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Plant Protection

Anthracnose intensity and physical and chemical characteristics of 'Prata anã' banana under different nitrogen doses

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Abstract - The balanced supply of nitrogen to fruit trees leads to higher fruit quality and reduced incidence of diseases. The aim of this study was to evaluate anthracnose intensity and the physical and chemical characteristics of 'Prata Anã' banana under different nitrogen doses. Regarding anthracnose intensity, N doses of 150, 200, 250, 400 and 600 kg ha^{-1} were used. The Area Under the Incidence Progress (AUIPC) and Severity Curves (AUSPC) were evaluated. The physical and chemical characteristics were: fruit and pulp mass; soluble solids; starch; total, reducing and non-reducing sugars; coloration and nutrient concentration in peel, evaluated every three days for 15 days. Plants fertilized with N doses of 200, 250 and 400 kg.ha^{-1} had lower AUIPC values and the application of 250 kg.ha^{-1} reduced AUSPC in fruits inoculated with *C. musae*. N, P and K increased in banana peel, fruit and pulp mass, soluble solids, starch, total sugars, reducing sugars, with increasing N doses. There was no difference in the contents of micronutrients in fruit peel. N dose of 600 kg.ha^{-1} reduced the shelf life of fruits and provided higher sugar content and higher disease severity.

Index terms: Nitrogen fertilization, *Colletotrichum musae*, *Musa* spp., postharvest pathology.

Intensidade de antracnose e características físicas e químicas em banana 'Prata anã' sob diferentes doses de nitrogênio

Resumo - O fornecimento equilibrado de nitrogênio para as fruteiras propicia maior qualidade dos frutos e redução de doenças. O objetivo foi avaliar a intensidade de antracnose e as características físicas e químicas em banana-'Prata-anã' sob diferentes doses de nitrogênio. Na intensidade de antracnose foram utilizados 150; 200; 250; 400 e 600 kg.ha^{-1} de N. Avaliaram-se a Área Abaixo da Curva de Progresso da Incidência (AACPI) e Severidade (AACPS). As características físicas e químicas foram: massa do fruto e polpa; sólidos solúveis; amido; açúcares totais, redutores e não redutores; coloração e concentração de nutrientes na casca, avaliadas a cada três dias, por 15 dias. Plantas adubadas com 200; 250 e 400 kg.ha^{-1} de N apresentaram menores valores de AACPI , e a aplicação de 250 kg.ha^{-1} reduziu a AACPS em frutos inoculados com *C. musae.* Obteve-se aumento de N, P e K na casca da banana, na massa do fruto e da polpa, sólidos solúveis, amido, açúcares totais, açúcares redutores, com o aumento das doses de N. Não houve diferença nos teores de micronutrientes na casca dos frutos. A dose de 001247600 kg.ha^{-1} reduziu a vida de prateleira dos frutos e proporcionou maior teor de açúcar e maior severidade de doença.

Termos para indexação: Adubação nitrogenada, *Colletotrichum musae*, *Musa* spp., patologia pós-colheita.

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Introduction

Banana anthracnose, caused by *Colletotrichum musae* (Berck.; Curt.) Von Arx, is the most important post-harvest disease (MAQBOOl et al., 2010; ALEMU, 2014), causing losses of up to 80% when fruits are not treated (BILL et al., 2014). Fruit maturation leads to high anthracnose incidence (SIVAKUMAR and BAUTISTA-BAÑOS, 2014) and rapid deterioration (AHMED and PALTA, 2016; CHEN et al., 2017). *C. musae* infection occurs in the field in the early stage of fruit development and the fungus remains quiescent until maturation (SIVAKUMAR and BAUTISTA-BAÑOS, 2014; DE COSTA and ERABADUPITIYA, 2005). Thus, losses occur to traders and consumers, who discard spoiled fruits.

