

Ability of *Haematostaphis barteri* Hook F. to air layering in the Sudano-Sahelian zone of Cameroon

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ABSTRACT

Haematostaphis barteri is a highly valued wild species of socio-economic interest that is under multifaceted pressure. Indeed, excessive use of this plant leads to a repressive dynamic in its population. The objective of this work is to evaluate the aptitude of *Haematostaphis barteri* for air layering. The layering technique used consists of girdling the branch in order to remove the bark over a length of 6 or 7cm until the wood appears. Then, the cambium is thoroughly scraped and the phloem is thoroughly removed. Three trials were conducted. The first and second were carried out in august 2023, they consisted of determining the favorable period for air layering by quantifying carbohydrates on the mother plants of the species during the year(trial 1). For experiment two, the substrates used consisted of back soil; the black soil/sawdust mixture (40/60%); the spent grain of traditional beer and the foam. the experimental unit consisted of 10 layers. That is, a total of 120 layers(10 x 4 x 3). The experimental devise used was a completely randomiwed block for the first, two trials. The last experiment was carriedout in june 2024. Thus, using the tailor's tape, three ranges of branch diameter [2-4[cm; [4-6[cm; [6-8 [cm were examined in the best substrate of the previous trial. That is, a total of 180 layers (10 x 3 x 2 x 3) were placed. The experiment design used is a Split-plot with 3 repetitions. The results of this study show that the quantification of sugars shows that the favorable period for conducting aerial layering of the species is the beginning of the rainy season. *Haematostaphis barteri* has a good aptitude for air layering. The type of substrate, the diameters of the branches and the aluminium foil have an influence on the rooting rate. The black earth/sawdust mixture with a rate of (63.33±15.27%) is numerically the best rooting substrate and the diameters [4-6]cm and [6-8]cm presented promising results for the same rooting parameter in this species. The aluminium foil improved and protected the sheaths against light rays. These tested parameters are important in developing a domestication strategy for this species.

Keywords : Aerial layering, *Haematostaphis barteri*, Branch diameter, Substrate, Rooting, Sudano-Sahelian zone, Vegetative propagation.

I. INTRODUCTION

The Congo Basin is ranked as the second largest tropical forest after the Amazon, with a total area estimated at approximately 200 million hectares, representing nearly 91% of Africa's dense rainforests (FAO, 2007). Indeed, this

forest area is abundant in woody plants that have played an important role in the food and socio-economic development of rural populations for centuries (Moupela *et al.*, 2011). Furthermore, it is recognized as an universal common good and a source of numerous services for humans in both rural and urban areas (Tankoano, 2017). Despite its role, this ecosystem faces significant natural anthropogenic, and edaphic threats. The pressure on forests does not spare the plant species found there, even though the populations who live there depend on them (Bwama *et al.*, 2007). Indeed, to combat the scarcity of woody plants in the tropical zone; forest reserves, sacred forests and several conservation zones have been established (Boissieu *et al.*, 2007). However, these measures unfortunately remain without considerable effect, agroforestry constitutes an alternative to this problem. Vegetative propagation is one of the fastest and low-cost techniques and appears to be a positive way to overcome this problem (Bellefontaine and Monteuiis, 2000). It is an exact copy of the parental characteristics and has several advantages including rapid production of plants, early fruiting, reduction in the size of individuals and especially the rapid propagation of species whose multiplication by seeds is difficult (Hannah *et al.*, 2003). Moreover, studies concerning it are rare for tropical species (Bellefontaine, 2005). The ecosystems of the Sudano-Sahelian savannahs of Cameroon are diverse and rich in species of socio-economic interest, including *Haematostaphis barteri*. It is a common and disseminated species found exclusively in the Sudano-Sahelian zone of tropical Africa (Arbonnier, 2002). This tree can reach an impressive height of around 15 meters. Its straight and slender trunk can measure up to 50 cm in diameter at the base (Tchiengue & Zapfack, 2010). It is one of the food species that provides the local community with great utility at the regional scale (Hamawa *et al.*, 2018). Various parts (leaves, fruits, bark, roots and wood) are used in the fields of pharmacopoeia, food and even ritual areas. In the northern part of Cameroon, the fruit of this tree fights against coughs. The decoction of the leaves; bark and roots treat several diseases such as: malaria, stomach ache, Typhoid (Tsobou *et al.*, 2022). Kubmarawa *et al.* (2007) report that traditional practitioners use the bark of the stems to treat cancers in Nigeria. Indeed, the excessive use of this plant makes it an endangered species, moreover this species still lives in the wild and sexual reproduction remains the only way to guarantee the vitality of this species. Young plants from natural sowing die in very large numbers during long dry seasons, which reduces their reproduction by seeds while reducing the diversity of this species. The general objective of this study is to evaluate the suitability of *Haematostaphis barteri* for vegetative propagation with a view to its domestication. More specifically, it is a question of determining: (i) the favorable period for layering this species; (ii) the effect of the type of substrates on the rooting of aerial layers; (iii) to evaluate the influence of the diameter of the branches on rooting; (iv) to examine the effect of aluminum foil on the rooting of layers.

