

# Agronomic Performance of Banana (*Musa* AAA) Cultivars of the Cavendish and Gros Michel Subgroups

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## ABSTRACT

The productive performance, postharvest characteristics, populations of nematodes and root content in low and intermediate cultivars of bananas (*Musa* AAA) of the Cavendish and Gros Michel subgroups were evaluated in sedimentary origin soil of the Caribbean of Costa Rica. At flowering, except for the similar (11.37 vs 11.0;  $P \geq 0.4540$ ) number of leaves of the mother plant between subgroups, plants and follower suckers of the Gros Michel subgroup showed higher ( $P < 0.0001$ ) plant height 3.6 and 1.85 vs 2.97 and 1.2 m) and pseudostem diameter (30.1 and 19.2 vs 25.2 and 13.8 cm) and more leaves (6.05 vs 1.97;  $P < 0.0001$ ) in the follower sucker. Rosetting mainly affected ( $P < 0.0001$ ) Gros Michel, where 30 vs 4.06 % of the plants presented some degree of symptoms. A lower ( $P \leq 0.0222$ ) ratooning was observed in Gros Michel, where it varied between 1.43 and 1.58 vs 1.59 and 1.78 bunches per production unit in Cavendish. At harvest, the Gros Michel plants presented fewer leaves (3.0 vs 3.3;  $P = 0.0005$ ), heavier bunches (29.0 vs 22.5 kg;  $P < 0.0001$ ), with more hands (9.35 vs 7.57;  $P < 0.0001$ ) and more fruits (between 17.1 and 20.7 vs 15.43 and 19.4;  $P \leq 0.0002$ ) by hand, fruits more ( $P \leq 0.0008$ ) thicker (between 39.25 and 42.55 vs 37.43 and 41.9 mm) in all the hands evaluated, fruits less ( $P \leq 0.0027$ ) long in the upper hands (second 23.0 vs 23.7; fourth 21.8 vs 23.2 cm) and longer ( $P < 0.0001$ ) in the lower hands (sixth 20.9 vs 20.37; eighth 19.9 vs 19.17 cm). A higher ( $P \leq 0.0002$ ) content (64.8 vs 34.9 g per sucker) and percentage (83.6 vs 76.0 %) of functional roots was determined in the Gros Michel. There were no differences ( $P \geq 0.0632$ ) in the number of *R. similis* (10018 vs 15126 per 100 g of roots by follower sucker) or in the absence/presence of *Helicotylenchus* spp. (43 vs 33 %) and *Meloidogyne* spp. (49.5 vs. 33.3 %). In *Pratylenchus* spp. its presence was higher ( $P = 0.0321$ ) in Gros Michel (15.4 vs 4.0 %). In the postharvest variables, greater ( $P \leq 0.0006$ ) fruit firmness was found in Cavendish (0.94 and 1.24 vs 0.85 and 1.09 newtons in Gros Michel) in both harvests. Although, the fruit peel was thicker in Gros Michel 3.65 vs 3.35 mm in Cavendish, the difference was not large enough to be significant ( $P = 0.6118$ ). No plants with *Fusarium oxysporum* race 1 symptom were registered in the susceptible cultivars, nor was their presence detected in the soil samples analyzed.

### Keywords

Growth, *Musa* AAA, nematodes, fruit quality, root system, varieties, yield

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## Introduction

In Costa Rica, the entire banana production as fresh fruit for export originates from materials belonging to the Cavendish subgroup, consisting mainly of the Grande Naine, Williams and Valery cultivars. These cultivars have high susceptibility to nematodes, weevils (*Cosmopolites sordidus*) and black Sigatoka (*Pseudocercospora fijiensis*), but are resistant to *Fusarium oxysporum* race 1, which causes Panama Disease. This pathogen caused between 1926 and 1930 that the current cultivar at that time belonging to the Gros Michel subgroup (cv. Gros Michel of tall growth), susceptible to Panama Disease (Champion, 1967, 1975) but more tolerant to nematodes, weevils and black Sigatoka, had to be replaced in its entirety (Campion, 1975; Shepherd *et al.*, 1986; Soto, 1992) by cultivars of the Cavendish subgroup.

However, on a much smaller scale, the Gros Michel cv continued in production basically under subsistence management in mountainous areas of the Atlantic zone, of Turrialba, altitude between 750 and 1200 meters above sea level and certain areas of the Caribbean (Eastern Zone of the Reventazón River) of the country in areas cultivated by small growers (Zapata *et al.*, 1998; Umaña, 2002; Salazar *et al.*, 2012). Currently it is marketed for local consumption, where its fruit is highly appreciated, or for the processed food industry. The traditional high-growing cultivar and low-growing materials (cvs. Highgate and Lowgate) are planted, the latter with much less propensity for plants to fall over.

Various authors (Champion, 1975; Geense *et al.*, 2015; Segura *et al.*, 2018) point out that the pathogen that causes Panama Disease (*Fusarium oxysporum* race 1) can be more aggressive in acidic soils and in those low in nutrients, especially in relation to potassium (Champion, 1975). This, in conjunction with poor drainage and abundant rainfall, intensifies the spread of the fungi. This description agrees with the banana producing areas of Turrialba (volcanic origin), where the presence of the pathogen causes a high plant mortality rate. However, in the soils of the eastern area of the Reventazón River (sedimentary origin) of the Costa Rican Caribbean region, considered younger and of high natural fertility (López and Solis, 1991), it is possible to observe long-lived plantations of the Gros Michel cultivar with a tall growth and, recently, of short growth, without visual manifestations of the disease. This even though it is known the presence of the pathogen on these soils.

This could suggest a differential behavior of the crop depending on the origin of the soil, an aspect that, together with production strategies developed under an organic concept or rational use of agrochemicals, given the greater tolerance to black Sigatoka and nematodes already mentioned, could compete successfully in yield, environmental, economic and fruit quality aspects, with those countries that base their organic or biological banana export on the use of cultivars of the Cavendish subgroup, in the event of an eventual arrival of the Foc R4T.

Different authors (Stover, 1962; Borges *et al.*, 1983; Beckman, 1990; Peng *et al.*, 1999; Stoorvogel and Segura, 2018) consider that the incidence and severity of Panama Disease may be directly or indirectly related to edaphic and nutritional factors of the plant, and that these act on the resistance mechanisms. Given the Gros Michel replacement in the first third of the 20<sup>th</sup> century by cultivars of the Cavendish subgroup, there is no current information available on the agronomic performance, nematode population and fruit quality characteristics of the cultivars of the Gros Michel subgroup, especially those of short growth, nor their comparison under current production technologies, with the cultivars of the Cavendish subgroup.

