitors.

METHODS AND MATERIALS

Banana plants

Two-month old tissue cultured banana saplings cv. "Embul" of uniform size were planted in a field site at Meewatura, in the premises of the University of Peradeniya, Sri Lanka (longitudes 080° 35' 42.3'' east and latitudes 07° 15' 06.3'' north). Planting pits (2 x 2 x 2 ft^3) were dug leaving a 3 m distance between rows and pits. Urea (110 g), muriate of potash (150 g) and rock phosphate (190 g) were applied to plants as recommended by the Department of Agriculture, 2, 5 and 9 months after planting. Plants were watered regularly and pesticides were not applied at any stage during the field trial.

Chemical elicitor treatment

Suspensions/solutions of Bion® (200 mg/L, acibenzolar-S-methyl; Syngenta), salicylic acid (500 mg/L, $C_7H_5O_3Na$, Sigma®) and K_2HPO_4 (20 mg/L) were prepared in sterile distilled water. Tween 20 (50 µL/L) was added as a wetting agent. The elicitor concentration for treatment was decided from the results of preceding postharvest treatment trials (Wanigasekara, 2009). The treatments were arranged in a randomized complete block design within a factorial scheme (4 treatments × 2 application times). The treatments were (i) Bion® (200 mg/L 500 mg/L), (ii) salicylic acid (500 mg/L), (iii) K_2HPO_4 (20 mg/L) and distilled water (control), applied at two stages (i) 2 and 4 weeks after bunch emergence (WABE), and (ii) 9 and 11 WABE. Banana bunches intact to the plant, were separately sprayed with chemical elicitor preparations or distilled water (control) using a domestic hand-held sprayer until run-off. Six replicate bunches were used per treatment.

Harvesting

Treated bunches were harvested 14 – 15 weeks after bunch emergence and brought to the Plant Pathology Laboratory, Department of Botany, weighed and de-

handed. The hands were washed in running tap water and allowed to dry. The number of hands and the weight of each hand were recorded. Ten fruits from the third hand of each bunch were weighed and the average fruit weight was calculated. Uniform sized fruits from the upper four hands without any defects were selected and used for inoculation experiments.

Inoculation and disease assessment

Colletotrichum musae isolated from an anthracnose lesion on a ripe banana fruit cv. "Embul", identified using the Commonwealth Mycological Institute (CMI) descriptions and maintained on potato dextrose agar at 26 °C ± 2, was used for all the experiments. A conidial suspension was obtained by flooding a 10 day - old culture with sterile distilled water (SDW) and scraping the mycelium. The suspension was filtered through glass wool and the concentration in the filtrate was adjusted to $1 × 10^6$ conidia mL^{-1}. Fifteen fruits from each treatment were wiped with cotton wool soaked with 70 % ethanol and allowed to air-dry. The fruits were challenge inoculated by placing three drops (20 µL) of a conidia suspension of *C. musae* along the long axis of each fruit. Inoculated fruits were incubated in moist chambers at 26 °C ± 2 for 10 days. The number of inoculations that developed into lesions was recorded daily and the percentage disease incidence was calculated. The diameter of lesions was measured at two positions right angles to each other and the average lesion area was calculated for each treatment. Percentage disease reduction in each treatment was calculated against controls.

Induced defence responses

Elicitor treated and control fruits from three replicate bunches were inoculated with *C. musae* on the day of harvest. Peel segments (1 – 2 mm thick, 15 g) were cut from beneath the drop of inoculum immediately after inoculation and again after three days.

Chitinase activity was assayed by the method described by Zou *et al*. (2002) with slight modifications. One gram of the tissue sample was homogenized in 5 mL of 0.067 M sodium phosphate buffer (pH 5) for 2 min, using an ultrasonic homogenizer (Ultra Turrax® T25 basic, IKA Labortechnik) at 11,000 rpm. The homogenate was clarified by centrifugation at 6000 g (Sigma 3K30 Laboratory centrifuge) and 4 °C for 10 min. The supernatant was used for the enzyme assay. Agarose-glycol chitin substrate was prepared (Trudel & Asselin, 1989) and poured into petri dishes (11 cm diameter). Aliquots (10 µL) of the extract or standard chitinase (from *Serratia marscescens*, Chitinase EC 3.2.1.14, Sigma)