II. MATERIALS AND METHODS

2.1-Study Site

The study was conducted in the Sudano-Sahelian zone, more precisely in the Northern region, Bénoué Department, Lagdo District, located between 8°53 North latitude and 13°58 East longitude (Fig. 1). The climate is Sudano-Sahelian, with two alternating seasons: a long dry season lasting approximately 7 months from November to May and a short rainy season lasting approximately 5 months from May to October. Average annual precipitation is around 950 mm. The region experiences high temperatures, estimated at an annual average of approximately 30°C (Wangbé *et al.*, 2024). The local terrain is dominated by hillocks, but mountain ranges and inselbergs are also present. This area is home to several vegetation variants. The hydrographic network is mainly composed of seasonal watercourses. In addition, there is an artificial reservoir built on the Bénoué River. This basin is subject to agricultural and pastoral activities to which are added fishing, small trade and crafts.

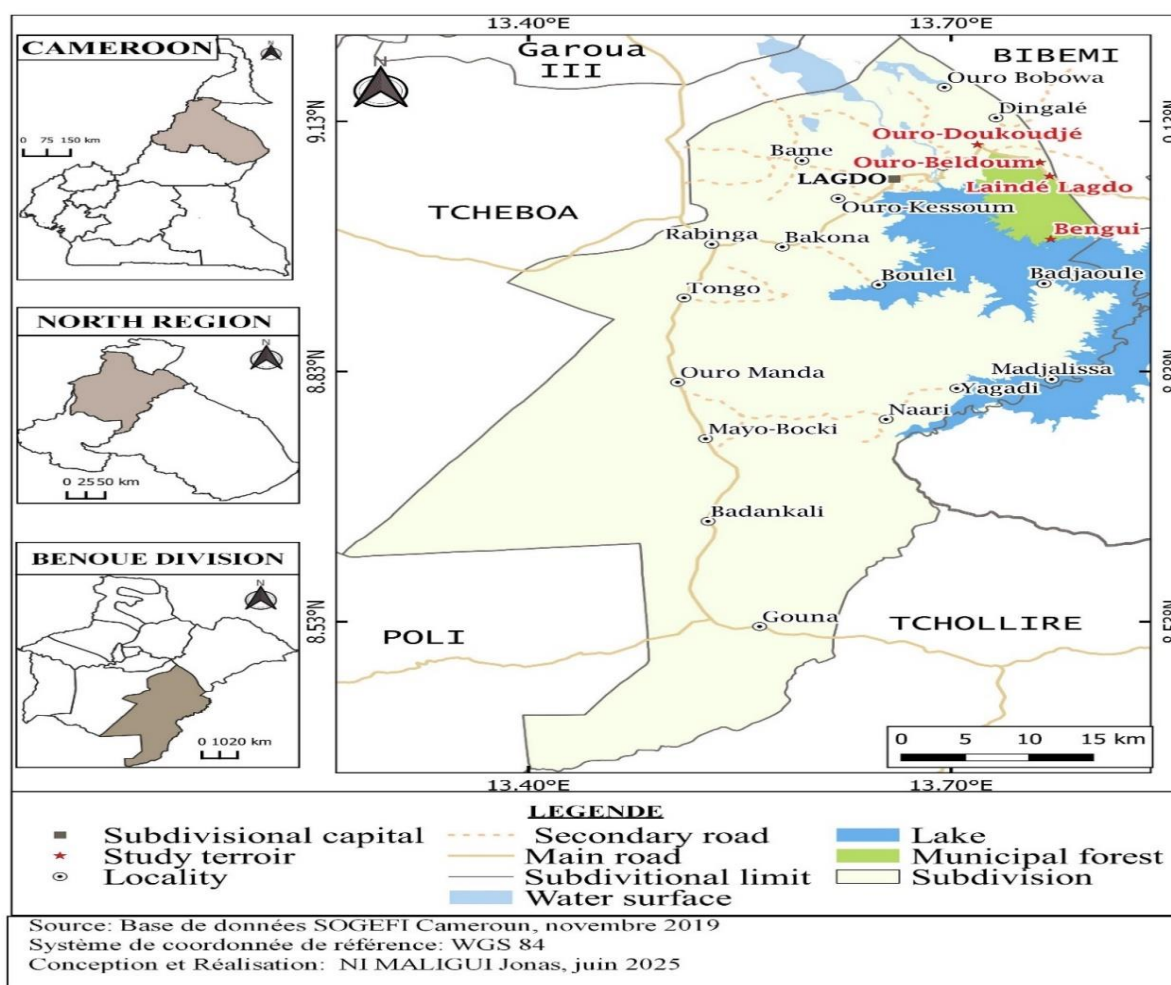


Fig.1. Location of the study area

2.2-Method

2.2.1-Air Layering Trials

2.2.1.1-Experiment 1: Determining the favorable period for layering

In northern Cameroon, particularly in the Sudano-Sahelian zone, the favorable period for layering is poorly understood. To do this, a fragment of the species' bark was collected to determine this period by quantifying the carbohydrates (soluble sugars, sucrose, cellulose, and glucose) contained in the mother plants. Thus, bark sampling of this species began in October 2023 and took place monthly for one year. Approximately 1 kg of bark was collected from 10 mother plants of the chosen species. After sampling, the bark samples were dried in the shade and then ground to obtain a powder that was bagged, labeled (date, month, and location of sampling), and stored. After obtaining the powder each quarter, chemical analyses were carried out at the Biochemistry and Organic Chemistry laboratory of the University of Maroua to determine the variations in carbohydrates, particularly soluble sugars, glucose, and sucrose, over 12 months. The choice of sugars was inspired by the work of Bartolini *et al.* (1996) on *Vitis ruggeri* and those of Stenvall *et al.* (2009) on *Populus tremula* and Oumarou, 2021. These authors mentioned that budding and rooting of cuttings are correlated with the availability of these carbohydrates. The determination of these sugars was carried out using the spectrophotometric method described by Dubois *et al.* (1956); Miller (1972). Once the period of maximum carbohydrate production in the species is determined, it is considered the best layering period. The quantity of carbohydrates is expressed in grams/100 grams of dry matter. The experimental design for determining the quantity of soluble sugars, sucrose, glucose, fructose, and cellulose in the species is a randomized complete block design where the 12 months were the only factor. The number of replicates is 2.

2.2.1.2-Experiment 2: The Influence of Substrate on Layering Rooting

