Nutrient status and vegetative growth of *Vanilla planifolia* Jacks plants as affected by fertilization and organic substrate composition

Estatus nutricional y crecimiento vegetativo de plantas de *Vanilla planifolia* Jacks afectadas por la fertilización y la composición de substrato orgánico

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Abstract

The cultivation of vanilla (*Vanilla planifolia* Jacks) is promissory in Colombia, however, its nutritional requirements are currently unknown. In this study the hypothesis was that vanilla plant nutrient status and growth depends on the substrate composition and fertilization applied. Experimental plots of 0.8 x 0.8 x 0.2 m were filled out each two months with additional 10 L of a substrate containing either woodchips (WC) or coconut fiber (CF) and leaf litter (LL) in different volumetric proportions (%) (75:25, 50:50, 25:75). Four annual doses of a chemical fertilizer (grade 27-11-11) were applied: 0, 20, 60, and 140 g per plant. Results indicate that there was a highly significant ($P \leq 0.01$) interaction between substrate and fertilization on plant growth. Shoot length of vanilla plants was significantly increased with the fertilization dose of 20 g per plant only in the substrates composed by 75% of either CF or WC and 25% of LL. The presence of CF produced foliar contents of P, K, Cu, Mg, and Mn significantly higher ($P \leq 0.05$) than those obtained when WC was used in the substrate. On the contrary, the presence of WC in the substrate significantly ($P \leq 0.05$) increased foliar N and Ca contents in respect to those levels observed with CF in the growth substrate.

Key words: Fertilization, coconut fiber, woodchips, leaf litter.

Resumen

El cultivo de la vainilla (*Vanilla planifolia* Jacks) es promisorio en Colombia, sin embargo, sus requerimientos nutricionales son desconocidos. En este estudio la hipótesis fue que el estado nutricional del cultivo de vainilla y su crecimiento dependen de la composición del sustrato y de la fertilización aplicada. En parcelas experimentales de 0.8 x 0.8 x 0.2 m se aplicaron cada 2 meses 10 lt adicionales de un sustrato que contenía fragmentos de madera (WC) o fibra de coco (CF) y hojarasca (LL) en diferentes proporciones volumétricas (%) (75:25, 50:50, 25:75). Se aplicaron cuatro dosis anuales de un fertilizante químico (grado 27-11-11): 0, 20, 60 y 140 g por planta. Los resultados indican que una interacción altamente significativa ($P \leq 0.01$) entre el sustrato y la fertilización sobre el crecimiento de las plantas. La longitud del tallo de las plantas de vainilla se incrementó significativamente con la dosis de fertilización de 20 g/planta solamente en los sustratos compuestos por 75% de CF o WC y 25% de LL. La presencia de CF produjo contenidos foliares de P, K, Cu, Mg y Mn significativamente más altos ($P \leq 0.05$) que aquellos obtenidos cuando WC fue utilizado en el sustrato. Por el contrario, la presencia de WC en el substrato incrementó ($P \leq 0.05$) los contenidos foliares de N y Ca con respecto a los niveles observados con CF en el sustrato de crecimiento.

Palabras claves: *Vanilla planifolia*, fibra de coco, chips de madera, hojarasca.

Introduction

Vanilla planifolia is a tropical orchid highly appreciated in the food flavoring industry (Ferrão, 1992; Ranadive, 2003; Damiron, 2004; Anandaraj *et al*., 2005). Its natural distribution occurs in Central America (from Mexico to Panama) to the North of South America (Colombia, Venezuela, Ecuador and Peru) (Moreno and Díez, 2011). Currently, it is cultivated in Mexico, South Pacific Islands, Indonesia, India, Comoros Islands, and Madagascar (Bory *et al*., 2008).