Control measures that prevent infection and anthracnose development play an important role in extending shelf life during fruit storage (MAQBOOL et al., 2010). Thus, post-harvest disease control begins with care in cultivation fields, harvesting and transportation. Among the various management methods that can be implemented in the field, chemical control (VILAPLANA et al., 2018) and cultural control (FERNANDES et al., 2019; VENTURA et al., 2012) stand out. Among cultural management methods to control diseases, soil fertility is one of the most important factors. Balanced N supply to fruit trees provides higher fruit quality, longer shelf life and reduced disease intensity. Balanced nutrition is essential to increase plant resistance to diseases. The nutritional status of plants is reflected in defense mechanisms against fungal diseases (MARSCHNER, 2012; WALTERS and BINGHAM, 2007). Adequate nitrogen levels are required for disease resistance, but excess or deficiency may promote increased severity (BALLY et al., 2009; WALTER et al., 2008; NAM et al., 2006; NGUYEN et al., 2004). When N supply is high, the synthesis of secondary metabolites through the shikimic acid pathway is compromised, reducing the production of phenolic (fungistatic) compounds. N also increases the concentration of amino acids and amides in the apoplast, which apparently have greater influence than sugars on germination and development of conidia, thus favoring the development of fungal diseases. In contrast, nutritional deficiency leads to the accumulation of low molecular weight organic substances that reduce plant resistance to diseases (MARSCHNER, 2012).

For banana crop, there are no studies in Brazil that relate nitrogen nutrition to anthracnose intensity. Thus, the aim of this study was to evaluate anthracnose intensity and the physical and chemical characteristics of 'Prata Anã' banana obtained from plants fertilized with different nitrogen doses.

Material and methods

The experiment was carried out in a ten-month-old 'Prata anã' banana orchard located in Janaúba, MG, Brazil, (Latitude -15 ° 48 '09' 'Longitude -43 ° 18' 32 '' Altitude 533 m a.s.l.; average annual temperature of 27.5 ºC and predominant tropical dry Aw type climate according to the Köppen classification). Plants were arranged in 3 x 2m spacing, single row and irrigated by micro sprinkler. Each plot consisted of nine plants, using the two central plants, with internal and external borders. Each family was conducted with three plants (mother, daughter and granddaughter), evaluating fruits of the mother plant in the first production year.

Initially, soil samples were collected for soil fertility characterization and liming and nutrient recommendation. In this sense, 150 kg ha^{-1} of dolomitic limestone were applied to the surface of the experimental area and then incorporated with the aid of a hoe in the 0-10 cm deep soil layer. Reference N, P_2O_5, K_2O doses corresponding to fertilization performed for banana trees in northern Minas Gerais corresponded to 250, 45, 700 kg ha^{-1} year, respectively. N and K doses were applied on 06/16/2016 and 07/16/2016 and phosphorus (P) was applied on 06/16/2016. All applications were performed before inflorescence on the soil surface in the form of semicircle within 30 to 40 cm from the pseudostem, always in front of the mother plant and followed by irrigation. N, P, K sources used were ammonium sulfate, simple superphosphate and potassium chloride. Micronutrients were applied at a dose of 50 g $clump^{-1}$ of commercial product FTE-BR 12®, in a single dose and also before inflorescence. The experimental design was randomized blocks, with five treatments in each block (150; 200; 250; 400 and 600 kg ha^{-1} N) and four replicates. Phosphorus and potassium contents were kept constant, 45 kg ha^{-1} and 700 kg ha^{-1}, respectively.

Anthracnose intensity evaluation

To evaluate anthracnose intensity in fruits, the hands of each plot were harvested with peel color 2 according to the Von Loesecke maturity scale (PBMH and PIF, 2006). Subsequently, central bunches were selected, for greater fruit uniformity during ripening.

In the laboratory, bunches were subdivided into bouquets with three fruits, washed with water, neutral soap and left under the bench for drying. Subsequently, they were atomized with a 2.5×10^6 conidia mL^{-1} suspension of *C. musae*, obtained from colonies grown in BDA (Potato, dextrose, agar) for seven days. After inoculation, bouquets were placed in expanded polystyrene trays and placed in chamber with 90% relative humidity for 24 hours. Then, they were taken to a climate chamber at 25 $\pm$ 1 ° C and relative humidity of 90 $\pm$ 5%. The design was completely randomized in a 5 x 5 factorial scheme, consisting of five doses and five evaluation periods (0, 3, 6, 9 and 12 days).

The experimental unit consisted of a bouquet of three fruits with four replicates, totaling 100 units.

Fruit evaluations were performed every three days for a period of 12 days and the Area Under the Incidence Progress Curve (AUIPC) and the Area Under the Severity Progress Curve (AUSPC) were calculated using the equation of Shaner and Finney (1977). The incidence was obtained by the number of fruits affected by repetition, which values were expressed as a percentage by treatment. Fruit anthracnose severity was evaluated using a diagrammatic scale developed by Moraes et al. (2008).