The sheaths were installed in August 2023 and monitoring continued until March 2024 on 24 adult *Haematostaphis barteri* individuals using as substrates : black soil; a black soil/sawdust mixture in the respective proportions of 40% and 60%; traditional brewery spent grain called bili bili; and sphagnum (moss harvested from rocky slabs). For each selected plant, 5 layers were placed on the middle part of the branches of the diameter randomly chosen for layering (Mapongmetsem *et al.*, 2010). The experimental unit consisted of 10 layers repeated three (3) times. For a total of 120 layers (10 x 4 x 3). The layering technique used is that described by many authors: Tchoundjeu *et al.*, 2010; Bellefontaine *et al.*, 2012 and Moupela *et al.*, 2013. It consists of removing the bark over a length of 6 to 7 cm until the wood appears using a sharp knife (Fig. 2). Then the cambium is carefully scraped and the bast is carefully removed in order to stop the flow of descending elaborated sap. The substrate ridge is held around the incised area using transparent polyethylene film and securely tied at the ends with string. The experimental design used is a completely randomized block design.



Fig .2: A ringed branch of *Haematostaphis barteri*

2.2.1.3-Experiment 3: The influence of branch diameter and the effect of aluminium foil on layering

Air layering was carried out in June 2024 and monitored until January 2025. This experiment tested the diameter ranges and the effect of aluminum foil on layering rooting. Using tailor's tape, three branch caliber ranges [2-4 [cm; [4-6 [cm; [6-8 [cm were examined in the best substrate from the previous trial. For this trial, 20 plants were targeted, with 6 layers installed on each plant in the middle of the branches. The trial consisted of covering half of all the installed sheaths with aluminium foil (Fig. 3a) to protect the layering from excessive temperatures in the event of solar radiation, and the other half of the layers were not covered with aluminium foil (Fig. 3b). The experimental unit consisted of 10 layers. This gives a total of 180 layers (10 x 3 x 2 x 3) placed. The experimental device used is a Split-plot with three repetitions where the diameter ranges constitute the main treatment, the covering mode (covered and not covered with aluminium foil) represents the secondary treatment. For each of these experiments, monthly watering of the layers was done using a 24 ml medical syringe filled with water to prevent the substrate from drying out. The hole left by the needle at the end of the operation was closed with a piece of tape. Each sleeve placed carried the following information: nature of substrate; diameter of the branch and date of sheath installation. Data collection began one month after the sheaths were installed and continued monthly (for 7 months). The data collected included the number of rooted layers, the number of destroyed layers and the number of dead layers. The number of layers that had formed calluses was counted at the end of the trial.



(a)



(b)

Fig. 3. Covered sleeve (a); uncovered sleeve with aluminium foil (b).

2.2.2-Data collection

The data collected for each trial were subjected to an analysis of variance. The significant separation of means was done using Duncan Multiple Range Test. The statistical software used for the analysis of variances is Statgraphics plus 5.0. The Excel spreadsheet of Microsoft Word 2016 was used to create graphs and tables.

