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Effect of Natural Flooding and Postharvest Gibberellic Acid Application on Banana Fruits

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ABSTRACT

The effect of natural flooding for a period of 144 h was evaluated on banana fruit (*Musa* AAA, cv. 'Grande Naine') resulting from fruiting plants with physiological bunch age at the moment of the flood event of 10, 9, 8 and 7 weeks, from flooded (F) or non-flooded (NF) areas. Banana fruit of the F area were treated with GA_3 (0, 400, 800, 1200, 1600 mg kg^{-1}) as a postharvest treatment. The variables measured were green and yellow life, pulp firmness (kg), soluble solids content (SSC; °Brix) and total titratable acidity (TTA; % of malic acid). A comparison of these variables between F and NF control areas determined the flooding effect. The effect of GA_3 evaluated only in the F area was determined by regression analysis. During the storage period of 21 days at 14°C (simulation transportation time) neither fruit from F nor NF areas showed any sign of natural ripening onset. The flooding event did not have any negative influence on the ripening process of the fruit. Flooding did not have any influence on firmness, SSC and TTA of harvested fruit. Independent of the GA_3 dose, the results showed an increment in SSC (19.04 to 23.17%) and TTA (0.31 to 0.42%) with an increase in the age of the fruit at the time of the flooding event. In general, different GA_3 rates applied to fruit from the F areas did not have an effect on the measured variables of yellow life, including the time to reach Grade 5 of maturity. These results suggest that the commercial practice of discarding bunches in the field after a flooding period may not be necessary in all cases.

Keywords: Cavendish subgroup, flood, gibberellic acid, Grande Naine, green life, *Musa* AAA, yellow life
Abbreviations: exp, experiment; **F**, flooded; **GA_3**, gibberellic acid 3; **NF**, non-flooded; **R-2**, second ratoon

INTRODUCTION

Costa Rica exports approximately 104 million boxes of bananas of 18.45 kg each, from a planted banana area of 44,313 ha, with an income of USA $674 million (Jiménez *et al*. 2009). The largest planted area is located on the Caribbean side. This region is classified as Tropical Moist Forest and Tropical Moist Forest Premontane Transition (Holdrige 1967).

The Caribbean zone has a high frequency of high intensity rainfall, with an annual average from 3,500 to 4,000 mm (Soto 2000). Some high rainfalls cause rivers to overflow, especially those rivers with meandering courses. This, in connection with a flat topography of the area, induces serious flooding which affects the banana plantations situated in this region. The decrease of soil oxygen levels caused by water excess, stops root growth, causes the roots to die, reduces plant photosynthesis and respiration rates, thus increasing the synthesis of foliar ethylene as a response to the stress condition. Symptoms of these alterations are primarily in the form of leaf chlorosis and wilting and the extent of damage depends on soil oxygen level (hypoxia, anoxia) reduction. Damage can vary from plant stunting and collapse, including the bunch (irreversible damage), up to loss in yield from reduced fruit dimensions or even the risk of fruit ripening during transport (Standard Fruit Co. 1975; Stover and Simmonds 1987; Soto 2000). Bananas may tolerate 72 h of flooding with flowing water, and 24 to 28 h under stagnant water or static flood water (Robinson 1992). Damage from flooding is more severe when water is standing than flowing (Israeli and Lahav 2000) which may be related with the lower oxygen content of standing water. Damage from flooding is more intense when high solar radiation occurs after the event (Sastry 1988).

In plants flooded for 48 h growth ceases and first yellowing symptoms become apparent on leaves at this time, longer periods of flooding (72 to 92 h) result in irreversible damage (Israeli and Lahav 2000). The flooding effect is classified as **light** - when plants are exposed for 12 h or less; **moderate** - when they are exposed from 12 to 24 h; or **severe** - when exposure is for more than 24 h (Soto *et al*. 2008). In Central American banana plantations, the practice is that after 48 h of flooding all bunches from affected areas are discarded in the field.