Vanilla crop is highly promissory in Colombia given the favorable environmental conditions and its expected economical impact in some regions of the country (Moreno and Díez, 2011; Gómez *et al*., 2011). The crop may offer new opportunities to increase income of poor farmers as well as to reduce the pressure on forests, especially in natural parks and biological reserves (Ramirez *et al*., 1999; Ledezma *et al*., 2006; Castro, 2008); however, the area cultivated in Colombia is very small (<100 ha) and the annual crop yield is commonly low (<1 kg per plant), which is below the yield obtained in other countries such as Madagascar, Mexico, and Reunion island (Damiron, 2004; Ledezma *et al*., 2006). Low productivity of vanilla crops in Colombia can be explained by the little experience of farmers in growing vanilla and the poor knowledge about its agronomical management, particularly its nutritional requirements (Moreno and Díez, 2011) and the effect of chemical fertilizers. Generally, fertilizers are not applied in vanilla crops because it is believed that vanilla plants are highly sensitive to them (Damiron, 2004; Osorio *et al*., 2011).

Under natural conditions, vanilla grows on decaying litter (Stehlé 1954; Anilkumar, 2004; Hernández and Lubinsky, 2010) and for its cultivation the most common substrates used are leaf litter, decomposed and rotting tree bark, coconut fiber, sawdust, and vermicompost (Anilkumar, 2004; Hernandez and Lubinsky, 2010; Hernandez, 2011). Because fertilization of vanilla crops is rare, nutrient supply is generally based on the decomposition of the organic substrate (Hernandez and Lubinsky, 2010). Therefore, the selection of organic materials for the growth substrate seems to be critical (Ramírez, 1999; Damiron, 2004; Ordoñez, 2012).

However, fertilization seems to be required because organic materials have low contents of certain nutrients that are essential for the growth of the plants (Damiron, 2004). Increases in the growth of vanilla plants after applying chemical fertilizers to the organic substrates have been reported (Domínguez 2005). The improvement of the vegetative growth of vanilla plants can have a major impact on the profitability of the crop; these plants only begin to flower and fructify two or three years after planting. Additionally, it has been reported that flowering only occurs after the plant reaches a certain size and quantity of accumulated resources, which allows it to endure the high nutrient demands of flower and fruit development (Leakey, 1970). For this reason, management practices that contribute to improve the vegetative growth in the early years of the vanilla plant may help to improve the success and expansion of a crop that only begins to yield economic benefits in the medium term.

In this study we evaluate the effect of the type of substrate and its composition, the fertilization applied and the interaction between these two factors on the vanilla plant nutrient uptake and growth. Because root growth and performance depend both on chemical and physical properties of the substrate, we hypothesize that vanilla growth is better in substrates with high nutrient contents and good physical properties, especially those related with good aeration for roots, and that plants will respond positively to fertilizers as much as the nutrient concentration of the substrate is low for any of the nutrients applied in the fertilizer.

Materials and methods

Site

This study was conducted in Sopetrán (Antioquia, Colombia) which has a mean temperature of 27 °C and an altitude of 1070 m (6º 29.9’ N, 75º 43.8’ W). Vanilla plants used were grown in a shade-house with a light interception of 65%.

Plant material

Seedlings of vanilla were obtained by clone-multiplication of selected individuals collected in San Pedro de Urabá (Antioquia, Colombia) from commercial crops of vanilla. Seedlings of 35 ± 2.5 cm length and four pairs of leaves were placed in an organic substrates to produce abundant terrestrial roots for 45 days and then they were transplanted in the experimental plots (two seedlings per sub-plot, thinned to one 30 days later). Plants were grown for 210 days after transplanting.

Treatments

The experimental design used was split-plots, substrate type was assigned to the main-plots and fertilization levels to the sub-plots (Figure 1). Each subplot (0.8 m x 0.8 m x 0.2 m) had a plastic stick (1.2 m height) in the center to support the shoot growth. Every other month each subplot received 10 L of an organic substrate composed by either coconut fiber (CF) or wood chips (WC) each mixed with leaf litter (LL) in a proportion 75:25, 50:50, and 25:75 (V:V), respectively. The elemental compositions of these organic materials are shown in Table 1.

