

The background of the entire page is a photograph of a forest. In the foreground, a plant with large, dark green, glossy leaves is prominently displayed. Some leaves show signs of being eaten, with small holes visible. The background is a soft-focus view of a forest floor with various plants and trees.

THE EBONY PROJECT

Developing an Integrative Program for Restoration,
Use and Community-based Livelihoods

Progress Report
2018 October

THE EBONY PROJECT

Developing an Integrative Program for Restoration,
Use and Community-based Livelihoods

Progress Report
2018 October

Vincent Deblauwe^{1, 2, 3}
Donald Eloumou^{1,5}
Giovanni Forgione²
Matthew LeBreton^{1,3,4}
Kevin Njabo^{1,3}
Eric Onguene^{1,2,6}
Scott Paul⁴
Thomas B. Smith^{1,3}
Zac Tchoundjeu^{1,5}
Alvine Tchouga^{1,2}
Virginia Zaunbrecher^{1,3}

1. Congo Basin Institute, Yaounde, Cameroon
2. International Institute of Tropical Agriculture, Yaounde, Cameroon
3. Center for Tropical Research, Institute of the Environment and Sustainability, University of California, Los Angeles, Los Angeles, United States of America
4. Taylor Guitars, San Diego, United States of America
5. High Institute of Environmental Sciences (HIES), Yaounde, Cameroon
6. National Forestry School Mbalmayo, Cameroon

PROJECT PARTNERS

The Ebony Project is coordinated by the Congo Basin Institute (CBI) in Yaoundé, Cameroon, and the work is implemented by CBI and the following project partners:



International Institute of Tropical Agriculture
Cameroon



University of California, Los Angeles
United States of America



Institut Supérieur des Sciences
Environnementales
Cameroon



Taylor Guitars
United States of America



Madinter International
Spain



Crelicam
Cameroon

The Ebony Project is developing activities in collaboration with institutions in Cameroon including:



Université de Yaoundé I, Yaoundé, Prof. N. Niemenak



Ministry of Environment, Nature Protection and Sustainable Development (MINEPDED),
Ministry of Forests and Wildlife (MINFOF), Cameroon, Conservation of the Dja Faunal Reserve
National Forestry School Mbalmayo, N. Kingsly

Table of Contents

Project Partners	3
What is The Ebony Project?.....	5
Working With Communities.....	8
Project Accomplishments to Date	8
Plans for 2019.....	9
Producing and Planting Trees.....	11
Project Accomplishments to Date	11
a. Cuttings	11
b. Seeds.....	11
c. Plant Nurseries.....	12
d. Transplantations.....	12
Plans for 2019.....	13
Understanding the Ecology of Ebony	15
Project Accomplishments to Date	15
e. Ebony's Range	15
f. Ebony Inventory and Threats.....	16
g. Ebony Reproduction	17
h. Growth Rates	19
Plans for 2019.....	20
Deploying Technology to Increase Production	21
Project Accomplishments to Date	21
i. Seed germination	21
j. Improving outcomes via cuttings.....	21
k. Microcutting	22
l. Somatic embryogenesis	23
Plans for 2019.....	23
m. Sapling growth and survival.....	23
n. Cuttings.....	23
o. Tissue Culture	23
A Vision for Expansion.....	25
Acknowledgements.....	26
Appendix A	27
References	33

WHAT IS THE EBONY PROJECT?

The Ebony Project is funded by Bob Taylor of Taylor Guitars and is a partnership where business, communities, and researchers work together to protect a valuable timber species, reforest degraded land, and improve rural livelihoods.

The Ebony Project was launched in 2016 and to date has been 100% financed by Bob Taylor of Taylor Guitars, co-owner of the Yaoundé based CRELICAM ebony mill. The majority of the work for this project takes place at Congo Basin Institute's (CBI) Yaoundé campus, the Higher Institute of Environmental Sciences (HIES) campus in Yaoundé, Bouamir Research Camp in the Dja Biosphere Reserve and communities in the natural range of the species. However, the project includes many field sites and partners (see Figure 1).

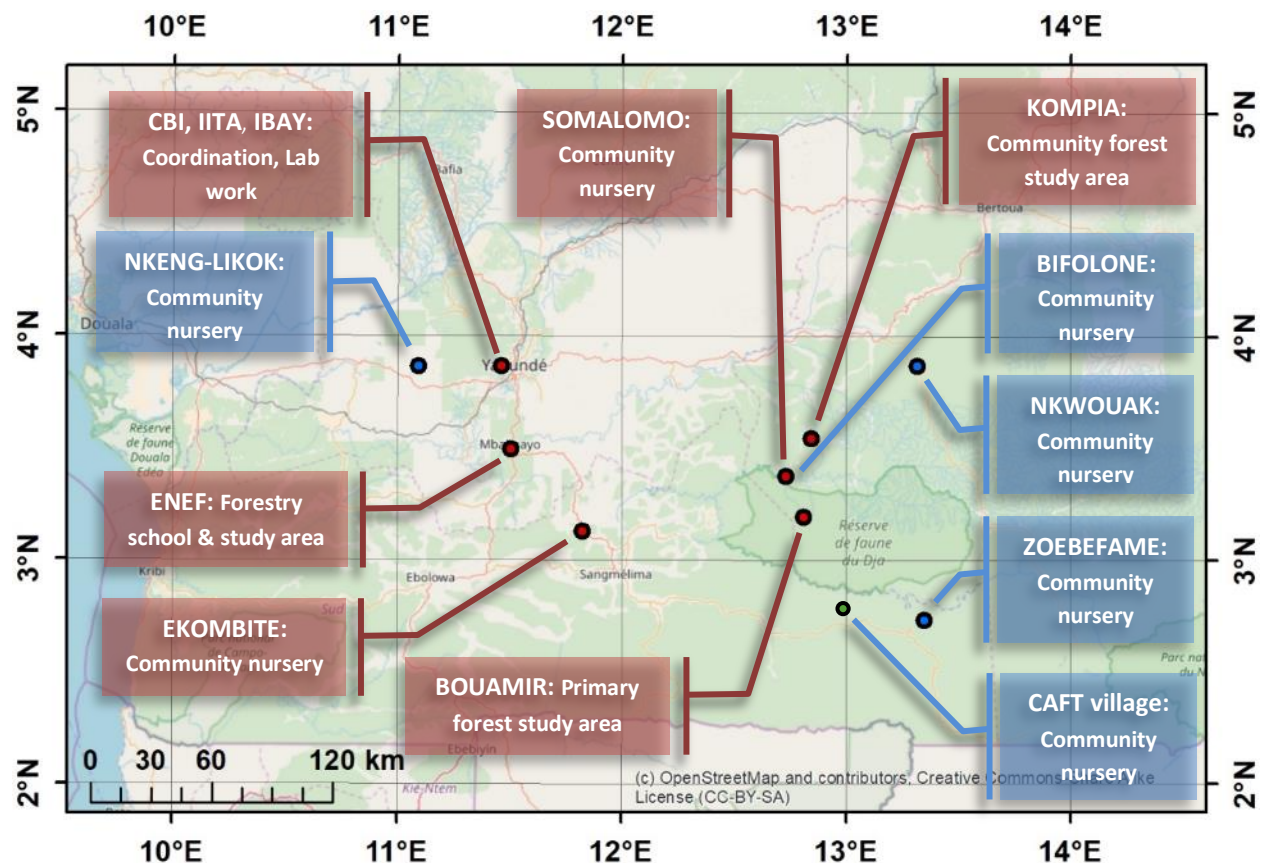


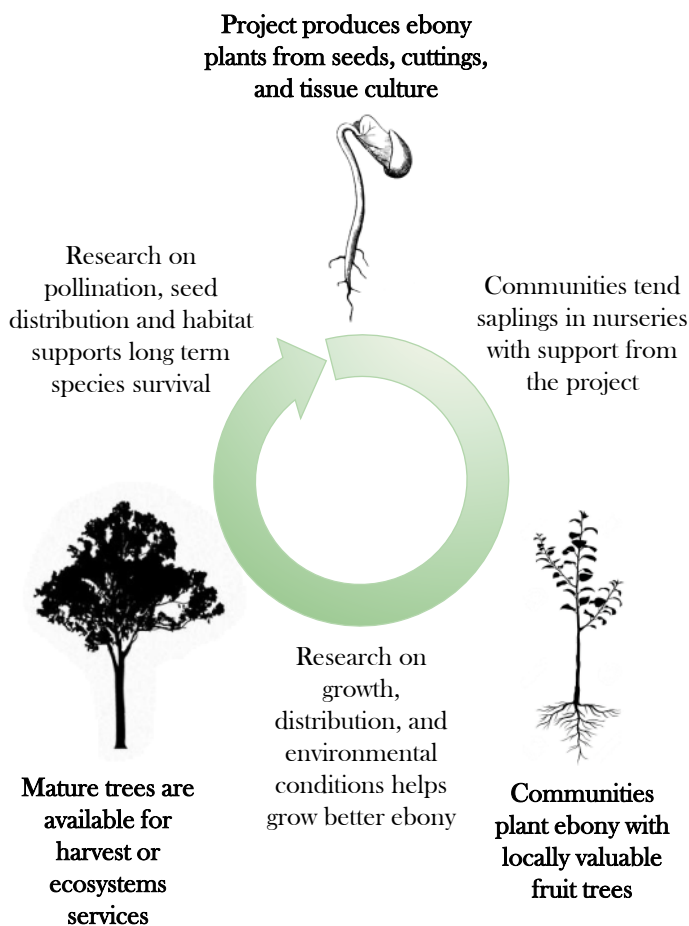
Figure 1. The ebony project working sites. Red and blue corresponds to present and expansion sites respectively.

The projects has four main objectives:

- 1) Work with rural communities to create a scalable program for the sustainable production and stewardship of ebony.
- 2) Model West African ebony distribution and assess harvesting rates and appropriate planting areas.

- 3) Understand the basic ecology of ebony to enhance natural reproduction and dispersal, and test restoration approaches.
- 4) Test alternative propagation approaches, including tissue culture, to identify optimal conditions for cultivating ebony.

The diversity of objectives demonstrate that the project is more ambitious than just planting trees. The Ebony Project seeks to create a model to enhance the sustainability of the species, its rainforest habitat, and participating communities. It has a quantitative target—to plant 15,000 ebony trees—but it is much more than that. It seeks to add capacity and knowledge at every stage of the life cycle to ensure that those trees, and the people who care for them, will not just survive but thrive (Figure 2).



UCLA leads the CBI team and is responsible for investigating the ecology of ebony and modelling its distribution. The International Institute of Tropical Agriculture (IITA) is leading efforts to develop tissue culture propagation for ebony. Cameroonian university the Higher Institute for Environmental Studies (HIES) works with villages to develop community-managed nurseries that produce and grow ebony and locally valuable fruit trees. Taylor Guitars, Madinter and Crelicam staff provide valuable business perspective and a deep knowledge of the ebony trade.