Postharvest applications of gibberellic acid 3 (GA_3) delay fruit ripening (McDonald *et al*. 1997). The most conspicuous effect is in the maintenance of fruit green color due to a delay in chlorophyll degradation (Khader 1991; McDonald *et al*. 1997; Zilkah *et al*. 1997). On bananas (*Musa* AAA), GA_3 induces vegetative elongation, abscission of floral parts, delay in fruit senescence and an increase in fruit size (Vendrell 1970; Lockard 1975; Mishra *et al*. 1981; Lahav and Gottereich 1984; Satyanarayama 1985; Shanmugavelu *et al*. 1992; Kumar and Reddy 1998).

The possibility of using GA_3 to delay ripening may help to postpone banana bunch harvest, and thus increasing dry matter accumulation. Also, as a postharvest treatment, there is evidence that AG_3 may affect the activity of starch degrading enzymes and therefore delaying the starch to sucrose conversion (Rossetto *et al.* 2003; Rossetto *et al.* 2004) although did not delay ethylene and CO_2 burst. This should happen also in fruits coming from bunches of plants stressed by biotic or abiotic factors like flooding. This approach can contribute to increase yield. The objective of the present work was to evaluate the effect of natural flooding and postharvest application of GA_3 on banana fruit green and yellow life and quality.

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Fig. 1 (**A**) Banana plants (*Musa* AAA, cv. Grande Naine) from flooded area (F) one week after flooding with the typical symptoms caused by the event. (**B**) Bunch with sediments residues in the cover that indicate the level reached by flood water.

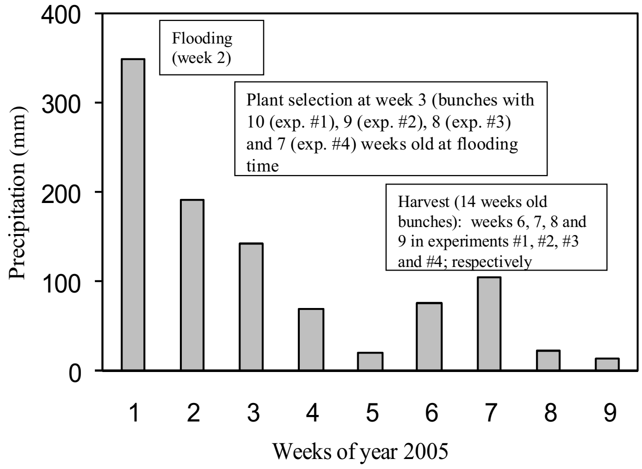

Fig. 2 Weekly evolution of precipitation as well the selection and harvest of the banana plants (*Musa* AAA, cv. Grande Naine) coming from non flooded (NF) and flooded (F) areas.

MATERIALS AND METHODS

Four experiments were carried out during the first quarter of 2005 on a commercial banana plantation in the Costa Rican Caribbean zone. Fruits from banana plants (*Musa* AAA, cv. 'Grande Naine') second ratoon (R-2) with 144 h of continuous flooding with flowing water during the second week of 2005, were used (**Fig. 1A, 1B**). The plants were sown in a soil shaped in domes to aid drainage. To determine the effect of flooding on fruit green and yellow life and quality, fruits from a flooded area (F) without GA_3 (control) were compared with fruits from similar nearby areas that had not suffered flooding (NF) and without GA_3 (control). To determine the effect of GA_3 on fruit´s green and yellow life and quality, fruits from the flooded area were treated with increasing doses of GA_3. Rainfall data (**Fig. 2**), temperature and solar radiation (**Table 1**) prior to and after the flooding, and during the experimental period, were obtained from a weather station located at the plantation.

One week after flooding, plants with 10 (experiment #1), 9 (experiment #2), 8 (experiment #3), and 7 (experiment #4) weeks at flooding time (from flowering) were selected and labeled for the experiments. Harvesting in experiments #1, #2, #3 and #4 was 4, 5, 6 and 7 weeks post flooding, respectively. In all experiments, the fruits were harvested at 14 weeks of age.

From the F area, 25 bunches were selected for each experiment and resulted at harvest (average ± standard deviation) in: 33.0 mm ± 1.9 fruit thickness and 24.3 cm ± 1.3 fruit length and 30.1 mm ± 1.9 fruit thickness in the second hand and 20.3 cm ± 1.2 fruit length in the last hand. In the NF areas 5 bunches for each experiment, were also selected with resulted in 33.8 mm ± 1.7 fruit thickness and 24.8 cm ± 0.8 fruit length in the second hand and 32.1 ± 1.5 fruit thickness and 21.0 ± 1.3 fruit length in the last hand. The R-2 plant in F areas reached at harvest 4.1 ± 1.8 leaves while in NF areas 5.8 ± 1.1 leaves.

