

Evaluation of the Effect of Four Levels of Shade on the Growth and Development of *Desmodium adscendens* with a View to Its Domestication as a Cover Crop in Côte d'Ivoire

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How to cite this paper: Kouame, K.D., Yao, K.J.-E., Kassi, K.F.J.-M., Kouadio, K.Y.H., Kone, K.N. and Kone, D. (2024) Evaluation of the Effect of Four Levels of Shade on the Growth and Development of *Desmodium adscendens* with a View to Its Domestication as a Cover Crop in Côte d'Ivoire. *Agricultural Sciences*, 15, 1179-1194.

<https://doi.org/10.4236/as.2024.1511064>

Received: August 12, 2024

Accepted: November 9, 2024

Published: November 12, 2024

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Abstract

Desmodium adscendens is a plant in the Fabaceae family that is very important to man, both agronomically and medicinally. It is an important cover crop that contributes to improving soil fertility, reducing the risks associated with crop pests and weeds. However, very little research has been carried out into its domestication for use as a cover crop in banana plantations in Côte d'Ivoire. The general objective of this study was to evaluate, under semi-controlled conditions, the effect of different levels of shade on the growth and development of *Desmodium adscendens*. The studies were carried out on the experimental site of the UP-Plant Physiology Laboratory. Four shading levels were evaluated: 0 (NO), 20% (N1), 40% (N2) and 60% (N3) light levels. The *Desmodium adscendens* cuttings used were collected from a 3-month nursery. For each treatment, 10 tubs containing sterilized soil were used, and 50 cuttings were planted. After planting, growth and development parameters were assessed weekly on 10 plants. In addition, yield and fresh and dry mass were measured. Finally, the reproductive cycle of *Desmodium adscendens* was determined. The results showed that plant growth and development were greatest in plants grown under the 40% shade level, followed by the 20% and 60% shade levels. In contrast, plants in full light showed poor growth and development. The 40% shade level was the most favorable for growing *Desmodium adscendens* cuttings, with a cycle length of 67 days. Growing *Desmodium adscendens* under shade with 20% and 40% light levels could be recommended to farmers.

Keywords

Desmodium adscendens, Shade Levels, Cover Crops, Domestication—Côte d'Ivoire

1. Introduction

The search for more effective ways of limiting weed competition was the starting point for new broad-spectrum herbicides such as glyphosate. Glyphosate is the most widely used herbicide on the planet, due to its effectiveness [1]. However, its use poses numerous dangers to human health, animals and the environment. This is why agronomists and ecologists have developed innovative cropping systems to ensure the sustainability of agriculture. These methods aim to modify energy-efficient cropping systems, drawing inspiration from ecology [2]. It is in this context that a marked interest has been placed on the introduction of cover crops into cropping systems [3] [4], in order to halve the amount of pesticides used, according to the BAGAP production and certification standard, and to produce organic produce over the long term [5] in line with quality standards, in terms of Maximum Residue Limits (MRLs) for pesticides and environmental preservation, in relation to chemicals. According to [6], a cover crop is a plant species that produces biomass during its life cycle and covers the soil surface. It is also known as a green manure and is an ecological and effective solution for improving soil health and crop productivity. They play a crucial role in sustainable soil management in organic farming and permaculture. In fact, according to the same author, green manures are plants or parts of non-woody plants that have grown after or at the same time as the main crop, a weed from the fallow period, or the leaves of a tree or shade plant that have been pruned or have fallen. The use of cover crops is widespread in tropical countries. In West Africa, and Côte d'Ivoire in particular, relatively large collections of this plant material have been maintained on station over the last ten years [7]. Cover crops play a variety of roles in agronomy, improving soil fertility [8], reducing the risks associated with crop pests [9] [10] and combating weed infestation. It is in this context that this study was initiated on the use of *Desmodium adscendens* as a cover crop in banana cultivation in Côte d'Ivoire. *Desmodium adscendens* is a tropical plant that thrives in the hot, humid climates typical of equatorial zones. It also prefers high temperatures and constant humidity. The ideal climatic conditions also help the plant to resist disease and parasites. The general objective of this study is to evaluate, under semi-controlled conditions, the effect of different levels of shade on the growth and development of *Desmodium adscendens*. More specifically, growth and development parameters were determined under different levels of shade.

2. Materials and Methods

2.1. Experimental Site

The studies were carried out at the Centre National de Floristique (CNF), located

within the Université Félix HOUPHOUËT-BOIGNY in Abidjan. It is located at longitudes 5°20'51" North and latitudes 3°59'01" West. The climate at the CNF experimental site is of the Atetian type, characterized by four seasons determined by rainfall [11]. The soil is essentially ferralitic [12]. The soil type is characterized by the presence of a thin humus horizon and a thin gravel horizon. The study area is home to most of the tree species of the dense evergreen rainforest. They are carefully preserved in the CNF botanical garden.

2.2. Plant Material

The plant material used in this study consisted of *Desmodium adscendens* cuttings with four buds. They were taken from three-month-old plants grown in nurseries. They were taken from nursery plants at the Société Agricole Kablan Joubin (SAKJ) site in the village of Akréssi in Ayamé department.