Evaluation of physical and chemical characteristics of fruits

To evaluate the physical and chemical characteristics, fruits of each plot were selected, washed and dried, immersed in imazalil fungicide solution for five minutes, placed in expanded polystyrene trays and kept in refrigerated chamber at 14 ± 1 ° C and 80 ± 5% relative humidity.

The experimental design was completely randomized in a 5 x 6 factorial scheme, consisting of five treatments (150, 200, 250, 400 and 600 kg ha^{-1} of N), six evaluation periods (0, 3, 6, 9 , 12 and 15 days) and four replicates containing a bouquet of three fruits per replicate. The evaluated variables were: fruit and pulp mass; soluble solids (CARVALHO et al., 1990); starch (NELSON, 1944); total sugars (DISCHE, 1962); reducing and non-reducing sugars (NELSON, 1944). The post-harvest shelf life was evaluated by observing the number of days elapsed for the occurrence of the first color changes from green to intense yellow. Macro and micronutrient analysis of the peel of ten fruits of each plot that were initially dried in forced air circulation at 65 ° C until constant weight was also performed. The dried peel was ground, sieved in 0.42 mm sieves (40 meshes) and sent to the Laboratory of Soils of the Minas Gerais Agricultural Research Company-EPAMIG for quantification of nutrient content in plant tissue.

Data obtained in the study were submitted to analysis of variance and regression. For AUIPC and AUSPC, Tukey test at 5% probability was used to compare dose effect, using the SAS software (SAS Institute, 2000).

Results and discussion

There was no adjustment of regression models, so that AUIPC and AUSPC averages of 'Prata Anã' banana anthracnose were compared by the Tukey test ($p < 0.05$).

Higher AUIPC values of anthracnose caused by *C. musae* were observed in fruits of plants fertilized with the lowest (150 kg ha^{-1} N) and highest nitrogen doses (600 kg ha^{-1} N). The increase in AUIPC compared to the reference dose (250 kg ha^{-1} N) was 28.75 and 29.9%, respectively. The lowest AUIPC values were obtained in plants fertilized at N doses of 200, 250 and 400 kg ha^{-1} and not significantly different from each other (Table 1).

According to Marschner (2012), high nitrogen doses decrease the production of phenolic compounds and increase the concentration of amino acids on the leaf surface, which influences conidial germination and germ tube development.

The resistance of immature fruits to post-harvest diseases is associated with the presence of phenolic compounds in the peel (BARKAI-GOLAN, 2001). Green banana peel has high tannin concentration (MAINA et al. 2012; ESPINOSA and SANTACRUZ, 2017) and is responsible for pathogen quiescence (JEFFRIES et al., 1990) and can directly act on pathogens (DROBY et al., 1986; BARKAI-GOLAN, 2001).

Bally et al. (2009) used different nitrogen doses in mango and observed that high doses mainly applied during flowering provided greater anthracnose severity in fruits. According to the authors, the probable cause may be related to reduction of antifungal compound resorcinol. According to Droby et al. (1986), resorcinol present in the peel of green mango fruits inhibits the development of *Alternaria alternata*.

Plants fertilized at dose of 250 kg ha^{-1} showed reduction of 63.93% of AUSPC when compared to dose of 150 kg ha^{-1} (Table 1). When fertilized with N doses of 200, 400 and 600 kg ha^{-1}, no significant differences were observed, but higher AUSPC values compared to dose of 250 kg ha^{-1} were observed, indicating that excessive or insufficient N doses lead to higher AUSPC values in fruits.

Plants fertilized with N dose of 150 kg ha^{-1} presented lower nitrogen content in 'Prata Anã' banana peel (Figure 5). The lowest nitrogen dose used provided higher anthracnose severity in fruits (Table 1).

N deficiency slows plant growth, makes them susceptibility to pathogens and influences the formation of various compounds important for plant growth and development. Thus, when N deficiency occurs, plants may not express their productive potential (CRUZ et al., 2006) and also the synthesis of fruit defense compounds (VENTURA et al., 2012).

Fruit and pulp mass were influenced by N doses, linearly responding to increasing doses. N dose of 600 kg ha^{-1} provided fruits with approximately 240g. Similar results were observed for pulp mass, with increase of approximately 140 g of fruit weight at the highest dose, 600 kg ha^{-1} (Figure 1).