The project is influenced by the seasonal cycles of Cameroon and of ebony itself (Figure 3). In the Center, South and East regions of Cameroon, ebony trees produce flowers in February to April and fruit and seeds are ripe approximately mid-August to September. The Cameroonian climate in these regions have a bimodal

precipitation regime, with a small rainy season in mid-March to end of June and a larger rainy season in September to mid-November. These seasons are separated by a short and cold dry season. The months of December to February correspond to the long dry and hot season. We have found that the transplantation of woody species to the field is more likely to be successful at the onset of the rains in March. This maximizes the time for the saplings to establish a root system before the start of dry and hot season in December. Unlike seedlings, ebony saplings from cuttings can be produced all year long.

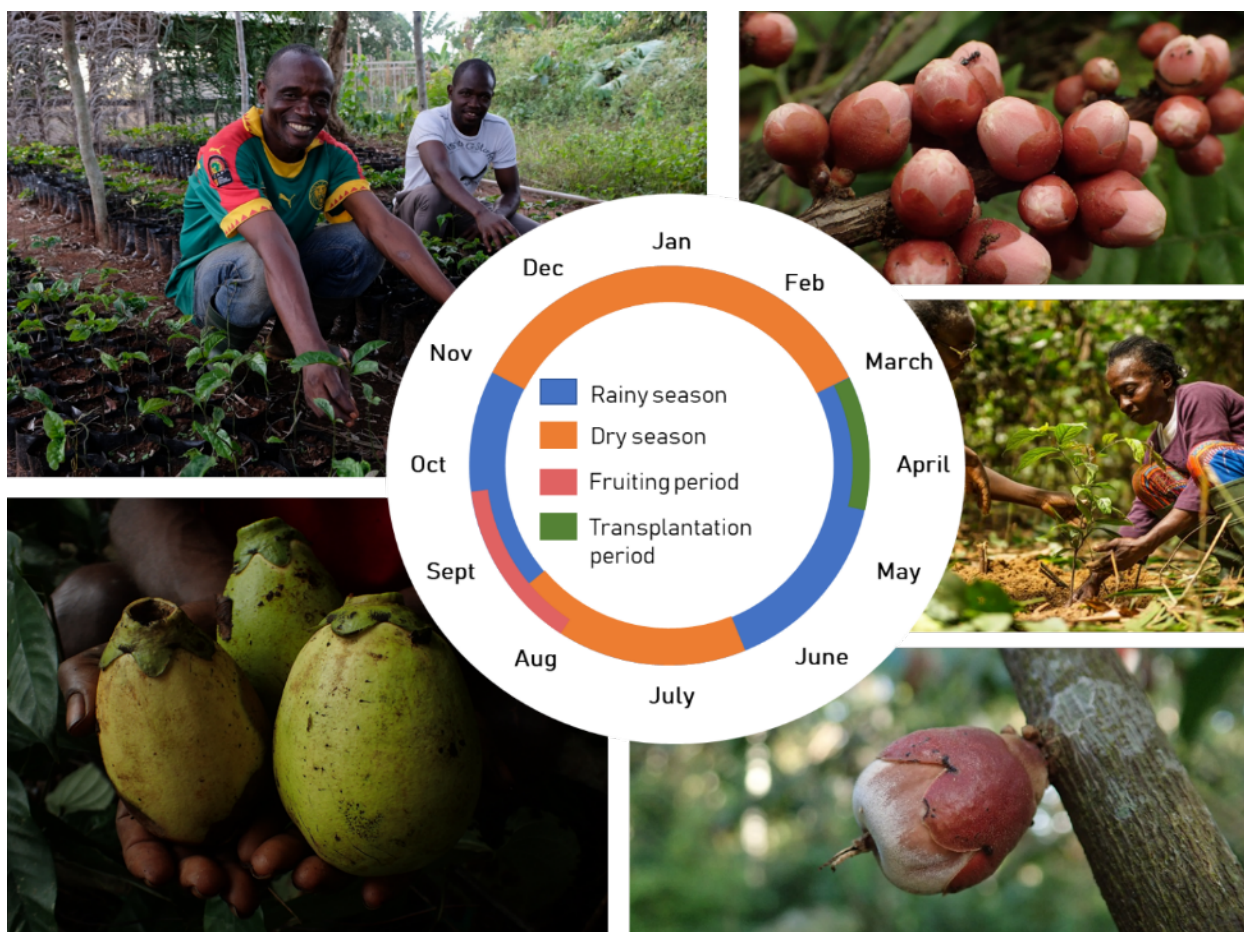


Figure 3. Farmer calendar for ebony multiplication in Cameroon. From Top left corner and going clockwise: M. Siel Siel & M. Ndjankoum in the community ebony nursery in Somalomo; flower buds in Ekombité; a woman planting an ebony sapling in Ekombité; an immature ebony fruit in ENEF, Mbalmayo; ripe fruits around Eseka.

WORKING WITH COMMUNITIES

Project Accomplishments to Date

The Ebony Project signed agreements and established community nurseries with two communities in 2017, the first in Ekombité (Dja-et-Lobo) in April and the second at Somalomo (Haut-Nyong) in December (see the maps in Figure 1, Figure A.1 and Figure A.2 Appendix A). Accordingly, the project provided materials and training to community members to construct and manage a small nursery. Community members were also involved in collection of seeds, vegetative propagation and ecological research.

The project is also developing a “Sylvicultural Booklet” to document where saplings are planted, what species they are, and who planted them. This is an effort to address the challenge of ownership of the resulting mature trees. Under Cameroonian law, all trees not planted by a person are part of the forest estate, which belongs to the State. However, there is no formal process for officially recording plants purposefully planted by humans. As the species involved in this project are all naturally occurring, without documentation it would be difficult for an individual to establish tenure rights for trees planted decades earlier. In lieu of an official mechanism to confer

ownership, the Sylvicultural Booklet developed by the project will seek to provide documentation to support ownership claims. This approach has not, to our knowledge, been attempted on this scale. As a non-State actor, the project cannot guarantee State acceptance of Sylvicultural Booklets, however, we believe it will support ownership



claims, and could feed into an official State mechanism should one be developed. The species, location, and owner of all saplings planted to date have been recorded in a database, pending the finalization of the Sylvicultural Booklet.

As per the community agreements signed to date, Crelicam will provide participants in the two pilot communities of Ekombité and Somalomo with their first incentive payments as each have cared for the ebony plants in the nurseries for approximately one year. UCLA and IBAY

researchers are currently working to agree on the appropriate amount for a maintenance payment.

Plans for 2019

The number of trees a single community can plant is limited by the surface of appropriate land owned by the participants. In spring 2018 the two pilot communities of Ekombité and Somalomo planted roughly 1,000 saplings each. Moving forward, we believe participating communities can plant a maximum of 2,000 trees per year, which requires about 10ha of available land. Future communities may have higher or lower planting capacity depending upon land availability and it is unknown how planting capacity may change over time, but we anticipate a gradual decrease in each project area as less land becomes available. To achieve the original goal of planting at least 15,000 ebony trees, we believe the project must be expanded from the current two communities to seven.

Fortunately, two additional communities that UCLA has worked with for many years have expressed interest in participating in the project. Their enthusiasm and their proximity to an existing site (Somalomo) are beneficial to the project, and it is hoped that agreements will be signed with the communities within the next six months. These communities are:

- Bifalone (Haut-Nyong) will be the first indigenous Baka community to join the project. While the Baka are traditionally hunter-gatherers, they are becoming increasingly sedentary. The members of this community are interested in developing agroforestry approaches to supplement the small scale farming they already do and to improve their livelihoods.
- Kompia (Haut-Nyong) already hosts a census plot used for the comparative ecology work and has a large number of mature fruiting ebony trees in a locally managed community forest. The community leaders already expressed interest about conservation programs seeking to enrich and help them manage their community forest.

In the next 6-18 months, we hope to add the following villages to the project:

- Zoebefame and Nkwouak are Baka and Bantu communities that have been working with UCLA researchers for many years.
- Nkeng-Likok (Nyong-et-Kéllé) already hosts a nursery initiated by ICRAF.
- Ndikinmeki is the ancestral village of Mme Eheth of the Ministry of Forestry and Fauna. We will set up a small demonstration there that will include the planting of a small number of ebony trees. The location allows us to collect data to help assess the likely impact of climate change on ebony.

The current approach for selecting communities—based on suitability for ebony growth and previous working relationships with members of the project team—has proven effective and appropriate given the size of the existing project, but would be unworkable at a larger scale. To address this, the project will identify key community traits that lead to success based on our experience with existing communities. This will guide us in the selection of future participants once more obvious candidates identified from previously existing relationships have been exhausted.

PRODUCING AND PLANTING TREES

Project Accomplishments to Date

The project currently supports the production of ebony and locally valuable fruit, medicine, such as safou (bush plum), avocado, and moabi grown from either seed or cuttings. This section focuses on the project's novel efforts to produce and care for slow growing ebony while simultaneously producing and transplanting faster growing local fruit and medicinal trees.

Cuttings

Communities can grow ebony plants from cuttings year-round with some basic materials and training. This technique can produce large numbers of plants and is not dependent on ebony flowering and fruiting. It also allows us to multiply selected elite trees that are genetically identical to the source plant. However, its usefulness is limited by a current mortality rate of 40 to 90% at rooting stage, the long rooting time (14 weeks), and slow growth rate. With cuttings, stem growth is observed after approximately one year, and successful saplings require at least 2 ½ years before they are ready to be transplanted. Farmers in the two pilot villages of Ekombité and Somalomo have been trained for vegetative propagation using cuttings and have grown approximately 200 ebony plants using this technique.



Seeds

The second source of saplings is from seed. In Cameroon, ebony trees produce flowers in February-April and fruit from mid-August to early October (see Figure 3) and the availability of seeds is dependent on the production of flowers and the successful maturation of fruits.

In 2017, the second year of the project, ebony trees in Cameroon produced very few fruits, which accordingly limited the number of seeds that could be collected. Complicating matters, given the predation rate from rodents, once the fruits dropped to the ground, the project was unable to collect adequate samples from nearby mature trees found at either Ekombité or at the Mbalmayo governmental experiment ebony plantation, and the project was forced to largely collect seed from a few known mature wild trees. From the fruits collected during 2017, 1,270 saplings were produced. In 2018, natural ebony fruit production was several orders of magnitude above the previous year, and provided the project with approximately 20,000 seeds.

From seed, stem growth is observed after two months and saplings can be transplanted approximately 18 months after germination. The mortality at transplantation stage is approximately 10%.

Table 1. Production of ebony plants per year in the project nurseries.