For each experiment, the harvested bunches were transported to the packing house for de-handing, selection, crown protection treatment and packing process. From each bunch, two clusters of 6 to 7 fingers from the central portion of each hand were selected from hand 2 through 6 (10 clusters in total) to fill one box and each box was considered as a replication, for five replications per treatment. Fruits from all treatments received a fungicide mixture applied to the crown using Lotos® 40SL (thiabendazole 200 mg kg^{-1} + imazalil 200 mg kg^{-1}) at 400 mg L^{-1} (LAQUINSA, Costa Rica) as a source of fungicide plus Alum (99% aluminium and ammonium sulphate) at 1% from Industrias Bochica, Colombia, as astringent applied directly to each cluster using a 5 cm wide brush. The GA_3 source (Ryzup® 40% WSG from Valent Biosciences Corp.) was applied in a solution mixed with the crown fungicides and Alum.

The effect of GA_3 application was evaluated in fruits of F areas. Four increasing doses of 400, 800, 1,200 and 1,600 mg kg^{-1} were tested in each experiment plus the untreated control (no GA_3). The effect of postharvest application of GA_3 on banana fruit from NF areas was not evaluated in the present study because published results confirm the delay of ripening (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). The GA_3 product used was RyzUp® 40% WSG (400 g of GA_3 mg kg^{-1}) from Valent BioSciences Corp. GA_3 treatments were applied with the fungicide treatment described above.

Two rows of fruits were packed in telescopic corrugated cardboard boxes of 12.7 kg capacity contained in a plastic bag (Banavac type) hermetically closed with a rubber band. Boxes were then carried to the University of Costa Rica Postharvest Laboratory and stored for 21 days in a cold room at 14-15°C to simulate normal shipment transport time required for European port destination (commercial green life). For all experiments, boxes were stored completely at random. During this period visual fruit color evaluations were performed every two days according to the Von Loe-

Table 1 Meteorological registration (temperature and solar radiation) previous and after flooding and during the period plants were evaluated in the field (2005).

Month	Week	Temperature (°C)			Solar radiation (W/m²)	
		Minimum	Maximum	Mean	Minimum	Maximum
January	1	21.83	27.91	23.98	253.07	341.13
January	2	21.86	27.80	23.97	242.92	284.36
January	3	20.15	23.60	21.80	148.19	169.29
January	4	20.77	27.10	23.39	302.96	408.16
February	5	20.31	27.39	23.43	321.79	454.70
February	6	21.61	27.74	24.14	286.66	350.81
February	7	22.71	28.77	25.19	320.11	339.41
February	8	22.91	29.19	25.76	371.99	403.57
March	9	23.44	30.04	26.26	473.49	530.74

Table 2 Accumulated percent (n = 5) of banana boxes (*Musa* AAA, cv. Grande Naine), which reached Grade 5 maturity, depending on the experiment, the number of days after the application of ethylene to fruit (fruit age at flooding) and the GA_3 treatment from non-flooded (NF) and flooded (F) areas.

Experiment¹	Days after ethylene application	Treatments (rate of GA_3 mg kg^{-1})					
		NF	F				
		0	0	400	800	1.200	1.600
		Accumulated percent of boxes with reach Grade 5 maturity					
1	7	80	0	20	40	20	0
	8	100	60	80	80	60	60
	9	---	100	80	80	60	60
	10	---	---	100	100	100	100
		Contrasts				**Pr> Chi square**	
		0 mg kg^{-1} GA_3 (NF) vs 0 mg kg^{-1} GA_3 (F)				0.0151	
		0 mg kg^{-1} GA_3 (F) vs rate GA_3 (F)				0.6624	
2	7	60	0	80	67	60	80
	8	100	100	100	100	100	100
		0 mg kg^{-1} GA_3 (NF) vs 0 mg kg^{-1} GA_3 (F)				0.0192	
		0 mg kg^{-1} GA_3 (F) vs rate GA_3 (F)				0.0015	
3	7	0	0	0	0	0	0
	8	100	100	100	100	100	100
		0 mg kg^{-1} GA_3 (NF) vs 0 mg kg^{-1} GA_3 (F)				1.0000	
		0 mg kg^{-1} GA_3 (F) vs rate GA_3 (F)				1.0000	
4	10	0	0	75	100	100	75
	11	100	100	100	0	0	100
		0 mg kg^{-1} GA_3 (NF) vs 0 mg kg^{-1} GA_3 (F)				1.0000	
		0 mg kg^{-1} GA_3 (F) vs rate GA_3 (F)				0.0002	

