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Effect of Dose Rate, Application Method and Commercial Formulations of GA_3 on Banana (*Musa* AAA) Fruit Green Life

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ABSTRACT

The postharvest effect of GA_3 on banana (*Musa* AAA, Cavendish subgroup) fruit green life was evaluated throughout four experiments in the Caribbean side of Costa Rica. Experiment #1 evaluated GA_3 dose rates of 0, 750, 1500 and 2000 mg kg^{-1}, experiments #2 and #3 dose rates of 0, 500, 750, 1000 and 1500 mg kg^{-1}; and experiment #4 dose rates of 0, 500, 1000 and 1500 mg kg^{-1}. Two application methods were implemented (brushing and spraying) using RyzUp® 40% WSG and were evaluated in experiments #1, 2 and 3, while two commercial formulations of GA_3 (RyzUp® 40% WSG and RyzUp® 4 LS) applied by brush only was evaluated in experiment #4. GA_3 was applied in combination with the fungicides and Alum used for crown rot protection. In all experiments, differences ($P < 0.0437$) were found between the untreated control and the remaining GA_3 group dose rates ($P < 0.0113$). With the exception of spray application in experiment #3 where a possible effect was noted ($P = 0.0506$), there was no increasing linear effect ($P > 0.2074$) nor quadratic ($P > 0.1923$) effect of GA_3 dose rate on fruit green life. No differences between application methods ($P > 0.3066$) nor between GA_3 formulations ($P = 0.8622$) were observed. GA_3 delayed maturation in comparison to the untreated control by 10.6, 9.5 and 12.4 days in experiments #1, #2 and #4, respectively; and 24.5 and 9.5 days, respectively for the brush and spray applications in experiment #3. There was no difference in banana fruit green life with either application methods ($P > 0.3066$) or either commercial formulations ($P = 0.8622$). Under packing house commercial process and storage conditions utilized in these studies it was possible to delay banana fruit ripening by the postharvest use of GA_3.

Keywords: brush application, Cavendish subgroup, gibberellic acid, postharvest, RyzUp® 4 LS, RyzUp® 40% WSG, spray application
Abbreviations: GA, gibberellin; **GA_1**, gibberellic acid 1; **GA_3**, gibberellic acid 3; **GA_4**, gibberellic acid 4; **GA_7**, gibberellic acid 7

INTRODUCTION

Gibberellins (GAs) possess the capacity to stimulate growth in many plant species (Salisbury and Ross 1994). More than 130 GAs are known, although only a few (Bethke and Jones 1998) are biologically active (GA_1, GA_3, GA_4 and GA_7) of which only gibberellic acid (GA_3) and a mixture of GA_4 + GA_7 (Gianfagna 1995) are available as commercially marketed products. They are produced commercially via fermentation of the fungi *Gibberella fujikuroi* (Díaz 2002), and GA_3 is the most extensively applied in agriculture. GA_3 is formulated (Devisetty *et al*. 2007) either in a liquid solution (2 to 5% of GA_3 in isopropyl alcohol or 10% of GA_3 in methyl alcohol), as a soluble powder (5 to 20% of GA_3), in effervescent tablets of 1 to 10 g of GA_3 and in soluble granules (40% of GA_3). The latter authors patented a new soluble granule extrusion process that produces a wettable soluble granule formulation of high stability and potency (40% of GA_3).

GA_3 controls multiple plant responses, including stem and petiole elongation (Damasco *et al*. 1996). In banana (*Musa* AAA), GA_3 induces elongation of the pseudostem, abscission of flower parts, increases fruit size and delays senescence of fruit (Lockard 1975; Mishra *et al*. 1981; Lahav and Gottreich 1984; Satyanarayama 1985; Kumar and Reddy 1998; Shanmungavelu *et al.* 1992; Díaz 2002).

In banana fruits of the Cavendish subgroup, the immersion of GA_3 after harvest at rates of 50 to 250 mg L^{-1} for 5 to 30 min resulted in prolonged green and yellow fruit life as a consequence of delayed maturation (Awad *et al*. 1975; Desai and Deshpande 1978; Rao and Chundawat 1984; Rao and Chundawat 1988; Rao and Chundawat 1991; Acharya and Kumar 1998; Patil and Hulmani 1998a, 1998b). Similar postharvest effects have been documented for the fruits of the Plantain subgroup AAB (George and Marriott 1983; George 1987) and for the Silk subgroup of AAB (Mary and Sathiamoorthy 2003).