2.3. Setting up the Shades

The shade structure was covered with layers of shade to attenuate solar radiation to the desired level and protect the plants from insect attack. These shelters were 5.29 m long and 3.10 m wide. The level of shading was determined using a luxmeter placed above and below the layer of mosquito netting. The measurement consisted of determining the amount of light able to pass through one, two and three layers of mosquito netting. A total of four shading levels were selected. Level 1 (N0) corresponds to a shelter with no shading, and is the control treatment. Levels N1, N2 and N3 correspond to 20%, 40% and 60% light, respectively.

2.4. Preparation and Planting of Cuttings

Three-month-old *Desmodium adscendens* mother plants from the nursery were selected on the basis of their physiological and health status [13]. Cuttings were obtained by cutting into 5 cm to 10 cm portions of the stem using pruning shears below a node. Cuttings with four nodes and leaves were taken [14] and underwent partial leaf removal [15]. Transplanting was carried out without removing the leaves inserted at the nodes to avoid damaging the buds in the leaf axils. This transplanting method has been described and used for cuttings of many ornamental species. The soil was taken from the National Centre de Floristique. The 10-litre trays were filled with soil previously sterilized in an oven set at 120°C for one hour. Holes were drilled in the substrate and the cuttings were inserted vertically below the third node. After planting, light pressure was applied around the cuttings.

2.5. Experimental Set-Up

The trial was carried out to study the effect of shade levels on the growth and development of *Desmodium adscendens* seedlings (Figure 1). The experimental set-up consisted of four defined shelters representing the four treatments: control N0 (no shading), N1 (20% shading), N2 (40% shading) and N3 (60% shading). For each treatment, ten trays were used in which five cuttings were planted, for a

total of 50 plants per treatment. All production factors, *i.e.* substrate, shade level, irrigation and manual weeding, were kept uniform and at their optimum for each treatment. Each tray was watered every other day with 1 liter of water.



Figure 1. Cuttings from 3-month-old plants used for planting.

2.6. Assessment of Morphological Parameters

2.6.1. Cuttings Recovery Time and Rate

The recovery time is the time elapsed between the planting of the cutting and the appearance of the first bud. The vegetative recovery rate (Tr) was obtained by dividing the number of cuttings that recovered, *i.e.*, those that produced buds (Nr), by the number of cuttings initially transplanted (Nt). The formula is as follows:

$$\text{Tr (\%)} = \text{Nr/Nt} \times 100 \quad (1)$$

The maximum recovery rate corresponds to the cutting recovery capacity of the species. The recovery rate is the variation in the recovery rate of cuttings as a function of time. The ratio between the cumulative number of cuttings that have given buds daily and the number of cuttings planted.

2.6.2. Cutting Mortality Rate

The cuttings mortality rate (Tm) was obtained by dividing the number of dead cuttings (Pm) by the number of cuttings initially transplanted (Nt), and multiplying by 100 [16]. In our case, this parameter was taken into account at the end of the trial and determined using the following formula:

$$\text{Tm (\%)} = \text{Pm/Nt} * 100 \quad (2)$$

2.6.3. Evaluating the Effect of Treatments on Stem Height and Diameter

Stem height (H) was the distance from the collar to the apical apex. It was measured every week for 8 weeks using a 50 cm-long graduated tape measure. These values were used to determine the evolution of cuttings height over time. The diameter of the plant stems was the volume or thickness of the stems. Measurements were taken every week using a caliper graduated to the millimeter. In the case of

overestimation of volume, the diameter reading was given without rounding down or up the values obtained.

2.6.4. Evaluating the Effect of Treatments on the Number of Leaves and Plant Spread

During the trial, the number of leaves (F) emitted per plant was counted every five days for 75 days. These measurements made it possible to evaluate the evolution of the number of leaves as a function of time. Spread was determined on the basis of the number of branches per plant. It was assessed every week for 3 months.

2.7. Evaluation of Production Parameters

2.7.1. Flower Emission and Number of Pods

Flowering rate is the monthly variation in the number of flowers as a function of time. The number of pods was counted every three days. The average number of pods per plant was determined by dividing the number of pods by the number of plants assessed. These values were used to determine changes in the number of pods per plant as a function of time.

2.7.2. Fresh and Dry Pod Mass and Moisture Content

The mass of pods obtained for each treatment was measured using a balance. The seeds were then oven-dried at 50°C, and regular measurements were taken to determine the dry mass. The water content of each pod was determined using the following formula:

$$\text{Water level (\%)} = (mf - ms)/mf \times 100 \quad (3)$$

2.7.3. Assessment of Above-Ground and Below-Ground Biomass

After 3 months, the plants were uprooted and transported to the UP Plant Physiology and Pathology Laboratory. Fresh mass (Mf) was assessed by weighing the roots, stems and leaves, which were then placed in a 50°C oven. Regular weighing determined the dry matter (Ms). The dry matter content of each organ was determined using the following formula:

$$Qs (\%) = Ms/Mf \times 100 \quad (4)$$

2.8. Growing Cycle Duration

The length of the *Desmodium adscendens* growing cycle was determined from the date of transplanting of the cuttings to harvesting. It takes into account the phenological phase of vegetative growth, flowering and fruiting. The vegetative growth phase begins as soon as the cuttings are transplanted and ends with flowering. The flowering phase was taken into account when the flower buds set and fruiting ended when the fruits ripened.