Then, the objective of the present work was to compare, under conditions of a sedimentary origin soil in the Caribbean Costa Rican region, the vegetative and yield performance, the post-harvest characteristics of the fruit, as well as the population of the phytoparasitic nematodes and content of roots of banana (*Musa AAA*) cultivars of low and intermediate growth of the Cavendish subgroup (cvs Shiroles, Enano Verde, and Williams) and Gros Michel (cvs Turrialba and Talamanca) subgroup. Additionally, the presence of symptoms of Panama Disease (*Fusarium oxysporum* f. sp. cubense race 1) was periodically monitored in the Gros Michel Subgroup cultivars.

## Materials and Methods

The study was carried out at the 28 Mile Agricultural Research Center of CORBANA, S. A. located in the Limón province, Matina County and included five production cycles. During this period, the highest and lowest annual precipitation was 3800 and 2608 mm, respectively. The highest average maximum and minimum temperatures were in the order of 31.8 and 20.5 °C. The highest and lowest average relative

humidity were respectively 93 and 90%. The experimental area was previously cultivated for a period of three years with plantain (*Musa* AAB, cv. Censa ¾). Additionally, a surrounding area previously planted with Gros Michel showed typical symptoms of Panama Disease (*Fusarium oxisporum* f. sp. cubense race 1) in one of its plants, which was confirmed by isolation in culture medium and observation of the structures under a microscope in the Phytopathology Laboratory of CORBANA S.A (pers. comm. Ricardo Villalta, Phytopathologist of CORBANA, S.A.).

Three cultivars of the Cavendish subgroup (Shiroles, Enano Verde and Williams) and two of the Gros Michel subgroup (Turrialba and Talamanca) were evaluated, referred to according to their commercial name (Enano Verde and Williams) or named according to their geographical location (Shiroles, Turrialba and Talamanca). In all cases the materials were of low or intermediate growth.

The planting material consisted of sword suckers without pruning the leaves, roots or stem, with a pseudostem height between 160 and 180 cm, sectioned from the mother plant and transplanted directly to the field. A population density of 1666 plants ha<sup>-1</sup> in an isosceles triangle (2.0 m between plants and 3.0 m between rows) was used. The chemical characteristics of the soil were: pH 6.3; extractable acidity 0.05; organic matter 3.3%; Ca 19.1; Mg 9.9 and K 1.2 cmol<sup>(+)</sup> L<sup>-1</sup>; P 17, Fe 279, Cu 8, Zn 2 and Mn 59 mg L<sup>-1</sup>. The physical characteristics, referring to the texture, were: 45% sand, 22% clay and 36% silt and it was classified as Loam.

The experimental design was a randomized complete block design with six repetitions. Each repetition consisted of a row of ten plants for each cultivar, eight of them useful, with a border plant at each end of the row, and lines of plants as side borders. De-suckering was carried out at flowering of the mother plant, retaining in the stool the sucker with the best position with respect to the sucker of the neighboring production units and looking at the best vigor within the stool. Fertilization was carried out by applying 390 kg of N, 57 kg of P<sub>2</sub>O<sub>5</sub>, 640 kg of K<sub>2</sub>O, 71 kg of S, 1.9 kg of B and 6.3 kg of Zn ha<sup>-1</sup> year<sup>-1</sup>, distributed in 17 cycles. Black Sigatoka (*Pseudocercospora fijiensis*) was combated with terrestrial applications of systemic and or protective fungicides in combination with mineral oil in a total volume solution (emulsion) per application of 50 L ha<sup>-1</sup>. The chemical control was complemented with weekly

leaf removal of the affected tissue. Weed control was carried out by applying glyphosate (90 ml in 16 L of water) and was complemented by sporadic hand cutting with machete.

Nematode control was carried out only in the cultivars of the Cavendish subgroup with applications in rotation every four months of the granular chemical nematicides Counter® 15GR (terbufos-AMVAC), Mocap® 15GR (ethoprophos-AMVAC), Namacur 15GR (Phenamiphos-AMVAC) and Rugby® 10GR (cadusafos -FMC) at the register rate on the label of 20 g cp in front of the follower suckers. In the cultivars of the Gros Michel subgroup, it was not applied since it was considered of interest to know the magnitude of the nematode populations and the root condition of the plants of this subgroup, considered as more tolerant to *R. similis* (Mateille 1992, 1994; Stoffelen *et al.*, 2000; Dochez *et al.*, 2000; Gaidoshova *et al.*, 2008) for a technical support for an eventual biological management of said material.

As a preventive measure against plant falling over, propping of fruiting stems of all cultivars was done with two poles of cane rods (*Saccharum robustum*) per plant. Bunch bagging was carried out under the semi-premature modality (two to three open bracts) with blue polyethylene covers impregnated with the insecticide bifenthrin. In none of the cases was there any deflowering or fruit removal. In the plant crop, bunches were not trimmed. The elimination of true hands was carried out for the following cycles where those with six, seven and eight hands, the last one was removed, those with nine and ten true hands, the last two and those with eleven or more true hands, the last three were removed. The bunch bell was removed one week after trimming. The harvest was carried out 12 weeks after bagging (13 weeks after flowering).

At flowering of the plant crop and its respective follower suckers, the number of leaves was recorded (in the suckers, a true leaf was that with at least 10 cm wide in the widest part of its leaf blade), as well as the height (m) and diameter (cm) of the pseudostem.

When these follower suckers became mothers, these variables were evaluated again and so on, so that there was an evaluation of 5 cycles of mother plants and their respective follower suckers. Additionally, in the plant crop the number of days from planting to flowering was recorded and then the period between flowering

(ratooning) and the degree of choke throat (rosseting) which was measured after the third production cycle (3, 4 and 5 cycle).

The pseudostem height was measured from the ground level to the union of the last two emitted leaves. The pseudostem diameter was measured at the plant base in cm with a caliper graduated in cm. The ratooning corresponds to the number of flowerings emitted by banana stools in a calendar year. It was calculated dividing the 365 days of the year by the days between successive flowerings. The degree of choke throat (rosseting) was determined according to the reduction in the normal leaf emergence between leaf sheath internodes based on the scale determined for bananas of the Cavendish subgroup (Valle *et al.*, 2009), where 0 corresponds to plant without symptoms and 3 to a plant with short internodes which have become compacted and congested at the opening, and grades 1 and 2 (in ascending order of severity) as intermediate values.