Nevertheless, the application method used in those previously cited studies are not feasible or easily adaptable to commercial treatments of fruit crown protection systems. The actual banana export commercial practice around the world utilizes the immediate application of a protective mixture (fungicides + astringent) on the crown (brush applied) and its fruits (spray applied) for all the clusters followed by immediate box packing (Soto 1985; Conie and Young 2000; Monge 2004; Sáenz 2004).

The prolonged green and yellow life is due to the delay in the degradation of starch, cellulose and hemicellulose, and conversion to soluble sugars (Desai and Deshpande 1978; Patil and Hulmani 1998b; Rossetto *et al*. 2004), since GA_3 affects both the degradation of complex carbohydrates as well as the synthesis of sucrose (Rossetto *et al*. 2003).

Bananas destined for distant markets are harvested when they reach a commercially marketable size. Plants stressed by disease or climatic conditions have smaller fruit at the date of normal 'green' harvest. Larger fruit are produced if the bunch hangs on the plant an additional 1 to 2 weeks; however, these fruit are more physiologically mature and pre-maturity can affect green life for shipment to distant markets. A delay in postharvest banana fruit maturity and the consequent increase of its green life could make it possible to hang the bunch on the plant and correspondently increase the fruit dimensions (Acharya and Kumar 1998).

Based on these potential advantages and with the recent availability of new a GA_3 formulation (RyzUp® 40% WSG, Valent Biosciences Corp.) and its easy adaptability to the

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Fig. 1 Processing of bunches and fruits to prepare the experimental units. (**A**) Bunch de-handed, (**B**) hand cutting into clusters, (**C**) clusters identification, (**D**) selected clusters deposited into concrete water tank were groups of treatments were partitioned by vinyl polychloride (PVC) tubes, (**E**) clusters treated by brush (**F**) clusters treated by spray (**G**) clusters packed in corrugated carton box and banavac bag (**H**) cold chamber at La Rita.

current commercial protection practices of the postharvest crown, the objective of the present studies was to evaluate the effect of dose rate and method of application, on the postharvest green life of banana (*Musa* AAA, subgroup Cavendish) fruit, which is the most widely consumed in the world.

MATERIALS AND METHODS

Four postharvest experiments were made with banana fruit (*Musa* AAA, Cavendish subgroup) from a commercial farm located in the Caribbean Coast of Costa Rica from 2004 to 2006. Bunches of bananas for experiments #1 to #3 came from plots established within a farm of 288 ha in a replanting area of 27.5 ha sown for first time in 1968 and replanted from 2003 to 2005. Replanting plant density was of 1710 plants ha^{-1} for cv. 'Williams' (experiment #1) and 'Valery' (experiments #2 and #3) replant stock originated from *in vitro* propagated plants. Bunches of experiment #4 were harvested from plots (8.0 ha) at the same farm from 'Valery' planted in 1968 (1690 plants ha^{-1}).

The average fertilization for the years 2004 to 2006 was 415 kg of N, 90 kg of P_2O_5, 590 kg of K_2O, 61 kg of MgO, 593 kg of CaO, 100 kg de S, 11 kg of Zn and 4 kg of B ha^{-1} $year^{-1}$, split equally into 17 applications through out the year. Black Sigatoka caused by *Mycosphaerella fijiensis* was controlled by aerial spraying of systemic (Calixin® 80 OL - BASF, Baycor® 30CE - Bayer Crop Science, Sico® 25 CE- Syngenta) and protective fungicides (Dithane® 60 SC - Dow Agroscience). Fungicides were applied alternately in mixture with miscible oil (Spraytex® M - Texaco) or water according to the Black Sigatoka infection level of the farm and weather conditions. Sigatoka infected and/or damaged whole or partial leaf parts were removed weekly as part of a normal control strategy.