2.9. Statistical Analysis of Data

The data obtained were analyzed using STATISTICA version 7.1 software. An analysis of variance (ANOVA) was used to study the effect of different shading

levels. In the event of a significant difference between the means, the Newman-Keuls comparison test at the 5% threshold was used to separate the means into homogeneous groups.

3. Results

3.1. Effects of Different Shading Levels on Agro-Morphological Parameters

The lag times were 2 days and recovery began on the 5th day and continued until the 11th day. The curves for treatments N1 and N2 showed an increasing trend. These curves were well above the other curves, fluctuating between 22% and 78% from day 3 to day 11. The N3 curve evolved intermediately between 16% and 64% from D3 to D11. The N0 curve fluctuated between 6% and 12% and evolved below the other curves. The analysis revealed significant differences between treatments. The cuttings recovery rate varied from 52% to 78% (**Figure 2**). Treatment N0 had the lowest recovery rate at 52%. Treatments N1 and N2 had high recovery rates of 74% and 78% respectively. Finally, intermediate values were observed for shade level N3 (66%). Analysis of variance showed significant differences ($F = 6.096$; $p = 0.002$) between mortality rates for the different treatments (**Figure 3**). The lowest mortality rates were observed in treatments N1 (28%) and N2 (26%). On the other hand, high mortality rates were observed in the control (N0) at around 56%. Treatment N3 had 56% dead cuttings (**Figure 4**). The effects of shade levels showed highly significant differences ($F = 3.780$; $p = 0.012$) between mean plant heights from week S4 recorded in **Table 1**. Comparison of the means showed that the mean height of the plants under shade was higher than that of the plants exposed without shade. Treatments N1, N2 and N3 showed the greatest growth. The values were 3.43 cm and 4.18 cm at S1 before rising to 56.23 cm and 49.04 cm at S12. The unshaded N0 plants, on the other hand, grew more slowly, recording heights of 3.419 cm at S1 and 21.568 cm at S12. The average height of plants in treatment N3 increased progressively to reach a value of 40.333 cm in week 12. Analysis of the data revealed a significant difference ($F = 4.884$; $p = 0.003$) between plant thickness for the different treatments. Shade level N2 (40%) gave the best plant diameter for *Desmodium adscendens* and the average plant diameter was 1.859 cm. On the other hand, small diameters of *Desmodium adscendens* plants were obtained for N0 and N3 with diameters of 1.48 and 1.59 cm respectively. Treatment N1 had intermediate *Desmodium adscendens* plant diameter values of 1.62 cm (**Table 2**). The analysis revealed a variation in the average number of leaves between treatments. Considerable changes were noted for treatments N1 and N2. The number of leaves varied from 0.58 to 85.48 between D0 and D75. With N3, the number of leaves ranged from 0.46 to 38.32. The lowest number of leaves was noted for treatment N0 with only 28 leaves at D75 (**Table 3**). The branching curves showed exponential and continuous growth. The first branches were observed from day 5. The N1 and N2 curves were well above the others, while the N0 curve was below. The N3 curve evolved intermediately (**Figure 5**). After 9

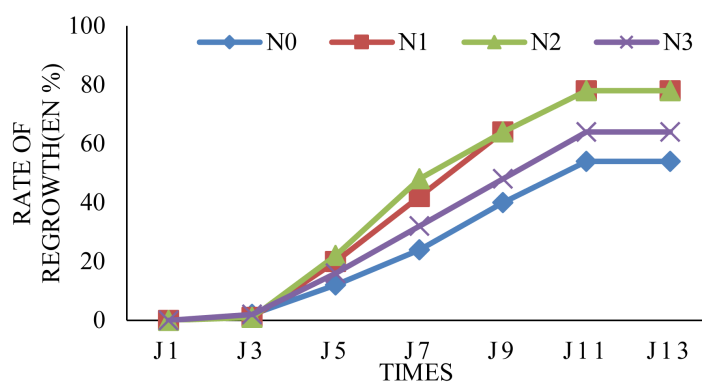


Figure 2. Cuttings recovery rate as a function of shading levels.

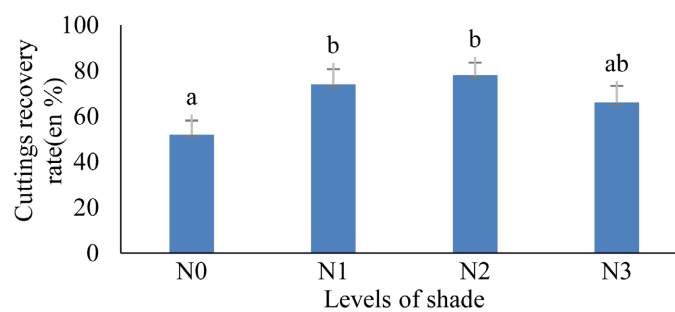


Figure 3. Cuttings recovery rate 11 days after planting.

