

Full Length Research Paper

Nitrogen effects on leaf gas exchange, vegetative growth and yield of black pepper plants using *Gliricidia sepium* as living support

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For the implantation of one hectare of black pepper (*Piper nigrum*), about 25 to 30 trees are removed from the Amazon Forest to produce tutors for black pepper. As an alternative to the dead wooden stake (WS), there is sustainable cultivation of black pepper with tree species, with the living supports (LS) of gliricídia (*Gliricidia sepium* L.). However, there is inadequate technical information on the effect of black pepper cultivation with tree species on the growth, physiology, and production of the culture. Therefore, the objective of this study is to evaluate the vegetative growth, physiology, and production of black pepper, cultivated in a LS subjected to doses of nitrogen (N). The experiment was carried in a randomized design blocks, in split plots, with three replications in a field. The plots consisted of two tutors (dead tutor and live tutor of *G. sepium*) and the subplots consisted of increasing doses of N (10, 20, 40 and 60g N plant⁻¹). The results indicated that black pepper plants in WS stand out in terms of initial growth; however, at the end of the first year of black pepper cultivation with LS tends to present a vegetative growth slightly like the cultivation with a WS. The production of green pepper was higher in cultivation with *G. sepium*, while in terms of dry pepper there was no distinction between the tutors, with only N doses affected, which was estimated at 37 g N plant⁻¹ to obtain the highest productivity of dry pepper.

Key words: Black pepper, Grain yield, Vegetative growth Nitrogen competition, Vegetative growth.

INTRODUCTION

Black pepper (*Piper nigrum* L., Piperaceae) is a perennial vine of relevant economic importance to the agribusiness due to the flavor and spiciness of their fruits, which are largely used worldwide for culinary purposes (Nair, 2004).

The main world producers of black pepper are Vietnam, Brazil, Sri Lanka, India, and Indonesia. Nevertheless, the annual yield of fruits is quite variable among those countries. For the period between 2015 and 2018, the

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annual yield of black pepper fruits ranged from 2,595 kg ha⁻¹ in Vietnam to 479 kg ha⁻¹ in Indonesia (FAOSTAT, 2020). In Brazil, black pepper has been cultivated in the states of Espírito Santo, Bahia, and Pará, the latter presenting the largest cultivated area (c.a. 16,531 ha) and the second average of dry pepper yield (c.a. 2,246 t ha⁻¹) for the period between 2017 and 2019 (IBGE, 2020). Since black pepper has a climbing habit, then their orthotropic growth needs to be guided by using some physical support (Nair, 2004). The conventional supports for black pepper growth are wooden stakes (WS) of around 2.5 to 3 m tall (Duarte 2004; De Menezes et al., 2013). Considering black pepper is generally planted in single rows spaced 2.5 m each other and considering the plants are 2.5 m spaced in the rows, thus c.a. 2,000 plants can be cultivated per each hectare, requiring, at least, an equal amount of wooden stake to guide the vegetative growth of plants (Duarte 2004; Brasil et al., 2020). In north Brazil, where largest areas of black pepper cultivation are found, the wooden stake have been obtained from different tree species logged from primary and secondary forest areas in the Amazon biome (Ishizuka et al., 2004). However, this logging negatively impacts the Amazon ecosystem and, for this reason, the use of stakes for black pepper cultivation has been questioned. In some black pepper cultivations worldwide, wooden stakes have been successfully replaced by living supports (LS), which consists of some tree species planted in the same hole of black pepper, so that both species are co-cultivated onwards (Dinesh et al., 2010; Kumar et al., 2021). In this cultivation system, the seedlings or stem segments of the LS species are planted prior black pepper and because the vegetative growth of the LS species is relatively fast, their stem will be used to anchor the tendrils of black pepper (De Menezes et al., 2013; Rodrigues et al., 2016). In India, more than 31 tree species have been used as LS (Salam et al., 1991), such as *Ailanthus triphysa*, *Erythrina variegata*, *Gliricidia sepium*, and *Garuga pinnata* (Gunaratne and Heenkenda 2004; Kunhamu et al., 2018). In Sri Lanka, Malaysia and the Philippines, the most common species used as LS are *Grevillea robusta*, *G. sepium* and *Erythrina variegata* (Dinesh et al., 2010). In the Dominican Republic (Central America), black pepper is cultivated under shading using *Azadirachta integrifoliola* and *Leucaena leucocephala* as LS (Ishizuka et al., 2004). In Brazil, particularly under the edaphoclimatic conditions of Eastern Brazilian Amazon, which is the main area producing black pepper in Amazon, the use of LS in black pepper cultivations is incipient. In this area, the grain yield of black pepper co-cultivated with *G. sepium* ranged from 2.5 to 3.5 kg dry grain plant⁻¹ and such variability was related to the type of cultivar used (De Menezes et al., 2013). On the other hand, it was observed that yield of grains of black pepper in the first two years of cultivation was lower in the co-cultivation system with *G. sepium* (1.26 kg plant⁻¹) than in the conventional system (2.54 kg plant⁻¹), however, in the

third year of cultivation, the grain yield was higher in the co-cultivation system with *G. sepium* (5.11 kg plant⁻¹) than in the conventional system (4.2 kg plant⁻¹) (Rodrigues et al., 2019). The authors speculate that adjustments in fertilization could attenuate the competition for nutrients between black pepper and *G. sepium* in the first year of cultivation under field conditions, inducing a greater vegetative growth and initial grain yield of black pepper (Rodrigues et al., 2019). Such inference has not been examined yet and considering that black pepper requires high amounts of nitrogen (Sim, 1971; Veloso et al., 1999; Veloso et al., 2000; Dalazen et al., 2020), particularly during the following 12 months after planting the seedlings in the field (Chiba and Terada, 1976; Rodrigues et al., 2019), one can hypothesize that adjustments in the nitrogen fertilizing in the first year of cultivation may attenuate the competition for nutrients between black pepper and *G. sepium* (LS), enhancing both vegetative growth and initial fruit yield of black pepper. The production of biomass depends on the photosynthetic activity, and this is directly related to the gaseous exchanges of the leaves. High rates of gas exchange and, consequently, high photosynthetic rates are associated with high concentrations of leaf nitrogen, since a large part of the absorbed nitrogen is invested in photosynthetic components (Trevisan et al., 2017; Tränkner et al., 2018; Yang et al., 2022). Then, this work aimed to evaluate the effects of different doses of nitrogen (10, 20, 40 and 60 g N plant⁻¹) on vegetative growth, leaf gas exchange and first yield of green fruits and dry grains black pepper cultivated with wooden stake and *G. Sepium* as LS in the edaphoclimatic conditions of the eastern Brazilian Amazon.

MATERIALS AND METHODS

Experimental set up

This work was set up in an area of about 0,65 ha located in the municipality of Castanhal (1°17'40" S, 47°49'35" W; and 47 m altitude above sea level), state of Pará, north Brazil. The local climate is A_w type according to Köppen classification characterized by a rainy with annual average temperature of 26°C, maximum and minimum of 28 and 22°C respectively, values of relative humidity of the air that vary between, 95 and 79% and annual average of precipitation around 2500 mm (Alvares et al., 2013). The soil in the experimental site is a Dystrophic Yellow Latosol (dos Santos et al 2018), whose physical and chemical properties accessed in samples collected between 0 to 20 cm deep are shown in Table 1.