The chemical fertilization was applied to the substrate as a granular fertilizer of the grade 27-11-11 in annual amounts of 0 (control), 20, 60, and 140 g per plant. These doses were divided in three equal applications

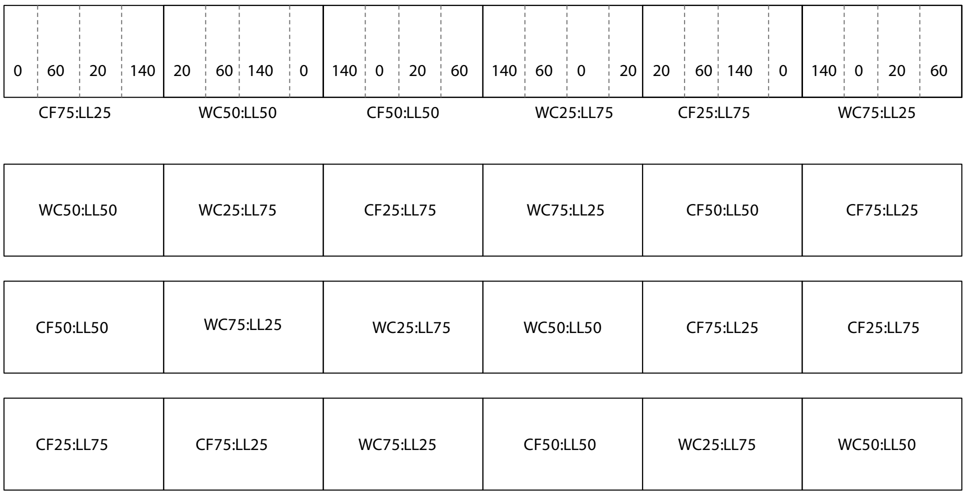

Figure 1. Schematic illustration of the split-plot experimental design used in this study. The main-plots consisted of certain substrate (percentage combination of either coconut fiber -CF- or Woodchips –WC- and leaf litter –LL); the subplots were constituted by a given dose of the fertilizer 27-11-11 (0, 20, 60, and 140 g per plant per year).

Table 1. Elemental composition of organic materials used in the root growth substrates for vanilla plants.

Nutrient	Coconut fiber	Woodchips	Leaf litter
N (g/kg)	49	51	129
P (g/kg)	10	9	11
S (g/kg)	4	6	11
Ca (g/kg)	19	123	218
Mg (g/kg)	14	13	37
K (g/kg)	160	30	40
Fe (μg/g)	712	502	770
Mn (μg/g)	19	163	459
Cu (μg/g)	5	12	16
Zn (μg/g)	12	22	48
B (μg/g)	22	20	26

at 90, 120, and 150 days after transplanting. This fertilizer was applied at a 3 cm depth in the substrate at certain points around each plant, taking care that grains did not directly touch the roots; afterwards, fertilizer was covered again with substrate.

Variables studied

Shoot length was monitored as a function of time at 120, 150, and 210 (harvest) days after transplanting. At harvest, the variables measured were shoot dry weight (60 °C for five days) and leaf nutrient content following standard procedures (nitrogen (N): Kjedahl method; phosphorus (P): molybdate-blue method; calcium (Ca), magnesium (Mg), potassium (K), and micronutrients Fe, Mn, Cu, and Zn: atomic absorption spectrophotometer; B determination by spectrophotometer). The analyses were carried out in the Laboratory of Biogeochemistry of the Universidad Nacional de Colombia at Medellin.

Experimental design and data analysis

As mentioned above, the experimental design used was split-plots; treatments had a factorial arrangement 6 x 4, i.e., six organic substrate combinations (CF:LL = 75:25, 50:50, 25:75; WC:LL = 75:25, 50:50, 25:75) placed in the main-plots and four annual doses of fertilization (0, 20, 60, and 140 g per plant) placed in the subplots. Initially each treatment had six replicates, however during the period of growth two replicates were lost because fungal diseases (mainly due to *Fusarium oxysporium* attacks). Four replicates were considered enough given the low variability of the clones of vanilla used as plant material.