III. RESULTS

3.1-Tree layring period in the Sudano-Sahelian zone of Cameroon

The carbohydrate content of the studied species varies throughout the year (Fig.4). Given that sugars regulate root growth and new formation, the period when their accumulation is high on the plant would be appropriate for layering. In this study, the soluble sugar concentration ranges from 6.42 g/100 g of dry matter (DM) to 14.95 g/100 g of DM on the mother plant throughout the year. This content is low in November and the optimum is in June. The sucrose content is high in August (8.03 g/100 g of DM) and low in December (1 g/100 g of DM). Over the 12 months of the year, glucose fluctuates from 3.47 g/100 g to 10.09 g/100 g of dry matter on the mother plant of this species. The optimal value is found in June, the lowest concentration is in November. Regarding cellulose, the peak is in March, the lowest amount is in July. The variance analyses of these carbohydrates evaluated on the plant show significant differences between the months of the year (0.0000 < 0.001)

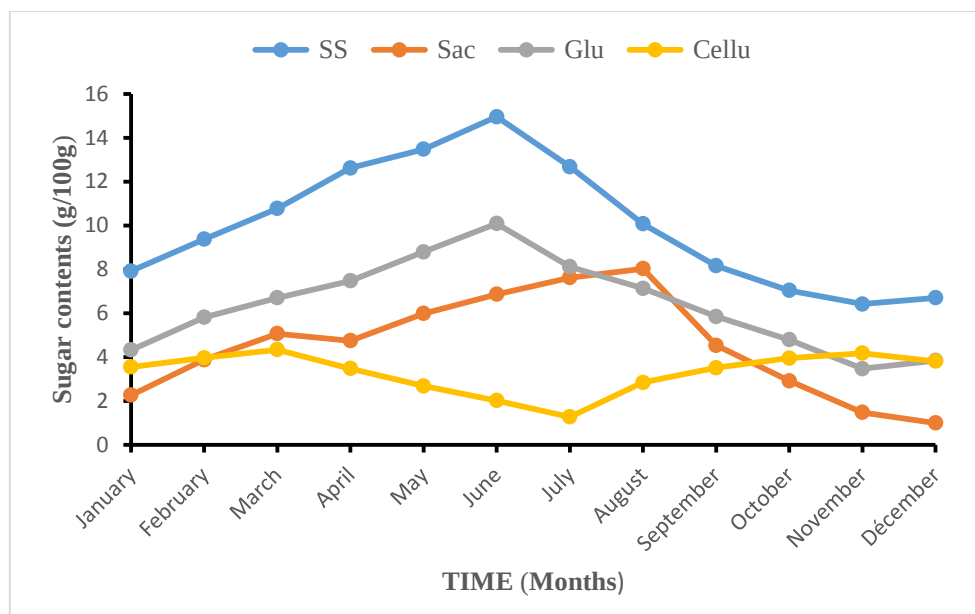


Fig. 4: Carbohydrate content over the year

SS : Soluble Sugar ; Sac : Sucrose ; Glu : Glucose ; Cellu : Cellulose

3.2-Reaction of *Haematostaphis barteri* to air layering

At the end of the trial (7 months of follow-up), the ability of *Haematostaphis barteri* to air layering revealed an average rooting rate of $53.33 \pm 18.33\%$. It should be noted that the roots were clearly visible on the sheaths mainly two months after the installation of the layers (Fig. 5).



Fig. 5: Rooted branch of *Haematostaphis barteri* after 3 months

For all the sleeves installed, $35.55 \pm 20.68\%$ formed a callus, while $6.56 \pm 5.27\%$ died naturally or due to the tree's physiological conditions. The rate of layers destroyed by curious people was $4.54 \pm 2.26\%$ (Table 1).

Table 1. Proportional distribution of *Haematostaphis barteri* responses to air layering

Type of reactions observed	Layers rooted	Layers forming the callus	Layers dead	Layers destroyed
Rooting (%)	53.33±18.33	35.55±20.68	6.56±5.27	4.54±2.26