According to Coelho et al. (2003), when there is an increase of N doses, there are increases in the average mass and fruit size. This increase is due to the influence of nitrogen on the processes involving growth and development, with a direct effect on the source-drain relations, altering the distribution of assimilates between vegetative and reproductive plant parts (MARSCHNER, 2012).

Soluble solids content increased from 5 to 25 ° Brix with increasing N doses and storage period (Figure 2). These results allow us inferring that the highest N doses (250, 400 and 600 kg ha^{-1}) promoted an increase in soluble solids content. The increase in soluble solids content in banana occurs because fruit has high starch content when green, so as it ripens, starch is converted into sugars to be used for fruit respiration (PIMENTEL et al., 2010).

Regarding variables starch and non-reducing sugars, there was a significant interaction between factors doses and storage days. As there was no adjustment of linear regression models for doses within days and days within doses, the results were presented as means of 5% Tukey tests (Table 2 and 3).

Higher starch percentage averages were found in green fruits in the first days of storage. Starch degradation occurred gradually during the storage period, reaching low levels on the last day. N doses of 150, 200 and 250 kg ha^{-1} provided on average 8% starch at the beginning of fruit storage and 4% on the last day of storage. N doses of 400 and 600 kg ha^{-1} presented average of 11% at the beginning of fruit storage and 2% at the end of storage (Table 2).

During the ripening process of climacteric fruits, starch degradation is one of the most striking characteristics, because as starch is hydrolysed, there is an increase in the total soluble sugar content (MOTA et al., 1997).

The increase in total sugar content was gradual over the storage days and N doses, concomitantly with the evolution of soluble solids content and decrease of starch content. On the fifteenth day of storage, N doses of 150, 200 and 250 kg ha^{-1} presented total sugar content of 20%, while N doses of 400 and 600 kg ha^{-1} presented total sugar content of 25% (Figure 3).

The results obtained for reducing sugars (glucose and fructose) behaved similarly to those obtained for total sugars, and values increased with increasing N doses (Figure 4). N doses of 150, 200 and 250 kg ha^{-1} presented contents of 10%, and N doses of 400 and 600 kg ha^{-1} provided reducing sugar contents of 15%. The banana-related characteristics that explain the increase in total sugar levels are the high starch levels found in green fruits and their consequent degradation during ripening and conversion into simple sugars (VILAS BOAS et al., 2001).

There was an increase in non-reducing sugar content in 'Prata anã' banana as fruits ripened (Table 3). On the first day of evaluation, fruits presented an average of 0.45% of non-reducing sugars in all tested doses. From the twelfth day, these levels increased to 8% and remained constant until the fifteenth day of storage. The low concentration of non-reducing sugars found in green fruits is mainly due to their low sucrose content, which is synthesized with fruit ripening (OLIVEIRA JUNIOR et al., 2003).

The increase in N doses provided increases in soluble solids, total sugars and reducing sugars. As a consequence, there was an increase in the incidence percentage and anthracnose severity in bananas. High N doses favored the disease as a consequence of the higher conversion of starch into soluble sugars and greater accumulation of amino acids on the leaf surface, thus favoring the development of fungal diseases (MARSCHNER, 2012; GANESHAMURTHY et al., 2011). In apple, Sitterly and Shay (1960) observed that the artificial increase in the level of soluble sugars increased rot caused by *Botryosphaeria ribis*. Green fruits do not provide nutrients necessary for pathogen development, but during ripening, the conversion from insoluble to soluble carbohydrates favors the establishment of the pathogen in fruit tissues (BARKAI-GOLAN, 2001).

Treatment with N dose of 600 kg ha^{-1} was the one that showed the fastest development of peel color, from green to intense yellow, reaching the coloration in six days of storage (Table 4). N doses of 150 and 400 kg ha $^{-1}$ delayed the onset of ripening by approximately 10 days. Higher N doses increase crop yield, but shorten post-harvest shelf life (GANESHAMURTHY et al. 2011). According to Dadzie and Orchard (1997), fertilizer application intensifies banana ripening due to increased respiratory rate and ethylene production.