The experiment was set up as a block design, in which the growth support types (wooden stakes, WS, as inert support and *G. sepium*, LS, as living support) were distributed over the plots and nitrogen doses (10, 20, 40, and 60 g N plant⁻¹) over the subplots, with three replicates per each treatment combination. Each replicate was formed by a planting line containing 4 plants, making a total of 12 plants per treatment combination and a total of 96 plants in the experiment as a whole (Figure 1). The control treatment consisted of the combination of black pepper cultivation in dead tutor at the standard dose of 20 g N plant⁻¹, this combination is commonly used in commercial black pepper plantations in the State of Pará (Brasil et al., 2020). For this, the

Table 1. Physical and chemical properties accessed in samples collected between 0 -20 cm deep at the experimental site (Castanhal, state of Pará, Brazil).

pH (in H ₂ O)	P* (mg dm ⁻³)	K* (mg dm ⁻³)	Ca (cmol _c dm ⁻³)	Ca + Mg (cmol _c dm ⁻³)	Al** (cmol _c dm ⁻³)	OM*** (g kg ⁻¹)	Sand (g kg ⁻¹)	Silt (g kg ⁻¹)	Clay (g kg ⁻¹)
4.16	9	12	0.38	0.68	1.24	7.44	733	127	140

* In available forms, ** in exchangeable form, *** OM means organic matter.

Source: Author

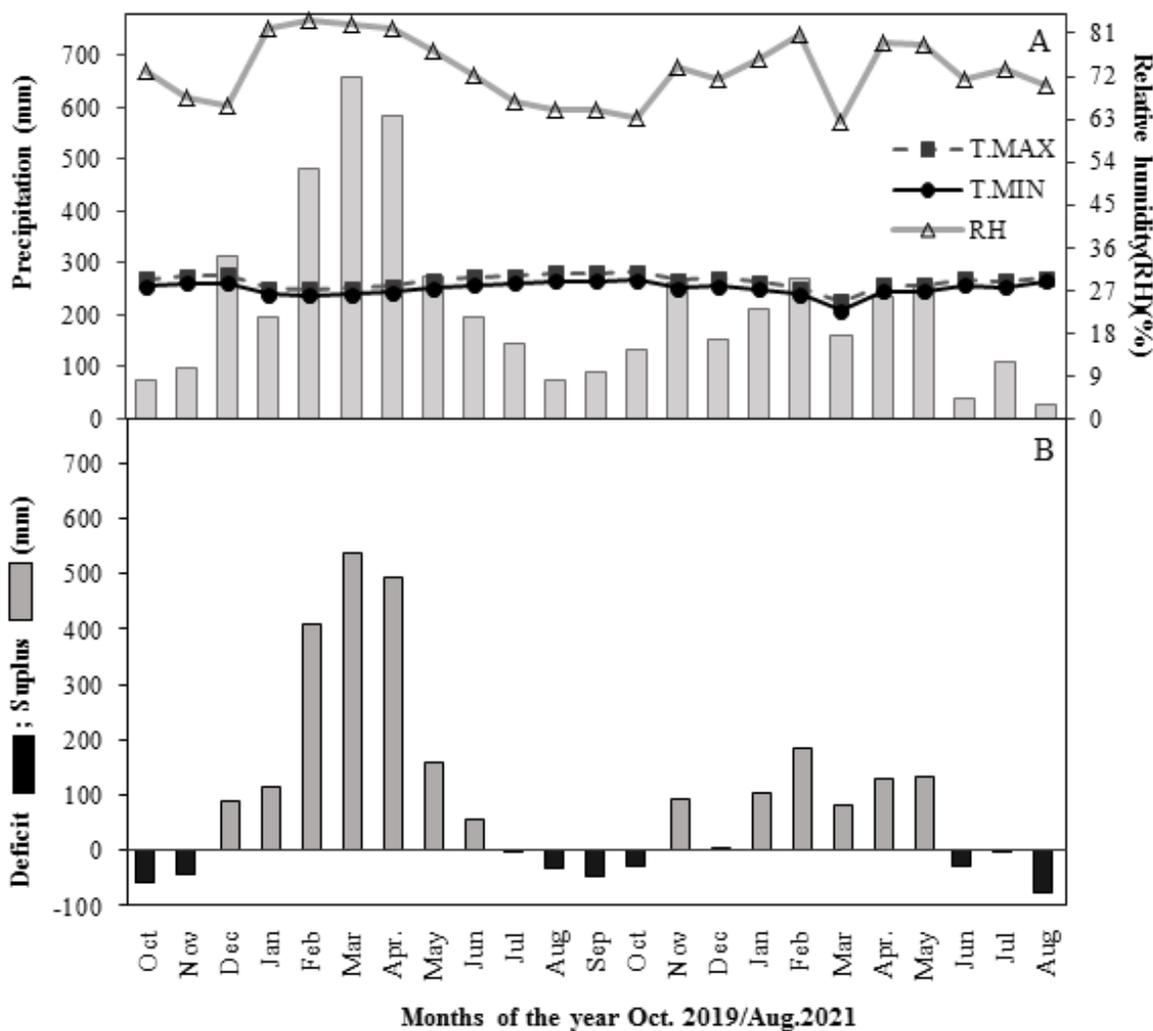


Figure 1. Maximum and minimum air temperature, relative humidity (RH) and precipitation (A) and extract of the water balance and timeline of the experiment (B) as at October 2020/August 2021 in Castanhal (PA), calculated according to Thornthwaite and Mather (1955), using an electronic spreadsheet developed by de Souza Rolim et al. (1998). Available water capacity (CAD) of 100 mm.

Source: Author

vegetation at the experimental site was removed in September 2019, and after cleaning the area the soil surface was braked up and smoothed out by using a set of plows and harrows. Dolomitic limestone (3.7 ton ha⁻¹) was applied to raise the base saturation to 60% and 30 days later, a total of 6 windrows (40 cm high, 4.5 m wide, 110 m length) spaced 4 m each other were prepared. In

December 2019, wooden stakes (3 m high and 5 cm in diameter) and stem segments of *G. sepium* (3 m length and 5 cm stem diameter), referring respectively to WS and LS supports for black pepper growth, were distributed over the windrows. Each windrow received two lines of WS or LS, in 2.2 m square arrangement (Figure 1). The WS and LS stem segments were fixed at 60 cm

deep, in 15 cmx15 cmx60 cm holes. *G. sepium* stem segments with fertilized with 100 g de single superphosphate (18% P₂O₅, FERTIPAR, São Luiz -MA, Brasil) (Ishizuka et al., 2004). In January 2020, 2 month old seedlings of black pepper (Bragantina, grow crops) were planted about 10 cm from WS or LS, in 40 cm * 40 cm * 40 cm (wide, length, and deep) holes previously filled with a mixture of soil, 5 L poultry bed, 30 g micronutrients (FTE BR-12, fertilizer containing: Sulfur (S): 3.9%, Boron (B): 1.8%, Copper (Cu): 0.85%, Manganese (Mn): 2.0% Zinc (Zn): 9.0%; nutriplant, São Paulo, Brasil), and 50 g triple superphosphate (45% P₂O₅, FERTIPAR, São Luiz -MA, Brasil). Nitrogen treatments were induced by applying different amounts of urea to provide 10, 20, 40, and 60 g N plant⁻¹. The total amount of urea for each N dose was fractioned in three equal parts and each part was applied manually around the black pepper plants at 30, 60, and 90 days after planting. All black pepper plants were equally fertilized with 50 g K₂O plant⁻¹, which was applied in three times similarly to N fertilization. In September 2020, a formation pruning consisting of cutting the orthotropic branch at 1 m from the apices of the black pepper plants was performed to stimulate the differentiation of the orthotropic and plagiotropic branches. In December 2020, all shoots on *G. sepium* plants were pruned, only black pepper remained on the stem of *G. sepium*. During the experimental period (January 2020 to January 2021), the monthly averages of precipitation rate, T_{air}, and RH data were obtained from a climatic station located at the data were obtained from the meteorological station in the municipality of Castanhal, about 14 km away from the experimental area. The descending climatological water balance considering available soil water capacity (AWC) of 100 mm was estimated according to De Souza Rolim et al. (1998). Climate data are presented in Figure 1.