3.3-Substrate effect

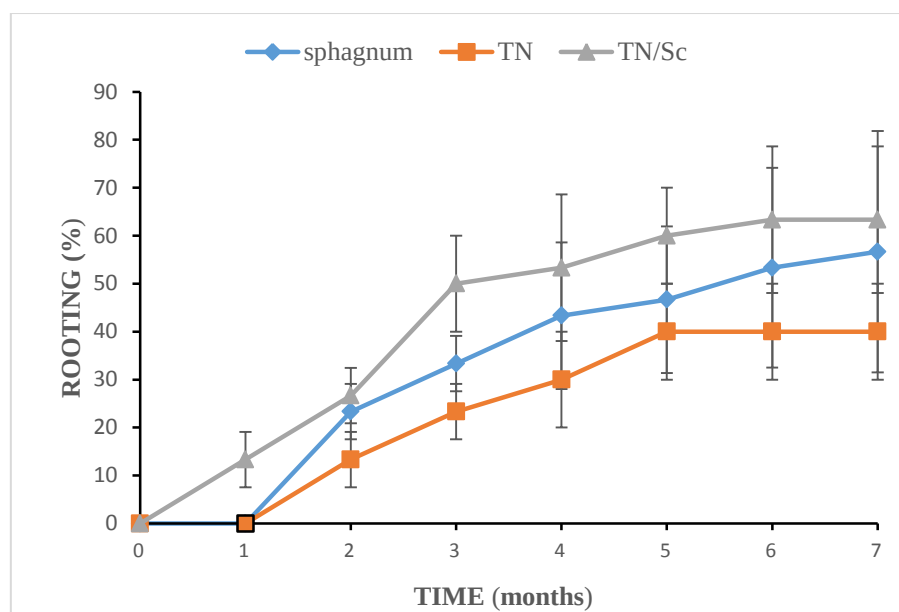
The success of air layering depends on the rooting substrate, the physiological state of the tree, and the technique used. In this study, the rooting percentage of layers after seven months ranged from 40±10% for topsoil to 63.33±15.27% for the topsoil/sawdust mixture (Table 2). Analysis of variance indicates a significant difference between the different substrates (0.0044 < 0.001).

Table 2. Rooting Rates by Substrate

Substrates	Sphagnum	Black soil	Mix soil/Sawdust	Brewer's grain traditional	Average
Rooting (%)	56.66±25.16b	40±10b	63.33±15.27b	0.00±00a	53.33

Means followed by the same letter are statistically identical ($p < 0.05$)

The success rate for all three substrates was consistent, peaking at the sixth month for the black earth/sawdust mixture and the black earth, and at the seventh month for the moss (Fig. 6). It should be noted that rooting began one month after the sheaths were placed in the black earth/sawdust mixture. For the other substrates, roots were visible from the second month. Traditional brewer's grains proved to be a poor substrate. Indeed, after the sheaths were placed, it was observed that this substrate, once moistened, decomposed and gave off an odor that attracted flies. For all the layers placed in this substrate, the branches either dried out or showed no reaction.

**Fig. 6: Percentage of rooted layers by substrate type over time**

TN: Black soil; TN/Sc: Black soil-sawdust mixture

3.4-Effect of diameter on layer rooting

The rooting rate at the end of the experiment ranged from 46.66±24.22% for sleeves placed on branches of [2-4[cm to 68.66±16.32% for those with a diameter between [4-6[cm (Table 3). Analysis of variance did not indicate a significant difference between diameters (0.2857 > 0.05). Rhizogenesis began one month after layering in branches with calibers

greater than [4-6[; [6-8[cm); in layers on small-diameter branches, roots were visible two months after their installation. The evolution was progressive for the three types of diameters and the optimal value was reached in the sixth month (Fig. 7).

Table 3. Rooting percentage according to branch diameters

Diameters (cm)	[2-4[	[4-6[	[6-8[	Average
Rooting (%)	46.66±24.22a	64.66±30.11a	68.66±16.32a	60

Means followed by the same letter are statistically identical ($p < 0.05$)

The delay in rhizogenesis and the low rooting rate at the layering level on the slices of small diameter class could be explained by stress, especially a mortality rate of 26.66% was observed in this diameter class. In light of the results observed in the layers of large diameters, the aerial layering of *Haematostaphis barteri* presents satisfactory results on the branches of higher calibers.