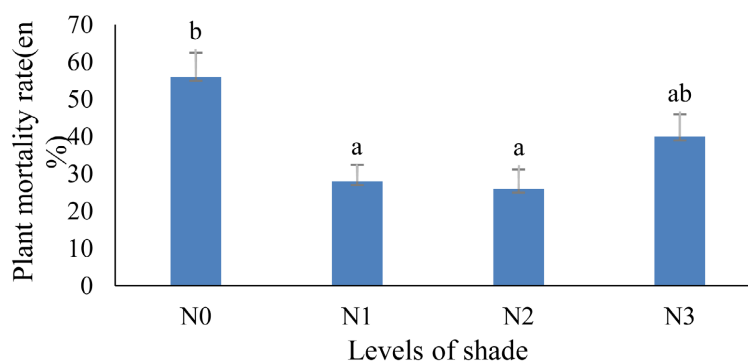


Figure 4. Plant mortality rate as a function of shading levels.

Table 1. Comparison of average plant height as a function of shading levels studied each month.

Levels of shade	Mean plant height (cm)			
	S1 (Mean ± standard error)	S4 (Mean ± standard error)	S8 (Mean ± standard error)	S12 (Mean ± standard error)
N0	3.41 ± 0.36a	5.76 ± 0.48a	11.09 ± 0.97a	21.56 ± 2.43a
N1	3.43 ± 0.27a	7.15 ± 0.57ab	23.82 ± 2.49b	49.04 ± 4.96b
N2	4.18 ± 0.28a	8.72 ± 0.62b	23.85 ± 1.75b	56.23 ± 4.07b
N3	4.30 ± 0.44a	7.54 ± 0.69ab	19.81 ± 5.93b	40.33 ± 5.93ab
P	0.23	0.02	0.036	0.024

Table 2. Average plant diameter as a function of shade level.

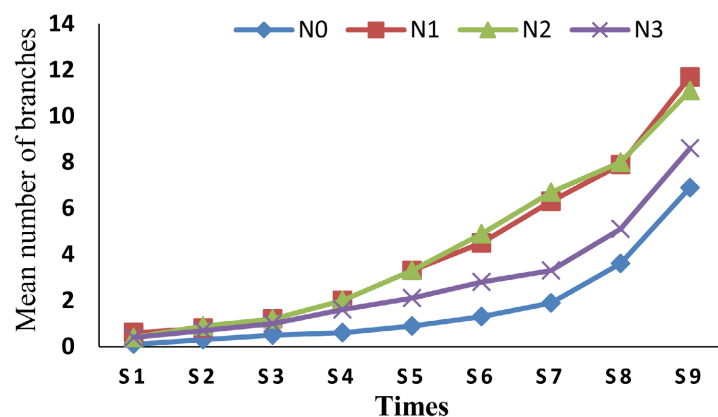
Levels of shade	Mean number of cuttings spread		
	S1 (Mean \pm standard error)	S5 (Mean \pm standard error)	S9 (Mean \pm standard error)
N0	0.10 \pm 0.07a	0.91 \pm 0.25a	6.95 \pm 1.02a
N1	0.67 \pm 0.11b	3.28 \pm 0.44b	11.75 \pm 11.11b
N2	0.41 \pm 0.13ab	3.29 \pm 0.54b	11.11 \pm 1.29b
N3	0.40 \pm 0.15ab	2.15 \pm 0.34ab	8.65 \pm 1.21ab
P	0.001	0.04	0.021

Table 3. Average number of leaves according to shade level.

Levels of shade	Mean stem thickness (in mm) (Mean \pm standard error)
N0	1.480 \pm 0.051a
N1	1.625 \pm 0.054ab
N2	1.859 \pm 0.102b
N3	1.594 \pm 0.048a
P	0.001

Table 4. Comparison of the average number of plant branches according to the shade levels.

Times	Mean number of leaves			
	N0 (Mean \pm standard error)	N1 (Mean \pm standard error)	N2 (Mean \pm standard error)	N3 (Mean \pm standard error)
D0	0.64 \pm 0.11ab	0.58 \pm 0.10ab	1.05 \pm 0.15b	0.46 \pm 0.17a
D15	3.76 \pm 0.52a	6.58 \pm 0.54b	7.0 \pm 0.71b	5.59 \pm 0.44b
D30	7.0 \pm 0.81a	11.24 \pm 0.72b	11.94 \pm 1.08b	9.70 \pm 0.84b
D45	10.52 \pm 0.89a	21.42 \pm 1.71bc	24.29 \pm 2.66c	16.33 \pm 1.72b
D60	18.31 \pm 1.24a	48.12 \pm 4.58b	51.64 \pm 5.33b	27.59 \pm 3.37a
D75	28.40 \pm 2.08a	85.48 \pm 8.84b	85.14 \pm 4.69b	38.32 \pm 5.17a

**Figure 5.** Changes in the average number of plant branches as a function of treatments.

weeks, analysis of the data showed a significant difference. Plant branching was greatest in treatments N2 (11% or 22%) and N1 (10% or 20%), followed by treatments N3 (8% or 16%). Treatment N0 (6% or 12%) showed low branching, with a total of 6.95 (**Table 4** and **Figure 6**).