Nursery	Origin	Type	Production in 2016	Production in 2017	Production in 2018
Crelicam	Crelicam	Cuttings	NA	900	
Ekombité	Ekombité	Cuttings	NA	100	
	Mbalmayo	Seeds	NA	1,120	
Somalomo	Crelicam	Cuttings	NA	83	
	Mbalmayo	Seeds	NA	816	
ICRAF	ICRAF	Cuttings	NA	146	
	Mbalmayo	Seeds	NA	437	
IBAY	ENEF	Seeds	NA	NA	20,000
IITA	ENEF	Seeds	1,270	2,000	
TOTAL		Cuttings	0	1,229	
		Seeds	1,270	4,373	

Plant Nurseries

In 2018, the project constructed or expanded nurseries in Ekombité, Somalomo and at CRELICAM. Additionally, to accommodate the large influx of seeds in 2018, the project recently completed the construction of a new nursery at the IBAYSUP Campus in Nkolbisson, Yaoundé. This nursery will serve two key functions:

1. Additional stewardship capacity of ebony and locally valuable fruit and medicine trees for the project necessary to maintain the influx of seeds made available during strong fruiting years (like 2018 for ebony).
2. Much needed practical access for university students from HIES of IBAY SUP providing a critical hands-on learning environment for the next generation of agro-foresters in Cameroon.

In total, at the time of writing, 1,200 plants from cuttings and approximately 2000 plants from seed collected in 2017 are growing at community nurseries, and 20,000 additional seedlings from the 2018 ebony fruit harvest are growing in the newly constructed IBAYSUP learning nursery.

Transplantations

Over the first two years of the project, a main constraint in the ability to transplant trees—i.e. take saplings from the nursery and transplant them into the ground—has been the supply of ready ebony saplings in the nursery. Project experience suggests that 18 months are required for the seedling to reach a size of about 40 cm. At that size, the saplings are large enough to survive with minimal maintenance but have not lingered in the nursery long enough to tax the community members who care for them. This year we are also testing the survival rate of the 6 months transplants in Ekombité.

In March 2017, as the first community agreement were signed, the project planted 50 ebony trees to test transplantation. In February 2018 the transplants were assessed, and in April 2018 project participant transplanted 2,170 ebony plants. These plants were mostly obtained from seed acquired in September 2016. In Ekombité, 900 seedlings produced from seeds acquired in September 2017 were planted as an experiment to determine survival rate of 6-month old transplants compared to the recommended 18-month old transplants. The project plans to assess the success of all the transplants after the end of the long dry season in March 2019, at which point we will be able to provide an estimated survival rate for the transplants in the field.

Table 2. Number of saplings transplanted to the field at the time of writing. Ebony (*Diospyros crassiflora* Hiern), avocado (*Persea americana* Mill.), andok (*Irvingia wombolu* Vermeesen), moabi (*Baillonella toxisperma* Pierre), cola (*Cola acuminata* (P. Beauv.) Schott & Endl.), djansang (*Ricinodendron heudelotii* (Baill.) Pierre ex Heckel), safou (*Dacryodes edulis* H.J. Lam), ayous (*Triplochiton scleroxylon* K.Schum.), soursop (*Annona muricata* L.), cacao tree (*Theobroma cacao* L.), cherry (*Dacryodes macrophylla* (Oliv.) Lam.).

Location	Ebony from seed	Avocado	Andok	Moabi	Cola	Djansang	Safou	Ayous	Soursop	Cacao tree	Cherry
Somalomo	596	22	0	36	2	20	0	0	0	0	0
Ekombite	1,574	78	15	0	0	0	7	3	52	16	2
Total	2,170	100	15	36	2	20	7	3	52	16	2

Ebony trees were planted at 10 x 5 m spacing (i.e. around 200 trees per hectare). In order to evaluate survival and growing rates, 20 ebony plants were tagged and measured in each of the eight plots established in the communities in 2018.

Plans for 2019

A phenomenal 2018 ebony fruit production coupled with the planned addition of new program communities will allow the project to significantly scale up planting activities in 2019-2020.

During the March – April 2019 planting season, the project plans to work with Ekombité and Somalomo and two new communities to plant approximately 3,500 18-month-old seedlings. During the March-April 2020 planting season, the project will have access to the saplings planted from seeds gathered in August-September 2018, which we expect will be in excess of 20,000. The cuttings planted in 2017 will be available for the same transplanting season. Thus, we plan to reach and exceed the goal of 15,000 ebony trees planted in March-April 2020. For the Year 2021, saplings grown from seeds collected in 2019 will be used. As we cannot guarantee that fruit production will be good in 2019, we will plant 15,000 cuttings in 2018 to secure the stock. A timeline for the planting of ebony trees is shown in Figure 5.

The project also plans to scale up the propagation and transplantation of valuable native fruit trees.

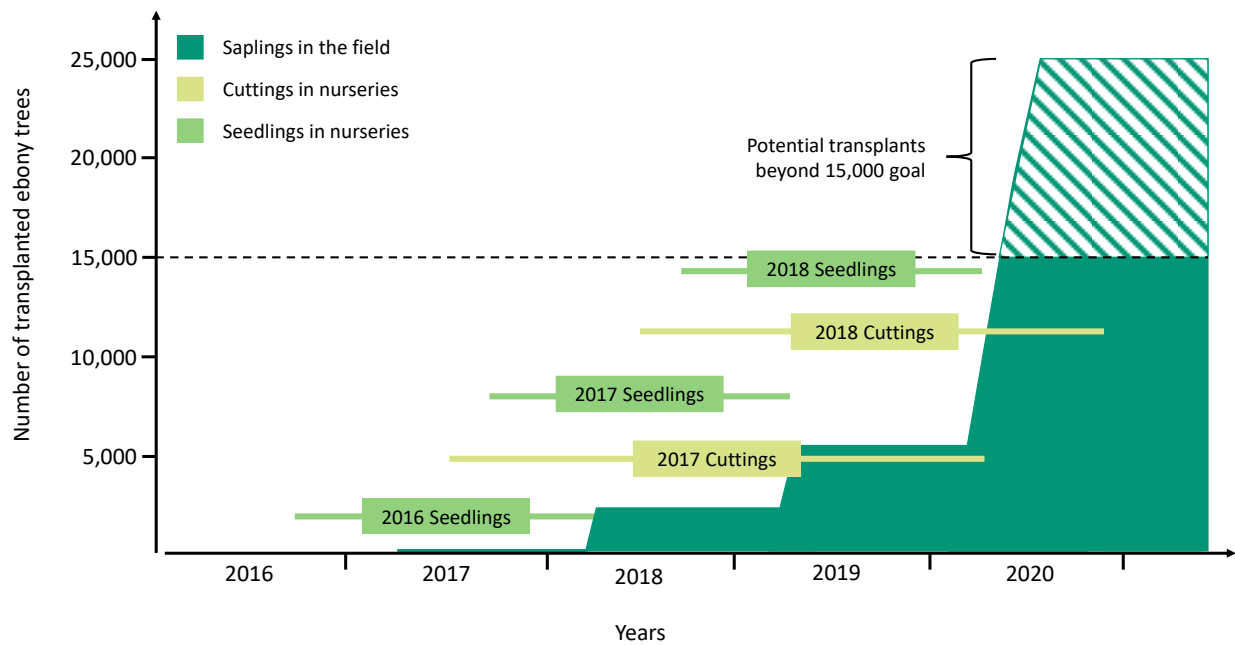


Figure 5. Existing (2016-2018) and planned (2019-2020) planting of ebony in the communities. The batches of plants in the nursery are represented as lines bounded between time of planting and transplantation to the field. The height of the green polygon corresponds to the number of transplants on the y-axis.

UNDERSTANDING THE ECOLOGY OF EBONY

Project Accomplishments to Date

Effective conservation and stewardship of ebony relies on a better understanding of the species. Such an understanding can identify the threats and opportunities, and inform conservation and management decisions. Unfortunately, information on the basic ecology of *Diospyros crassiflora* Hiern is extremely limited. As an acknowledgement of the important role research plays in thoughtful conservation and commerce, the project focuses on improving our basic understanding of ebony ecology focusing on four key questions:

- 1) Where does ebony grow?
- 2) How much ebony is there, and what are the threats to its survival?
- 3) How does ebony reproduce?
- 4) How fast does ebony grow, and what conditions are most conducive to its growth?

To address these questions, the project established three comprehensive ebony census plots of 400 ha each in the Dja Biosphere Reserve (Figure A.3, Appendix A). Further, the project studies two ebony stands planted circa 2003 in the ENEF, Mbalmayo, arboretum (Figure A.4, Appendix A), and also ebony planted in an agroforestry setting 1988 in the village of Ekombité. Trees have also been planted at IITA in March 2018 for an experiment on sapling survival and growth as a function of transplanting time. Table 3 summarizes the research plots.

Table 3: Summary of the research plots used for Ebony Project. All plots are located in Central Cameroon.

Location Name	Location Type	Hunting Status	# of Trees	Research Utility
Sim River – Dja Reserve	Protected area	Low	128	Pristine forest growth rate; seed dispersal in forest with low hunting pressure; pollination
Kompia	Community forest	High	486	Degraded forest growth rate; seed dispersal in forest with high hunting pressure; pollination
Bouamir – Dja Reserve	Protected area	Very low		Pristine forest growth rate; seed dispersal in forest with very low hunting pressure; pollination
Mbalmayo	Mono-cultural research plantation	N/A		Plantation growth rate; pollination
Ekombité	Community agro-forest	N/A		Agro-forestry growth rate; pollination

Ebony's Range

To determine the geographical distribution of *D. crassiflora*, the project collated geographical coordinates of known populations from several sources: (i) data from Crelicam and other private

timber companies; (ii) herbarium specimens collected during the project; (iii); data shared by Kew Gardens and Botanic Garden Meise (iv) the RAINBIO dataset, a comprehensive database of georeferenced records of vascular plants in Africa (Dauby *et al.* 2016); (v) tree inventory data from old-growth forest without signs of recent human disturbance collated by Gilles Dauby and Ferry Slik (Slik *et al.* 2015); (vi) the ebony inventories we made for the project in the Dja Biosphere Reserve.

The distribution data acquired from several herbaria institutes (BR, BRLU, K, LISC, MO, P, and WAG (incl. AMD, L & U)) goes back to the 19th century, include plants of all diameter and give the highest possible level of identification accuracy because voucher specimens are freely available for re-identification. However, the size of trees and the density of the population is not known. Tree inventories were sampled relatively recently (after the 1950s) but only include trunk diameter at breast height (DBH) above 10 cm and could not be verified for taxonomic accuracy. The project's three census plots are well identified, include all diameters from seedlings to mature trees and provide information about tree density but are restricted to the Dja Biosphere Reserve.

This dataset was mainly used to produce an ebony distribution model across its native range (Figure 4). This work was extensively reported in a technical submission to the IUCN committee re-evaluating ebony's conservation classification. The project used newly gathered ecology data to update the distribution model and improve estimated inventories of the species.