N, P and K contents in banana peel increased with increasing N doses (Figure 5), and did not influence S, Ca, Mg, Fe, B, Cu, Fe, Mn, Zn and Na contents. The higher N, P and K content found in banana peel was attributed to higher N availability and synergistic effect of nitrogen fertilization on P and K absorption. Plant nutrient absorption process is specific and selective, and there is some competition among them, which may have a synergistic or antagonistic effect (PRADO, 2008). Similarly, synergistic interaction between P and N occurs, whose effect on crop production, at appropriate doses, is better than when applied separately (SILVA and TREVISAM, 2015).

Table 1-Area Under the Incidence Progress Curve (AUIPC) and Area Under the Severity Progress Curve (AUSPC) of anthracnose in 'Prata anã' banana fertilized with nitrogen doses.

N doses (kg ha^{-1})	AUIPC	AUSPC
150	750.00 b	292.00 c
200	421.87 a	166.56 b
250	534.37 a	69.25 a
400	556.25 a	177.94 b
600	762.00 b	298.62 c
VC (%)	37.04	50.33

* Means followed by the same letter in the column do not differ significantly by the Tukey test at 5% probability level.

Table 2- Average starch values (%) of 'Prata anã' banana fertilized with different N doses and stored for 15 days.

N doses	Storage days					
(kg ha^{-1})	0	3	6	9	12	15
150	7.27 Aa	6.81 Aa	5.98 Aa	5.57 Aab	6.75 Aa	5.75 Aab
200	8.78 Aa	8.19 Aa	4.59 Aab	6.00 Aa	3.50 Ab	3.25 Ab
250	9.16 Aa	9.16 Aa	4.44 Abc	5.75 Ab	6.00 Ab	3.50 Ac
400	11.56 Ba	9.58 Aa	11.52 Ba	7.75 Aa	6.50 Aa	2.00 Aa
600	10.00 Ba	9.34 Ab	8.44 Ba	3.75 Abc	5.50 Ab	2.75 Ac
VC (%)	47.53					

* Averages followed by the same uppercase letter in the column and lowercase in the row do not differ by the Tukey's test at 5% probability level.

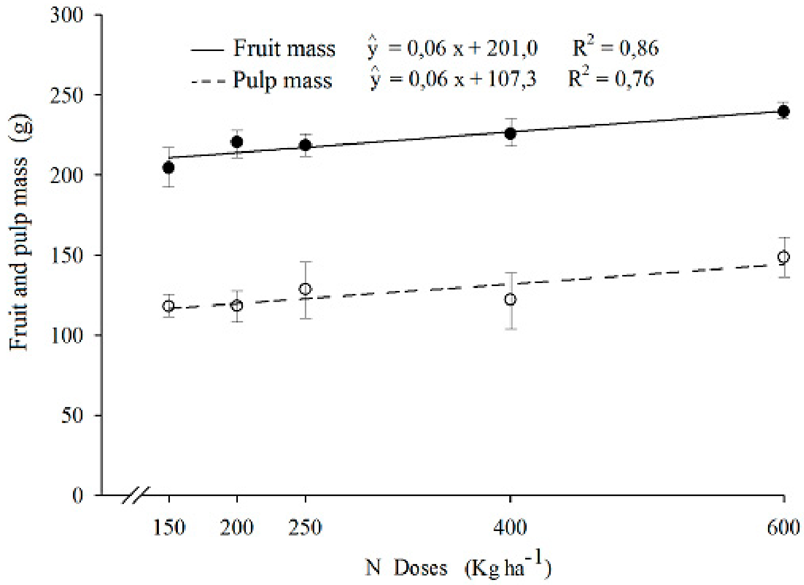

Figure 1- Fruit and pulp mass of N fertilized plants.