Root sampling was carried out at the pseudostem base and in front of the follower suckers of the recently flowered (3 to 6 days after the inflorescence was emitted) plants during the 5 cultivation cycles. A hole 15 cm wide by 15 cm long and 30 cm depth was made and all the roots found were collected. For cultivar and repetition, three to four suckers were sampled, and the collected roots were placed in labeled polyethylene bags for shipment to the CORBANA S.A. Nematology Laboratory. Nematode extraction was done by the maceration-sieving method (Taylor and Loegering, 1953) with its modifications carried out in CORBANA (Araya, 2002). The content of total roots (g), functional roots (g), non-functional roots (g) and percentage of functional and non-functional roots and the population of *Radopholus similis* per 100 g of roots and the presence or absence of *Helicotylenchus* spp., *Meloidogyne* spp. and *Pratylenchus* spp. were determined.

At harvest, the number of leaves, bunch weight (kg), number of hands present, number of fruits per hand, thickness (mm) and length (cm from pulp to tip) of the central fruit of the outer row of the second, fourth, sixth and eighth hand when present were evaluated. Fruit thickness was measured with a caliper graduated in mm and fruit length with measuring tape graduated in centimeters.

The post-harvest evaluation was carried out with fruits from second and fourth generation plants. By repetition,

a box of 18.14 kg (the commercial export standard) was packed with the fruits, and they were transported to the cold chambers of La Rita Agricultural Experimentation Center in Guápiles where they remained in shipping simulation at 14 °C for a period of 14 days. Subsequently, temperature was increased to 18 °C and ethylene was applied to promote ripening, and it was evaluated when the fruits reached grade 5, according to the ripening scale of Von Loesecke (1950). The processing of these fruits was carried out following the general methodology available (Dadzie and Orchard, 1996).

The evaluations were carried out on the central fruit of the outer row of a cluster representative of ripening degree 5 of each box and were: firmness (newtons), brix (%), titratable acidity (% malic acid) and peel thickness (mm). The firmness of the pulp was measured in both the basal and apical sections of each fruit using a flat-toothed Chatillon® penetrometer and the determination of Brix degrees with an Atago® model Palette refractometer. In the fourth harvest, except for cv. Enano Verde, and with the help of a vernier, the thickness (mm) of the peel was measured. The procedure was carried out at the CORBANA S.A. facilities. located in the Research Center La Rita.

During the experimental period, the presence of *Fusarium* symptoms in the Gros Michel cultivars was monitored and at the end of the experimental time, soil sampling was carried out in each of the repetitions of the Gros Michel subgroup cultivars with the purpose of determining by PCR the presence or absence of *Fusarium* sp. and *Fusarium oxysporum*. The sample for each replicate came from five subsamples taken alternately along the respective treatment row to make up six samples per cultivar. One gram of each sample was placed in a 50 ml falcon tube containing 5 ml of extractant solution, later it was transferred to the Molecular Biology laboratory of CORBANA S.A. in La Rita. The genetic material was extracted according to the protocol (Brandfass and Karlovsky, 2008) with modifications made by CORBANA. For molecular detection, primers for the genus *Fusarium* sp. ITS-Fu-f/ITS-Fu-r (Wei *et al.*, 2012) and for the species *Fusarium oxysporum* POF2/POF3 was used (Edel *et al.*, 2000).

The variables measured at flowering and harvest were analyzed through a repeated measurements analysis of the averages of their plots (cycles = repeated

measurements) with a composite symmetry model of the covariance structures in the SAS Proc Mixed program. The time interval between successive flowerings (ratooning) was analyzed using an analysis of the variance of plot averages in Proc GLM of SAS. Post-harvest variables were analyzed using analysis of variance in Proc GLM of SAS. In all cases, the cultivars were compared using contrasts.

*Radopholus similis* plot averages were analyzed with a generalized linear model using the log transformation as the link function and assuming a negative binomial distribution of the errors or residuals. Repeated measurements (cycles = repeated measurements) were modeled with a compound symmetry covariance structure in Proc Genmod of SAS. The presence/absence of *Helicotylenchus* spp., *Pratylenchus* spp. and *Meloidogyne* was analyzed in the samples using the Chi-square test with Proc. Freq. of SAS. Root-related variables (total and functional) were analyzed using repeated measurements (cycles = repeated measurements) with composite symmetrical model of covariance structure in Proc Mixed of SAS.

## Results and Discussion

### Variables Measured at Flowering

Differences were found in the number of leaves, height and diameter (Table 1) of the mother plant ( $P \leq 0.0005$ ) and its respective follower sucker ( $P < 0.0001$ ) among the cultivars of the Cavendish subgroup. The Williams cv presented the highest number of leaves in the mother plant with 11.8 differing from the Enano Verde where 11.6 were recorded and both differed from the Shiroles cv which presented 10.7 leaves. That is, the last cultivars emitted 1.1 and 0.9 fewer leaves, respectively. In their follower sucker, the number of leaves was greater in the Williams cv with a difference of 1.9 and 1.1 more leaves, compared to the Shiroles cv (1.4) and Enano Verde (1.2), respectively, which were statistically like each other.

In the mother plants, the highest pseudostem height was recorded in the Shiroles cv with 3.2 m followed by the Williams cv with 3.1 m and lastly the Enano Verde cv with 2.6 m. In their follower sucker, the trend was different. The highest height of the pseudostem was observed in the Williams cv with 1.4 m, followed by the Shiroles cv with 1.2 m and lastly the Enano Verde cv with 1 m. The pseudostem diameter in the mother plants was greater in the Williams cv (25.9 cm) without

differing from the Shiroles cv (25.4 cm) and both surpassed the Enano Verde cv which registered 24.3 cm. The same trend was observed in their follower sucker, where the greatest pseudostem diameter was recorded in the Williams cv with 15.4 cm, then the Shiroles cv with 13.8 cm and lastly the Enano Verde with 12.4 cm.