were added to wells (2 mm diameter) cut in the gel and the plates were incubated for 20 – 22 h at 26 °C ± 2. The gel plate was stained with 0.1 % (w/v) calcofluor dye (Fluorescent Brightner 28, Sigma) for 20 min and washed with SDW for 2 h in an orbital shaker (IKa-Vibrax-VXR). The hydrolyzed zones were visualized by placing the gel under UV light [Mineralight® Lamp (365 nm), model UV GEL-58; Chromato- VUE Cabinet, model cc-10] and the diameter of the darker hydrolyzed zones was recorded. Sample enzyme activity was determined using a regression equation obtained from chitinase standards.

For β-1,3-glucanase assay, peel samples (1 g) were homogenized at 11,000 rpm in a pre-cooled centrifuge tube with 1 % (w/w) polyvinyl polypyrrolidone and 5 mL potassium acetate buffer (50 mM, pH 5), containing 1 mM EDTA and 5 mM reduced glutathione (Dann & Deverall, 2000). The extracts were centrifuged at 9000 g for 5 min at 4 °C and the supernatant was used for the enzyme assay. Potassium acetate buffer (1.6 mL, 10 mM, pH 5) and the crude extract (0.4 mL) were allowed to equilibrate to 30 °C for 3 min. The reaction was initiated by adding 0.4 mL of the substrate (Pachyman) suspension and was stopped after 10 min by adding 2.8 mL of 20 % (w/v) Tris. The tube was vortexed for 5 min and centrifuged at 9000 g for 3 min. Aliquots (3.0 mL) of the supernatant were transferred to cuvettes (Optiglass Ltd., England) and the absorbance was measured at 610 nm against a blank containing the substrate devoid of enzyme extract, using a spectrophotometer (Cam Spec M302, Spectronic Camspec Ltd., UK). The enzyme activity was expressed as absorbance at 610 nm. Both experiments were repeated.

Antifungal activity: Peel tissues (10 g) cut from the fruits treated with each elicitor and controls 3 days after inoculation with conidia of *C. musae*, were extracted separately in ethyl acetate (50 mL) under vacuum with constant magnetic stirring for 1 h. The extract was filtered through Whatman No. 1 filter paper. The residue was subjected to repeated extraction thrice with fresh ethyl acetate and the extracts were pooled and evaporated to dryness in a rotary evaporator at 40 ºC (Stuart RE 300, UK) (Adikaram & Bandara, 1998). The crude residue was collected in 500 µL of ethyl acetate. The extract (100 µL) was spotted on a thin layer chromatography (TLC) plate coated with silica gel (Kieselgel GF$_{254}$, 13 % CaSO$_4$, BDH). The plate was developed in chloroform: methanol (95:5 v/v) and air-dried overnight. The plate was carefully sprayed with a suspension of conidia of *C. cladosporioides* in Czapek-Dox nutrient solution and incubated for 2 – 3 days in a moist chamber at RT (26 ºC ± 2). The size and Rf values of areas lacking mycelia growth were recorded (Klarman & Stanford, 1968).

Previous studies (Abayasekara *et al.,* 2013) have shown that there was no difference in the number and the size of antifungal zones between the TLC plates bioassayed with *Cladosporium cladosporioides* and *C. musae*.

Postharvest quality of chemical elicitor treated fruits

The postharvest quality was assessed when the harvested fruit reached table ripe stage. Six fingers from the third hand of six replicate bunches were used to determine the fruit firmness, percentage titratable acidity (% TA) and total soluble solids (TSS). Fruit firmness was measured using a pocket penetrometer (Forestry Suppliers Inc., UK) at three points along the long axis of each fruit and the values were averaged. The pulp (30 g) was homogenized in 90 mL of distilled water in a Waring blender (Christison Laboratory Blender, Germany) for 1 min at high speed. The homogenate was centrifuged at 3000 g for 5 min and filtered through a muslin cloth. A few drops of the filtrate was placed on a hand-held refractrometer (Leica, model 10430) and the reading was multiplied by the dilution factor (× 3) to obtain the TSS (Dadzie & Orchard, 1997). A sample (10 mL) of the filtrate was titrated against 0.03 M NaOH with phenolphthalein as the indicator and % TA was calculated (Dadzie & Orchard, 1997).