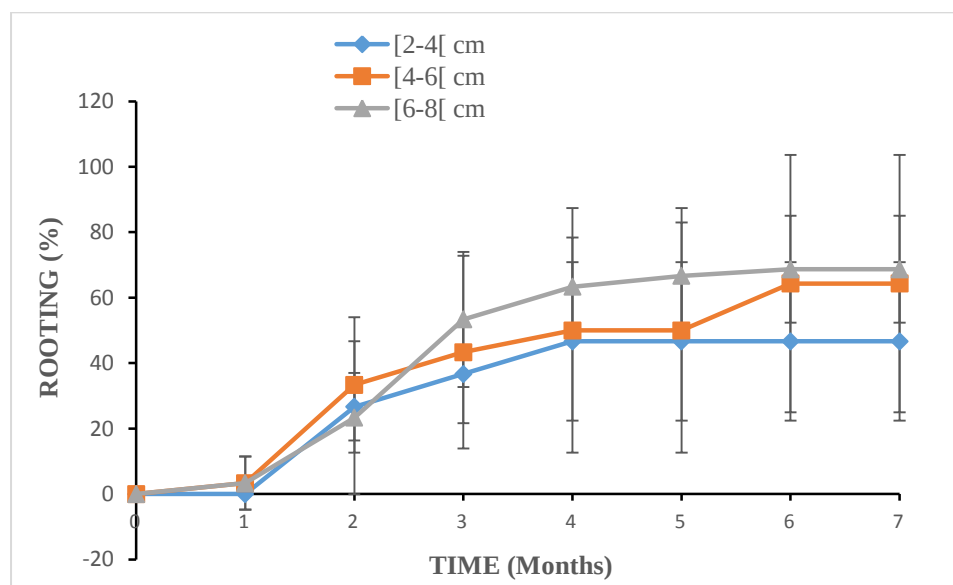


Fig. 7: Percentage of rooted layers by diameter class as a function of time

3.5-Effect of aluminium foil

Seven months after installation, the rooting percentage ranged from 51.11±26.66% for uncovered layers to 68.88±20.27% for those covered with aluminium foil (Fig. 8). The rooting rate began to change one month after the installation of the sleeves for the layers protected with aluminium foil. The saturation plateau was reached in the fifth month, but for the layers not covered with aluminium foil, roots were not observable until the second month after installation, and the optimum was reached in the sixth month. Although the analysis of variance did not show a significant difference in the light exposure mode ($0.1309 > 0.05$), the aluminium foil proved effective in that it maintained humidity in the sleeves and mitigated the harmful effects of light rays on the layers.

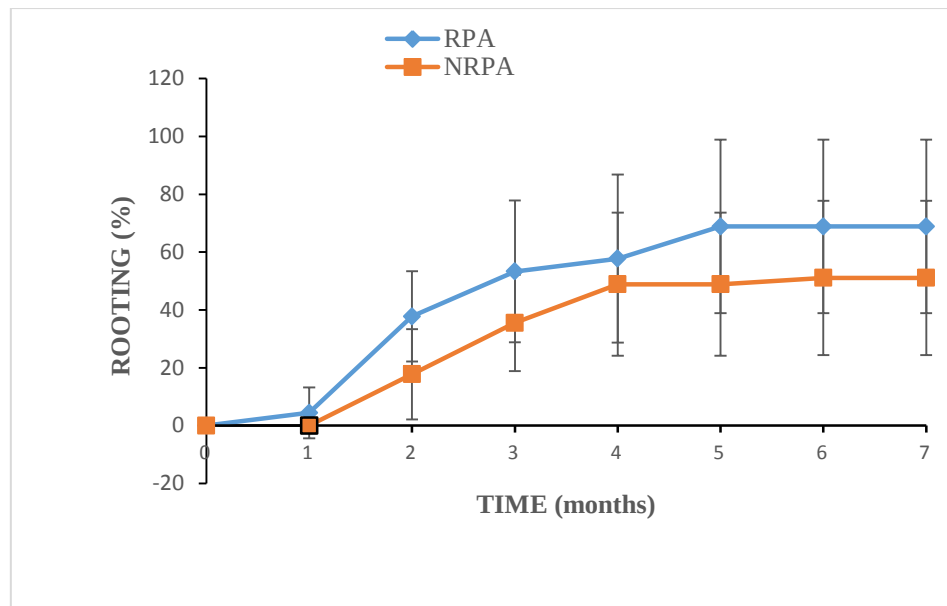


Fig. 8: Evolution of rooting rate following layer exposure.
RPA: Covered by aluminum foil; **NRPA:** Not covered by aluminum foil

3.6-Diameter*covering method interaction effect

The rooting percentage ranges from 33.33% for [2-4[cm diameters unprotected by aluminium foil to 73.33% for [6-8[cm diameter layers covered by aluminium foil (Fig. 9). The diameter/covering method interaction on layer rooting is not significant ($0.5076 > 0.05$). It is important to note that layers protected by aluminum foil exhibited the best rooting rates. Combining the covering method and branch diameter factors shows that the rooting percentage increases when moving from one type of branch diameter to another.