Between the cultivars of the Gros Michel subgroup, differences were found in the height ( $P < 0.0001$ ) and pseudostem diameter ( $P = 0.0047$ ) of the mother plant. The pseudostem of Turrialba cv was 0.4 m taller and 0.1 cm thicker than that of the Talamanca cv. Between subgroups, except for the similar number of leaves between both ( $P = 0.4540$ ) from the mother plant to flowering, the plants of the Gros Michel subgroup were taller and thicker, and their follower suckers had more leaves and greater height and diameter of the pseudostem than those of the Cavendish subgroup ( $P < 0.0001$ ).

The ratooning among cultivars of the Cavendish subgroup ( $P \geq 0.3375$ ) and between cultivars of the Gros Michel subgroup ( $P \geq 0.2028$ ) were similar in each of the flowering intervals (F) evaluated (Table 2). In the Cavendish subgroup it varied between 1.54 and 1.74 and in the Gros Michel subgroup between 1.40 and 1.58 bunches by production unit by year through the 5 production cycles. Between subgroups, the Cavendish outperformed ( $P \leq 0.0222$ ) the Gros Michel in the 4 intervals evaluated with averages of 1.59 vs 1.50 in F1-F2, 1.72 vs 1.47 in F2-F3, 1.63 vs 1.43 in F3-F4 and 1.68 vs 1.58 in F4-F5 (Table 2).

Choke throat (Table 3) was similar ( $P = 0.4258$ ) among cultivars of the Cavendish subgroup where over 90% of the plants did not present any symptoms. In the Gros Michel, the morphological alteration was greater ( $P < 0.0001$ ) in the Talamanca cv (Grade 2= 15.2% and Grade 3= 10.7%) vs. the cv. Turrialba, (Grade 2= 7.5% and Grade 3= 0%). Between subgroups, the Gros Michel had a greater ( $P < 0.0001$ ) predisposition to said morphological alteration.

The total root weight (between 37.4 and 53.7 g by follower sucker) and functional root weight (between 33.8 and 41.1 g by follower sucker) and the percentage of functional (between 75.1 and 76.6% by follower sucker) and non-functional (between 23.4 and 25.1% by follower sucker) were similar ( $P \geq 0.1279$ ) among cultivars of the Cavendish subgroup (Table 4). Only in non-functional root, the Williams cv presented a higher ( $P = 0.0112$ ) content with 12.5 g per follower sucker vs. 8.8 g in

Shiroles cv and 7.4 g by follower sucker in Enano Verde. A similar trend was observed between the Gros Michel, where the contents of total root, functional root, percentage of functional and non-functional roots were similar ( $P \geq 0.1677$ ) between the cultivars. Again, only in non-functional roots, its content was higher ( $P = 0.0308$ ) in the Turrialba cv with 13.9 g vs. 10.1 g by follower sucker in the Talamanca cv. Between subgroups, the Gros Michel had higher ( $P \leq 0.0328$ ) content of total roots, functional roots, non-functional roots, percentage of functional roots and a lower ( $P = 0.0002$ ) content of non-functional roots.

The *R. similis* population was similar ( $P = 0.3710$ ) among the cultivars of the Cavendish subgroup where it varied between 12088 and 19930 by 100 g of roots by follower sucker (Table 5). The number of samples with the presence of *Helicotylenchus* spp. ( $P = 0.1054$ ), *Meloidogyne* spp. ( $P = 0.1184$ ) and *Pratylenchus* spp. ( $P = 0.3522$ ) were similar among cultivars and varied between 25 and 50%, 29 and 54% and 4 and 8%, respectively. In the Gros Michel the number of *R. similis* was similar ( $P = 0.9018$ ) with 10198 in the Turrialba cv and 9837 by 100 g of roots by follower sucker in the Talamanca cv. The number of samples with the presence of *Helicotylenchus* spp. ( $P = 0.8411$ ), *Meloidogyne* spp. ( $P = 0.1023$ ) and *Pratylenchus* spp. ( $P = 0.8260$ ) were similar between both cultivars with 43 and 43%, 62 and 37% and 14 and 17% in the Talamanca and Turrialba cultivars, respectively. Between subgroups, there were no differences ( $P = 0.0632$ ) in the number of *R. similis* or in the presence of *Helicotylenchus* spp. ( $P = 0.2274$ ) and *Meloidogyne* spp. ( $P = 0.2245$ ), while in the Gros Michel a higher presence ( $P = 0.0321$ ) of *Pratylenchus* spp. was found.

### Variables measured at harvest

Among the cultivars of the Cavendish subgroup, the highest ( $P < 0.0001$ ) bunch weight was recorded in the Shiroles cv (24.4 kg), exceeding the Enano Verde cv by 1.8 kg and the Williams by 3.9 kg (Table 6). The number of hands was greater ( $P < 0.0001$ ) in the Williams cv with 8.1 per bunch, but with a lower ( $P < 0.0001$ ) number of fruits per hand, in each of the evaluated hands (second, fourth, sixth and eighth hand) where it decreased from 17.8 in the second hand to 14.4 fruits in the eighth hand. Figure 1 shows a bunch at harvest of each of the evaluated cultivars in each subgroup. The number of leaves at harvest was higher ( $P < 0.0001$ ) in the Enano Verde cv with 3.9, followed by the Shiroles cv with 3.2 and lastly the Williams cv with 2.9 leaves per plant.