***Fruit ripening*:** Four clusters each containing six banana fingers, removed from six replicate bunches were used for each treatment. The fruits were allowed to ripen at room temperature and the ripening was assessed daily using a colour index, 0 - green, 1 - colour-break, 2 - more greener than yellow, 3 - more yellow than green, 4 - yellow with green tip and stalk, 5 - yellow (fully ripe), 6 - yellow with brown spotting (over ripe). Number of days taken to reach the colour-break stage after harvest was determined and used to compare the ripening of fruits subjected to different treatments.

Test for any inhibitory effect of defence elicitors on the pathogen

A series of solutions containing 0, 10, 25, 400, 1000 and 2000 mg/mL of each elicitor was prepared. Equal quantities of elicitor solution and a conidia suspension (10^6 conidia /mL) of *C. musae* were mixed. Drops (20 µL) were placed on clean glass slides (2 drops per slides and 2 slides per treatment). The slides were incubated in moist chambers at 26 °C ± 2 and at the end of 8 h a drop of lactophenol was added to each drop to stop germination. One hundred randomly selected conidia from each drop were counted for germination under high power (×400) light microscope and percentage germination for each elicitor concentration was determined.

Statistical analysis

Fruit ripening data were analyzed by Kruskal Wallis test followed by mean separation by Mood Median (p = 0.05) test using MINITAB Release 13.1 and all the other data were analysed by ANOVA using general linear model (GLM) procedure. Means were separated by Duncan's multiple range test (DMRT) using SAS Release 6.12 and differences at 5 % level were considered as significant.

RESULTS

Effect of pre-harvest elicitor application on postharvest anthracnose development in *C. musae* inoculated fruits

There was a greater and significant (p < 0.05) reduction in anthracnose development in fruits when they were treated with Bion® or SA elicitor preparations at a later stage of fruit development (9 and 11 WABE) than at an early stage (2 and 4 WABE) (Table 1a). Two way ANOVA showed that there was a significant (p = 0.0001) difference in disease severity between the two application times and among the three different chemical elicitors used. The interaction between the two factors, the application time and elicitor type, was significant (p = 0.0001) (Table 1b). The fruits treated with Bion® or SA at 9 and11 WABE

stage showed delayed development and reduced severity of anthracnose lesions compared to the untreated controls (Table 2). Therefore the subsequent studies were carried out using the fruits treated at 9 and11 WABE.

In SA treated fruits, the anthracnose lesions commenced one day later than in the fruits treated with Bion®. The lesions in SA treated fruits expanded much slower compared to Bion® treated fruits and the controls (Table 2). There were no anthracnose lesions in the fruits treated with salicylic acid until the 8th day after inoculation while in the controls, the lesions could be seen on the 6th day (Table 2). The lesions developed 9 and 10 days after inoculation in SA treated fruits were significantly (p < 0.05) smaller compared to that of the Bion® treated fruits. Treatment with Bion® or SA at 9 and 11 WABE, while delaying the disease incidence, significantly reduced (p < 0.05) the severity of the disease by 43 % and 65 %, respectively compared to the control. SA (500 mg/L) treatment was 22 % more effective than Bion® (200 mg/L) while the treatment with K_2HPO_4 (20 mg/L) increased the disease severity by 5 %. K_2HPO_4 treated fruits developed anthracnose lesions, which were similar in size to that of the controls until the 8th day and increased in size thereafter. The disease incidence in fruits treated with Bion®, SA or K_2HPO_4 was not, however, significantly different from that of the control (Table 2).