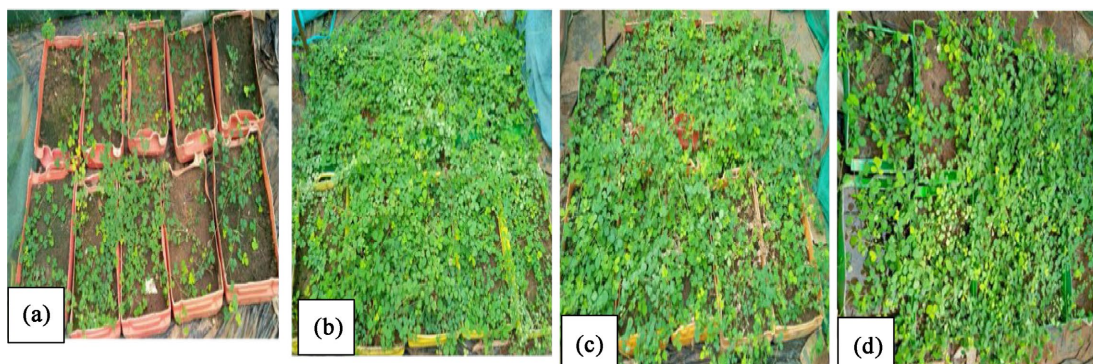


Figure 6. Level of cover as a function of shading levels. (a) unshaded plant; (b) 20% shade; (c) 40% shade; (d) 60% shade.

3.2. Effect on Reproduction Parameters, Material Production and Cycle

Analysis of variance showed significant differences ($F = 3.041$; $p = 0.0347$) between the number of flowers in the different treatments from day 72 onwards. The rate of flower emergence is shown in **Figure 7**. The curves for N1 and N2 reached their optimum at 101 days. The gradual evolution of the number of flowers reached a peak at day 132 for N0 and N3. The analysis revealed significant differences between treatments. Plants under shade N1, N2 and N3 had the highest number of flowers, with values ranging from 14.8 to 17.4 flowers (**Table 5**). The curves show three parts for all treatments. Between days 175 and 165, the N1 and N2 curves moved well above the other curves. The N0 and N3 curves showed little growth from day 105. Pod production was statistically identical for the treatments between days 45 and 75, while differences were observed between days 105 and 135. The number of pods was greater for treatments N1 and N2, with 39.93 and 36.76 pods on day 135 (**Figure 8** and **Table 6**). Analysis of variance revealed no significant difference ($F = 1.131$; $p = 0.366$) between treatments. Total fresh weight was greater in N1 (20.58 g) and N2 (24.26 g) than in N0 (7.6g). The fresh weight of the above-ground part was lower. There was no difference in plant mass or dry matter content between treatments (**Figures 9-11**). The effect of shade levels on pod and seed weight showed a significant effect ($p = 0.000$). The fresh weight of pods per plant was low for the N0 treatments (0.128 g) followed by the N3 (0.55 g). On the other hand, the highest weights were noted for N1 and N2 with 0.96 g and 1.09 g respectively. For dry weight, the highest values were obtained for treatments N1, N2 and N3 with 0.38 g, 0.41 g and 0.27 g, respectively. As for the weight of dry seeds per pod, the results showed that N1 (3.29%) and N2 (4.99%) had the highest values (**Table 7**).

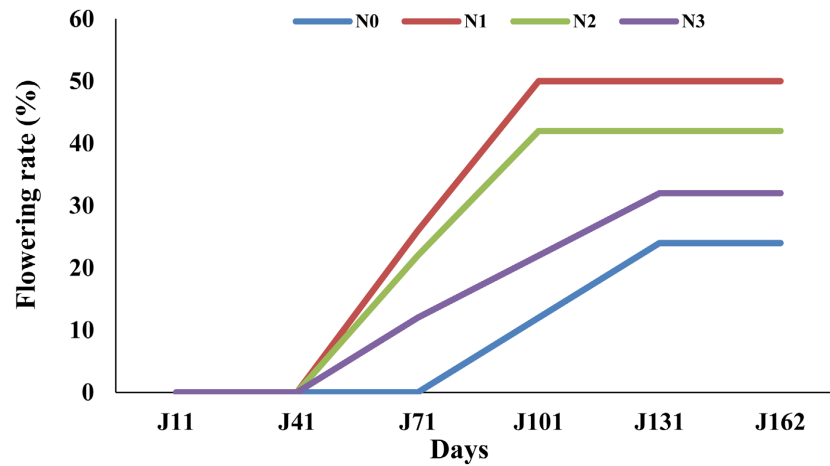


Figure 7. Evolution of flowering rate according to treatments.