Data were subjected to analysis of variance and LSD and Duncan tests for mean separation. In both cases a level of significance ($P \leq 0.05$) was used. Statistical analyses were conducted with the software SAS System for Windows 9.0. The types of substrates and leaf nutritional contents were subjected to ordination by a principal component analysis (PCA), which allowed to simplify the data set and identify positive and negative correlations among variables.

Results and discussion

There were significant ($P \leq 0.05$) effects of treatments on plant nutrient uptake and growth (Table 2). The main effects of organic substrate type were significant on leaf nutrient contents, but it did not affect the shoot length and dry weight. On the contrary, the main effects of fertilization treatment were significant on shoot length and dry weight, but it did not influence significantly the leaf nutrient content (Table 2).

Table 2. Levels of significance (*P*) of the ANOVA´S (NS= non significant).

Variable	Organic substrate (A)	Fertilization (B)	Interaction (AxB)
Shoot length			
(120 day)	NS	<0.01	<0.001
(150 day)	NS	<0.001	<0.001
(210 day)	NS	<0.001	<0.001
Shoot dry weight	NS	<0.001	<0.01
Leaf content			
N	<0.01	NS	NS
P	<0.01	NS	NS
K	<0.01	NS	NS
Ca	<0.01	NS	NS
Mg	<0.05	NS	NS
Mn	<0.01	NS	NS
Cu	<0.01	NS	<0.01
Zn	NS	NS	NS
Fe	NS	NS	NS

A significant interaction between organic substrate type and fertilization was detected on shoot length and dry weight. At all dates of observation (even as early as 90 days after transplanting) the shoot length of vanilla plants was significantly increased with the fertilization dose of 20 g per plant only in the substrates composed by 75% of either CF or WC and 25% of LL (Figure 2). Under such conditions, at harvest the shoot length reached a peak of 229 cm when CF was included in the substrate (CF75:LL25), which was 41% and 27% above those found when the substrate had 50% and 25% of CF, respectively. In the case of WC (WC75:LL25) the peak was 217 cm, which was 58% and 38% higher than when the WC had a proportion in the substrate of 50 and 25%, respectively.

Similarly, interactive effects of both factors were detected in the shoot dry weight (Table 2), thus, it was significantly higher when the fertilizer dose of 20 g per plant was applied in the organic substrate composed by 75% of either CF or WC and 25% of LL (Figure 3). The increase respect to the unfertilized treatment was 95% and 105% for CF