Table 1a: Development of anthracnose during ripening of fruits pre-treated with different elicitors at 2 and 4 weeks after bunch emergence (WABE) or 9 and 11 WABE, following inoculation with *C. musae*

Treatment stage	Disease severity (lesion area, cm²/fruit)			
	Bion®	SA	K_2HPO_4	Control (SDW)
2 and 4 weeks after bunch emergence	0.64 [a] (- 23 %*)	0.53 [a] (- 1.9 %)	0.74 [a] (- 42 %)	0.52 [a]
9 and 11 weeks after bunch emergence	0.23 [b] (43 %)	0.14 [b] (65 %)	0.42 [b] (- 5 %)	0.40 [a]

* Percentage disease reduction
Means within each column followed by the same letter are not significantly different (DMRT, p < 0.05)

Table 1b: Two–way ANOVA of disease severity data showing interaction effects among treatments

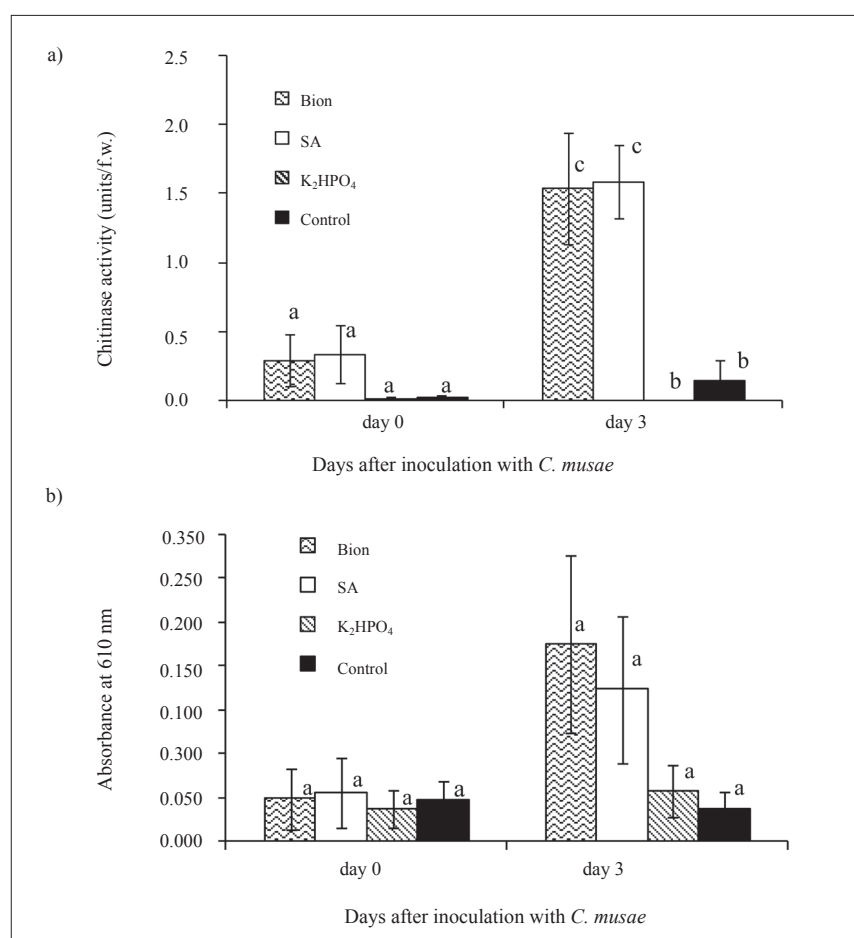| Source	df	F	p
Elicitor application time	1	233.92	0.0001
Elicitor	3	16.84	0.0001
Interaction	3	13.58	0.0001

Defense-related enzymes

The fruits treated with Bion® or SA upon challenge inoculation with *C. musae* after harvest showed a significant increase in chitinase activity on the 3rd day (Figure 1a). The increase of chitinase activity in fruits treated with either elicitor three days after inoculation, was 10 fold compared to the controls. An increase in β–1,