1/ Bunch age (weeks) at flooding time: 10, 9, 8 and 7 weeks old in experiments #1, #2, #3 and #4; respectively.

secke scale (1950) in all treatments. Twenty-one days after storage, ethylene (Madurex®, from Madurex Industrias, S.A, Costa Rica). was applied at 100 µl L^{-1} through an ARCO® ethylene generator Model 100, serial # 5972 (American Ripener Co. Inc., Charlotte, North Carolina, USA) for 24 h at 18°C in the storage room this is, in general these conditions simulated commercial ripening practices (Kader 1996). The elapsed time in days, from the application of ethylene up to the time the fruit reached Grade 5 (Von Loesecke scale) of maturation, was determined. When the fruit reached this maturity stage (Grade 5), pulp firmness (kg), soluble solids content (SSC; °Brix) and total titratable acidity (TTA; as % of malic acid) were measured. Pulp firmness was measured both in basal and apical section using a penetrometer (McCormick Fruit Tech Co., Yakima, WA) with a convex probe of 5/16″ diameter. Then, six central slices from the same number of fruits per treatment and repetition were used for determination of SSC and TTA. Each group of slices was weighed on an analytical balance (Sartorious®) and the set was deposited with six times its weight of distilled water in a blender for three minutes at maximum speed. The mixture was filtered in a beaker through a Whatman 41®, from the filtrate some drops were used for the determination of SSC with a digital refractometer (Atago, Tokyo, Japan). TTA determination was made by titration with 0.01 M NaOH solution of a mixture of 5 g of the remaining filtrate with 10 g of distilled water by an automatic titrator (Orion 960, Thermo Fisher Scientific Inc., Beverly, MA, USA). The dilution factor was determined using the formula: weight (pulp + water weight) / weight of the pulp, the SSC was obtained by multiplying the value in the refractometer by the dilution factor and the TTA with the formula: {(NaOH concentration × ml used of NaOH) × milliequivalent} / sample weight in grams} × 100, where the concentration of NaOH used was 0.01 M and the milliequivalent to indicate the TTA as a percentage of malic acid is equal to 0.067. All analytic methods were based on those described by Dadzie and Orchard (1997).

The effect of flooding and GA_3 on the elapsed number of days from ethylene application until fruit reached Grade 5 of maturation (a multinomial response variable) was analyzed separately for each experiment through the Genmod Procedure of SAS (SAS 2005). Pulp firmness, soluble solids content and total titratable acidity data were analyzed as a 4 × 6 factorial (IV experiments and six treatments) with ANOVA. The comparison between F and NF control areas for those variables determined the flooding effect. The GA_3 effect (F area), on tested variables was determined by regression analysis.

RESULTS

In the four experiments, fruit green life was similar in F and NF areas. Also, no differences were observed among GA_3 doses in F area. During the simulation transport of 21 days, there were no visual changes, and the fruit color remained in Grade 1.

Fruits without GA_3 (control) from F area for exp #1 (bunches of 10 weeks old, $P = 0.0051$) and #2 (bunches of 9 weeks old, $P = 0.0192$) reached Grade 5 of maturation later than those from NF control area (**Table 2**). For exp #3 (bunches of 8 weeks old) and #4 (bunches of 7 weeks old), fruits reached Grade 5 of maturation at the same time ($P = 1.0000$).

In the F area (**Table 2**) when contrasting the average of the non-treated fruits with those with GA_3 doses in exp #1, the number of days to reach Grade 5 of maturation was similar ($P = 0.6624$), and identical ($P = 1.0000$) in exp #3. For exp #2 ($P = 0.0015$) and #4 ($P = 0.0002$) fruits without

Table 3 Effect of experiments (fruit age at the moment of flooding) of banana plant (*Musa* AAA, cv. Grand Naine) on firmness, SSC and TTA (one fruit from each five clusters per replicate).