Fruits from similar sized bunches (10 true hands at flower emergence) were utilized in all experiments. They were harvested at 14 weeks (experiments #1 and #2) and 13 weeks (experiments #3 and #4) from flower emergence. In experiments #1, #2 and #4 the bunches were of similar fruit thickness for the second hand (means ± standard deviation): 34.5 ± 0.5, 34.8 ± 0.8 and 34.7 ± 0.4 mm). In experiment #3, in each treatment there were equal number of bunches of the following fruit thickness in the second hand (means ± standard deviation 38.5 ± 0.1, 38.1 ± 0.2, 37.3 ± 0.2 and 36.6 ± 0.3 mm. This was done to get a treatment balance in fruit thickness among bunches. The harvested bunches were transported from the plantation to the packing house by a cableway system. Once at the packing house, each bunch was de-handed and the individual selected hands were cut smoothy from the peduncle using a curved blade and leaving part of the crown attached to the hand (**Fig. 1A**). Each hand was cut with a hooked knife into clusters of 5 to 6 joined fruits each and specifically identified for the treatment (**Fig. 1B**). The selected clusters (**Fig. 1C**) were deposited into concrete circulating water tanks to remove latex exudates for 15 min and each of the treatment groups were partitioned in the tank by floating vinyl polychloride (PVC) tubes (**Fig. 1D**). Fruit clusters were removed from the wash tank and placed on plastic trays and treated by brush directly to the cluster crown surface (**Fig. 1E**) or spray over the crown surface and cluster fruit (**Fig. 1F**) with the mixed solution (fungicides + astringent + GA_3). Each tray held 10 clusters and was treated with 50 ml of the mixed solution. Next, clusters were packed (**Fig. 1G**) and stored in a cold chamber (**Fig. 1H**).

The GA_3 dose rates evaluated were: 0, 750, 1500 and 2000 mg kg^{-1} (experiment #1); 0, 500, 750, 1000 and 1500 mg kg^{-1} (experiments #2 and #3) and 0, 500, 1000 and 1500 mg kg^{-1} (experiment #4). For experiments #1, #2 and #3 the formulation utilized was RyzUp® 40% WSG (400 g of GA_3 mg kg^{-1}) applied with brush or

spray and, in experiment #4, the formulations evaluated were: RyzUp® 40% WSG and RyzUp® 4SL (32.12 g of GA_3 L^{-1}), applied by brush only, both from Valent Biosciences Corp.

The growth regulator was applied in all experiments in a solution combined with the fungicides thiabendazole (400 mg kg^{-1}) and imazalil (600 mg kg^{1}) from Syngenta and Makhteshin-agan, respectively, with the astringent and crown sealed Alum at 1% (99% aluminum and ammonium sulfate) from Industrias Bochica. In the brush method (utilized a brush width of 3.0 cm) the solution was applied directly to and around the cluster cut crown area. In the spray method (hand sprayer) the solution coated all the cluster fruit and crown surfaces. Both methods are commonly used in standard commercial banana packing house postharvest production systems.

In experiments #1 and #2, 10 clusters selected from the two central clusters from the second and third hands of each bunch were utilized. In experiments #3 and #4, the two central clusters from the second through the sixth hand of each bunch were utilized. Additionally, part of the packing material was a non-perforated polyethylene plastic bag (Banavac) lining (104 cm wide, 122 cm long and 20 µm thick) closed with a rubber band. Each box represented one experimental unit (replication). Experiments #1, #2 and #3 included four replications per treatment, and experiment #4 had five replications per treatment.

The packaged fruits were then transport to La Rita Experimental Research Center at CORBANA, S.A.) and transferred to cold storage chamber (12 m^3 from Thermotec de Centroamérica, 1/15 HP BOHN® condensing unit model BHT010H2BS, from Heatcraft Refrigeration Products and automatic temperature control (± 1°C) where the boxes were distributed in a completely randomized experimental design. Chambers were set up a (mean ± standard deviation) of 19.1 ± 0.2; 17.8 ± 1.8; 15.7 ± 1.0 and 18.4 ± 0.7°C for experiments #1, #2, #3, and #4 respectively, and at relative humidity of 91.2 ± 1.6; 89.3 ± 3.8; 99.3 ± 0.8% and 92.9 ± 2.4 for experiments #1, #2, #3 and #4 respectively. Temperature and relative humidity were monitored every hour with a LTC-3PN sensor, Eagle-Picher Technologies.

All individual boxes and fruit clusters were inspected daily. On the date that a finger or cluster reached Grade 3 of the Von Loesecke (Von Loesecke 1950) maturation scale and/or exhibited softness to manual touch, the box or boxes of those fruits were removed from storage. The main variable measured was the interval in days between the harvest date and through to the maturation stage (natural green life). The data sets were analyzed using the SAS statistical program (SAS Institute 2005) considering the factorial structure of rates × method of application for Experiments #1, #2 and #3 and rates × formulation for experiment #4. Regression and contrast analysis were performed.