Table 2: Incidence and severity of anthracnose disease in fruit cv. "Embul" treated with elicitors at 9 and 11 weeks after bunch emergence, following artificially inoculation with *C. musae* after harvest

| Treatment | Disease severity (lesion area cm^2/fruit) | | | | | Disease incidence (%) | | | | |
| | Days after inoculation | | | | | Days after inoculation | | | | |
	6 d	7 d	8 d	9 d	10 d	6 d	7 d	8 d	9 d	10 d
Bion ®	0.00 [b]	0.03 [b]	0.19 [b]	0.41 [c]	0.79 [c]	0.00 [a]	13.3 [a]	65.4 [a]	65.4 [a]	89.4 [a]
SA	0.00 [b]	0.00 [b]	0.13 [b]	0.28 [d]	0.48 [d]	0.00 [a]	00.0 [a]	66.6 [a]	70.6 [a]	96.0 [a]
K_2HPO_4	0.00 [b]	0.12 [a]	0.34 [a]	0.80 [a]	1.67 [a]	0.00 [a]	46.7 [a]	86.8 [a]	90.8 [a]	98.6 [a]
* Control	0.03 [a]	0.12 [a]	0.39 [a]	0.65 [b]	1.13 [b]	6.66 [a]	24.0 [a]	94.8 [a]	94.8 [a]	96.0 [a]

Means within each column followed by the same letters are not significantly different (DMRT, $p < 0.05$).
* Treated with SDW and inoculated after harvest with *C. musae*

Figure 1: Chitinase (a) and ß-1,3-glucanase (b) activity in the peel of banana treated with chemical elicitors 9 and 11 weeks after bunch emergence and challenge inoculated soon after harvest with *C. musae.* Columns with same letters, at each time point, are not significantly different (DMRT, $p < 0.05$).
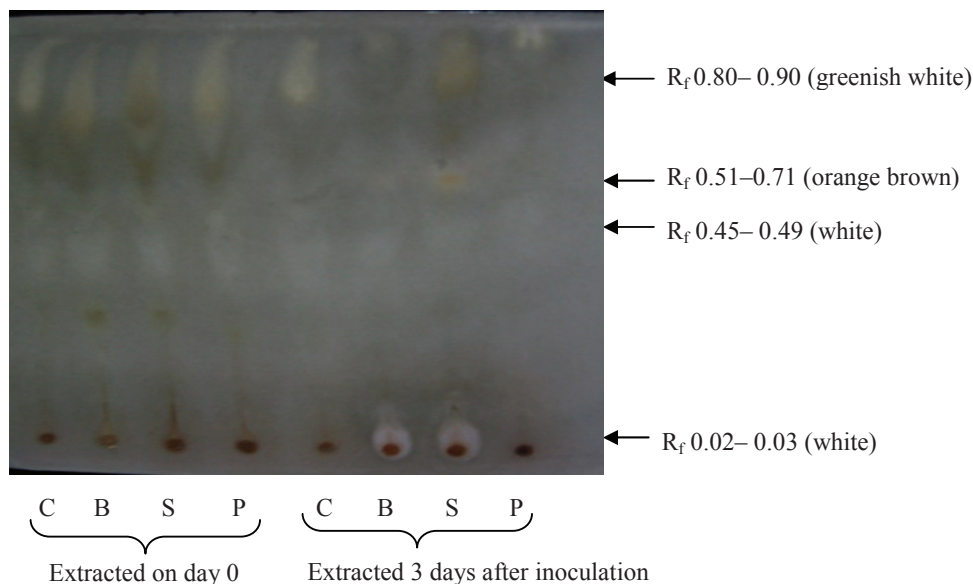
3-glucanase activity was also observed in treated fruits following inoculation, however, the increase was not significant (Figure 1b). K_2HPO_4 treatment did not induce defense related enzymes (Figure 1a & b).

Antifungal activity

The extracts of peel tissues taken on the day of harvest from all treated and control fruits produced two slight antifungal areas at R_f 0.80 – 0.90 and R_f 0.45 – 0.59. The antifungal activity of these zones diminished after three days of inoculation and/ or harvest (Figure 2) to relatively less prominent antifungal areas. Three days after inoculation with *C. musae*, the fruits treated with Bion® or SA showed two additional antifungal zones at R_f 0.02 – 0.03 and R_f 0.51 – 0.71. The latter was more prominent in SA treated fruit peel extract compared to that of the Bion® treated fruits.