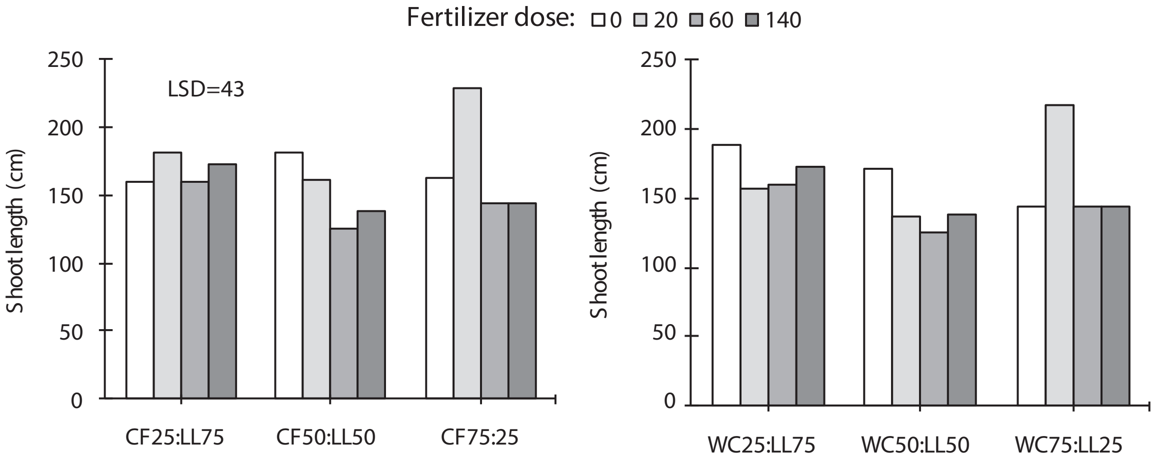

Figure 2. Shoot length of vanilla plants at harvest as a function of interactive treatments with two types of organic substrates (Left: coconut fiber –CF- and leaf litter –LL- in different percentage proportions; Right: woodchips –WC- and leaf litter –LL- in different percentage proportions) and four fertilizer doses.
Legend numbers indicate annual fertilizer dose (0, 20, 60, 140 g per plant). Each value is the mean of four replications. The bar represents the value of LSD (P ≤0.05).

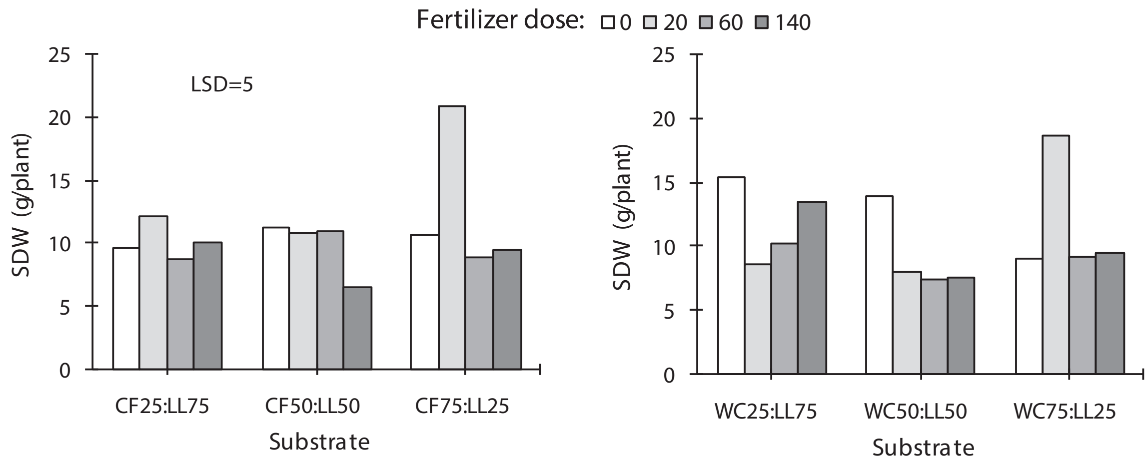

Figure 3. Shoot dry weight (SDW) of vanilla plants at harvest as a function of interactive treatments with two types of organic substrates (Left: coconut fiber –CF- and leaf litter –LL- in different percentage proportions; Right: woodchips –WC- and leaf litter –LL- in different percentage proportions) and four fertilizer doses.
Legend numbers indicate fertilizer dose (g/plant). Each value is the mean of four replications. The bar represents the value of LSD (P ≤0.05).

and WC, respectively, (Figure 3). With the other substrates (richer in LL) there were not responses on shoot dry weight to fertilization treatments.

Likely, the high proportion of CF or WC in the growth substrate provided favorable physical conditions for root growth (aeration, water retention) (Ramirez *et al*., 1999); however, this material had a slow decaying rate and probably was unable to provide enough nutrients. Under such conditions, fertilization could provide the nutrients required for vanilla plants (Osorio *et al*., 2011). Given the sensitivity of vanilla roots to high doses of fertilizers, it makes sense that the positive response was only detected with the lowest level of the fertilizer 27-11-11. On the other hand, it seems that when the growth substrate was richer in leaf litter the decomposition process produced an environment poorly aerated which is probably restrictive for root functioning. It is also probable that under such conditions some plant pathogens may cause diseases in vainilla crops (Pinaria *et al*., 2010; Santa *et al*., 2012).