RESULTS

No interaction between rate × method of application was observed in experiments #1 and #2 ($P > 0.3345$), although an interaction was observed in experiment #3 ($P = 0.0002$). There was no interaction between rate × formulation in experiment #4 ($P = 0.8697$). Thus in experiments #1, #2 and #4 the overall means for rates are presented ignoring the method of application, although in experiment #3 both application methods are illustrated (**Table 1**).

In all experiments (**Table 1**), differences ($P < 0.0437$) were found among rates between the untreated control and the GA_3 rates ($P < 0.0113$) with the exception of green life whose increase was linear ($P = 0.0506$) for the GA_3 dose rate observed with the spray application in experiment #3, no linear ($P > 0.2074$) or quadratic ($P > 0.1923$) effect due to rate were found. No differences between application methods ($P > 0.3066$) or between GA_3 formulations ($P = 0.8622$) were observed. Treatments with GA_3 delayed maturation compared to the untreated control in 10.6, 9.5 and 12.4 days in experiments #1, #2 and #4, respectively; and at 24.5 and 9.5 days respectively for the brush and spray applications in experiment #3. There was no difference in green life of banana fruit (**Table 2**) with the two application methods ($P > 0.3066$) or between the two commercial formulations ($P = 0.8622$).

Table 1 Days from harvest to ripening (natural green life) of banana fruits (*Musa* AAA, Cavendish subgroup) under different GA_3 dose rates applied post harvest. Experiments 1, 2 and 3 considered two application (brush or spray) methods and experiment 4 two commercial formulations (RyzUp® 40% WSG and RyzUp® 4SL) applied with brush.

Rates (mg kg^{-1})	Experiment				
	1	2	3		4
	Brush/ Spray	Brush/ Spray	Brush	Spray	Brush
0	50.9	43.7	57.5	67.9	63.2
500	-----	52.2	83.0	76.0	73.4
750	58.0	54.3	81.8	68.8	-----
1000	-----	52.4	81.8	82.0	77.3
1500	63.2	53.3	81.3	83.0	76.1
2000	63.2	-----	-----	-----	-----
Standard error	2.8	1.3	2.2	2.2	1.7
ANOVA (probabilities)					
Rate[a]	0.0437	0.0110	0.0001	0.0001	0.0001
Rate 0 vs. GA_3 rates[b]	0.0113	0.0013	0.0001	0.0001	0.0001
Lineal rate GA_3	0.2074	0.8146	0.5170	0.0506	0.4174
Quadratic rate GA_3	0.5261	0.8129	0.7242	0.6578	0.1923
	Difference (days)				
Rate 0 vs. rates GA_3	-10.6	-9.5	-24.5	-9.5	-12.4

[a] Includes the rate of 0 mg L^{-1} of GA_3
[b] Excludes the rate of 0 mg L^{-1} of GA_3
Number of experimental units per treatment: four in experiments 1, 2, and 3; five in experiment 4.

Table 2 Days from harvest to ripening (natural green life) of banana fruits (*Musa* AAA, Cavendish subgroup) under different GA_3 methods of application and commercial formulations applied postharvest. Experiments 1, 2 and 3 considered two application (brush or spray) methods and experiment 4 two commercial formulations (RyzUp® 40% WSG and RyzUp® 4SL) applied with brush.

Test type	Experiment			
	1	2	3	4
Method				
Brush	57.7	50.9	77.1	-----
Spray	60.0	50.8	75.5	-----
Standard error	2.0	0.9	1.7	-----
Probability	0.4286	0.9164	0.3066	-----
Commercial formulations				
40% SG (Soluble Granule)	-----	-----	-----	72.4
4 SL (Liquid Solution)	-----	-----	-----	72.7
Standard error	-----	-----	-----	1.2
Probability	-----	-----	-----	0.8622

DISCUSSION

In general, independently of the treatments, natural fruit green life was related with storage temperature and fruit age at harvest. Then, older fruits which were storage in higher temperature (experiment #1 and #2) had a lesser green life than younger (experiment #3 and #4) fruits which were stored at lower (experiment #3) temperatures.

The storage conditions utilized in these studies were at 16-19°C and 91 to 99% relative humidity, and based on the evaluation of natural green life, it was possible to delay the maturation of banana fruits by adding GA_3 to the fungicide + Alum solution of the postharvest treatment to the banana crown and/or fruit.