Experiment[1]	Firmness (kg)	SCC (%)	TTA (%)
1	0.85	23.17	0.42
2	1.07	20.74	0.42
3	0.77	20.35	0.36
4	1.17	19.04	0.39
Standard error	0.02	0.34	0.01
	ANOVA (probabilities)		
Experiment	0.0001	0.0001	0.0001
Area	0.6146	0,0993	0.0007
Experiment x Area	0.0367	0.1706	0.3436
Experiment linear effect	0.0001	0.0001	0.0009
Experiment quadratic effect	0.0001	0.0873	0.1084

1/ Bunch age (weeks) at flooding time: 10, 9, 8 and 7 weeks old in experiments #1, #2, #3 and #4; respectively.

GA_3 reached Grade 5 later. No linear nor quadratic effect (data not shown) was found for GA_3 rates (400 to 1.600 mg kg^{-1}) in any experiment ($P > 0.0803$) meaning that fruits ripened uniformly and without observable ripening abnormalities.

Fruit firmness, SSC and TTA at stage of maturation 5 differed ($P < 0.0001$) among experiments. Pulp firmness ($P = 0.0001$) increased and SSC ($P < 0.0001$) level and TTA ($P < 0.0009$) decreased linearly as the age of the fruit (Experiments) at the flooding time decreased (**Table 3**). The natural flooding did not affect fruit firmness ($P = 0.6146$) nor SSC ($P = 0.0993$) but a higher TTA ($P = 0.0007$) was observed in the NF area (**Table 3**). When the interaction of Experiments × Area was considered, this was significant only for fruit firmness ($P = 0.0367$), which should be associated with random variation. However, for a better interpretation, both interaction factors were considered in those variables.

With exception of higher SSC ($P = 0.0184$) in Exp #2 and TTA ($P = 0.0331$) in Exp #4 in fruits from F control area, no differences in pulp firmness ($P > 0.3134$), SSC ($P > 0.4359$) and TTA ($P > 0.2890$), at Grade 5 of maturation were observed in any experiment, compared to NF control area (**Table 3**). The application of GA_3 induced a quadratic effect on pulp firmness ($P = 0.0004$) for experiment #4, SSC ($P = 0.0008$) for exp #1, and II ($P = 0.0502$) and TTA ($P = 0.0252$) for exp #2 (**Table 4**).

DISCUSSION

The fact that fruit from F and NF areas reached similar green life indicates that the flooding event did not affect this variable for any fruit age at the time of the flood. Considering a transport simulation period of 21 (transport time from Costa Rica) days, fruits would reach Western European Markets with similar maturity grade and evidence of spontaneous ripening, Chillet *et al.* (2010) found similar behavior for fruit harvested after a simulated flooding event under greenhouse conditions.

The flooding event did not have any negative effect on the fruit ripening process. On the contrary, fruit of 10 and 11 weeks of age from F area showed a delay on ripening as compared to fruit from NF area of similar ages. There was no effect of the flooding event on fruit internal quality (firmness, SSC and TTA) regardless of the fruit age.

In general, the GA_3 rates applied to fruits from F areas did not cause an effect on the measured variables of yellow life, including the time to reach Grade 5 of maturation. The differences that were determined did not appear to correspond with any specific behavior directly tied to the treatments or to the age, and could be associated with random natural variations of the fruit. Independently of the GA_3 dose, SSC and TTA increased as the age of the fruit increased at the time of the flooding event. These values agree with those parameters established for export in the Cavendish banana subgroup described by Dadzie and Orchard (1997). This finding suggests that the stress induced by the flooding did not have a direct effect on sugar transport to the fruit. This suggests an effect of bunch age on those variables.