Table 5. Comparison of the average number of flowers according to the shade levels studied.

Levels of shade	J42	J72	J102	J132
N0 (Mean ± standard error)	0a	0.083 ± 0.083a	0.333 ± 0.333a	8.583 ± 1.909a
N1 (Mean ± standard error)	0.24 ± 0.24a	8.600 ± 1.930b	14.640 ± 2.316b	16.750 ± 1.715b
N2 (Mean ± standard error)	0.143 ± 0.143a	8.048 ± 2.021b	14.809 ± 2.684b	17.458 ± 1.971b
N3 (Mean ± standard error)	0.214 ± 0.214a	7.214 ± 2.283b	10.857 ± 2.799b	14.823 ± 1.639b

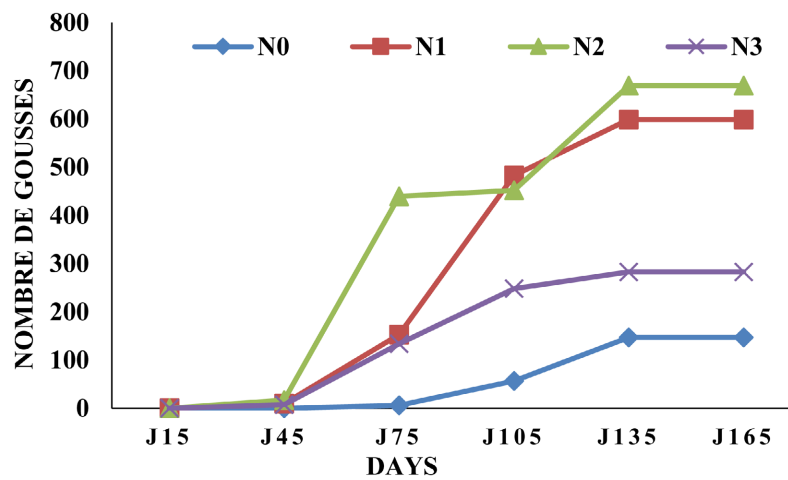


Figure 8. Change in number of pods as a function of treatments.

Table 6. Comparison of average number of pods according to Levels of shade d'ombrage

Levels of shade	J45	J75	J105	J135
N0 (Mean ± standard error)	0a	0.67 ± 0.66a	6.33 ± 3.294a	16.33 ± 4.61a
N1 (Mean ± standard error)	0.66 ± 0.54a	10.20 ± 2.68a	32 ± 3.955b	39.93 ± 4.02b
N2 (Mean ± standard error)	0.68 ± 0.31a	17.60 ± 5.48a	20.60 ± 4.11ab	36.76 ± 4.50b
N3 (Mean ± standard error)	0.47 ± 0.31a	7.88 ± 2.79a	14.58 ± 2.789a	16.647 ± 2.34a

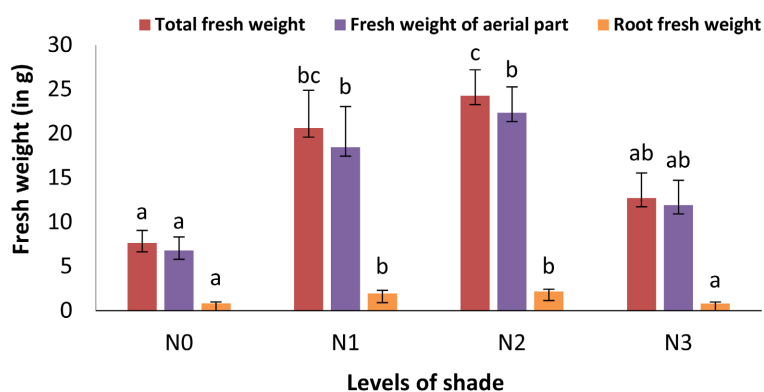


Figure 9. Fresh weight by treatment.

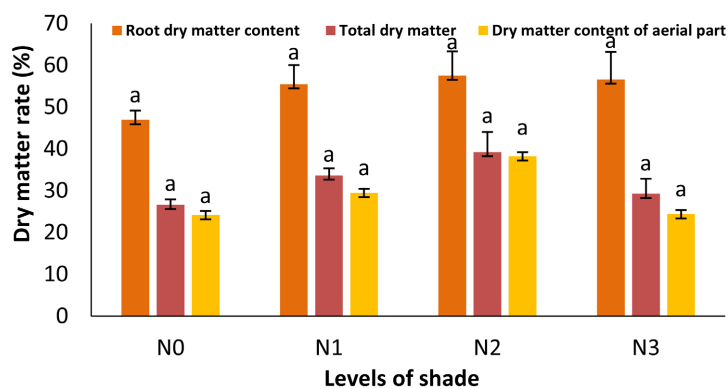


Figure 10. Dry weights by treatment.