These results are in agreement with several experiments made in Cavendish banana (*Musa* AAA) subgroup (Awad *et al*. 1975; Desai and Deshpande 1978; Rao and Chundawat 1984; Rao and Chundawat 1988; Rao and Chundawat 1991; Acharya and Kumar 1998; Patil and Hulmani 1998a; Patil and Hulmani 1998b; Osman and Abu-Gouk 2008) and of the more perishable (Lizada 1994) banana Silk (*Musa* AAB) subgroup (Mary and Sathiamoorthy 2003). Nevertheless, the cited studies only tested one rate of GA_3, either 50 or 150 (most of the studies) or 250 ppm for conditions, suggesting a preliminary and/or an exploratory character of those experiments.

Also, in those studies the treatment methods consisted

of dipping banana fruit in a GA_3 dose solution of 50 and 250 mg L^{-1} for relatively long periods, from 2 min (Awad *et al*. 1975), to 3 min (Osman and Abu-Gouk 2008), 10 min (Patil and Hulmani 1998a), 14 min (Rao and Chundawat 1991) and 30 min (Desai and Deshpande 1978) which resulted in a 3 to 4 days of extension of green life and which were significantly lower than those observed with the dose rates, formulations and commercial procedures used in this study. Additionally, the dipping methods utilized in previous studies are not adaptable for implementation in current commercial banana packing house practices for crown protection. In contrast, in these research studies GA_3 was combined with the fungicides and Alum as commercially implemented to protect the cluster crown and applied through current commercial postharvest banana fruit packing practices, totally implementable to current practices.

Although no differences in fruit green life were observed between the GA_3 doses tested, the problem is related to the variable fruit cold storage temperature during the post packed fruit transfer from the packing house to the shipping port and the ship loading processes (Vargas and Pineda 2008); this encourages the use of the intermediate doses tested in our studies. The problem caused by the inadequate or variable storage temperature could be overcome with GA_3. Additionally, GA_3 can increase the level of green life security under the existing export fruit production conditions and reduce the inherent risk of fruit ripening during the shipping process. For fruits harvested from unstressed plants the recommended dose range from 750-1000 mg kg^{-1} of GA_3 is suggested as a minimal response margin.

Both formulations (RyzUp® 40% WSG and RyzUp® 4SL) were effective in delaying ripening, then both are suitable for this use. However, according to Devisetty *et al*. (2007), the liquid formulations based on isopropyl alcohol and methanol exhibit severe disadvantages such as flammability, VOC (volatile organic compounds) related to an air pollutant, toxicity as well as restrictions in their manufacturing, packing, labeling, transport and storage. Our experiences agrees with the claims of these same authors that the new 40% WSG (water-soluble granule) formulation of GA_3 dissociates spontaneously and instantly when mixed with water and forms a clear, thoroughly dispersed solution. Additionally, this granular formulation appears to be highly stable in storage, highly resistant to decomposition, easy to use and few restrictions for its handling, transport and storage.

The results obtained in this study offer very positive alternatives for the postharvest handling of banana fruits for the subgroup Cavendish grown under conventional farm management. Under these conditions, GA_3 can be implemented as a green life preventive factor to cover logistical problems arising from fruit transport and/or during the storage period. Furthermore this approach could prove to be very useful for improving the postharvest life and fruit quality grown under abiotic stress conditions. Small fruit size caused by adverse climate, abiotic and/or biotic stresses such as foliar disease or nematodes are a significant limiting factor in banana production. This experimental approach could be expanded to include fruit from longer hang time or later fruit harvest, although its green life benefit needs to be scientifically confirmed. If GA_3 can successfully prolong green life of these fruits, it can potentially allow for incremental lengthening of the growing period resulting in potentially enhancing higher export fruit sizes thus its production volumes and/or the opportunity for shipments to more distant markets. Although the effect of GA_3 on banana postharvest life has been extensively studied, these recent studies of the present paper are innovating and practical to implement as it is readily adaptable to the commercial packing house conditions and the new formulation (soluble granules) is feasible for the two different application methods.

The primary variable to measure for these experiments was the natural fruit green life (days from harvest to natural ripening). For these studies it was considered irrelevant to evaluate the stored fruit condition parameters of soluble solids (°Brix), fruit firmness and titratable pulp acidity as these variables are most important evaluation parameters for fruit treated by post exogeneous ethylene application for de-greening and at Grade 5 stage (Von Loesecke maturation scale).

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