Between the cultivars of the Gros Michel subgroup, the highest bunch weight ( $P = 0.0002$ ) was observed in the Turrialba cv with 30.5 kg vs. 27.5 kg in the Talamanca cv (Table 6). Similarly, the Turrialba cv had a greater ( $P = 0.0102$ ) number of hands per bunch with 9.6 vs. 9.1 hands in the Talamanca cultivar. Although the number of fruits in the second, fourth, sixth and eighth hands was greater in the Turrialba cultivar, where it decreased from 20.8 in the second hand to 17.3 in the eighth hand, the differences did not reach significance ( $P \geq 0.0995$ ). The number of leaves at harvest was similar ( $P = 0.0925$ ) between cultivars with 3.1 in the Turrialba and 2.9 in the Talamanca. Between subgroups, cultivars of the Gros Michel subgroup presented on average bunches with greater ( $P = 0.0001$ ) weight, more ( $P = 0.0001$ ) hands and greater ( $P \leq 0.0002$ ) number of fruits in the second, fourth, sixth and eighth hands, and less ( $P = 0.0005$ ) number of leaves at harvest. Among cultivars of the Cavendish subgroup, the thickness of the central fruit of the outer row of the second, fourth, sixth and eighth hands was greater ( $P < 0.0001$ ) in the Shiroles cv, followed by the Enano Verde cv and lastly the Williams cv (Table 7). These measurements varied between 32.4 and 34.0 mm in the second hand, between 31.4 and 33.0 mm in the fourth, between 30.2 and 31.7 mm in the sixth and between 28.7 and 30.5 mm in the eighth hand. In the cultivars of the Gros Michel subgroup, there were only differences ( $P = 0.0086$ ) in the thickness of the fruit of the eighth hand where the Turrialba cv with 31.5 exceeded the Talamanca cv by 0.7 mm. Between subgroups, the Gros Michel fruits presented a greater ( $P \leq 0.0008$ ) thickness in the four hands evaluated. In the Cavendish subgroup, the cultivars Shiroles and Enano verde showed the longest fruit length in all hands evaluated ( $P \leq 0.0001$ ). Fruit length varied between 22.6 and 24.5 cm in the second hand, between 21.0 and 23.0 cm in the fourth hand, between 19.1 and 21.1 cm in the sixth hand and between 17.7 and 19.7 cm in the eighth hand (Table 8). Between the Gros Michels, the Turrialba cv presented the longest fruit ( $P \leq 0.0430$ ) in all hands evaluated, decreasing from 22.8 cm in the second hand to 19.5 cm in the eighth hand (Table 8). Between subgroups, the Cavendish had longer fruits in the second (0.8 cm;  $P < 0.0001$ ) and fourth hand (0.5 cm;  $P = 0.0027$ ) and shorter length in the sixth (0.5 cm;  $P < 0.0001$ ) and eighth (0.6 cm;  $P < 0.0001$ ) hand.

### Variables measured postharvest

Except for the fruits from the fourth harvest where the Shiroles cv showed greater ( $P = 0.0414$ ) firmness with

1.28 Newtons than the Williams cv with 1.20 Newtons, the brix degrees, which varied between 17.2 and 19.6 % and the titratable acidity of the fruit, which ranged between 0.36 and 0.78% were similar ( $P \geq 0.2900$ ) among cultivars of the Cavendish subgroup (Table 9).

In the Gros Michel, the firmness (0.82 vs 0.87 Newtons) and titratable acidity (0.63 vs 0.70%) in the fruits from the second harvest and the brix degrees (18.4 vs 18.6%) in the fruits from the fourth harvest were similar ( $P > 0.2633$ ). In contrast, in the fruits of the fourth harvest, firmness was higher ( $P = 0.0029$ ) in the Turrialba cv with 1.14 Newtons vs 1.03 Newtons in Talamanca cv. The brix (19.4 vs 17.6%;  $P = 0.0490$ ) in the fruits of the second harvest and the titratable acidity (0.45 vs 0.39%;  $P = 0.0043$ ) in the fruits of the fourth harvest was higher in the Talamanca cv.

Between subgroups, the Cavendish presented greater firmness ( $P \leq 0.0006$ ) and a similar percentage of Brix degrees ( $P \geq 0.2416$ ) than the Gros Michel in both harvests. The titratable acidity was similar between both subgroups in the fruits of the second harvest ( $P = 0.1766$ ) and was higher ( $P < 0.0001$ ) in the fruits of the fourth harvest of the Gros Michel. The peel thickness was greater ( $P = 0.0161$ ) in the Shiroles cv with 3.6 mm vs 3.1 mm of the Williams cv. In the Gros Michel, even though the Turrialba cv presented a thicker peel 3.8 mm vs 3.5 mm in the Talamanca cv, the difference did not reach significance ( $P = 0.4741$ ). Between subgroups, there were also no differences ( $P = 0.6118$ ) although the peel of the Gros Michel was thicker with 3.65 mm vs 3.35 mm in the Cavendish.

### **Presence/absence of *Fusarium***

There was no mortality of plants due to Panama disease in the Gros Michel subgroup cultivars during the five successive crop generations. Of the soil samples taken at the end of the experiment, only 8.3% tested positive for *Fusarium* sp. and none of them were found to have the presence of *Fusarium oxysporum*.

The height of the pseudostem of the mother plant crop at flowering in the materials of the Cavendish subgroup determined the presence of a short-growing cultivar (Enano Verde) and two intermediate-growing cultivars (Talamanca and Williams), which was also reflected in their follower suckers. The same trend was observed in the growth variables measured in the mother plant crop (greater pseudostem thickness) and in their successive

follower suckers (more true leaves, height and thickness of the pseudostem) in the Talamanca and Williams cultivars with respect to the Enano Verde cv.

In the Gros Michel subgroup materials, which were identified based on their different geographic origin, the differences in height and thickness between both suggest, based on the dimensions of the tall cultivars of the same subgroup present in the country, that they could be two cultivars with different heights, one of low height (Talamanca) and another of intermediate height (Turrialba). Likewise, the greater development of their successive follower suckers is a characteristic of the subgroup (Champion, 1967). Under integrated management of Black Sigatoka control (aerial), the materials of the Gros Michel subgroup in a commercial plantation would require maintaining an appropriate number of leaves, half of the aerial applications of fungicides than those of the Cavendish subgroup (Pers. Com. Gilbert Murillo researcher of CORBANA, S.A. Costa Rica). However, the difference in leaves was only observed in the follower suckers, where the cvs of the Gros Michel subgroup registered more.

In the mother plants, the number of leaves was similar between both subgroups, which could be influenced by the greater height of plants of the Gros Michel subgroup, which despite being defined as short and intermediate in height, the terrestrial black Sigatoka chemical control and phytosanitary leaf removal would be more difficult.

The similar ratooning among the Cavendish subgroup cvs and between the Gros Michel is an indication of the homogeneity of the materials within the same subgroup. However, between subgroups, the Cavendish has a greater ratooning, which indicates that the Gros Michel have a longer cycle and a lower precocity, which agrees with observations by other authors (Champion, 1975; Stover and Buddenhagen, 1986).

The degree of choke throat (rossetting) in the Cavendish subgroup plants was low (grades 0 and 1), which contrasted with the expression of more severe degrees of choke throat (grades 2 and 3) in the cultivars of the Gros Michel subgroup, where the Talamanca cv was identified with the greatest problems of this morphological alteration. This suggests a greater sensitivity of the Gros Michel subgroup to adverse climatic factors that favor the expression of this abnormality. The homogeneity within each subgroup was also manifested by the similar root content between both the Cavendish and Gros

Michel subgroup materials. However, between subgroups, the better radical development of the Gros Michel was evident. It is indicated (Champion, 1975) that, during plant development, the banana emits a certain number of roots that would have a direct relationship with its vegetative potential and with the volume of the rhizome or corm.