Vegetative growth

Plant height (PH), number of orthotropic branches per plant (OB), and number of internodes per orthotropic branches (IOB) of black pepper plants were determined in June 2020, September 2020 and January 2021, representing respectively the above-ground morphology prior the formation pruning (that is, 5 and 8 months after planting) and just before first flowering period (that is, 12 months after planting).

Leaf gas exchange

Net CO₂ assimilation rate (*A*), stomatal conductance to water vapor (*g_s*), transpiration (*E*), and substomatal to ambient CO₂ ratio (*C_i/C_a*) were determined monthly between March 2020 to January 2021 using a portable infrared gas analyzer (LI-6400 XT; LICOR Biosci. Inc., Nebraska, USA). A third leaf from plagiotropic branches selected from the median portion of the above ground was used as sample. Measurements were performed between 9 and 11 h, under ambient CO₂ concentration (c.a. 398 μmol CO₂ mol⁻¹) and under saturating photosynthetically active radiation of 1,000 μmol m⁻² s⁻¹ (Oliveira et al., 2018; Silvestre et al., 2017). The instantaneous water use efficiency (WUE) was estimated as the quotient between *A* and *E* (Hatfield et al., 2011).

Fruit yield

Fruit yield of black pepper was determined at 19 months after planting (August 2021). For this, green fruits were harvested manually and weighted to determine yield on fresh matter basis (that is, kg green fruits plant⁻¹). The green fruits were dried in an agricultural greenhouse for 72 hours and yield on dry matter basis was determined (that is, kg dry fruits plant⁻¹).

Statistical analysis

For analysis of variance (F test), we used data on plant height, number of internodes of the main branch and number of main branches obtained at five (June 2020), eight (September 2020) and 12 months (January 2021) and production data obtained at 19 months (August 2021) were subjected to analysis of variance and regression and the means compared by the Scott-Knott test (*P* < 0.05). In the regression analysis, the regression equations that best fit the data were chosen based on the significance of the regression coefficients at a significance level of 1% (***) and 5% (*) by the F test and at the highest coefficient of determination (*r*²). The data by period of the year of *A*, *g_s*, *E*, and WUE were submitted to ANOVA and the means compared by the Scott-Knott test (*P* < 0.05). For the analysis of principal components (PCA) growth variables (height, number of internodes of the main branch and number of main branches) were used at five, eight and twelve months after planting, production variables (green pepper, dry pepper and yield) and gas exchange variables (*A*, *g_s*, *E*, WUE and *C_i/C_a*) for the months prior to five (June 2020), eight (September/2020) and twelve months after planting (January/2021). All statistical analyzes were performed using the SISVAR software (Ferreira, 2011).

RESULTS

Vegetative growth

At 5 months after planting, the transition month between winter and summer, there was an effect of the interaction support x N doses for plant height and number of internodes of the main branch and an isolated effect of N doses for the number of main branches (Table 2). In LS, there was no significant difference in heights of black pepper plants supplied with 10 and 20 g N plant⁻¹ and these were higher by 28 and 42%, respectively, at doses of 40 and 60 g N plant⁻¹ (Figure 2a). Regardless of N doses, the height of black pepper plants in WS remained unchanged. In both supports there was a linear adjustment of the plant height data. With supply of 10 g N planta⁻¹ there was no difference in height of black pepper plants grown in WS or LS, however, at doses corresponding to 20g N plant⁻¹, 40g N plant⁻¹ and 60g N plant⁻¹ WS was superior to LS, respectively, by 29, 67, and 98%. For the number of orthotropic branches (OB) per plant of black pepper, the data fit the linear regression model (Figure 2d). For the number of internodes per orthotropic branches (IOB) in black pepper cultivated with WS, the highest value of was obtained with the supply of 60 g N plant⁻¹; on the other hand, with LS, the highest values were verified with the supply of 10gN plant⁻¹ and 20g N plant⁻¹. Within the same N doses, both supports promoted similar number of orthotropic branch internodes at doses of 10 and 20 g N plant⁻¹; however, when evaluated under 40 g N plant⁻¹ and 60 gN plant⁻¹, WS was superior, respectively, by 38 and 59% to LS (Figure 2g). Except for OB, at eight months after planting, period immediately before black pepper pruning, all biometric variables evaluated were significantly affected by the interaction support x N doses (Table 2). In LS, there was no significant difference in heights of black

Table 2. F values and significance levels for the isolated effects of support type (ST) and nitrogen doses (ND) and their interaction (ST and ND) on vegetative growth of black pepper plants.

Date of evaluation	Parameters	Factors		
		ST	ND	ST x ND
June 2020	Plant height	464.673 [*]	0.436 ^{ns}	4.222 ^{**}
	Number of orthotropic branches per plant	3.449 ^{ns}	3.023 [*]	0.108 ^{ns}
	Number of internodes on orthotropic branches	13.596 ^{ns}	1.666 ^{ns}	6.942 ^{**}
September 2020 (Prior formation pruning)	Plant height	225.537 ^{**}	0.309 ^{ns}	5.256 ^{**}
	Number of orthotropic branches per plant	2.813 ^{ns}	3.963 [*]	0.301 ^{ns}
	Number of internodes on orthotropic branches	194.678 ^{**}	0.297 ^{ns}	5.106 ^{**}
January 2021 (Prior first flowering)	Plant height	0.723 ^{ns}	0.935 ^{ns}	3.109 [*]
	Number of orthotropic branches per plant	28.00 ^{ns}	6.825 [*]	1.520 ^{ns}
	Number of internodes on orthotropic branches	9.633 ^{ns}	3.626 [*]	0.300 ^{ns}

^{ns} non-significant; ^{*} $P < 0.05$; ^{**} $P < 0.01$.