Leaf nutrient content was significantly influenced by the type of organic substrate used, but not by the fertilizer level (Table 2). For instance, leaf nitrogen (N) and calcium (Ca) contents were significantly higher ($P \leq 0.05$) when the organic substrate included WC than when CF was used (regardless the proportion used) (Table 3 and Figure 4). By contrast, foliar concentrations of phosphorus (P), potassium (K), magnesium (Mg), copper (Cu), and manganese (Mn) were significantly higher ($P \leq 0.05$) when CF was used instead of WC (regardless the proportion used) (Table 3 and Figure 4). The ability of these organic

Table 3. Leaf nutrient content of vanilla plants as a function of organic substrate composition at harvest time. Legend numbers indicate the percentage proportion of leaf litter -LL- in the organic substrate respect to the percentage proportion of coconut fiber -CF- and woodchips -WC-).

Nutrient	Substrate					
	CF25:LL75	CF50:LL50	CF75:LL25	WC25:LL75	WC50:LL50	WC75:LL25
N (mg/g)	18.60 c*	20.7 b	21.10 b	21.4 b	22.7 ab	25.00 a
P (mg/g)	0. 83 a	0. 82 a	0. 82 a	0. 80 b	0. 77 b	0. 76 b
K (mg/g)	50.40 a	50.30 a	45.10 b	32.7 c	31.8 c	29.40 c
Ca (mg/g)	24.40 b	22.70 b	22.10 b	32.2 a	29.4 a	33.10 a
Mg (mg/g)	7.40 a	7.40 a	6.8 00 ab	6.40 b	6.60 b	6.80 ab
Fe (μg/g)	52.27 ns	52.31 ns	52.96 ns	47.42 ns	53.46 ns	55.85 ns
Cu (μg/g)	8.03 a	8.94 a	7.27 a	6.22 b	4.92 c	5.66 b
Mn (μg/g)	89.85 a	84.22 a	83.35 ab	70.48 bc	64.55 c	57.92 c
Zn (μg/g)	25.25 ns	29.62 ns	30.48 ns	25.24 ns	26.06 ns	29.98 ns

* Means with different letters are significant different (LSD test, $P \leq 0.05$). Each value is the mean of fifteen data.

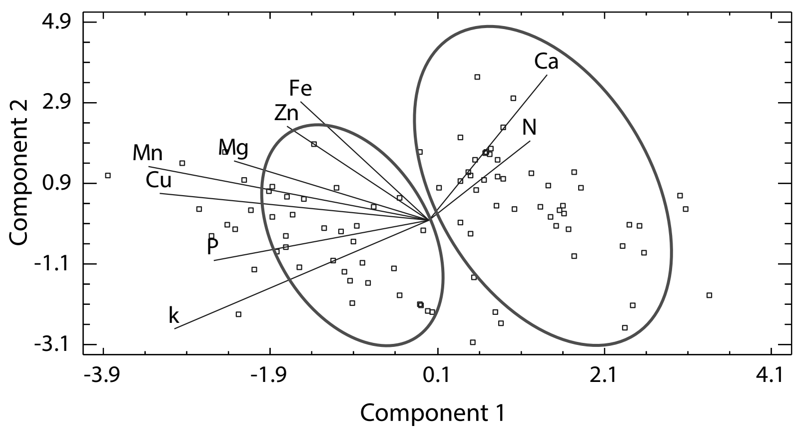

Figure 4. Biplot of principal component analysis for nutrient contents of leaves of vanilla plants growing in two organic substrates. On the right side are concentrated the data for woodchips and on the left side the data for coconut fiber. The first principal component explained 80.7% of total variability and the second component explained 15.1% (i.e. the first two components accounted for 95.8% of data variability).

materials to supply Ca and K is extremely important because vanilla plants extract more K and Ca than other elements, including N (La *et al.*, 1998). The leaf content of N, Ca, and K ranged in satisfactory levels according to values reported by Cibes *et al.* (1947). Unfortunately, no critical levels were available in the literature for Mg, Cu, and Mn.