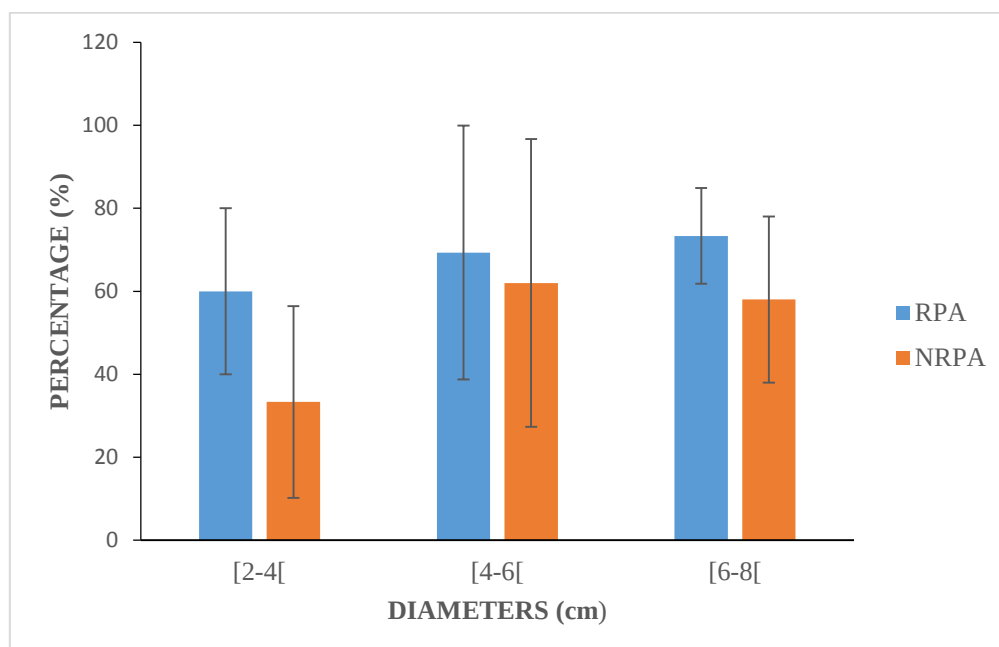


Fig. 9: Percentage of rooting following the diameter*coverage mode interaction

At the end of the trial, twenty (20) layers were weaned and acclimatized for three months in the rehabilitation propagator (Fig. 10a). During this process (35 days) after recovery, some plants flowered (Fig. 10b) but sometime later lost their flowers. This loss of flowers could be due to trauma or the lack of pollinators. It should be noted that the layers resulting from the acclimatization will be planted in fields in order to monitor their development. The

recovery in the nursery is not homogeneous within the population of weaned layers, after weaning the recovery rate varies from $53.33 \pm 51.6\%$ to $66.66 \pm 48.79\%$ (Table 4). The maximum value is observed in the black earth/sawdust mixture. The analysis of variance does not indicate a significant difference between the different substrates ($0.7704 > 0.05$).

Table 4. Layer recovery rate after weaning.

Substrates	Sphagnum	TN	TN/Sc	Average
Recovery rate (%)	$53.33 \pm 51.63a$	$60 \pm 50.77a$	$66.66 \pm 48.79a$	61 ± 27.35

TN: Black soil; TN/Sc: Black soil-sawdust mixture

It should be noted that the layers resulting from acclimatization will be planted in fields in order to monitor their development.

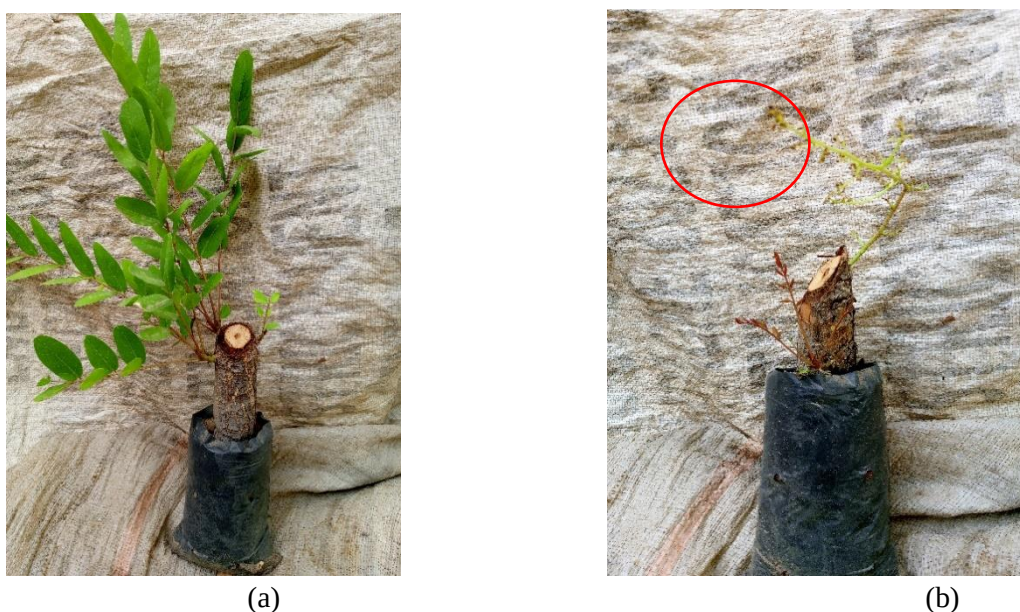


Fig. 10: Layering after 3 months of acclimatization (a); layering having flowered after three months of acclimatization (b).