Source: Author

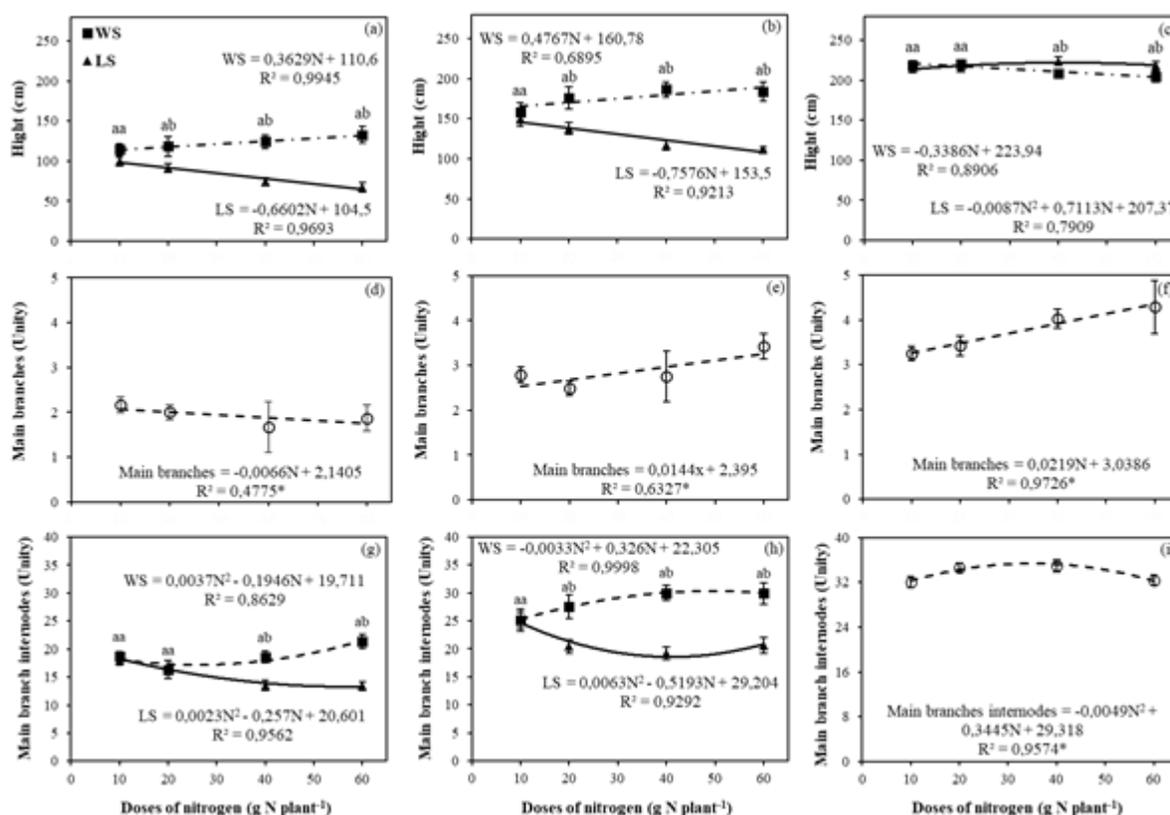


Figure. 2 Height of plants, main branches of plants and internodes of the main branch and black pepper (*Bragantia* grow crops) in conventional support (WS) and live support (LS) at five (A, D and G), eight (B, E and H) and twelve (C, F and I) months after planting. Lowercase letters indicate difference ($P < 0.05$; Scott-Knott) between tutors and non-significant (ns).

Source: Author

pepper plants supplied with 10 and 20 g N plant⁻¹ and these were higher by 19 and 22%, respectively, at doses

of 40 g N plant⁻¹ and 60 g N plant⁻¹ (Figure 2b). Regardless of N doses, the height of black pepper plants

Table 3. F values and significance levels for the isolated effects of support type (ST) and nitrogen doses (ND) and their interaction (ST and ND) on yield parameters of black pepper plants.

Parameters	Factors		
	ST	ND	ST x ND
Green fruits (kg plant ⁻¹)	9.039 [*]	9,160 [*]	2,02 ^{**}
Dried fruits	0,030 ^{ns}	0,488 ^{**}	0,056 ^{**}

^{ns} non-significant; **P* < 0.05; ** *P* < 0.01.
Source: Author

in WS remained unchanged. Furthermore, it was verified that, depending on the N doses, there was an adjustment to the linear regression model of the plant height data in both supports. The height of black pepper plants did not differ between the supports compared under 10 g N plant⁻¹, but it was 28, 60 and 64% higher in WS than in LS under 20 g N plant⁻¹, 40 g N plant⁻¹ and 60g N plant⁻¹, respectively. The number of OB was affected only by the doses of N, the highest value was obtained with the dose of 60 g N plant⁻¹, which was 19, 28 and 20% higher than the doses of 10 g N plant⁻¹, 20 g N plant⁻¹ and 40 g N plant⁻¹, respectively; in addition, there was a quadratic response as a function of N doses, with a maximum value (3) obtained at the estimated dose of 25 (Figure 2e). In WS, the number of IOB did not differ between the doses of N applied, but in LS the application of 10g N plant⁻¹ was higher by 10, 24 and 18%, respectively, than the doses of 20 g N plant⁻¹, 40 g N plant⁻¹, 60g N plant⁻¹. Within the same N doses, both supports promoted similar number of IOB at the dose of 10 g N plant⁻¹; however, the number of IOB was 35, 57 and 45% higher in WS plant than in LS evaluated under 20 g N plant⁻¹, 40 g N plant⁻¹ and 60 g N plant⁻¹, respectively (Figure 2h).

At twelve months after planting, the month corresponding to the end of the vegetative period and immediately before the reproductive period (flowering), plant height was significantly affected by the interaction support x doses of N (Table 2). In LS, plant height did not differ between the doses of N applied, while in WS, the doses corresponding to 10 g N plant⁻¹ and 20 g N plant⁻¹ did not differ and were, on average, 10% higher than the doses of 40 g N plant⁻¹ and 60 g N plant⁻¹ (Figure 2c). In cultivation with WS, there was a linear response to the height of black pepper plants, on the other hand, for cultivation with LS, the height data were adjusted to the quadratic regression model with maximum height (211 cm) obtained at the dose corresponding to 37 g N plant⁻¹. Height of black pepper plants in LS did not differ from WS with supply of 10 g N plant⁻¹ and 20 g N plant⁻¹, but with the application of 40 g N plant⁻¹ and 60g N plant⁻¹ it was higher in 7 and 6% in LS compared to WS. Regarding the number of OB and the number of IOB, it was found that, regardless of the type of support used, there was a response only to the doses of N, with adjustment to the linear regression model for the number of OB (Figure 2f)

and the quadratic regression model for the number of IOB (Figure 2i) with maximum value (35) obtained at the estimated dose of 35 g N plant⁻¹.

There was an effect of the interaction type of support x doses of N (Table 3) both for the production of green pepper and for the production of dry pepper. The mean values of green pepper from black pepper plants submitted to doses of 20 g N plant⁻¹ and 40 g N plant⁻¹ did not differ significantly from each other and were 44 and 22% higher than doses of 10 g N plant⁻¹ and 60 g N planta-1 in LS and WS, respectively (Figure 3a). In both supports the green pepper data fitted the quadratic regression model. In LS and WS the maximum production of green pepper (5.3 kg/plant⁻¹; 3.5kg/plant⁻¹, respectively) was obtained at the estimated doses of 36 g N plant⁻¹ and 40g N plant⁻¹, respectively. Non-significant differences in green pepper were observed between LS and WS compared under 10 g N plant⁻¹ or 60 g N plant⁻¹; however, with the supply of 20 g N plant⁻¹ and 40 g N plant⁻¹ in LS, there was, respectively, an increase of 51 and 47% in green pepper in relation to WS. In relation to the control treatment (WS + 20 g N plant⁻¹), the treatments LS + 20 g N plant⁻¹ and LS + 40 g N plant⁻¹ promoted an increase of 51 and 60%, respectively, in green pepper.