In general, the response to waterlogging obtained in this investigation could be due to the more favorable post-flooding climatic factors (low solar radiation and low rainfall), which did not produce substantial changes in the plant's metabolism, in contrast to those mentioned by Sastry (1988), related with high solar radiation and hot weather, where the damage by flooding, according to that writer, is usually high. Agronomic management factors such as locating the plants in areas formed by domes, as well as a condition of the plant's natural resistance to hypoxia and anoxia mentioned by Castro *et al.* (2007) could have mitigated the effect of the flooding. On the other hand, the roots can adapt to oxygen deficiency through changes in their anatomy and metabolism (Turner 2003). In this respect, the roots of the banana plant are capable of developing aerenchyna, thus permitting a continuous flow of gas all along the root and, in waterlogged conditions, enabling the oxygen to move from the shoot to the roots (Aguilar *et al.* 2003). It has been reported (Bradford 1983; Angelov *et al.* 1996) that plants under waterlogged conditions have an initial reaction of reduction of almost all metabolic activity like nutrient uptake, photosynthesis, transport from root to

Table 4 Treatment means ± standard error (left side of table) and statistical significances (Pr>F) of the 0 mg L^{-1} GA_3 (NF) vs 0 mg L^{-1} GA_3 (F) contrast and of the linear and quadratic estimated effects (right side of table), for firmness, SSC and TTA of banana fruit (*Musa* AAA, cv. Grande Naine), from plants located in non-flooded (NF) and flooded (F) areas. Number observations =10 (two observations per repetition).

Experiment[1]	Treatments (rate GA_3 mg L^{-1})						Contrast 0 mg L^{-1} GA_3 (NF) vs 0 mg L^{-1} GA_3 (F)	Linear GA_3 effect[2] (in F area)	Quadratic GA_3 effect[2] (in F area)
	NF	F							
	0	0	400	800	1200	1600			
Pulp firmness (kg)									
1	0.90	0.84	0.84	0.82	0.82	0.86	0.3134	0.9622	0.6422
2	1.08	1.02	1.21	1.02	1.04	1.03	0.3435	0.3186	0.2550
3	0.80	0.74	0.77	0.75	0.80	0.77	0.3753	0.5840	0.7458
4	1.03	0.96	1.20	1.32	1.28	1.24	0.3388	0.0002	0.0004
SSC (%)									
1	21.09	21.97	25.61	24.75	23.61	22.0	0.4359	0.4657	0.0008
2	20.01	22.81	20.20	18.67	22.20	20.40	0.0184	0.2838	0.0502
3	20.39	20.81	20.37	20.40	19.80	20.37	0.7230	0.5811	0.6530
4	19.25	20.00	19.38	19.25	18.13	18.25	0.5560	0.1067	0.8861
TTA (% malic acid)									
1	0.38	0.40	0.46	0.43	0.42	0.47	0.4287	0.1694	0.8681
2	0.39	0.43	0.44	0.33	0.46	0.46	0.2890	0.2699	0.0257
3	0.35	0.37	0.38	0.35	0.37	0.34	0.3936	0.2200	0.7649
4	0.32	0.39	0.44	0.43	0.38	0.38	0.0331	0.2627	0.1562

1/ Bunch age (weeks) at flooding time: 10, 9, 8 and 7 weeks old in experiments #1, #2, #3 and #4; respectively. 2/ Does not consider the 0 mg L^{-1} rate.

aerial part of the plant, but after a certain time plants may recover normal metabolic levels provided oxygen can be transported from aerial parts to the root by, in most cases, newly formed aerenchyma like described for banana.

The results suggest that discarding of bunches from fields under flooding events similar to or less than tested here with a similar post flooding (weeks 3 to 9 of 2005) climatic conditions (20-23 and 24-30°C minimum and maximum temperature, respectively; 148-473 and 169-531 W/m^2 minimum and maximum solar radiation, respectively and 451 mm of rainfall) could be an unnecessary commercial practice. This is employed primarily as a commercial security measure rather than an objective evaluation of the impact caused by the flooding on the development of the bunch, the characteristics of the fruit at harvest and the useful life of the fruit.

However, the available information on flooding effects is scarce or oriented to the physiological effects of flooding on the plant (Stover and Simmonds 1987; Sastry 1988; Robinson 1992; Israeli and Lahav 2000; Soto 2000; Soto *et al*. 2008) and although it is indirectly related to the bunch and the fruit, the data available did not justify the discarding of the fruit.

Therefore, considering the potential economic impact, it is necessary to increase research efforts on the effects flooding has on banana plant physiology and to re-evaluate the criteria to determine when the fruit should be discarded or when the fruit can be harvested and shipped to market without risk of losses or reduction in commercial fruit quality.

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