IV. DISCUSSION

4.1-Tree layering periods in the Sudano-Sahelian zone of Cameroon

Generally, the time of year when the levels of these carbohydrates are high is the rainy season. Apart from their role as substrates in carbon or energy metabolisms, as well as in polymer biosynthesis, sugars are considered crucial molecules in the transduction of signals governing the growth and development of the plant. The results obtained in this study agree with those of Stenvall *et al.* (2009) in Finland who found a maximum value in soluble sugars in autumn (rainy season) in *Populus tremula*, Binwe *et al.* (2024) arrived at the same conclusion by obtaining mainly high quantities of carbohydrates in the rainy season on the cutting of *Ximenia americana*. Similarly, Oumarou (2021) reports that the presence of water in the rainy season seems to accentuate the photosynthetic activity of *Bombax costatum* and *Securidaca longepedunculata*. Furthermore, (Weyand and Schultz, 2006; Smith and Holzapfel, 2009) show that climatic condition, water stress, the management system and the variety also influence the assimilation of sugars. Zufferey *et al.* (2012) report that carbohydrates undergo conversion into macromolecules (starch, soluble sugars, etc.) during the year in different parts of the plant (branches, trunk, roots), then these reserves are redistributed in the form of simple sugars (glucose, fructose, galactose, etc.) depending on the functioning of the plant later. In this sense, Murisier *et al.* (1994) explains that the quantity of carbohydrates stored in the perennial parts of the plant (vine) is correlated with the leaf-fruit ratio.

4.2.1-Reactions of trees to layering

The callus formation on the upper part of the girdle during air layering is due to an accumulation of photosynthetic products (carbohydrates, auxins) in this area of the incision, which then differentiates into roots. With an average rooting rate of $53.3 \pm 18.33\%$ and layer survival until weaning, *Haematostaphis barteri* exhibits good air layering ability. This capacity may be explained by the species' ability to form scar ridges on the upper part of the girdled branch. This observation is in close alignment with Nanson (2004), who reported that successful vegetative propagation must take into account intrinsic species variations, environmental, climatic, and experimental conditions.

4.2.2- Substrate effect

The rooting substrate is a key factor in the success of air layering (Agarwal *et al.*, 2021). In our study, statistical analyses reveal a significant difference between the substrates. Traditional brewer's spent grain appears to be a poor substrate from the point of view of observations and findings. However, the black earth/sawdust mixture substrate proved to be numerically efficient with a rooting rate of $63.33 \pm 15.27\%$. This result is consistent with those of Nganjouong *et al.* (2022); Fawa *et al.* (2023) who obtained appreciable results with this type of substrate. The differences between our results could be explained on the one hand by the climatic conditions of our study areas and on the other hand by the intrinsic qualities of our studied species which lend themselves differently to air layering. It should be noted that to successfully layer woody plants, the substrate used must be loose and more porous to allow the emergence and growth of roots.

4.2.3-Effect of diameter on layer rooting

Branch diameter would also be an indicator of layering success. In the said study, the diameter ranges [4-6[and [6-8[cm, presented the best results. These observations corroborate those of Moupela *et al.* (2013); Mapongmetsem and Dicksia (2014) and Nganjouong *et al.* (2022) who obtained satisfactory results on large branches. Branch size has an influence on the layering of species which justifies the low rhizogenetic potential of young branches in this study. Furthermore Kengue *et al.* (1997) emphasize that young branches barely withstand the trauma suffered by the plant during bark girdling.

4.2.4- Effect of aluminium foil

There is a significant difference between the results obtained on sleeves covered with aluminium foil and uncovered. Aluminium foil proved to be an element to consider in air layering in that the humidity in the covered sheaths was maintained and is considerable. The result corroborates that of Elomo *et al.* (2014) on *Dacryodes edulis*; Fawa *et al.* (2023) on the species *Lophira lanceolata* in the Guinean savannah highlands of Cameroon. The authors made identical observations.

4.2.5- Diameter*covering method interaction effect

The combination of the factors of coverage mode and diameters does not present a significant difference in light of statistical analyses. However, the results obtained and direct field observations show that the rooting rate increases when moving from one branch size to another.

V. CONCLUSION

This study shows that *Haematostaphis barteri* has a good aptitude for vegetative propagation by air layering. Domestication of this species is possible by air layering. The substrate, the diameter of the branches and the aluminum foil have an influence on the rooting of the layers. The favorable period for layering of this species is the rainy season in the Sudano-Sahelian zone with regard to the quantification of sugars. The black earth/sawdust mixture proved to be the best rooting substrate and the branches of size [4-6[cm and [6-8[cm proved to be more efficient in terms of average rooting percentage. In addition, the aluminium foil improved the humidity on the sleeves favoring a promising result. It should be noted that air layering is a credible and simple method to multiply this species at a lower cost. This would be an asset for vegetative propagation and its integration into production systems by farmers in the area as part of agroforestry practices and the development of their land. The rooting rate obtained in this study could be improved.

Therefore, it is desirable to carry out further studies to test the branch positions (orthotropic and plagiotropic) on the rooting of this species, to plant and monitor the layers in the field, and to study other vegetative propagation techniques on *Haematostaphis barteri*.

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