In cultivation with LS, the highest values of dry pepper were obtained with the supply of 20 g N plant⁻¹ and this was superior in 29, 8 and 29% to treatments with 10 g N plant⁻¹, 40 g N plant⁻¹ and 60 g N plant⁻¹; in WS, mean values of dry pepper did not differ between black pepper plants submitted to 10 g N plant⁻¹ and 20g N plant⁻¹, but increased by 23 and 18%, respectively, in plants submitted to 40 and 60 in relation to the control (Figure 3b). Both in LS and WS there was an adjustment to the quadratic regression model of the dry pepper data. The maximum dry pepper production (2.3 kg/plant⁻¹; 2.1 kg/plant⁻¹, respectively) was obtained at the estimated rates of 34 g N plant⁻¹ for LS and 62g N plant⁻¹ for WS. There was no difference in dry pepper production between LS and WS with a supply of 10 g N plant⁻¹ and 40 g N plant⁻¹, but with a supply of 20 g N plant⁻¹ LS promoted a 40% increase in the production of green pepper compared to WS, however when subjected to 60 g N plant⁻¹ black pepper plants in WS produced about 16% more black pepper than pepper in LS. In relation to

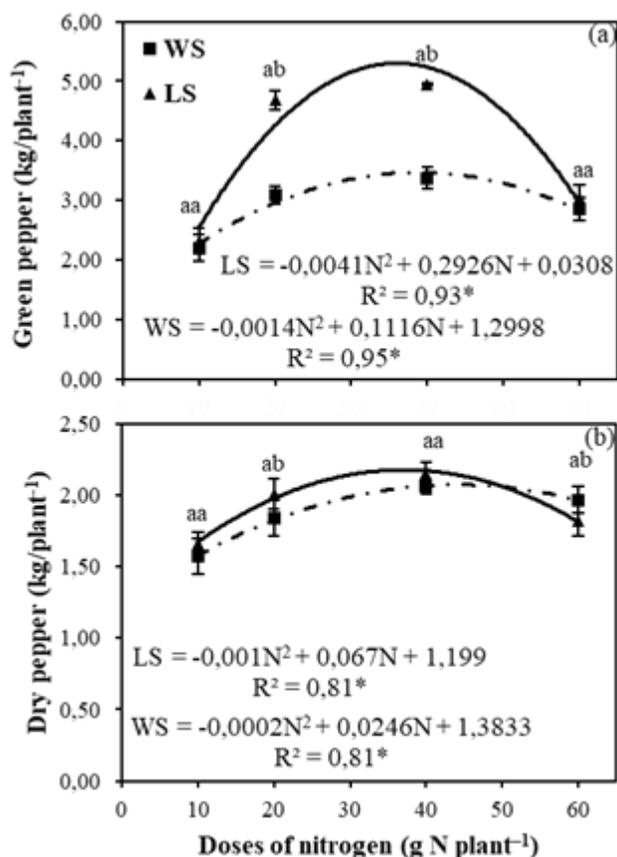


Figure 3. Green pepper (A) and dry pepper (B) of black pepper (Bragantina, grow crops) in conventional support (SC) and live support (LS) at nineteen months after planting. Lowercase letters indicate difference ($P < 0.05$; Scott-Knott) between tutors and non-significant (ns). Source: Author

the control treatment (WS + 20 g N plant⁻¹), the treatments LS + 20 g N plant⁻¹ and LS + 40 g N plant⁻¹ promoted an increase of 40 and 29%, respectively, in dry pepper.

Leaf gas exchange

For all gas exchange variables (A, gs, E, WUE and Ci/Ca) there was an effect of the interaction type of support x doses of N in the three evaluation periods during the first year of black pepper cultivation (Table 4). During the rainy season, regardless of the dose of N, A, and WUE, they were about 29 and 45% higher, respectively, in pepper plants grown in WS than in LS. In relation to the standard dose (20 g N plant⁻¹), the supply of 40 g N plant⁻¹ promoted an increase of 12% in A in pepper plants with WS, while the WUE values did not differ between the N doses (Figure 5). On the other hand, in LS there was no significant difference between the

dose of 40 g N plant⁻¹ and the standard dose, and these were superior to the other treatments, and, for WUE, the dose of 60 g N plant⁻¹ promoted an increase of 30% of this variable in relation to the standard dose (Table 5). The gs of pepper cultivated in WS was superior to the cultivation in LS about 23, 16 and 48% under 10 g N plant⁻¹, 40 g N plant⁻¹ and 60 g N plant⁻¹, respectively, but LS was superior to WS by 17% at the standard dose; the mean values of gs did not differ in pepper plants with WS submitted to 10 g N plant⁻¹, 40 g N plant⁻¹ and 60 g N plant⁻¹ and these were, on average, 15% higher than the standard dose; however, in LS, the standard dose was about 34% higher than the other doses of N (Table 5). Non-significant differences in E were observed between pepper plants grown in WS and LS at the dose corresponding to 10 g N plant⁻¹; however, this variable was 28 and 22% higher in LS than in WS under 20 g N plant⁻¹ and 40 g N plant⁻¹, respectively. In WS, E values did not differ between N doses, but in LS the highest E rates were observed in the standard dose and 40 g N plant⁻¹ and these were higher in 23 and 38% at doses of 10 g N plant⁻¹ and 60 g N plant⁻¹, respectively (Table 5). The Ci/Ca values did not differ between WS and LS under 10 g N plant⁻¹ and 60 g N plant⁻¹, but LS was superior to WS in 8 and 9% with application of 20 g N plant⁻¹ and 40 g N plant⁻¹, respectively. Both in cultivation with WS and LS, the highest values of Ci/Ca were obtained with the supply of 10 g N plant⁻¹ (Table 5).

In the dry period, A was significantly higher in pepper with WS than with LS with application of 10 g N plant⁻¹ (57%) and 20 g N plant⁻¹ (36%), on the other hand, at doses of 40 g N plant⁻¹ and 60 g N plant⁻¹ LS was superior to WS by 23 and 36%, respectively (Table 5). The value of A in pepper plants with WS decreases as a function of increasing N doses, a behavior contrary to this was observed in cultivation with LS. The gs did not differ significantly between pepper plants grown with WS and LS under 10 g N plant⁻¹ and 20 g N plant⁻¹, but it was significantly higher in LS than in WS, either under 40 g N plant⁻¹ (36%) or 60 g N plant⁻¹ (22%); both in WS and in LS the N doses positively influenced gs, in relation to the standard dose there was an increase of 61% with the supply of 40 g N plant⁻¹ in cultivation with LS; however, in WS the other doses were slightly similar to the standard dose (Table 5). Peppers in WS showed E values around 69, 65 and 18% higher than in LS under 10 g N plant⁻¹, 20 g N plant⁻¹ and 40 g N plant⁻¹, respectively; this variable, regardless of the N dose, remained unchanged in WS, on the other hand, in LS there was an increase in E rates as a function of N doses, with a higher value observed in the dose of 40 g N plant⁻¹, which was higher in 72% the standard dose. In terms of WUE, non-significant differences between pepper plants grown in WS and LS were observed under 10 g N plant⁻¹ and 20 g N plant⁻¹; however, this variable was 19 and 57% higher in LS than in WS under 40 g N plant⁻¹ and 60 g N plant⁻¹, respectively. In relation to the standard dose, the

Table 4. F values and significance levels for the isolated effects of support type (ST) and nitrogen doses (ND) and their interaction (ST and ND) on leaflet gas exchange of black pepper plants.