The susceptibility of the Cavendish subgroup cultivars to nematodes, especially *Radopholus similis*, makes it necessary to combat them with a certain number of annual applications of granular or liquid chemical nematicides to prevent yield losses. In contrast, the tolerance of Gros Michael to nematodes is known (Mateille, 1992, 1994; Speijer and De Waele, 1997; Dochez *et al.*, 2000; Gaidashova *et al.*, 2008; Gonzales *et al.*, 2012) and although in this study the difference was not significant, less *R. similis* was observed in the Gros Michel subgroup cultivars. The Gros Michel plants were not treated with nematicide, while those of the Cavendish subgroup received three annual applications of chemical nematicide.

The absence of statistical differences between subgroups for the number of samples with presence of *Helycotylenchus* spp. and *Meloidogyne* spp. shows the existence of a tolerance of the Gros Michel to these two nematodes. Similarly, it occurred with *Pratylenchus* spp., where despite there being a smaller number of samples with presence of this nematode in the Cavendish subgroup and these differences reaching to be significant with respect to the Gros Michel subgroup, their magnitude was negligible, considering the management strategies used. This behavior could have its origin in the dominance of the populations by *R. similis* both in the Cavendish subgroup and in the Gros Michel, which could have influenced the populations of the other nematodes.

The bunch weight and number of fruits by hand in the Shiroles cv of the Cavendish subgroup reaffirms its difference with the Enano Verde and Williams. The differences in these variables between the Gros Michel subgroup cvs indicate that the Turrialba cv outperformed the Talamanca cv, which would be consistent with the different varietal classification suggested for these two cultivars.

The greater vigor of the Williams cv of the Cavendish subgroup was not reflected in a better bunch weight and fruits by hand among the Cavendish subgroup cvs. On the contrary, in the Gros Michel cvs, the best vegetative

performance at flowering coincided with the best productive performance at harvest.

The greater thickness and length of the fruit of the Shiroles cv. of the Cavendish subgroup compared to the Enano Verde and Williams cvs., the latter with the lowest values, reaffirms their different varietal origin and does not support the performance of the Williams cv. as planting material in banana cultivation under the agroclimatic conditions of the site where the study was conducted. In the Gros Michel subgroup cvs, there were no differences in fruit thickness between cvs, but there were differences in fruit length, where the Turrialba cv was superior. The averages in thickness and length between both subgroups and their statistical differences in all the hands evaluated were influenced, in the case of the Cavendish subgroup, by the lower values obtained by the Williams cv. The fruit dimensions of the Shiroles and Enano Verde cvs and their comparison with the Gros Michel subgroup cvs indicated that there are no major differences between the two subgroups. This, especially in the lower hands, where in both variables, the length is the one that most influences the rejection of fruits (Vargas and Blanco, 2004) within the context of quality standards for export.

Postharvest data highlights the fruit quality of the Gros Michel subgroup bananas, a condition that makes them locally preferred (Zapata *et al.*, 1998), a characteristic also noted by other authors (Champion, 1967, 1975). The information obtained in this work allows us to infer that there is no marked difference in flavor and texture between bananas of both subgroups evaluated.

In the country, there is a widespread perception of a sweeter flavor with a different color and texture in the Gros Michel with respect to the Cavendish. These differences are given by virtue of the origin of the fruits that are marketed locally where most Gros Michel comes from high altitudes. According to Ramírez (2010) and Salazar *et al.*, (2012) under high altitude conditions, the fruit has a sweeter flavor because its production cycle is longer and there is a greater accumulation of starches that subsequently break down into sugars during ripening.

Additionally, there may be a greater accumulation of fructose, which is five times sweeter than glucose. However, if the fruits come from the same altitude and geographical location, as in the present study, the flavor and texture characteristics between both subgroups would be expected to be very similar, which was



previously demonstrated for export as fresh fruit, now under a packaging concept in corrugated cardboard telescopic boxes and not in bunches. The absence of *Fusarium oxisporum* in soil samples from the experimental site, as well as the very low incidence of the disease on plants of the Gros Michel subgroup (also

of the Turrialba and Talamanca cvs) determined in their surrounding areas, is consistent with the behavior of this subgroup in soils of high natural fertility of sedimentary origin (López and Solis, 1991) with higher Ca and Mg contents, higher pH values and located east of the Reventazón River (East zone).

**Table.1** Growth variables at flowering of the banana (*Musa* AAA) plant crop and its respective follower sucker of cultivars belonging to the Cavendish and Gros Michel subgroups in a soil of sedimentary origin.

Subgroup / cultivars	Flowering of the plant crop			Follower sucker		
	Leaves	Height (m)	Diameter (cm)	Leaves	Height (m)	Diameter(cm)
<b>Cavendish Subgroup</b>						
<b>Shiroles</b>	10.7c	3.2a	25.4a	1,4b	1.2b	13.8b
<b>Enano Verde</b>	11.6b	2.6c	24.3b	1,2b	1.0c	12.4c
<b>Williams</b>	11.8a	3.1b	25.9a	3.3a	1.4a	15.4a
<b>Average</b>	11.37	2.97	25.2	1,97	1.2	13.87
<b>Gros Michel Subgroup</b>						
<b>Talamanca</b>	11.0	3.4	30.1	6.1	1.8	19.2
<b>Turrialba</b>	11.0	3.8	30.2	6.0	1.9	19.2
<b>Average</b>	11.0	3.6	30.15	6.05	1.85	19.2
<b>Contrasts</b>			Probability			
<b>Among Cavendish</b>	< 0.0001	< 0.0001	0.0005	< 0.0001	< 0.0001	< 0.0001
<b>Between Gros Michel</b>	0.8376	< 0.0001	0.0047	0.8046	0.2045	0.9594
<b>Cavendish vs. Gros Michel</b>	0.4540	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Each value is average of 30 observations (5 production cycles \* 6 repetitions and in each repetition the data was the average of 8 plants).

**Table.2** Ratooning of banana (*Musa* AAA) cultivars belonging to the Cavendish and Gros Michel Subgroups in a soil of sedimentary origin during five production cycles.