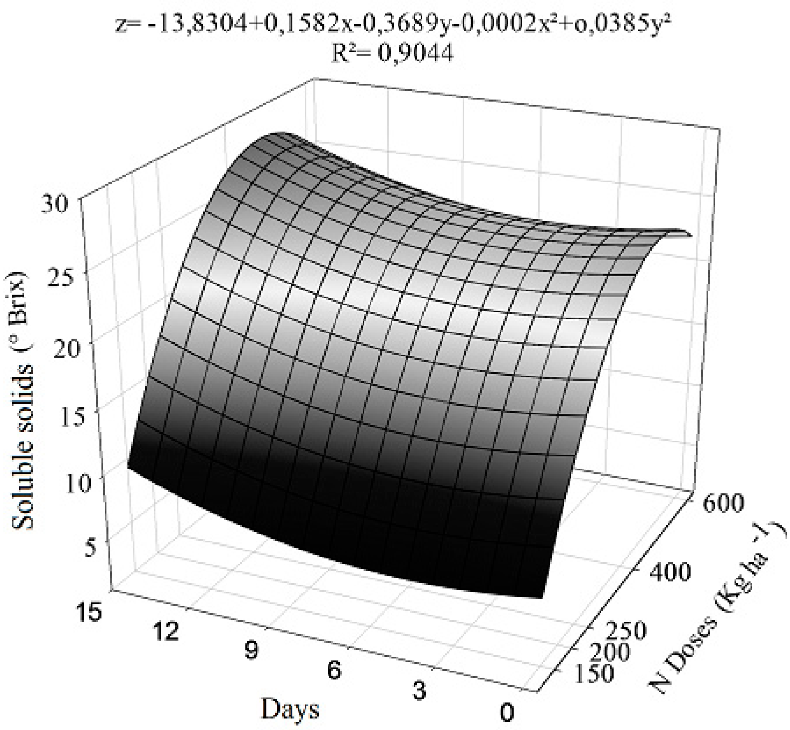

Figure 2- Soluble solids of 'Prata anã' banana fertilized with different N doses and stored for 15 days.

Table 3- Average non-reducing sugar content (%) of 'Prata anã' banana fertilized with different N doses and stored for 15 days

N doses (kg ha^{-1})	Storage days					
	0	3	6	9	12	15
150	0.25 Aa	0.50 Aa	5.00 Ab	7.25 Ab	8.25 Ab	8.75 Ab
200	0.50 Aa	3.00 Aab	6.50 Ab	2.50 Aab	8.75 Ab	8.75 Ab
250	0.50 Aa	3.25 Aab	3.50 Aab	5.75 Ab	7.50 Ab	7.00 Ab
400	0.75 Aa	5.00 Aa	8.25 Aab	3.75 Aa	7.50 Ab	7.00 Ab
600	0.25 Aa	5.25 Ab	2.75 Aab	3.75 Aab	7.25 Ab	8.00 Ab
VC (%)	45.37					

* Averages followed by the same uppercase letter in the column and lowercase in the row do not differ by the Tukey's test at 5% probability level.

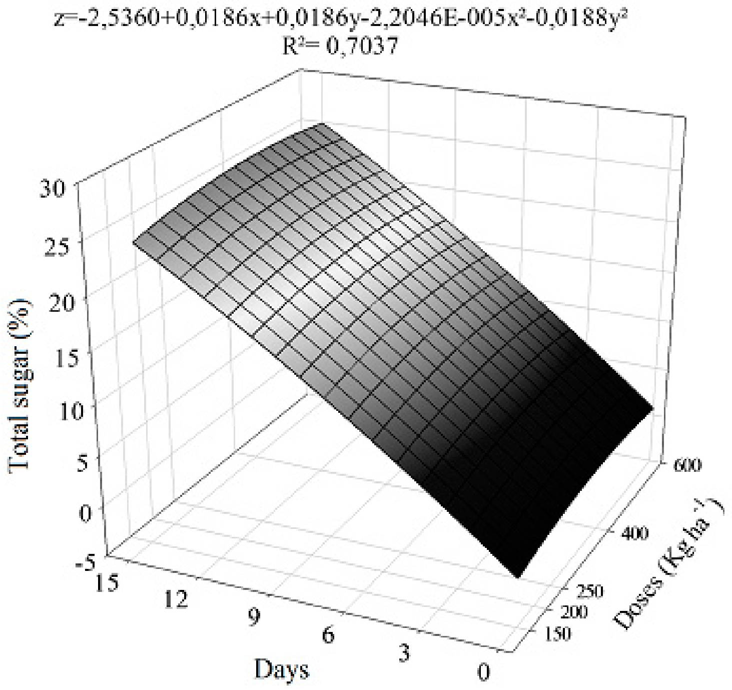

Figure 3- Analysis of total sugars of 'Prata anã' banana fertilized with different N doses and stored for 15 days.