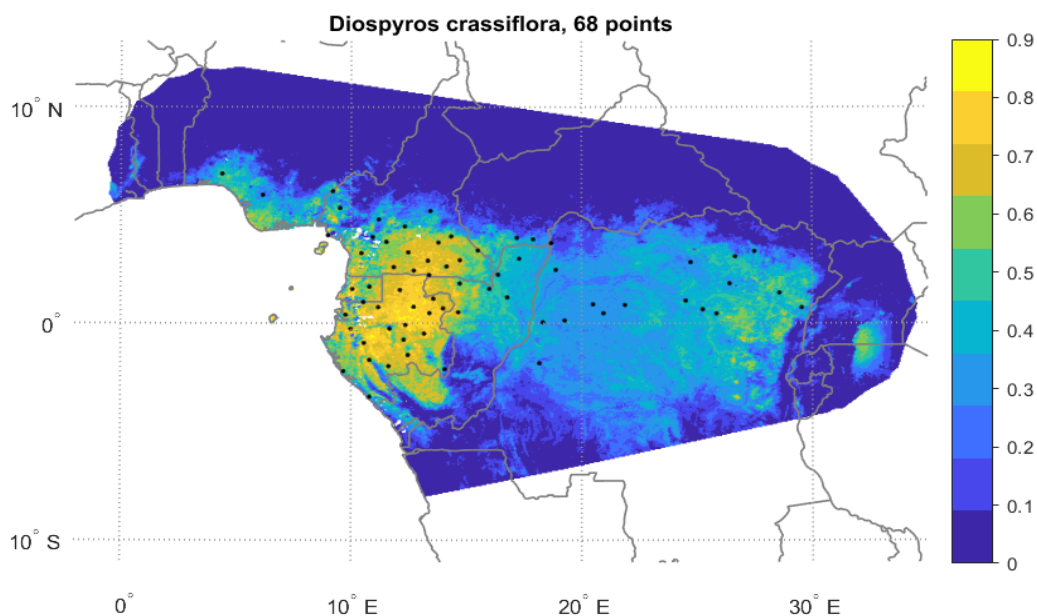


Figure 4. *D. crassiflora* suitability map built using MaxEnt algorithm. Black dots represent 68 known localities used for model training.

Ebony Inventory and Threats

The project's modelling work suggests there are approximately 30 million trees growing across the species' range. Given the significant inventory of ebony and the relatively small international

demand for the species, the major threats to the species appear to be land conversion for agriculture and the systematic elimination of traditional seed dispersal agents due to poaching and bushmeat hunting.

Indeed, a comparison of ebony population in hunted (i.e. Kompia) vs. protected (i.e Dja) forests may reflect the important consequences of removing mammalian fauna from the forest. Despite the considerable number of ebony trees found in the hunted site, tree regeneration was lower possibly due to the removal of traditional mammalian seed dispersal agents. The project recorded 57% of ebony trees were 1–10 cm in diameter in the hunted zone while in the protected forest, this same size class constituted 76% of the trees.

In addition, in the hunted site, many saplings below 1cm in diameter (28% of total number of ebony), were found immediately below the canopy of mother trees. In the protected site, no seedlings of that size were identified below the canopy of mature trees. This suggests that fruit and seed predation and dispersal is much higher in the protected area.

The relatively rare 1-10cm trees in the hunted zone were significantly clustered in space at distance of 40-60 m from the mother tree. In the less hunted sites in the Dja, the aggregation was comparatively weak and occurred at a scale of 120-140 m (Figure A.7, Appendix A). The lack of clustering among the numerous larger trees in Kompia suggests the recent removal of long distance dispersers in this hunted forest. This could have happened several decades ago given the slow rate of growth of that species. The small clustering radius observed in Kompia match the distances of dispersion observed for the native rodents (Rosin & Poulsen 2017), which are still present in this forest. These results support the hypothesis that *D. crassiflora* is becoming seed dispersal limited as a consequence of hunting-induced defaunation.

Ebony Reproduction

Successful reproduction of ebony depends on three things. First, the trees must flower. Second, the flowers must be pollinated. Third, the resulting fruits must be dispersed away from each other and from the mother tree.

Flowering

D. crassiflora, like most other ebenaceae, produce unisexual flowers. Staminate and pistillate flowers are always found on different plants, a condition known as dioecy. For convenience, we here refer to “male” and “female” respectively for staminate and pistillate flowers and the plants that bear them.

In 2018 the project has observed ebony flowering in sites with differing human intervention such as: the 15-year-old trees in the monospecific plantation, the 30-year-old trees mixed with cocoa and other crops in Ekombité, the community agroforestry site, and the trees near the Sim River in the Dja reserve. This, and prior observations, seems to confirm the thesis that ebony seed production varies considerably by year.

Observations of flowers further showed that in every field site, there were more male flowering trees than female flowering trees in 2018 (Figure A.5, Appendix A). The fact that the male trees are more frequent than the females in the smallest classes of diameter (Figure A.6, Appendix A) supports



the hypothesis that male trees start to produce flowers at a smaller size than females. The male trees also tend to dominate the largest diameter classes which suggest either a higher mortality or slower growth rate of females. Another possible explanation is that seeds are not produced at a 1:1 sex ratio. Out of 165 flowering trees the project identified two androgynodioecious trees (i.e. trees producing mostly male flowers but nonetheless sometimes producing fruits from fertile ovary).

The project investigated the effect of increasing the level of competition for light and resources at the Mbalmayo plantation site compared to the Ekombité agroforestry setting and the Dja reserve. The proportion of flowering trees were 98, 80 and 46% respectively. The threshold tree diameter between flowering and non-flowering trees computed through nonlinear logistic regression were 4.8, 8.8 and 13.9 respectively. This suggests that competition tends to decrease the proportion of flowering trees and increase the diameter at which trees become sexually mature. This variable is important when estimating the number of mature individuals in any given population.

Pollination

Prior to this project, very little was known about what organisms pollinated ebony flowers, a key step in successful reproduction. In order to capture video of the entire life of ebony flowers, the project developed in collaboration with V. Droissart (IRD, Montpellier) specific cameras out of Raspberry Pi Zero computers (Figure A.9, Appendix A). The cameras are powered by inexpensive power banks and can function in difficult operating environments. This equipment was used in April 2018 to film flowers at Mbalmayo arboretum from opening to wilting. We discovered that a large variety of insects visited the flowers from anthesis (around midnight) to wilting (the next night). This included Helicidae nocturnal bees, moths and flies. We also incidentally observed a gliding squirrel, *Anomalurus pusillus*, feeding on mature flowers during a night (see example images in Figure A.10, Appendix A).

Seed Dispersal

The above average ebony fruit production in 2018 provided the project an opportunity to further investigate the role of animals who disperse seeds (“seed dispersers”) in the reproduction of ebony. We are processing this data presently.

Growth Rates

Understanding how quickly an ebony trees grows to a size where it can (a) reproduce and (b) be harvested is critical to understanding and conserving the species. Prior to this project, there was very limited data about ebony growth rates available.

Growth rates vary significantly by forest type and study, but show a relatively slow growth rate either in forests or plantations. A growth rate of 0.8 cm yr⁻¹ was reported for the three largest trees in the *D. crassiflora* plantation of the Mbalmayo arboretum (Owona Ndongo 2009). In Ekombité, *D. crassiflora* trees were planted in a 4 ha cocoa field in 1988. About half of the site was maintained by periodically removing regrowth and small trees while the other half was left as a closed forest. In July 2017, 300 trees were measured among which 270 had no malformation or injuries on the main stem. We found that the intact trees in the cleared agro-forest had an annual diameter growth rate of 0.40 cm yr⁻¹ on average since their plantation and reaching up to 0.65 cm yr⁻¹ (95th percentile). Growth rate increases with age and between July 2017 and May 2018, the average growth of 100 of these intact trees was 0.68 cm.

In the shade and competition of the forest, however, these optimal conditions are rarely encountered and most trees would grow much more slowly. The median growth rate since plantation in the closed forest of the Ekombite agro-forestry community, i.e. the conditions that best match with natural forests, was 0.18 cm yr⁻¹. The trees planted in closed undisturbed forest had an annual diameter growth rate of 0.21 cm yr⁻¹ on average and reaching up to 0.43 cm yr⁻¹ (95th percentile) (Figure A.8, Appendix A).

Growth rates reported for trees in natural stands also show a large variability. Radiocarbon dating of individual tree rings of 14 trees in a late secondary stand in Central Cameroon showed an average growth rate of 0.46 cm yr⁻¹ (Worbes *et al.* 2003). In 10 permanent 4-ha plots followed annually since 1982 at the Mbaïki experimental station in Central African Republic, the annual diameter growth rate was estimated to be 0.13-0.14 cm yr⁻¹ on average and reaching up to 0.64 cm yr⁻¹ (95th percentile) (Gourlet-Fleury *et al.* 2011; Fayolle *et al.* 2012).

These growth rates have implications for both reproduction and harvesting of ebony. Table 4 summarizes the time to fecundity and harvest based on growth rates in agroforest and natural forest settings.

Table 4: Growth rates, size at fecundity, time to fecundity, and time to harvest for ebony trees in agroforestry and natural closed canopy forest settings.

Forest Type	Avg growth rate	Max growth rate	Diameter at fecundity	Avg time to fecundity	Earliest time to fecundity	Avg time to harvest	Earliest time to harvest
Agroforest	0.40 cm/yr	0.65 cm/yr	4.8 cm	12 yrs	7.4 yrs	150 yrs	92.3 yrs

Natural closed canopy	0.21 cm/yr	0.43 cm/yr	13.9 cm	78.2 yrs	32.2 yrs	199.2 yrs	139.5 yrs
-----------------------	------------	------------	---------	----------	----------	-----------	-----------

Plans for 2019

Thanks to the continued financial support of Bob Taylor, the project will enhance its research into ebony's ecology continuing to collect data on the growth, survival and natural regeneration of ebony at the existing field sites, which include primary forests (the Dja reserve), a community agro-forestry project (Ekombite), and mature trees in plantation settings (the State run Mbalmayo research plantation). In 2019, the project will also focus research on: (1) deepening our understanding of growth rate, geneflow, and the impact of anthropogenic activity on ebony genetic diversity, reproduction, and demographics, and (2) open a new line of inquiry about the potential use of ebony as a tool for reforestation.

Purposely grown ebony as well as the three plots in a natural habitat will be measured again to obtain:

- A better idea of growth rate by age class and treatment (trees grow first slowly, then fast, then slowly again as they become old). This will allow us to reliably estimate the age of trees in the forest and to make predictions regarding wood production in plantation settings.
- Determine whether or not the female tree grows slower than males. Such an effect is predicted by theory (physiology of dioecious species) and have been observed in preliminary study in Ekombite. This information could inform whether the project should actively manage the gender of planted trees. It is also important to model ebony demography.

Using the cameras developed within the project, the project will collect data about pollination by insects in primary forests. Because preliminary data has shown that ebony may be pollination limited in natural settings, we will follow the fruit and seed production.

The project will assess how far away from the mother trees the rodents disperse the seeds using telemetric thread tags. During this experiment we will assess the seed fate (consumed, store in larder or germinated). This will give us an idea of efficient will be the dispersal in hunted forests where only the rodents remain.

The frugivore and seed predator data collected in the fall of 2018 with camera traps showed that seed dispersers are absent in Kompia and rarely visit the trees in the Dja Faunal reserve. In 2019 we will focus our effort on the sampling of fecal material from the largest mammalian dispersers (elephants, gorillas and chimpanzees) to determine if they are effective dispersers of ebony seeds. Evidence from other study points to these species being able to disperse the large ebony seeds and we made casual observations of germinating ebony seeds inside elephant dung. Dung will be inspected for the presence and quantity of ebony seeds. Seed status (germinating, entire or damaged) will be recorded and a sample will be taken to try identifying the parents. A separate set of seeds will be placed in a germination substrate to determine the viability of the seeds after gut passage.