Figure 2: Thin layer chromatograph of peel extract of banana treated with elicitors, 9 and 11 weeks after bunch emergence, and inoculated soon after harvest with *C. musae* (developing solvent chloroform: methanol 95:5 v/v) bioassayed with *C. cladosporioides.* C = control, B = Bion ®, S = SA, P = K_2HPO_4

Table 3: Postharvest quality of banana cv. `Embul' treated with elicitors 9 and 11 weeks after bunch emergence after ripening

Treatment	Average fruit wt (g)	Firmness [1] (Kg/cm²)	TSS[y] (°Brix)	% TA[y]	[2] Days to reach colour break stage
Bion®	112.7 [a]	1.21 [a]	17.0 [a]	0.71 [a]	*5 [a]
SA	86.8 [a]	1.40 [a]	16.3 [a]	0.74 [a]	6 [a]
K_2HPO_4	101.4 [a]	1.06 [a]	16.8 [a]	0.72 [a]	5 [a]
*Control	99.9 [a]	1.41 [a]	16.1 [a]	0.71 [a]	5 [a]

[1] Assessed at table ripe stage (colour index 1)
[2] Colour index 1
Means within each column followed by the same letters are not significantly different (DMRT, p < 0.05). Each data point represents mean of six fingers from six replicate bunches.
* Devoid of elicitor treatment treated with SDW

Postharvest quality of elicitor-treated fruit at late fruit development stage

There was no significant difference in the fruit weight between elicitor-treated and control (Table 3). The treatments neither had any apparent phytotoxic effect on fruits at harvest nor after ripening. The postharvest quality of treated fruits evaluated in terms of the change in fruit firmness, TSS and % TA was not altered due to elicitor treatment. All parameters tested were not significantly different from those of the controls (Table 3).

Test for any inhibitory effect of elicitors on the pathogen

There was a slight reduction in the germination of conidia in both Bion® and SA, which is not, however, significant ($p = 0.05$). In K_2HPO_4 the percentage germination reduced progressively with the increase of the concentration and there was no germination at 1000 mg/L (Table 4).

Table 4: Germination of conidia of *C. musae* in elicitor solutions at different concentrations

	% germination of conidia		
Concentration (mg/L)	Bion®	SA	K_2HPO_4
0 (control)	21.5	21.5	21.5
10	15.5	15.5	15.1
25	16.7	NA	12.5
400	22.2	13.2	10.0
1000	12.5	14.1	0.0
2000	NA	10.0	0.0
p	0.497	0.226	0.003
z	- 0.68	-1.21	-2.93

NA - Not Assessed
p values and z values were calculated using binary logistic regression

DISCUSSION

Pre-harvest spray treatment of developing bananas with SA or Bion® delayed the incidence and reduced the severity of anthracnose disease in the harvested fruits during ripening. The treatment applied at a later stage of fruit development i.e. 9 and 11 weeks after bunch emergence (9 and 11 WABE) was found to be more effective. A similar treatment at an earlier stage of fruit development, 2 and 4 WABE, was less effective, indicating that the selection of an appropriate development stage of the fruit is crucial for the elicitor treatment to be effective. The data analysed by two-way ANOVA revealed that the type of chemical elicitor, time of their application and their interactions had a significant influence on the severity of anthracnose disease. The interaction was also evident from the outcome of the experiment where the application of elicitors at a late maturity stage had a greater effect on disease development than that of an early maturity stage. The timing of elicitor treatment and the selection of plant developmental stage have been important in determining the efficacy of Bion® treatment in strawberry against grey mould disease (Terry & Joyce, 2004).

In the present study, the time interval between the elicitor treatment and pathogen challenge in bunches was shorter when the treatment was given 9 and 11 WABE compared to that of 2 and 4 WABE. The period between the application of elicitor and the induction of defence responses has been found to be rather short in herbaceous plants (Benhamou & Bélanger, 1998). Of the elicitors used in the study, both Bion® and SA were effective while K_2HPO_4 treatment tended to increase the anthracnose severity. Neither the pre- nor postharvest application of potassium phophonate (0.1– 1.0 mg/L) induced mango fruit resistance against *C. gloeosporioides* (Zainuri *et al.*, 2001). However, there are reports of enhanced resistance obtained with phosphate salts in wine grapes (Reuveni & Reuveni, 1995). This shows that all elicitors are not equally capable of inducing resistance in every fruit species and that the selection of an appropriate elicitor for treatment is vital.