In all cases, the content of leaf P was very low (< 0.1%) which is far lower the range considered as satisfactory (0.29 - 0.37% according to Cibes *et al.* (1947)) and might be a limiting factor for vanilla productivity (La *et al.* 1998). In fact, foliar contents of Ca, K, P, and Fe were positively correlated with biometric variables, specifically shoot length, leaf area and aerial biomass (data not shown). Consequently, despite P leaf content was very low, this element seems to be important for plant development, which suggests that fertilization of vanilla crops should focus in increasing the content and availability of this element. Hence, the use of P fertilizers, mycorrhizal fungi (Otero *et al.*, 2000; Valadares, 2009) and other beneficial rhizosphere microorganisms (Tsavkelova *et al.*, 2007; Surendra *et al.*, 2009) have great potential and should be considered for further studies. The greater contents of leaf foliar P in plants growing in CF (Table 3) were unexpected because the three organic materials had similar contents (9 - 11 g/kg, Table 1). This result could be explained by the much better growth of roots observed in plants growing in CF (data not shown) which was also reported by Hernández and Lubisnky (2011), and it is known that root length is the main factor controlling P absorption (Barber, 1995).

The higher level of Ca in WC as compared to CF and the higher level of K in CF (Table 1) could explain the results for leaf content of these nutrients, but not for the other ones. Apparently, uptake of N and Ca was antagonist to uptake of K (Figure 4) which is consistent with the results of leaf nutrient content reported by Cibes *et al.* (1947). Since WC contained high levels of Ca and CF contained high levels of K (Table 1), an organic substrate containing these two materials should present optimal results for this crop. Furthermore, K seems to be a key nutrient to reduce the incidence of stem rot (Zaubin *et al.*, 2011) generated mainly by *Fusarium oxysporum*, which is one of the major problems encountered in vanilla crops (Tombe and Liew, 2011), the addition of coconut fiber could be very convenient not only for nutritional purposes, but also as pathogen control.

The fact that organic substrates did not have significant effects on plant growth parameters is explained by the substrate x fertilizer interaction; i.e. the effect of substrates was different depending on the dose of fertilizer used. Therefore, there is no a unique and consistent effect of substrates. Similar results of variable effects of substrates depending on the dose of fertilizer have been reported in other orchid species (Sheehan, 1961). Nevertheless, the results of best growth in substrates with high proportion of whether WC or CF combined with a low dose of fertilizer (20 g per plant) suggest that under such porous substrates, high doses of fertilizer are not well retained or even could be deleterious for vanilla roots.

On the other hand, organic substrates had significant effects on leaf nutrient contents regardless of fertilizer dose (no interaction substrate x fertilizer). We speculate that in the period of growth considered (seven months after transplanting) vanilla plants were accumulating nutrients for further growth stimulation. It is probable that more time would be needed to detect effects on plant growth with these treatments.

Conclusions

- Results suggest that the type of organic substrate was more important than fertilization treatment on the nutritional status of vanilla plants.
- When vanilla plants grew in a porous substrate, with favorable physical conditions (but poor in nutrients), fertilization was relevant for plant growth.
- Vanilla plant growth was significantly higher when the fertilization dose of 20 g/plant was added in a growth substrate composed by 75% of either coconut fiber or woodchips and 25% of leaf litter, higher doses did not promote plant growth and nutrition.

- Leaf N and Ca contents were significantly higher when woodchips were used in the substrate, while leaf P, K, Mg, Mn, and Cu contents were significantly higher when coconut fiber was used.
- P was one of the most limiting nutrients for vanilla growth under our experimental conditions. However, P content in the substrate does not seem to be a good indicator of the availability of this nutrient; other factors such as porosity, aeration, and hydric content, which favor root growth, seem to have more influence on P absorption for the plant.
- Because of the differential effects on leaf content of important nutrients when vanilla plants grow on WC or CF, recommend the use of both materials in the organic substrates for vanilla cultivation.

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