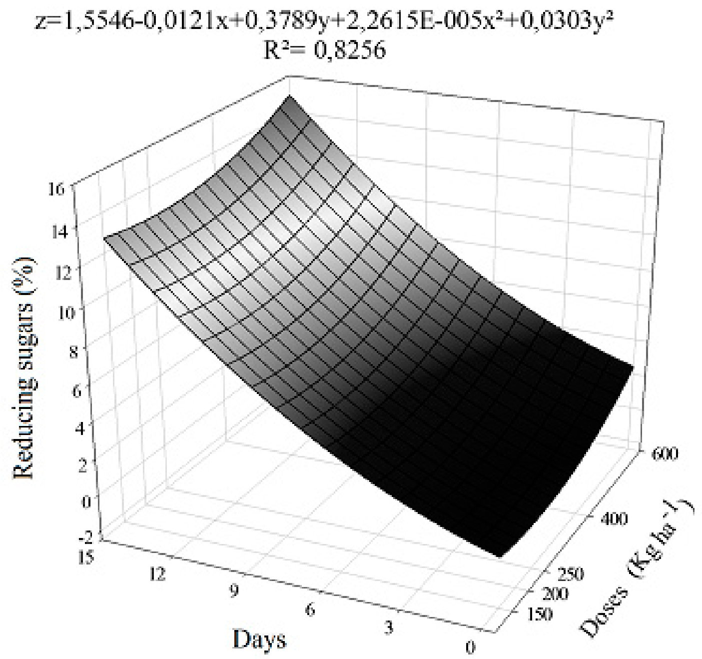

Figure 4-Analysis of reducing sugars on 'Prata anã' banana fertilized with different N doses and stored for 15 days.

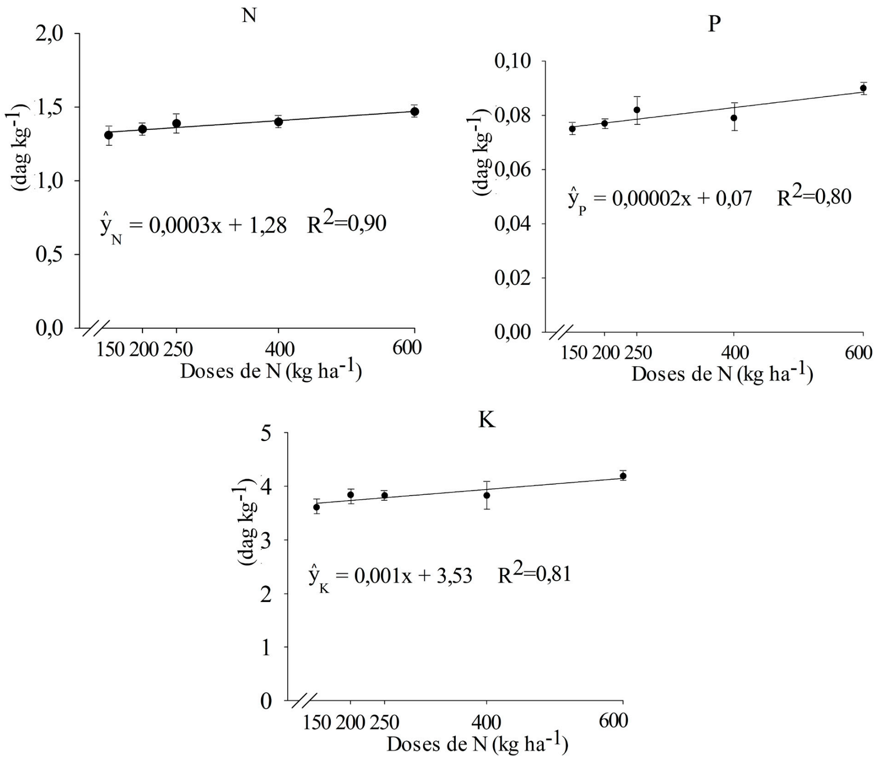

Figure 5-N, P and K content in ‘Prata anã’ banana peel in plants fertilized with different N doses.

Table 4-Number of days for the occurrence of color change in ‘Prata anã’ banana peel from green to intense yellow, fertilized with N doses

N doses	Days
150	10.50 a
200	9.00 a
250	9.75 a
400	10.50 a
600	6.00 b
VC (%)	15.5

* Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% significance.

Conclusions

‘Prata Anã’ plants fertilized with N doses of 150 and 600 kg ha^{-1} presented higher anthracnose incidence and severity.

As N doses increase, there is an increase in fruit mass, pulp mass, soluble solids, total sugars, reducing sugars and starch in ‘Prata Anã’ banana.

N dose of 600 kg ha^{-1} reduces the post-harvest shelf life of ‘Prata Anã’ banana.

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