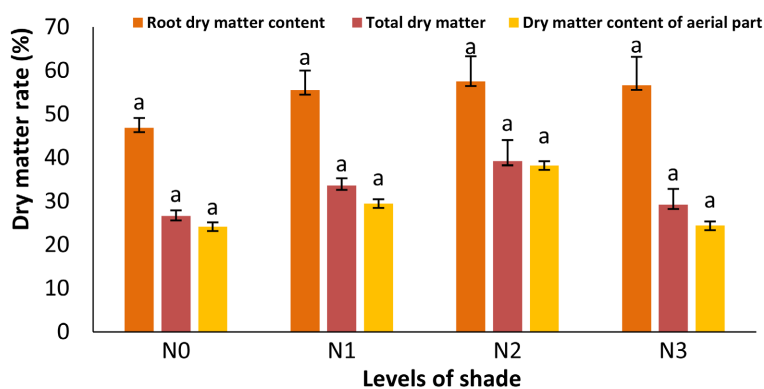


Figure 11. Dry matter content according to treatments.

Table 7. Pod production and yield by treatment.

Levels of shade	Pod production and yield		
	Pod weight per plant (in g)		Dry seed weight per pods (in %)
	Fresh weight	dry weight	
N0 (Mean ± standard error)	0.128 ± 0.01a	0.048 ± 0.006a	0.63 ± 0.12a
N1 (Mean ± standard error)	0.96 ± 0.06c	0.38 ± 0.01b	3.29 ± 0.14b
N2 (Mean ± standard error)	1.09 ± 0.08c	0.41 ± 0.03b	4.99 ± 0.49c
N3 (Mean ± standard error)	0.55 ± 0.10b	0.27 ± 0.13b	1.73 ± 0.4d

3.3. Growing Cycle of *Desmodium adscendens*

Figure 12 shows the growing cycle of *Desmodium adscendens* as a function of shade levels. The length of the growing cycle is not the same for all shade levels.

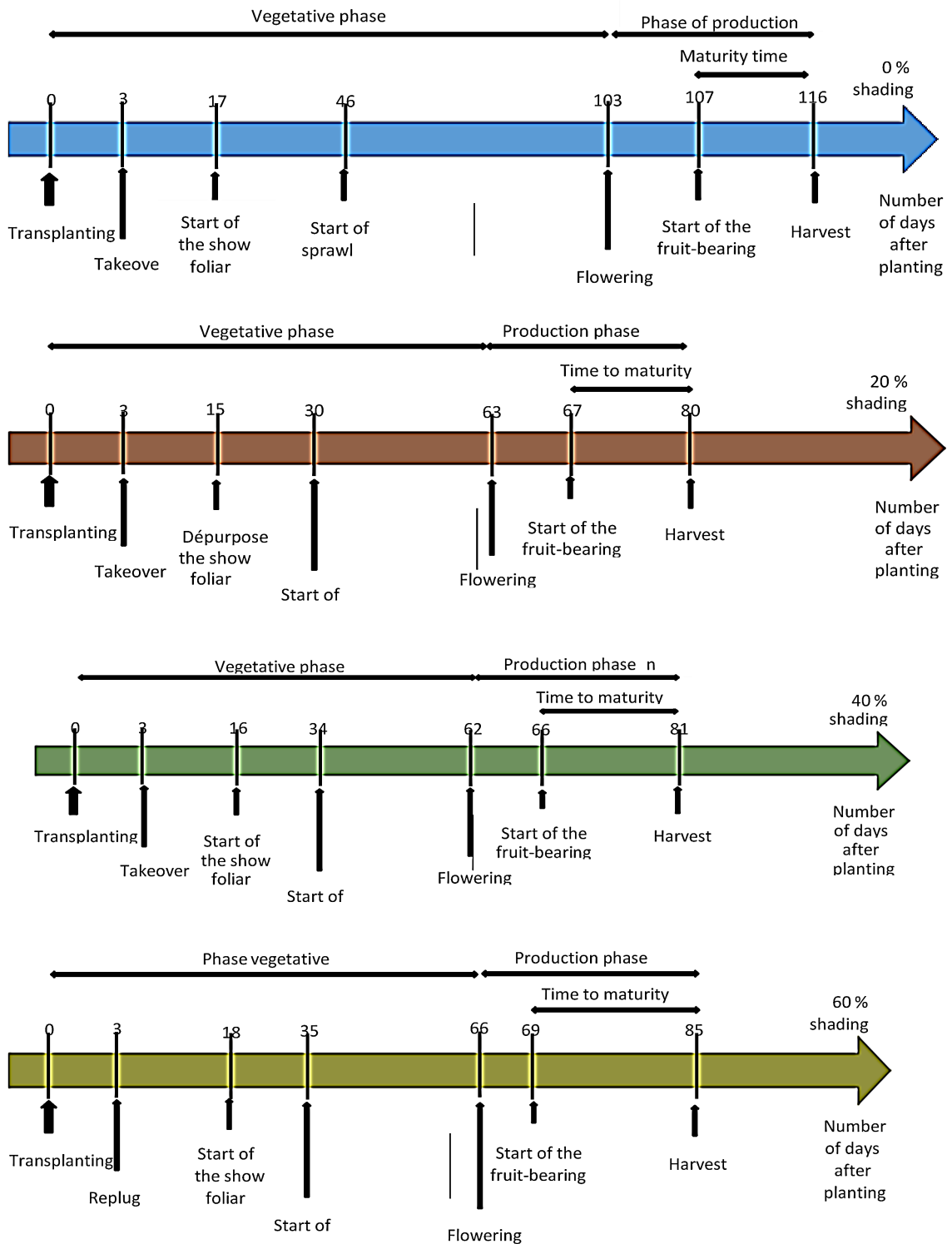


Figure 12. *Desmodium adscendens* growing cycle as a function of levels.