To assess the impact of hunting on reproduction and geneflow, the project will perform parentage analysis using the vegetal samples collected from every tree found in our three 400-ha research plots, which represent natural forest stands with (a) minimal hunting; (b) moderate hunting; and (c) high levels of hunting. This will tell us the actual distance of pollen and seed dispersion, and how different they are between protected and hunted places. Because the effect of hunting needs decades to be observable on the tree diameter distribution due to the slow growth rate of ebony trees, we will sample an additional 400 ha plot located in an area with a longer history of hunting activities than in Kompia. We are presently considering the Mbalmayo forest reserve due to its strategic location between large cities (Mbalmayo, Yaoundé, Ebolowa, Sangmélina). The forest is heavily disturbed by human interventions, but several patches of mature forest still remains. Given our previous observations, we expect to find a lack of regeneration in such a forest.

It is well known that ebony trees are quite variable in terms of wood characteristics, in particular for the color pattern. In coordination with several of CRELICAM suppliers we are currently collecting data across Cameroon about the quality of wood from felled trees. The ultimate purpose is to document three points:

1. Describe how the fruits/leaves/wood-quality vary together and explore any geographical patterns,
2. Understand how these patterns could relate to limitation in gene flow.
3. Detect possible cryptic species and understand the biogeographical history of the species.

In 2019, the project will also begin testing the potential of the ebony project as a tool of reforestation using randomized block design, where randomly selected plots either receive fruit trees planting or do not. Ebony and other fruit crops will be tested, and we will record the effect on bird abundance and dispersal of forest seeds inside the experimental plots after several years.

DEPLOYING TECHNOLOGY TO INCREASE PRODUCTION

Project Accomplishments to Date

Seed germination

Seed germination experiments showed that seeds kept at ambient temperature and humidity start to lose their germination power 15 days after being removed from the fruits. The project also found that ebony seeds do not display dormancy and a germination rate above 95% could be obtained 10 days after a one day soaking in water.

Improving outcomes via cuttings

Trials involving juvenile cuttings via vegetative propagation techniques have been successful. We now confirm that juvenile cutting harvested from the stumps of so called “Plus or best” trees

by CRELICAM (quality of wood, color of wood) could be multiplied using vegetative propagation techniques. This was already observed in a paper published by Tsobeng *et al* in 2011. If the color and quality of wood observed in these plus trees are genetically controlled we will capture these traits through rooting system. The team plans to work with CRELICAM to collect cuttings from the stumps of their best trees in order to multiply them vegetatively. Because of long period taken by Ebony to mature (80-200 years) it will be important to multiply and plant well known materials.

Our work on clonal variation suggests that the success rate of vegetal propagation can vary significantly by clone. Of the four clones we collected from CRELICAM, harvest sites in Attsieck village in the East region of Cameroon, one rooted better (60%) than other three clones collected in the same village. The poorest rooting clone demonstrated no rooting over 16 weeks. Although preliminary results, it indicated that rooting ability could be influence by clonal material in Ebony (Figure 5).

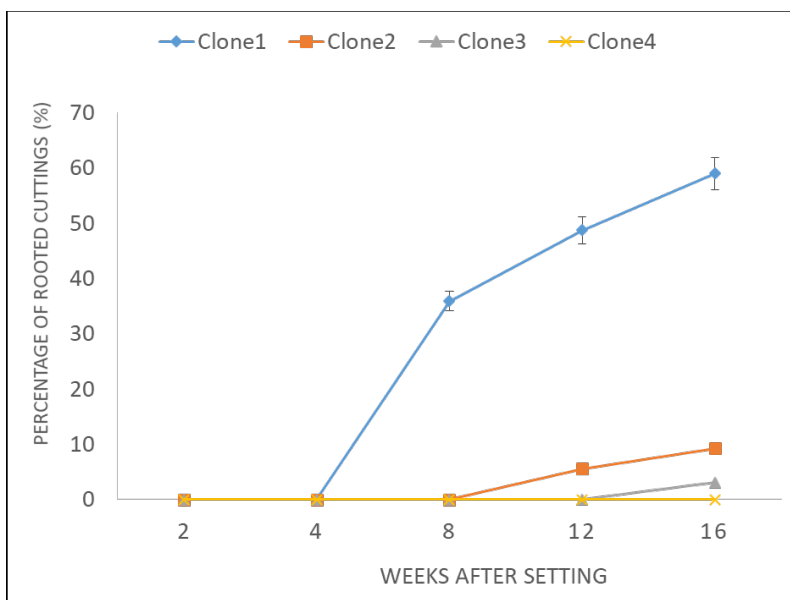


Figure: 5. Effects of clonal materials on rooting ability of Ebony leafy stem cutting.

Given the challenges and variability of producing ebony plants from seeds and cuttings, the project has also explored the possibility of employing tissue culture to generate ebony saplings. If successful, tissue culture would allow for the production of large numbers of plants at very low cost. The plants could be selected for beneficial traits, including wood quality, disease resistance, and growth rate. There are no models for hardwood production from tissue culture, so this aspect of the project has led to cutting-edge work. There are two possible tissue culture methodologies available: microcutting and somatic embryogenesis. The project is exploring both options in a quest for the optimal protocol for mass producing ebony plants at the lowest possible cost.

Microcutting

Microcutting consists of producing leafy rooted plants from meristems and nodes sampled on plants that grow in the nursery. It requires two steps—rooting and shoot production, which are achieved in different cultivation medium—and both must be successful to produce viable plants.

For shoot production, Zeatin, Adenine, Kinetin and BAP (benzyl adenine) hormones at different concentration were tested for bud burst and growth of explants sampled in the nursery. The best results were obtained with BAP which triggered bud burst on 90% of plantlets and the production of more than two shoots per explant. For rooting, we found that the mixture of phloroglucinol and IBA (indole-3-butyric acid) trigger rooting of up to 40% of the explants sampled in the nursery.

Preliminary efforts to combine these two steps have been successful. Micro cuttings first treated with Kinetin and BAP mediums to produce shoots, were then tested for root production using a mix of phloroglucinol and IBA. Rooting rates of up to 70% were achieved for plantlets using both hormones.

Somatic embryogenesis

Somatic embryogenesis consists of initiating callus (unorganized plant tissue) growth using vegetal hormones and inducing the production of an embryo (essentially a seed) from the callus. Leaf explants have produced mixed results in 2017. We are currently exploring the somatic embryogenesis using staminodes from female flowers and cotyledons of seeds collected in 2018.

Plans for 2019

The project will continue to explore three main avenues for producing ebony: germination from seeds, growth from cuttings, and production from tissue culture. In addition to producing the inputs needed for the project, this work will increasingly assess the feasibility of using these methods to produce a range of commercially and environmentally valuable species at a larger scale. We hope to parlay the methodological improvements pioneered by this project to support larger scale reforestation efforts.

Sapling growth and survival

Optimal saplings growth and survival is a key aspect for the project. The project will continue monitoring the plants already transplanted in the communities to achieve a better understanding of the determinant factors and assess the success of the project.

Cuttings

Initial testing suggests that it will be possible to multiply ebony trees with highly desirable traits such as quality or color of wood via cuttings if the characteristics are genetically controlled. In the coming phase, the project will investigate the ability to propagate clones of highly valuable trees. The project will also explore whether ebony saplings obtained from cuttings grow faster than plants raised from seedlings, as has been observed in high-value indigenous fruit trees.

Tissue Culture

The tissue culture efforts will focus on refining the microcutting protocol to produce healthy plantlets with roots and leafy stems and begin assessing the success rate of the acclimatization

to ex situ conditions. Our efforts on somatic embryogenesis will focus on the use of alternative tissues as a starting substrate.

Microcutting: The slow growth rate of the in vitro plantlets, in vitro and ex vitro, is limiting the usefulness of in vitro propagation. We will try to stimulate the elongation of the inter-nodes using gibberellins plant hormones.

The rooting of *in vitro* grown plantlets remain the most limiting step in our propagation protocol. Substantial improvement in rooting rate of difficult-to-propagate woody species have been reported by replacing the conventional fluorescent light source with a combination of single-wavelength LED lights. The sugar composition and concentration in the medium is also known to affect the rooting. We will test different combination of light source and sugar content of the medium. The effect of the growing medium used in the previous in vitro stages will be assessed as well.

Callogenesis: To explore the full range of possible alternatives for the successful generation of embryos from vegetative tissues, we will explore the production of callus from cotyledons (October 2018 onward) and from floral organs (March 2019 onward).

Acclimatization: The *in vitro* cultured plants resulting from microcutting are very tender and delicate owing to high humidity in the culture vessel, controlled temperatures, low light intensities and mixotrophic mode of propagation. *In vitro* plants lack protective mechanisms and are vulnerable to desiccation once exposed to ambient temperature, wind and solar radiation. The successful establishment of *in vitro* raised plants under *ex vitro* conditions is therefore a critical step in micropropagation techniques. As we have recently developed method to produce rooted *in vitro*-plants, we are now able to test different approaches for their acclimation to the field conditions. Different timing, substrates and fertilizers during hardening and acclimatization will be tested. The effect of the physical and chemical conditions used during the production of the plants will also be tested.

A VISION FOR EXPANSION

The Ebony Project model has garnered considerable interest from governmental and inter-governmental institutions raising questions about the possibilities for expanding the project into other geographies and land use settings. There is universal agreement within the team that this is possible. At its core, the project is simple and straightforward yet somewhat unique in its business-centric origins, step-wise approach and bottom-up community-based focus. Adapting the Ebony Project model, including substituting *Diospyros crassiflora* for another site-specific appropriate hardwood species, in a community, secondary forest or larger agroforestry setting should be achievable as long as the basic approach is followed.

Within Cameroon, the possibility of considerably expanding the Ebony Project is captured in the public-private-partnership (PPP) signed by Taylor Guitars and the Government of Cameroon in Bonn, Germany in November 2017. Facilitated by the World Bank, this PPP pledges Taylor to continue funding the project, the Government of Cameroon to commission a “Feasibility Study” on the potential expansion of the project, and a commitment to expand the project if it is deemed feasible.

A Feasibility Study examining the challenges and opportunities to scale up the exiting project will include:

- *Expanding the model:* An assessment of expanding the current model to additional participants and villages.
- *Examination of other tropical hardwood species:* Ebony is just one of many valuable African hardwood species that could be incorporated into community based agroforestry. For example, Bubinga (*Guibourtia arnoldiana*) and sapelli (*Entandrophragma cylindricum*) are two species of interest not only to Taylor Guitars but other commercial entities. The feasibility study will assess such species to better understand which are most appropriate in different geographies, and which provide enhanced intercropping values in different application.
- *Inclusion of commercial value chains:* The Ebony Project currently focuses on intercropping ebony with locally valuable fruit and medicine trees in a community setting. However, the region is also home to significant cocoa, coffee and oil palm industries which may themselves benefit in the long run from being co-cropped or grown in a less monocultural, more diverse shade environment. Accordingly, the feasibility study will examine key aspects of the Ebony Project to explore their applicability to other smallholder farmers, in some cases, a more industrial setting.
- *Expansion to other locations:* The current and planned project sites are all in Central Cameroon, but the Ebony Project model has the potential to operate throughout Central and West Africa, with different locations focusing on restoration of different hardwood species and different mix of local fruit and medicinal plants. As such, the feasibility study will identify and examine associated pertinent issues toward this aim.