Date of evaluation	Parameter	Factors		
		ST	ND	ST x ND
peak rainy period (March and April)	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	51.854**	10.092**	2.959*
	g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	27.868**	11.261**	19.140**
	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	7.025 ^{ns}	12.227**	10.840**
	WUE ($\text{mmol m}^{-2} \text{s}^{-1}$)	60.522**	1.733 ^{ns}	3.250*
	C_i/C_a (mol mol^{-1})	4.639 ^{ns}	7.996**	3.316*
Peak of the dry period (August and September)	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.129 ^{ns}	7.570**	14.324**
	g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	5.900 ^{ns}	14.997**	8.952 ^{ns}
	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	17.449*	6.246**	5.269**
	WUE ($\text{mmol m}^{-2} \text{s}^{-1}$)	11.379*	7.566**	5.020**
	C_i/C_a (mol mol^{-1})	94.592**	12.741**	0.990 ^{ns}
resumption of rains (December and January)	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	21.758**	12.710**	9.321**
	g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	17.393 ^{ns}	44.932**	23.262 ^{ns}
	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	21.963**	8.477**	7.141**
	WUE ($\text{mmol m}^{-2} \text{s}^{-1}$)	7.126 ^{ns}	3.601*	10.964**
	C_i/C_a (mol mol^{-1})	0.361 ^{ns}	3.348*	18.837**

^{ns} non-significant; * $P < 0.05$; ** $P < 0.01$.

Source: Author

application of 10 g N plant⁻¹, 40 g N plant⁻¹ and 60 g N plant⁻¹ induced, respectively, an increase of 26, 14 and 33% in WUE in the cultivation with LS. On the other hand, side in WS, only the dose of 10 g N plant⁻¹ promoted an increase (33%) of WUE in relation to the control. Regardless of the N dose, WS was on average 24% superior to LS in terms of C_i/C_a ; both in LS and WS, the highest value of C_i/C_a was observed at the dose of 40 g N plant⁻¹, which was 14 and 11% higher than the standard dose, respectively (Table 5).

In the flowering period, there were no differences between WS and LS for A when applied 10 g N plant⁻¹, 20 g N plant⁻¹ and 40 g N plant⁻¹, however with application of 60 g N plant⁻¹ WS was higher by 20% to LS. In WS, A rates tend to increase as a function of N doses, with a 15% increase in the dose of 60 g N plant⁻¹ in relation to the standard dose; in LS it was observed that there was no significant difference between the standard dose and that of 40 g N plant⁻¹ and these were higher by 14 and 7% than the doses of 10 g N plant⁻¹ and 60 g N plant⁻¹, respectively (Table 5). g_s was higher in WS than in LS with supply of 10 g N plant⁻¹ (35%) and 40 g N plant⁻¹ (23%), on the other hand at the dose of 20 g N plant⁻¹ there was no significant difference between supports and at the highest dose, 40 g N plant⁻¹, LS increased by 8% g_s compared to WS. Within the same support, higher g_s rate was observed at doses of 40 g N plant⁻¹ in WS and 60 g N plant⁻¹ in LS (Table 5). LS tends to have greater transpiration at most N doses compared to WS, on average LS showed a 17% increase in E

compared to WS. Both in LS and WS the highest value of E was observed in the standard dose. WUE was higher by 15, 10 and 32% with cultivation with WS than with LS at doses corresponding to 10 g N plant⁻¹, 20 g N plant⁻¹ and 60 g N plant⁻¹, but with 40 g N plant⁻¹ LS increased by 22% WUE compared to WS. In WS, WUE did not differ between the standard dose and doses of 10 g N plant⁻¹ and 40 g N plant⁻¹, but it was slightly increased (23%) with supply of 60 g N plant⁻¹ in relation to the standard dose (Table 5). In LS, WUE did not differ between 10 g N plant⁻¹, 60 g N plant⁻¹ and the standard dose but was 28% higher at 40 g N plant⁻¹ than at the standard dose. In terms of C_i/C_a , non-significant differences between pepper plants in LS and WS were observed at doses of 10 g N plant⁻¹ and 20 g N plant⁻¹, on the other hand, with supply of 40 g N plant⁻¹ WS was higher in 15% to LS and, at the highest dose, 60 g N plant⁻¹ LS was superior to WS by 19%. In WS there was no difference between the doses of 40 g N plant⁻¹ and the standard and these were 12% higher than the doses of 10 g N plant⁻¹ and 60 g N plant⁻¹. In LS, the dose corresponding to 60 g N plant⁻¹ was 13% higher than the standard dose, 19 and 13% higher than the doses of 40 g N plant⁻¹ and 10 g N plant⁻¹, respectively (Table 5).

Principal component analysis

From the set of growth variables (taken at five, eight and twelve months after planting) and gas exchange

Table 5. Net CO₂ assimilation rate (A), stomatal conductance to water vapor (g_s), Transpiration (E), Water use efficiency (WUE) and Ci/Ca ratio of black pepper plants (CV. Bragantina) in conventional support (WS) and live support (LS) in three periods of the year (winter, summer, autumn) at different nitrogen rates (10g N plant⁻¹, 20g N plant⁻¹, 40 g N plant⁻¹, and 60g N plant⁻¹).