Subgroups/ cultivars	Interval between flowering (Ratooning)			
	F1-F2	F2-F3	F3-F4	F4-F5
<b>Cavendish Subgroup</b>				
<b>Shiroles</b>	1.61	1.74	1.62	1.67
<b>Enano Verde</b>	1.62	1.74	1.62	1.66
<b>Williams</b>	1.54	1.67	1.66	1.71
<b>Average</b>	1.59	1.72	1.63	1.68
<b>Gros Michel Subgroup</b>				
<b>Talamanca</b>	1.46	1.47	1.45	1.58
<b>Turrialba</b>	1.54	1.47	1.40	1.57
<b>Average</b>	1.50	1.47	1.43	1.58
<b>Contrasts</b>		Probability		
<b>Among Cavendish</b>	0,3375	0,4437	0,6496	0,5253
<b>Between Gros Michel</b>	0,2028	0,9755	0,3287	0,7506
<b>Cavendish vs. Gros Michel</b>	0,0222	< 0,0001	< 0,0001	0,0042

Each value is an average of 6 repetitions and in each repetition the value was the average of 8 observations.

**Table.3** Percentage (%) of plants with choke throat of banana cultivars (*Musa AAA*) belonging to the Cavendish and Gros Michel subgroups in a soil of sedimentary origin in the third, fourth and fifth productive cycles.

Subgroups/ Cultivars	Choke throat (degree)			
	0	1	2	3
<b>Cavendish Subgroup</b>				
Shiroles	100	0	0	0
Enano Verde	97.0	2.3	0.7	0
Williams	90.8	5.4	3.8	0
Average	95.9	2.57	1.5	0
<b>Gros Michel Subgroup</b>				
Talamanca	55.1	19.0	15.2	10.7
Turrialba	73.9	18.6	7.5	0
Average	64.5	18.8	11.3	5.3
<b>Contrasts</b>	Probability			
Among Cavendish	0.4258			
Between Gros Michel	< 0.0001			
Cavendish vs. Gros Michel	< 0.0001			

Each value is average of 18 observations (3 crop cycles x 6 repetitions and in each repetition it was the average of 8 plants).

**Table.4** Root content (g) per plant and percentage of functional and non-functional roots in banana cultivars (*Musa AAA*) belonging to the Cavendish and Gros Michel subgroups in a soil of sedimentary origin.

Subgroup / Cultivar	Root weight (g)			Root (%)	
	Total	Functional	Non-functional	Functional	Non functional
<b>Cavendish Subgroup</b>					
Shiroles	42.6	33.8	8.8	75.1	25.1
Enano Verde	37.4	30.0	7.4	76.6	23.4
Williams	53.7	41.1	12.5	76.5	23.5
Average	44.57	34.97	9.57	76.07	24.0
<b>Gros Michel Subgroup</b>					
Talamanca	70.8	60.9	10.1	84.4	15.6
Turrialba	82.4	68.8	13.9	82.9	17.1
Average	76.6	64.8	12.0	83.6	16.3
<b>Contrasts</b>	Probability				
Among Cavendish	0.1279	0.2843	0.0112	0.8228	0.7881
Between Gros Michel	0.1677	0.2970	0.0308	0.5837	0.5395
Cavendish vs. Gros Michel	< 0.0001	< 0.0001	0.0328	0.0002	0.0002

Each value is average of 30 observations (5 crop cycles \* 6 repetitions and in each repetition the value came from the roots of 3 or 4 follower suckers).

**Table.5** Number of *Radopholus similis* per 100 g of roots and percentage (%) of absence/presence of *Helicotylenchus* spp., *Meloidogyne* and *Pratylenchus* in samples of banana cultivars (*Musa* AAA) belonging to the Cavendish and Gros Michel subgroups in a soil of sedimentary origin.

Subgroup/ Cultivar	<i>Radopholus similis</i>	<i>Helicotylenchus</i> spp.		<i>Meloidogyne</i> spp.		<i>Pratylenchus</i> spp.	
		No	Si	No	Si	No	Si
<b>Cavendish Subgroup</b>							
<b>Shiroles</b>	12088	75	25	46	54	96	4
<b>Enano Verde</b>	19930	75	25	71	29	100	0
<b>Williams</b>	13360	50	50	71	29	92	8
<b>Average</b>	15126	66.6	3.33	62.6	37.3	96	4
<b>Gros Michel Subgroup</b>							
<b>Talamanca</b>	9837	57	43	38	62	86	14
<b>Turrialba</b>	10198	54	43	63	37	83	17
<b>Average</b>	10018	55.5	43	50.5	49.5	84.5	15.5
<b>Contrasts</b>		Probability					
<b>Among Cavendish</b>	0.3710	0.1054		0.1184		0.3522	
<b>Between Gros Michel</b>	0.9018	0.8411		0.1023		0.8260	
<b>Cavendish vs. Gros Michel</b>	0.0632	0.2274		0.2245		0.0321	

Each value is average of 30 observations (5 crop cycles \* 6 repetitions and in each repetition the value came from the roots of 3 or 4 follower suckers).

**Table.6** Production variables of banana cultivars (*Musa* AAA) belonging to the Cavendish and Gros Michel subgroups in a sedimentary origin soil during 5 cultivation cycles.

Subgroup/ Cultivars	Bunch weight (kg)	Number of hands	Number of leaves	Number of fruits by hand			
				2 <sup>da</sup>	4 <sup>ta</sup>	6 <sup>ta</sup>	8 <sup>ta</sup>
<b>Cavendish Subgroup</b>							
<b>Shiroles</b>	24.4a	7.2b	3.2b	20.5a	17.0a	16.0a	16.4a
<b>Enano Verde</b>	22.6b	7.4b	3.9a	19.9a	16.5a	15.6a	16.1a
<b>Williams</b>	20.5c	8.1a	2.9c	17.8b	15.6b	14.7b	14.4b
<b>Average</b>	22.5	7.57	3.3	19.4	16.3	15.4	15.6
<b>Gros Michel Subgroup</b>							
<b>Talamanca</b>	27.5	9.1	2.9	20.6	18.8	17.6	16.9
<b>Turrialba</b>	30.5	9.6	3.1	20.8	19.2	18.1	17.3
<b>Average</b>	29.0	9.3	3.0	20.7	19.0	17.8	17.1
<b>Contrasts</b>		Probability					
<b>Among Cavendish</b>	< 0.0001	< 0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<b>Between Gros Michel</b>	0.0002	0.0102	0.0925	0.7342	0.1070	0.4227	0.0995
<b>Cavendish vs Gros Michel</b>	< 0.0001	< 0.0001	0.0005	0.0002	<0.0001	<0.0001	<0.0001

Each value is average of 30 repetitions (5 crop cycles \* 6 repetitions and in each repetition is average of 8 bunches, plants or hands).