ACKNOWLEDGEMENTS

The Ebony Project has benefited and is grateful for the help and support provided by many local and international partners. First of which are the participants to the projects in the communities of Ekombité and Somalomo. The project is grateful to the MINFOF conservation staff of the Dja Biosphere Reserve for their support on the ecological work undertaken in the Bouamir research station. Through a fruitful collaboration with the National Forestry School Mbalmayo, the project was able to develop a long-term research protocol and to train a student in the arboretum of the school and in the Dja area. The development and implementation of ambitious research protocols in the Dja was also greatly facilitated by our partners: Pof. B. Sonké of the Ecole Normale Supérieure in Yaoundé; O. Hardy of the Université Libre de Bruxelles (Belgium) and Prof. Jean Louis Doucet of Université de Liège (Belgium). Crucial data about the ecology of ebony were also obtained from Kew Gardens curators (UK), Steven Janssens of Botanic Garden Meise (Belgium), Sylvie Gourlet-Fleury from CIRAD (France), Nicolas Barbier and Gilles Dauby from IRD (France). The project is grateful to David Roubik from the Smithsonian Tropical Research Institute for his kind help on identifying the insects visiting ebony flowers. Thanks to the help of Crelicam and its wood suppliers, important ecological data were, and still are, obtained from all their felling sites. Finally, we would like to thank Bob Taylor, without whose support this project would not exist.

APPENDIX A

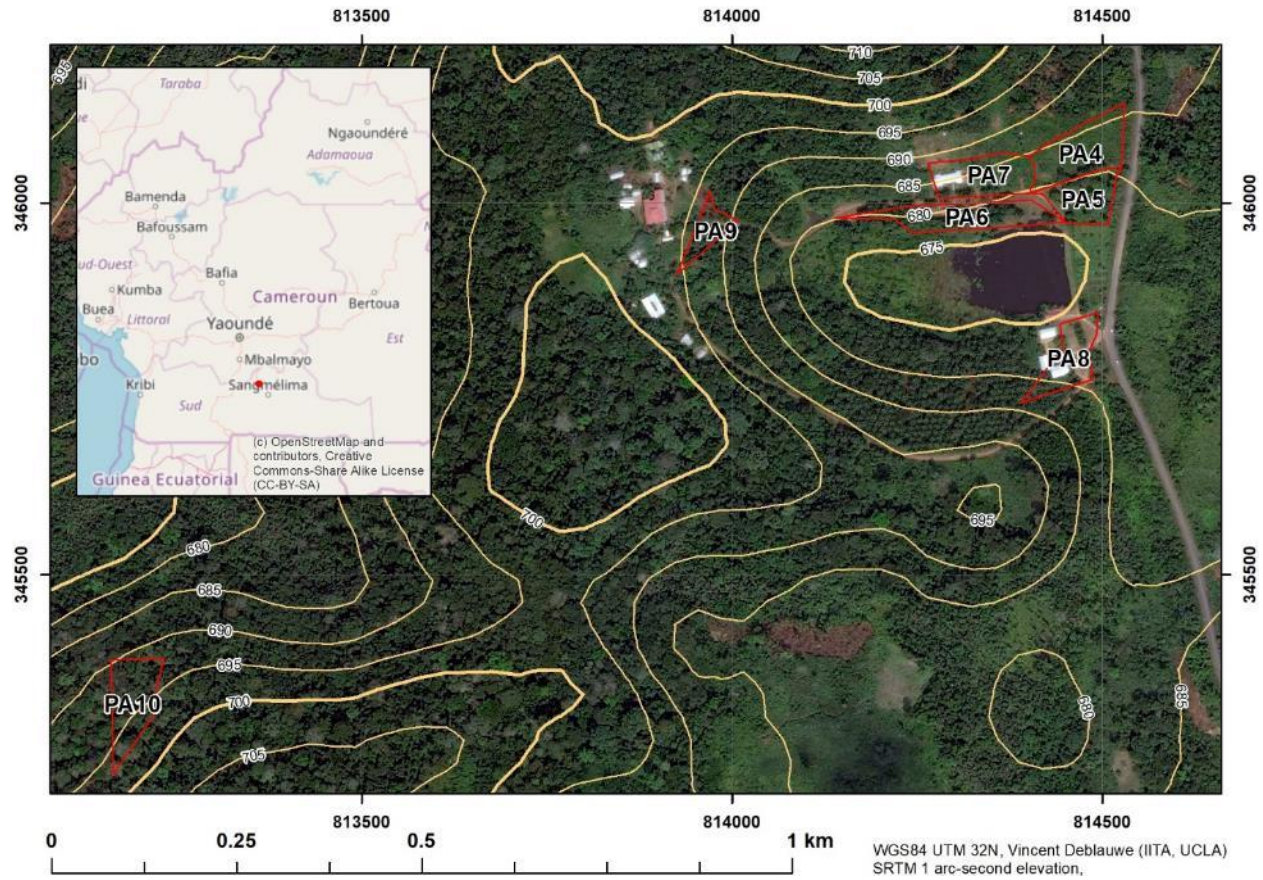


Figure A.1. Map of the ebony and other trees planted in Ekombité community. Plantations sites are represented as red polygons numbered from PA4 to PA8.

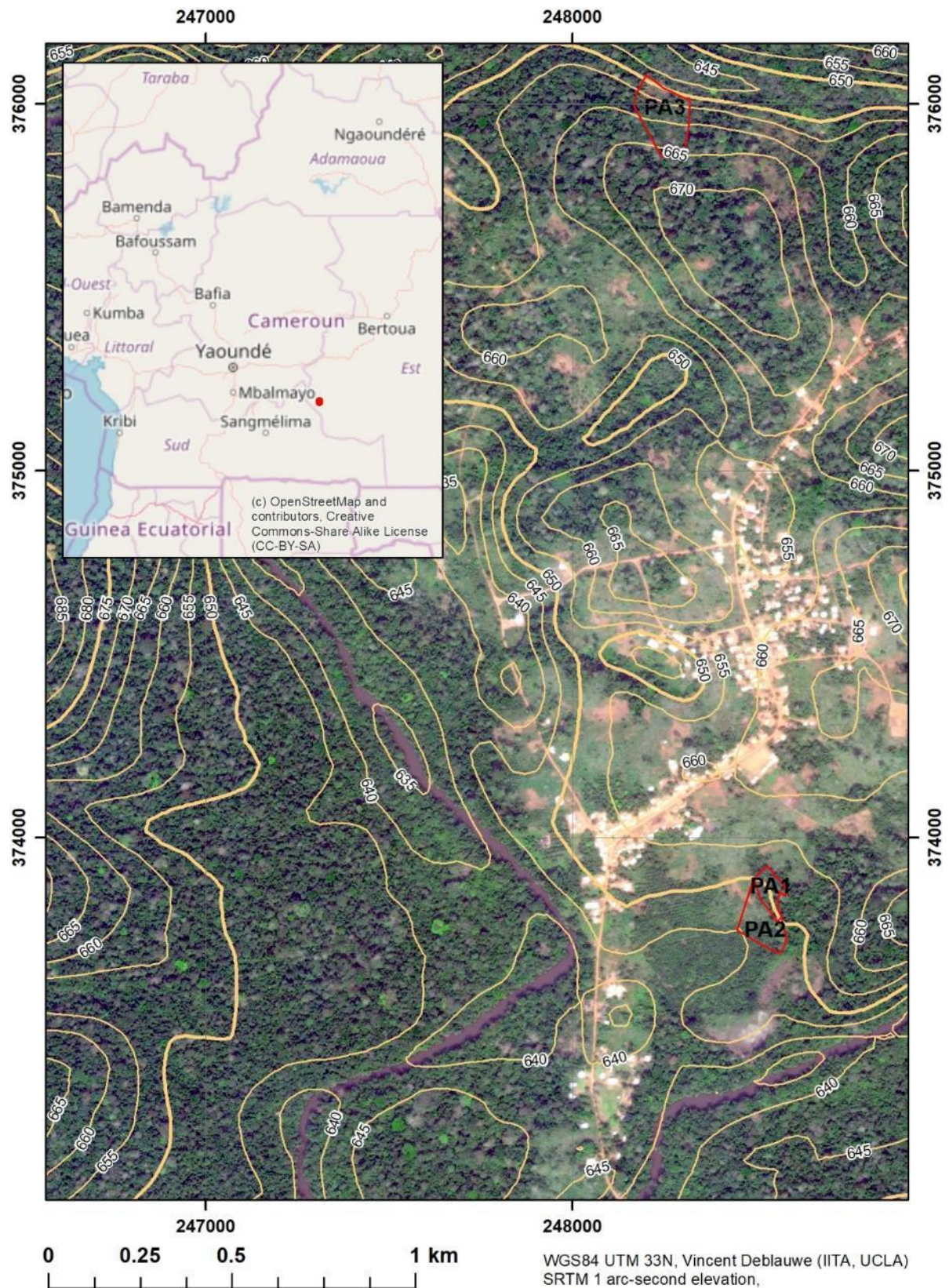


Figure A.2. Map of the ebony and other trees planted in Somalomo community. Plantations sites are represented as red polygons numbered from PA1 to PA3.

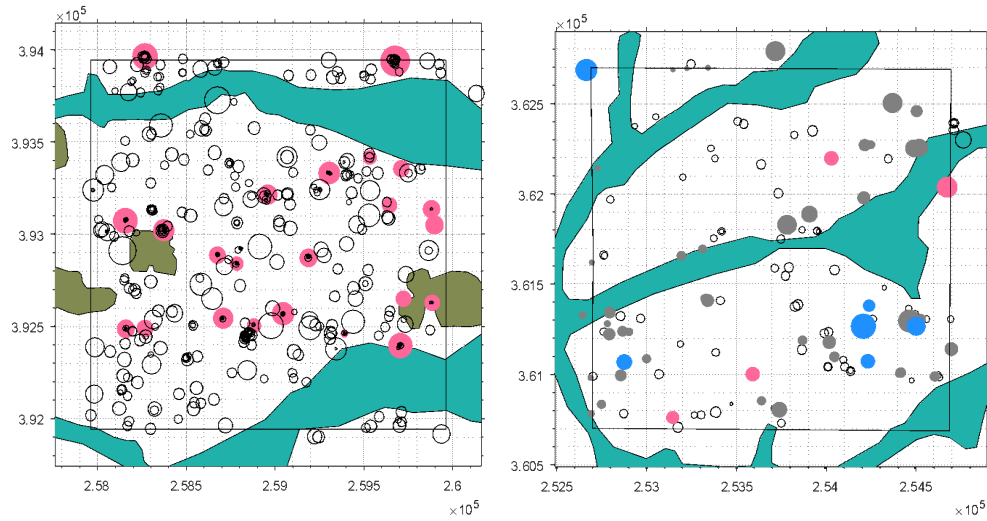


Figure A.3. The permanent 400 ha ebony census established in Kompia (left) and the protected area of the Dja Faunal Reserve (right). Circle size are relative to diameter at breast height. Colors indicate the sex of the tree: female (pink), male (blue), not flowering (gray) and unknown (open circle). Swamps and farmlands are represented as cyan and green polygons respectively. Coordinates and projection system are UTM 33 N, WGS 1984.