In treatment N0, from transplanting of the cuttings to flowering, the cycle lasted 103 days, while in treatments N1, N2 and N3, it varied from 62 to 66 days. The lag time and leaf emission were 2 days for treatments N1, N2 and N3. Spreading of the N1 (20%) shade plants began at 30 days, the N2 (40%) at 34 days, followed by the N3 (60%) at 35 days, and finally the N0 plants at 46 days. The duration of the phase from flowering to harvest was 19 days for the N2 and N3 shading levels, and that of the N1 and N0 treatments was 13 and 17 days, respectively. The ripening time for pods produced in N0 was 9 days. It was 13 days for treatment N1, 15 days for treatment N2 and 18 days for treatment N3. Overall, the crop cycle lasted 116 days for treatment N0. On the other hand, for treatments N1, N2 and N3, it lasted practically the same number of days.

4. Discussion

This study was conducted with the aim of developing the use of *Desmodium adscendens* as a cover crop in banana plantations in Côte d'Ivoire. The general objective of this study was to evaluate, under semi-controlled conditions, the effect of different levels of shade on its growth and development. Evaluation of the growth parameters as a function of the different shading levels revealed that the recovery time was very short for all levels. This proves that *Desmodium adscendens* is a flowering species that is easy to cut. This relatively short time may be due to the fact that the cuttings grow under favourable conditions. This good ability to take cuttings was highlighted by the high recovery rate of the cuttings for the 20% and 40% shade levels. This recovery rate was relatively low at 0%, whereas at 60% shade, the recovery of cuttings slowed down. This can be explained by the fact that light intensity influences the budburst of *Desmodium adscendens* cuttings. This shows that the domestication of *Desmodium adscendens* has been a success, as the plants can develop easily by taking cuttings. This ability to take cuttings was revealed to be identical in *Thunbergia atcorensis* by the work of Asseh [17] and in *Lovoa trichilioides*. The results showed that the growth and development of the seedlings were strongly linked to the type of shading, ranging from 20% to 40%. The greatest average heights, the highest number of leaves and the most extensive branching were observed at these shading levels. The effect of shading levels favoured the growth of plant diameters but also the leaf emission of *Desmodium adscendens* plants. These results show that *Desmodium adscendens* can be used as a cover plant in banana plantations, as the shade level will be between 30% and 40%. Our results are elucidated by the plant's high shade-covering capacity. These results corroborate those of who showed that cover crops grow better in the shade than in the sun. Plant spread was very high under shade. This may be due to the fact that shading accelerates shoot growth after bud break. Our results are contrary to those of [18] and [19]. The results revealed the effect of shade levels on pod production and plant cycle length. Shade levels of 20% and 40% showed a favourable effect on the number of flowers and pods obtained. This is linked to plant growth and strong plant branching. Above-ground, root and

total biomass were highest in plants grown from N1 and N3 cuttings. This is explained by the abundant presence of leaves, which are responsible for photosynthesis, which requires light, CO₂ and mineral elements to function, resulting in high biomass production. With regard to the growing cycle, the results showed that the plants from the seeds used completed their cycle earlier. These results are corroborated by the observations of [20]-[21] on *Jatropha curcas*, which show that the plants derived from cuttings have the same genetic programme as their mother plant and enter production relatively early, following the cultivation calendar of the original plant. With regard to domestication, it should be noted that these results show that shade plays an important role in the development of *Desmodium adscendens* plants.

5. Conclusion

This study was carried out with the aim of highlighting the suitability of *Desmodium adscendens* as a cover crop in banana plantations. It emerged that under semi-controlled conditions, different levels of shade had an effect on the growth and development of *Desmodium adscendens*. Fresh production and root biomass were not influenced by the different shading levels. On the other hand, the 20 and 40% levels favoured pod production. The development cycle was shortened to 116 days. So, to better combat weediness in banana plantations, *Desmodium adscendens* plants can use 20% and 40% shade levels to propagate cuttings. Further studies could be carried out in the field to determine the performance of *Desmodium adscendens*.

Authors' Contributions

This work was carried out with the collaboration of all the authors. The corresponding author of the article YKJE carried out the work, analysed the data and drafted the manuscript. KKD, KKFJL, KKYE, KKN and KD contributed to the interpretation of the data and the critical revision of the content of the article. KKD, the initiator of the research activity, read and corrected the manuscript. The final manuscript was approved by all the authors.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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