Variety	WS				Regression	LS				Regression
	10 g N plant ⁻¹	20 g N plant ⁻¹	40 g N plant ⁻¹	60 g N plant ⁻¹		10 g N plant ⁻¹	20 g N plant ⁻¹	40 g N plant ⁻¹	60 g N plant ⁻¹	
Winter										
A (μmol m ⁻² s ⁻¹)	10.46±0.37 Ac	11.48±0.17 Ab	12.89±0.14 Ab	11.50±19 Aa	WS = -0,0028N ² + 0,2193N + 8,4274 R ² = 0,9706	8.71±0.28 Bb	9.36±0.43 Ba	9.57±0.13 Aa	8.34±0.23 Ab	LS = -0,0018N ² + 0,1185N + 7,7068 R ² = 1
g _s (mmol m ⁻² s ⁻¹)	142.60±0.87 Aa	125.13±0.89 Bb	150.53±0.61 Aa	138.15±1.30 Aa	WS = -0,0028N ² + 0,2193N + 8,4274 R ² = 0,9706	113.23±0.74 Bc	146.98±0.99 Aa	129.30±0.57 Bb	93.35±1.07 Bd	LS = -0,057N ² + 3,4318N + 90,349 R ² = 0,8691
E (mmol m ⁻² s ⁻¹)	2.83±0.09 Aa	2.72±0.15 Ba	2.93±0.21 Ba	2.81±0.15 Aa	WS = -0,0028N ² + 0,2193N + 8,4274 R ² = 0,9706	2.87±0.14 Ab	3.47±0.15 Aa	3.59±0.06 Aa	2.56±0.16 Bc	LS = -0,0015N ² + 0,1015N + 2,0288 R ² = 0,9971
WUE (mmol m ⁻² s ⁻¹)	4.01±0.27 Aa	4.29±0.15 Aa	4.48±0.28 Aa	4.28±0.22 Aa	WS = -0,0028N ² + 0,2193N + 8,4274 R ² = 0,9706	3.03±0.20 Bb	2.67±0.15 Bc	2.64±0.06 Bc	3.49±0.33 Ba	LS = 0,0011N ² - 0,0691N + 3,6113 R ² = 1
C _i /C _a (mol mol ⁻¹)	0.70±0.08 Aa	0.61±0.05 Bb	0.59±0.06 Bc	0.64±0.02 Ab	WS = -0,0028N ² +0,2193N+ 8,4274 R ² = 0,9706	0.67±0.02 Aa	0.66±0.06 Aa	0.64±0.04 Ab	0.64±0.05 Ab	LS = 2E-05N ² 0,0022N + 0,6919 R ² = 0,9916
Summer										
A (μmol m ⁻² s ⁻¹)	5.63±0.32 Aa	4.86±0.34 Aa	5.11±0.79 Ba	4.41±0.21 Bb	WS = -0,0028N ² + 0,2193N + 8,4274 R ² = 0,9706	3.60±0.15 Bb	3.57±0.18 Bb	6.28±0.61 Aa	6.00±0.22 Aa	LS = -0,0013N ² + 0,1532N + 1,7981 R ² = 0,8471
g _s (mmol m ⁻² s ⁻¹)	39.08±0.15 Ab	45.91±1.95 Aa	46.00±0.36 Ba	41.13±0.32 Bb	WS = -0,0028N ² + 0,2193N + 8,4274 R ² = 0,9706	36.34±0.47 Ac	38.88±0.64 Bc	62.49±0.40 Aa	50.13±0.54 Ab	LS = -0,0232N ² + 2,0029N + 14,973 R ² = 0,806
E (mmol m ⁻² s ⁻¹)	1.15±0.18 Aa	1.28±0.20 Aa	1.22±0.22 Aa	1.24±0.19 Aa	WS = -0,0028N ² + 0,2193N + 8,4274 R ² = 0,9706	0.68±0.09 Bc	0.77±0.04 Bc	1.32±0.27 Aa	1.05±0.05 Bb	LS = -0,0006N ² + 0,0488N + 0,1685 R ² = 0,8463
WUE (mmol m ⁻² s ⁻¹)	5.13±0.32 Aa	3.86±0.36 Ab	4.09±0.38 Bb	3.65±0.40 Bc	WS = -0,0028N ² + 0,2193N + 8,4274 R ² = 0,9706	5.44±0.17 Aa	4.32±0.28 Ac	4.87±0.36 Ab	5.74±0.18 Aa	LS = 0,0016N ² - 0,1007N + 6,0758 R ² = 0,7767
C _i /C _a (mol mol ⁻¹)	0.50±0.04 Ac	0.54±0.03 Ab	0.62±0.06 Aa	0.56±0.02 Ab	WS = -0,0028N ² +0,2193N+ 8,4274 R ² = 0,9706	0.41±0.03 Bc	0.43±0.06 Bc	0.48±0.10 Ba	0.45±0.02 Bb	LS = -6E-05N ² +0,0054N + 0,3609 R ² = 0,9306
Autumn										
A (μmol m ⁻² s ⁻¹)	10.82±0.10 Ac	11.78±0.24 Ab	11.68±0.15 Ab	13.55±0,17 Aa	WS = 0,0008N ² - 0,0088N + 11,111 R ² = 0,8741	10.55±0.11 Ac	12.19±0.13 Aa	11.93±0.28 Aa	11.24±0.10 Bb	LS = -0,0021N ² + 0,1514N+9,5041 R ² =0,741
g _s (mmol m ⁻² s ⁻¹)	134.60±0.50 Ad	151.00±0.44 Ab	162.80±0.79 Aa	142.90±0.76 Bc	WS = -0,0028N ² + 0,2193N + 8,4274 R ² = 0,9706	99.85±0.85 Bc	154.10±0.83 Aa	132.10±0.25 Bb	154.55±0.78 Aa	LS = -0,0227N ² +2,3173N + 92,115 R ² = 0,458
E (mmol m ⁻² s ⁻¹)	2.45±0.12 Bb	2.76±0.17 Ba	2.84±0.12 Aa	2.54±0.09 Bb	WS = -0,0028N ² + 0,2193N + 8,4274 R ² = 0,9706	2.67±0.13 Ab	3.25±0.17 Aa	2.67±0.15 Bb	3.13±0.04 Aa	LS = 0,0001N ² - 0,0035N + 2,8984 R ² = 0,0801
WUE (mmol m ⁻² s ⁻¹)	4.59±0.17 Ab	4.42±0.23 Ab	4.19±0.07 Bb	5.43±0.20 Aa	WS = -0,0028N ² + 0,2193N + 8,4274 R ² = 0,9706	4.00±0.19 Bb	4.01±0.18 Bb	5.13±0.36 Aa	4.10±0.03 Bb	LS = -0,0014N ² +0,1049N + 2,8758 R ² = 0,6703
C _i /C _a (mol mol ⁻¹)	0.59±0.04 Ab	0.64±0.03 Aa	0.69±0.08 Aa	0.60±0.03 Bb	WS = -0,0028N ² + ,2193N+ 8,4274 R ² = 0,9706	0.63±0.04 Ab	0.63±0.04 Ab	0.60±0.04 Bc	0.71±0.03 Aa	LS = 0,0001N ² - 0,0065N + 0,6904 R ² = 0,8933

(*) significant (P <0,05; Scott-Knott) e (ns) not significant.

Source: Author

(taken during the first year of cultivation), two main components were extracted that accounted for more than 59% of the global variation (Figure

4). The PCAI corresponded to 40.4% of the data variability and the PCAII corresponded to 18.9% (Figure 4). Evaluating the main components there is a clear differentiation regarding the type of tutor used, both in relation to biometric characteristics and gas exchange. However, similar behavior was observed between the types of tutors at the dose of 10g N plant⁻¹ dose of N used (Figure 4). Furthermore, a high correlation was obtained between A, g_s and the growth variables, especially plant height and number of main branch internodes (EN) both at eight and twelve months after planting.