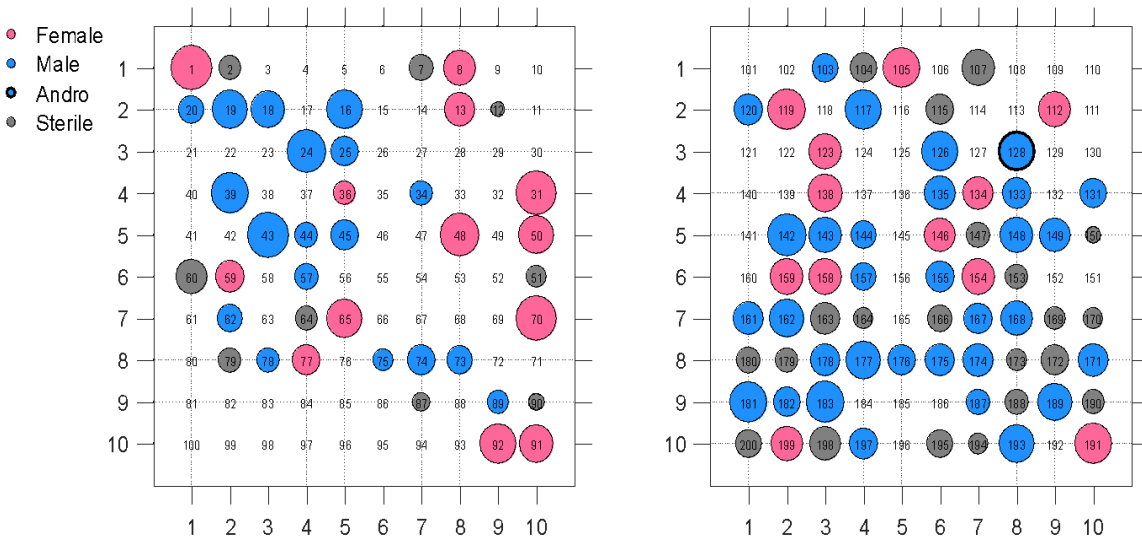


Figure A.4. Map of the two ebony plantations followed for reproductive and vegetative phenology at ENEF, Mbalmayo. The two plots were established around 2003 on a 2.5 m spacing square grid. Disc size is proportional to the tree diameter at breast height. The largest tree (slot 43) is 15.3 cm in diameter. The color indicate the sex of trees following flower observation in April 2018. Andro stands for androgynodioecious. Empty slots correspond to missing trees.

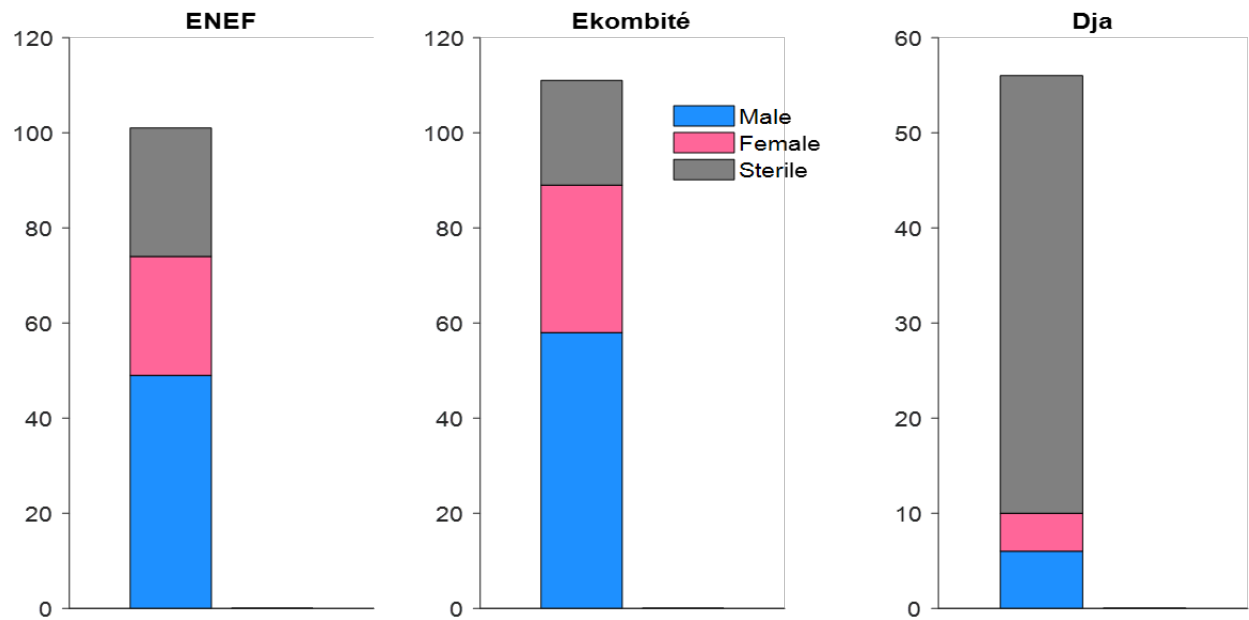


Figure A.5. Number of staminate (male) and pistillate (female) flowering individuals in each study site. No flowers were observed in 2018 on 'sterile' trees.

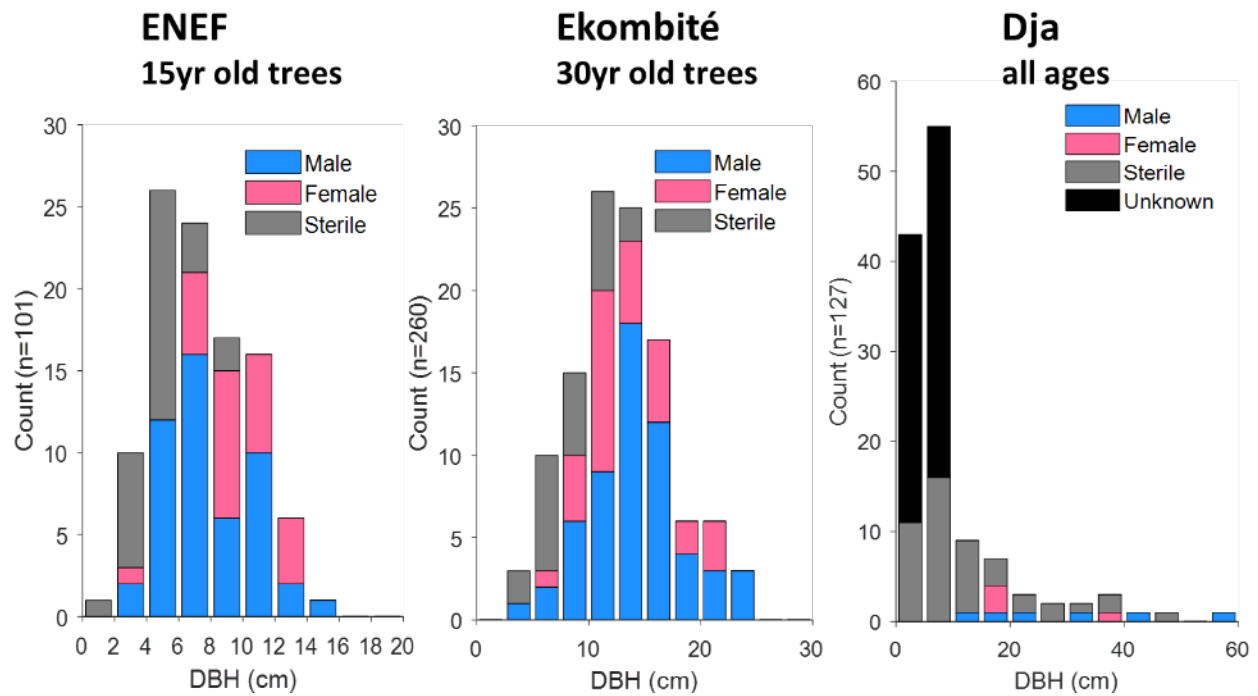


Figure A.6. Number of staminate (male) and pistillate (female) flowering individuals as a function of tree diameter at breast height (DBH). No flowers were observed in 2018 on 'sterile' trees.