The treatment of developing banana fruits with SA or Bion®, while delaying the anthracnose development during ripening reduced the rate of expansion of lesions but did not totally control the disease. Plants induce resistance to pathogens when treated with a variety of biotic and abiotic inducers, yet the resistance induced rarely provides complete disease control with most inducing agents reducing the infection by 20 – 85 % (Walters, 2009). Pre-harvest SA application has enhanced resistance in mango (Zainuri *et al*., 2001) and cherry (Yao & Tian, 2005). Bion® has shown a good potential of eliciting resistance to postharvest diseases in melon (Huang *et al*., 2000) and peach (Liu *et al*., 2005).

SA or Bion® treatment induced greater chitinase and *β*-1,3-glucanase activity in harvested banana fruits when they were challenge inoculated with *C. musae*. The enhanced disease resistance observed during this study could at least in part be due to the induction of chitinase and *β*-1,3-glucanase. These enzymes can be directly antifungal to the invading pathogen by lysing fungal cell walls. Alternatively, the breakdown products of pathogen and/or plant cell wall components released by the activity of these enzymes can act as elicitors of plant defence responses (Van Loon, 1997). Increases in chitinase and glucanase have been common in other fruits such as tomato (Baysal *et al*., 2003), sweet cherry (Yao & Tian, 2005) and cucumber (Alkahtani *et al*., 2011). In addition, the treatment with SA or Bion® in the present study showed accumulation of phytoalexins in the fruit peel. Phytoalexins too play a role in localized defense against fungal infections in plants. Brown and Swinburne (1980) demonstrated the accumulation of at least five phytoalexins in the peel tissue beneath the drops of conidia of *C. musae* applied on the surface of green bananas by using thin layer chromatography bioassay. Several phytoalexins were later chemically characterized from *C. musae*-infected banana fruit tissues (Kamo *et al*., 2001; Abayasekara *et al*., 2013).

These defense-related compounds were observed only after challenge inoculation of banana fruits with *C. musae*. The defense responses were assayed 3 days after inoculation of the fruit with *C. musae,* by which time the lesion initiation had just started as brown spots beneath the inoculation sites. Usually the treatment with a chemical elicitor does not necessarily produce apparent biochemical changes typical of systemic acquired resistance as long as the plant is not challenged with a pathogen. The elicitor treated plant is primed to react more efficiently than without the elicitor treatment (Gozzo, 2003).

The concentrations of elicitor solutions used in the treatment were only slightly inhibitory to *C. musae*. Bion® has been developed as a novel crop protection agent, which does not itself have antimicrobial properties (Górlach *et al*., 1996). However, there have been contradictory observations regarding SA. Yao and Tian (2005) reported direct fungi toxicity in SA treated sweet cherry fruits against *Monilinia fructicola*, while Reglinski *et al*. (1997) stated that the reduction of *Sclerotinia sclerotiorum* infection in Kiwi fruit was attributed to the induction of host defense mechanisms.

Elicitor treatments had no apparent toxicity on fruits at harvest. There was no significant difference in fruit weight between the control and the elicitor treatments. However, Lopez and Lucas (2002) noted phytotoxicity when cashew leaves were sprayed with 0.3 mM ASM. It has been reported that SA was phytotoxic when applied as a pre-harvest spray and caused injury at leaf margins where the runoff accumulates (Reglinski *et al*., 1997). The application of chemical elicitors often provides adverse effects on the crop yield. Although bell pepper plants sprayed with the chemical acibenzolar-*S*-methyl showed resistance to subsequent infections by *Xanthomonas axonopodis* pv. *Vesicatoria*, the treatment resulted in an unpredictable loss in fruit yield (Romero *et al*., 2001). The elicitors tested in the current study did not adversely affect fruit ripening or any other physicochemical parameter of the banana fruit.

In conclusion the present study has shown that SA or Bion® treatment, given at a late fruit development stage in the field enhanced resistance of harvested fruits when challenge inoculated with *C. musae,* reducing anthracnose development without altering the postharvest fruit quality.

Acknowledgement

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