DISCUSSION

The results of height and number of internodes of the main branch at five and eight months after planting indicate a strong influence of LS on the growth of black pepper when compared to cultivation with WS. Such influence can be attributed to the competition for nutrients between pepper and LS, since in research on the influence of different concentrations of NO₃⁻:NH₄⁺ and types of supports on NPK absorption and growth of pepper plants kingdom, under field conditions, it was found that black pepper plants, over the first eight months after planting, showed a low capacity to absorb nutrients in cultivation with LS, due to the shorter length and root surface of the pepper plant. -do-black, which were on average 57.99 and 55.74%, respectively, lower when compared to black pepper plants grown in WS, so that the growth of the culture associated with LS was lower (Issukindarsyah et al., 2021). *G. sepium* is a leguminous plant capable of performing biological nitrogen fixation (BNF). However, nitrogen fertilization in amounts greater than 30 kg N ha⁻¹ (equivalent to 12g N plant⁻¹) can delay or inhibit the nodulation process in legumes (Da Silva et al., 2011). Therefore, on this occasion the legume tends to absorb the available N from the soil solution and compete with the black pepper plant for the nutrient, affecting its vegetative growth. However, during cultivation, the nitrogen available in the soil solution from nitrogen fertilization tends to decrease, so in this situation *G. sepium* tends to nodulate and perform BNF and, when performing BNF, conditions are created in the rhizosphere that promote an increase in the easily mineralizable inorganic N reservoir (Dinesh et al., 2010) which could benefit both *G. sepium* and black pepper which, at least in part, would explain the results observed in height at twelve months after planting with the superiority of the black pepper plant with LS at the lowest dose of N (10 g N plant⁻¹) and a slight similarity to the cultivation with WS in the other doses of N. In general, the black pepper plant responded positively to the use of N, being recommended doses ranging from 25 g N plant⁻¹ to 37 g N plant⁻¹ to allow maximum vegetative growth. N is the nutrient most required by black pepper, in terms of

nutrient extraction, the crop follows the following order: N > Ca > K > Mg > P (Chiba and Terada, 1976; Veloso et al., 1999). The results of black pepper production indicated that the application of increasing doses of N favored obtaining the best responses of black pepper plants both in LS and WS. It also appears that the adoption of LS as a sustainable alternative to cultivation with WS is promising, since in terms of production of green pepper and dry pepper LS promoted similar or superior performance to black pepper plants cultivated with WS, in most doses of N applied. Furthermore, the average production of green and dry pepper can be considered satisfactory for a first year of cultivation, when compared to other studies such as the one by Oliveira et al (2007) that in research carried out in the State of Paraíba (northeast region of Brazil) with doses of cattle manure and different genotypes of black pepper, they obtained the following maximum yields of green and dry pepper, respectively, per grow crops: bragantina(1,012 g /plant-1; 358 g/plant-1), laçará (11,269 g/plant⁻¹ and 793 g/plant⁻¹) and Singapore (627 g/plant⁻¹ and 204 g/plant⁻¹). Furthermore, Rostiana et al (2017), in a study conducted for two years in East Kalimantan, province of Indonesia, reached an average production of 2.94 kg/plant⁻¹ of green pepper, a value much lower than that obtained in the present research, especially when compared to cultivation with LS. It is worth mentioning that the production data indicated a sign of good production in subsequent years, since the one obtained in the present research corresponds to the first production of the crop's production cycle, and this cycle does not reflect the maximum production potential, which is reached from of the third year of cultivation.

During the first year of black pepper cultivation, the effect of the type of support adopted on the gas exchange parameters of the plants became clear, especially with seasonal changes, with a consequent effect on the vegetative growth of the crop. Black pepper, when cultivated in WS, does not compete for nutrients or light with the support, unlike what happens in cultivation with LS, especially in the first months after planting (Issukindarsyah et al., 2021). In this context, the competition for nutrients allied to the slight shading caused by the shoots of *G. sepium* may have caused both a decrease in nutrient absorption and the amount of radiation absorbed by the photosynthetic apparatus and, consequently, have reduced the conversion of light energy into chemical energy affecting the physiological variables, growth, and development of pepper during the first months of cultivation. Gas exchange is closely associated with the opening and closing of leaf stomata, that is, the stomatal conductance of the plant (Taiz and Zeiger, 2006), thus the highest values of A in black pepper plants cultivated in WS in the winter, at least in part, is due to the higher values of g_s observed in the cultivation in WS in this period. Likewise, in autumn, the values of A close between LS and WS may reflect the

results of g_s which were similar between them, especially at doses corresponding to 10g N plant^{-1} and 20g N plant^{-1} (Table 5). Considering that the WUE is obtained through the A/E ratio, it was to be expected that values close to A and E between the supports would reflect similar WUE rates between them; which, at least in part, may have contributed to the similar results between the two supports in terms of height growth observed at the end of the first year of cultivation (Table 5).

In general, the rates of A, g_s and E, decreased significantly in the months of August and September (Table 5), months in which there was water deficit (Figure 1), both in black pepper cultivated with LS and WS. However, it is noteworthy that black pepper, when cultivated with LS, at the time of water deficit, presented higher gas exchange parameters, especially at higher doses of N (40g N plant^{-1} and 60g N plant^{-1}), compared to cultivation with WS, which suggests that LS favors black pepper in situations of lower water regime and tends to attenuate the deleterious effects of soil water deficit. In this context, changes in gas exchange parameters to avoid excessive water loss is considered a defense strategy for plants against environmental factors such as: decreased water availability, increased radiation, and temperature (Palmer, 2012; Li et al., 2015; Ferraz et al., 2016). Furthermore, N can promote greater plant adaptation to water stress (low water availability), minimizing its effect, either through the regulation of enzyme activity related to the carbon reductive cycle (Sugiharto et al., 1990; Taiz and Zeiger, 2013) or by regulating stomatal conductance to water vapor (Guidi et al., 1998; Zhang et al., 2017). Thus, from the results of the present research, it is inferred that, at least in part, the cultivation with a live tutor of *G. sepium* associated with the supply of adequate amounts of N can benefit the black pepper in periods of the year of lower water regime. Oliveira et al. (2018), when evaluating physiological responses to photosynthesis in black pepper plants in intercropping with rubber trees, observed that in summer, there was lower temperature and higher A value in treatments with rubber trees 2 and 5 meters away from the pepper tree, when compared to A values in plants in full sun, suggesting that during the warmer periods of the year shade can improve the photosynthetic functioning of black pepper, as previously reported for other species (Dai et al., 2009; Zhu et al., 2012). It is well known that shading causes microclimatic changes in many agricultural crops, especially in plants located in rows closest to trees, resulting in physiological and growth changes (Partelli et al., 2014; Araújo et al., 2016; Oliosi et al., 2016), which agrees with the findings of the present study since the SL, due to its crown, promotes certain shading of the black pepper tree.

In addition to the benefits observed in the present research, in terms of plant growth, gas exchange and grain production, the adoption of *G. sepium* as a support for black pepper tends to reduce the cost of implementing the pepper plant, bringing numerous benefits to the

environment as a contribution to the conservation of biodiversity, because with the use of *G. sepium* in the production system, the pressure from the exploitation of endangered tree species that are routinely used as support is reduced. It has positive effects on environmental recovery, since the systems (open areas, primary and secondary forests, permanent preservation areas-APPs, among others) around the areas covered by the technology on the properties suffer less impact.

Furthermore, as it is an arboreal species and sequesters carbon from the atmosphere, the cultivation of black pepper in *G. sepium* can generate carbon credits for the producer, who will have access to more and new lines of credit in front of financial institutions.

Conclusion

The results indicated that pepper plants in WS stand out in terms of initial growth; however, at the end of the first year of black pepper cultivation in LS it tends to show a slightly similar vegetative growth to the cultivation with WS. Furthermore, Green pepper production was higher in LS cultivation; on the other hand, dry pepper production was slightly similar between black pepper cultivated in LS and WS. In cultivation with LS, the maximum production of green pepper per plant, 5.3 kg/plant^{-1} , was estimated under a nitrogen dose of 36 g/plant^{-1} ; in cultivation with WS the maximum production, 3.5 kg/plant^{-1} , was estimated under a nitrogen dose of 40 g/plant^{-1} . Therefore, LS promotes a 51% increase in green pepper production compared to WS.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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