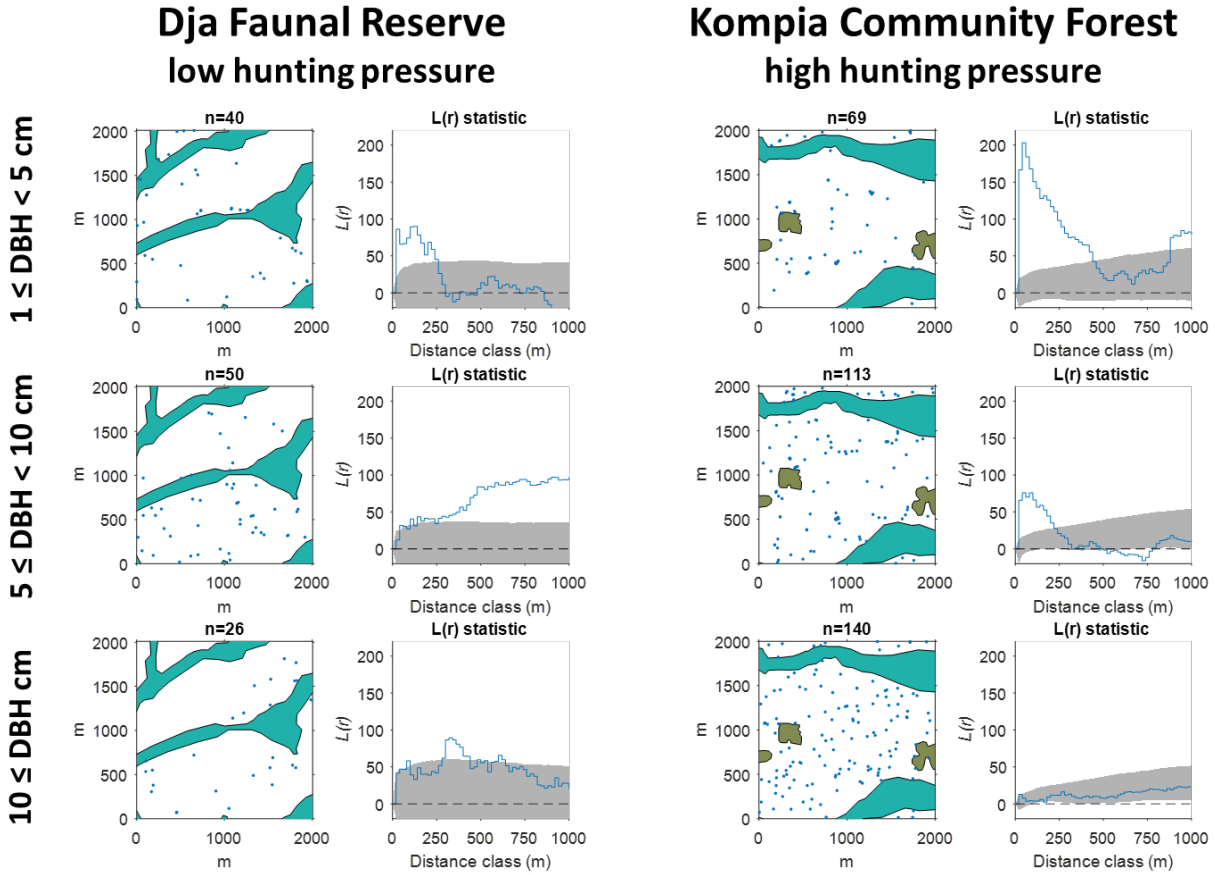


Figure A.7. Spatial clustering of the ebony saplings as a result of bushmeat hunting pressure. In Kompia community forest, saplings show peak clustering at 40–60 meters and clustering decrease with age/diameter. In the Dja faunal reserve, sapling clustering is relatively weaker and peak at 120–140 meters. Magnitude of clustering and over-dispersion as a function of the diameter at breast height (DBH) is show by distance class (Ripley's L statistic). Positive and negative values correspond to clustering and over-dispersion respectively. The confidence interval at 5% for a complete spatial randomness was computed through the simulation of 1000 Poisson processes excluding swamps (cyan) and farmlands (kaki) and is shown as gray area.

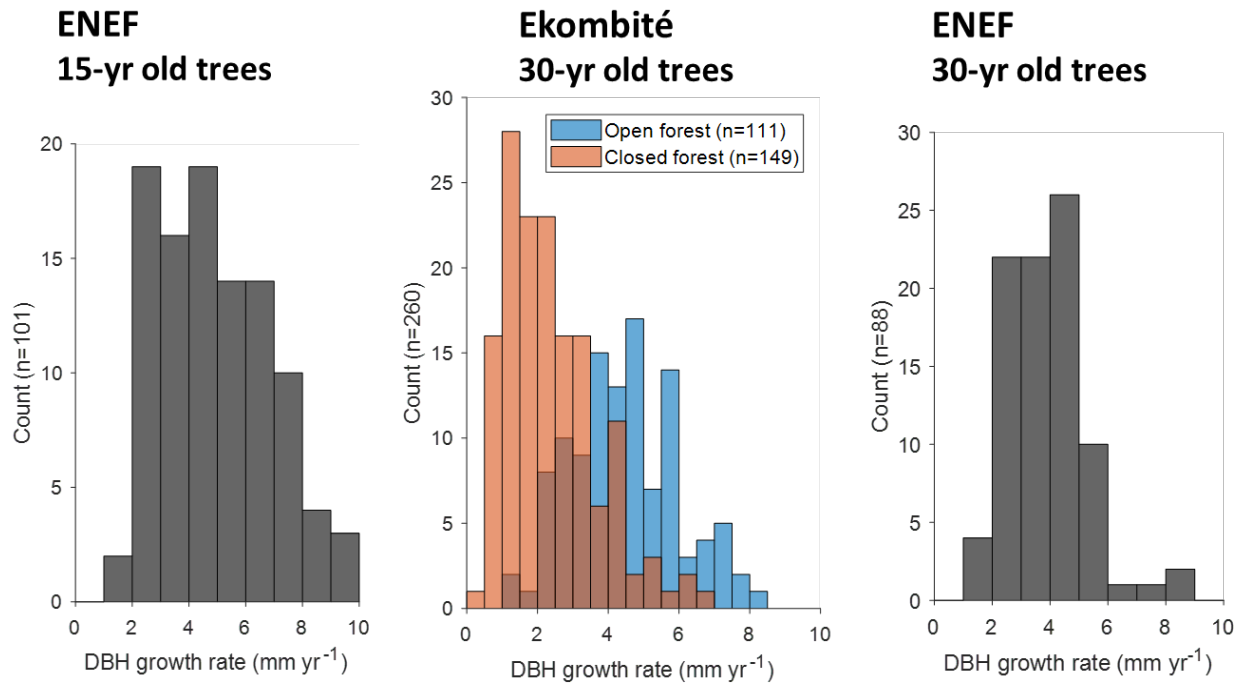


Figure A.8. Average growth rate inferred from the measurement of diameter of planted trees of known age. The closed forest condition in Ekombité is a mature secondary forests.



Figure A.9. The camera developed during the project to record movies of insect pollinating the flowers. The camera is mounted on the tree, focusing on a flower from before its anthesis until its dead. Image by V. Deblauwe.

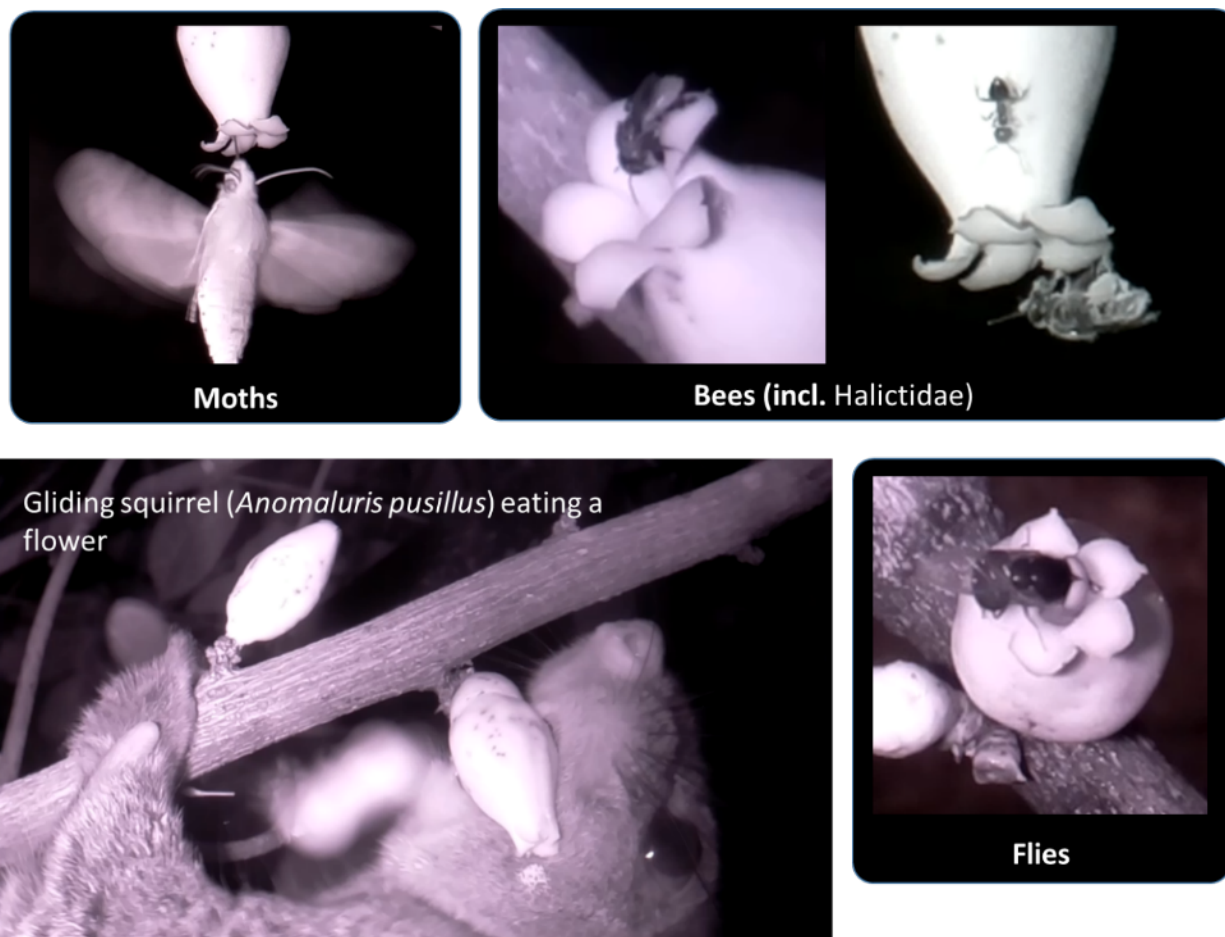


Figure A.10. Few selected images of animal interactions with ebony flowers recorded with our cameras in the ebony trees planted in ENEF, Mbalmayo, arboretum. Images by V. Deblauwe.

REFERENCES

- Dauby, G., Zaiss, R., Blach-Overgaard, A., Catarino, L., Damen, T., Deblauwe, V. *et al.* (2016). RAINBIO: a mega-database of tropical African vascular plants distributions. *PhytoKeys*, 74.
- Fayolle, A., Engelbrecht, B., Freycon, V., Mortier, F., Swaine, M., Rejou-Mechain, M. *et al.* (2012). Geological Substrates Shape Tree Species and Trait Distributions in African Moist Forests. *Plos One*, 7.
- Gourlet-Fleury, S., Rossi, V., Rejou-Mechain, M., Freycon, V., Fayolle, A., Saint-Andre, L. *et al.* (2011). Environmental filtering of dense-wooded species controls above-ground biomass stored in African moist forests. *J. Ecol.*, 99, 981-990.
- Owona Ndongo, P.-A. (2009). Plantations de bois d'oeuvre en zone équatoriale africaine : cas de l'arboretum de l'Enef de Mbalmayo au sud du Cameroun. *Bois et Forêts des Tropiques*, 299, 37-48.

- Rosin, C. & Poulsen, J.R. (2017). Telemetric tracking of scatterhoarding and seed fate in a Central African forest. *Biotropica*, 49, 170-176.
- Slik, J.W.F., Arroyo-Rodriguez, V., Aiba, S., Alvarez-Loayza, P., Alves, L.F., Ashton, P. *et al.* (2015). An estimate of the number of tropical tree species. *P Natl Acad Sci USA*, 112, 7472-7477.
- Tsobeng, A., Tchoundjeu, Z., Kouodiekong, L. & Asaah, E. (2011). Effective propagation of *Diospyros crassiflora* (Hiern) using twig cuttings. *International Journal of Biosciences*, 1, 109-117.
- Worbes, M., Staschel, R., Roloff, A. & Junk, W.J. (2003). Tree ring analysis reveals age structure, dynamics and wood production of a natural forest stand in Cameroon. *For. Ecol. Manage.*, 173, 105-123.
- Poulsen, J.R., Clark, C.J. & Smith, T.B. (2001). Seed dispersal by a diurnal primate community in the Dja Reserve, Cameroon. *J. Trop. Ecol.*, 17, 787-808.