**Table.7** Fruit thickness (mm) of selected banana (*Musa* AAA) hands belonging to the Cavendish and Gros Michel subgroups in a soil of sedimentary origin.

Subgroup/ Cultivars	Thickness <sup>1</sup> of the central fruit of selected hands			
	Second	Fourth	Sixth	Eighth
<b>Cavendish Subgroup</b>				
<b>Shiroles</b>	34.0a	33.0a	31.7a	30.5a
<b>Enano Verde</b>	33.4b	32.3b	31.1b	29.9a
<b>Williams</b>	32.4c	31.4c	30.2c	28.7b
<b>Average</b>	33.3	32.2	31.0	29.7
<b>Gros Michel Subgroup</b>				
<b>Talamanca</b>	33.7	32.7	32,0	30,8
<b>Turrialba</b>	33.9	33.1	32,4	31,5
<b>Average</b>	33.8	32.9	32,2	31,2
<b>Contrasts</b>	Probability			
<b>Among Cavendish</b>	< 0.0001	< 0.0001	< 0.0001	< 0.0001
<b>Between Gros Michel</b>	0.1828	0.1496	0.0938	0.0086
<b>Cavendish vs. Gros Michel</b>	0.0008	0.0003	< 0.0001	< 0.0001

Each value is an average of 30 repetitions (5 crop cycles \* 6 repetitions and in each repetition is an average of 8 hands).

**Table.8** Fruit length (cm from pulp to tip) of selected banana (*Musa* AAA) hands belonging to the Cavendish and Gros Michel subgroups in a soil of sedimentary origin.

Subgroup/ Cultivar	Length of central fruit from selected hands			
	Second	Fourth	Sixth	Eighth
<b>Cavendish Subgroup</b>				
<b>Shiroles</b>	24.5a	23.0a	21.1a	19.7b
<b>Enano Verde</b>	24.2a	22.9a	20.9a	20.1a
<b>Williams</b>	22.6b	21.0b	19.1b	17.7c
<b>Average</b>	23.7	22.3	20.3	19.1
<b>Gros Michel Subgroup</b>				
<b>Talamanca</b>	22.8	21.6	20.7	19.5
<b>Turrialba</b>	23.2	22.1	21.1	20.1
<b>Average</b>	23.0	21.8	20.9	19.8
<b>Contrasts</b>	Probability			
<b>Among Cavendish</b>	< 0.0001	< 0.0001	< 0.0001	< 0.0001
<b>Between Gros Michel</b>	0.0430	0.0150	0.0253	0.0016
<b>Cavendish vs. Gros Michel</b>	< 0.0001	0.0027	< 0.0001	< 0.0001

Each value is an average of 30 repetitions (5 crop cycles \* 6 repetitions and in each repetition is an average of 8 hands).

**Table.9** Post-harvest characteristics (degree 5 of ripeness) of banana fruits (*Musa* AAA) belonging to the Cavendish and Gros Michel subgroups in a soil of sedimentary origin in the second (2<sup>nd</sup>) and fourth (4<sup>th</sup>) crop cycle harvest.

Subgroup/ Cultivar	Firmness (newtons)		Brix (%)		Titratable acidity (malic acid %)	
	2 <sup>a</sup>	4 <sup>a</sup>	2 <sup>a</sup>	4 <sup>a</sup>	2 <sup>a</sup>	4 <sup>a</sup>
	Crop harvest					
	2 <sup>a</sup>	4 <sup>a</sup>	2 <sup>a</sup>	4 <sup>a</sup>	2 <sup>a</sup>	4 <sup>a</sup>
<b>Cavendish Subgroup</b>						
Shiroles	0.90	1.28	18.6	18.9	0.78	0.36
Enano Verde	0.96	ND	17.2	ND	0.66	ND
Williams	0.97	1.20	18.2	19.6	0.70	0.36
Average	0.94	1.24	18.0	19.25	0.71	0.36
<b>Gros Michel Subgroup</b>						
Talamanca	0.82	1.03	19.4	18.4	0.63	0.45
Turrialba	0.87	1.14	17.6	18.6	0.70	0.39
Average	0.85	1.09	18.5	18.5	0.67	0.42
<b>Contrasts</b>	Probability					
Among Cavendish	0.2993	0.0414	0.2900	0.4600	0.3278	0.8615
Between Gros Michel	0.2633	0.0029	0.0490	0.8904	0.7825	0.0043
Cavendish vs. Gros Michel	0.0006	<0.0001	0.3489	0.2416	0.1766	<0.0001

Each value is an average of 6 repetitions and in each repetition one fruit was evaluated. ND: non-data available.

**Figure.1** Bunches of the cultivars evaluated in each subgroup at harvest. Note the size of the bunches, number of hands and the dimensions of the fruits.



However, it contrasts with the high severity of the disease observed in soil of low natural fertility of volcanic origin (López and Solis, 1991), with lower Ca and Mg contents, lower pH values and located west of the Reventazón River (West zone). The differences between both soil types, given mainly by pH, were determined in nursery plants established at a lower pH, where a greater presence of *Fusarium* wilt was found (Segura *et al.*, 2019, 2021a, 2021b). Since the Gros Michel subgroup, unlike the Cavendish subgroup (in this case, low-growing), is cultivated by small growers in steep, low-fertility areas, with limited technology, and for the domestic market, the implementation of technologies that reduce the risk of *Fusarium* infection should be a priority. These considerations, in addition to their productive performance and fruit quality, should be assessed based on their tolerance to nematodes and black Sigatoka, the first three already evaluated in this research.

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### Author Contributions

Alfonso Vargas: investigation, resources, writing original draft, Eduardo Salas: validation, methodology, writing, reviewing, Eduardo Corrales: analysis, writing, reviewing, Mario Araya: writing, reviewing and editing.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable

**Consent to Participate** No applicable

**Consent to Publish** Not applicable

**Conflict of Interest** The authors declare no competing interests.

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