Sustainable Ebony Project:

Developing an Integrative Program for Sustainable Production and Harvesting

September 2017

EXECUTIVE SUMMARY

Despite its recognition, the African Ebony (*Diospyros crassiflora* Hiern) remains poorly understood. There is no sustainable agroforestry or plantation-based production of *D. crassiflora* in Central and West Africa. Approaches for production exist, but the essential basic ecology and life history characteristics such as phenology, pollinators, seed dispersers, and environmental requirements for optimal growth of *D. crassiflora* remain largely unknown. Answering these questions for ebony are key to developing a sustainable value chain for ebony, and may pave the way for larger restoration and sustainable forest management.

Project Goals

The Sustainable Ebony Project enhances *D. crassiflora* stocks through community-driven planting efforts while collecting crucial data to (1) identify key threats to the long-term sustainability of the species in its natural setting and (2) refine techniques for sustainable production and management of the species in Central Africa. The Ebony Project is a pilot for larger scale restoration initiatives and public-private partnerships and has four objectives:

1. Create a scalable program for the sustainable production and stewardship of ebony seedlings in rural areas
2. Model West African ebony distribution to identify a sustainable harvesting rate and appropriate planting areas
3. Understand the basic ecology of ebony necessary to enhance natural reproduction and dispersal, and test restoration approaches to determine the most successful methods
4. Test alternative propagation approaches, including tissue culture, to identify optimal conditions for cultivating ebony

The Sustainable Ebony Project was launched in 2016 with financial support from 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, Bouamir Research Camp in the Dja Biosphere Reserve and communities in the natural range of the species.

1. Create a scalable program for the sustainable production and stewardship of ebony seedlings in rural areas.

2. Model ebony distributions to identify suitable harvesting and planting areas.

We used a maximum entropy modelling approach to develop a map of suitable locations for *D. crassiflora* based on (1) locations known to have ebony, (2) climate data, and (3) ecological data. The area environmentally suitable for *D. crassiflora* covers 1.36 million square kilometers. However, in 2000, 14% of this area comprised degraded forests or cropland (Mayaux *et al.* 2004), leaving 1.17 million square kilometers of intact forests where *D. crassiflora* are likely to be found (Figure 1). Based on this model and existing forest inventories, we estimate there are 30.9 million ebony trees with trunk diameter at breast height (DBH) above 10 cm at the regional level. These numbers should be taken with caution as our current knowledge of *D. crassiflora* density and distribution is only based on a limited and geographically biased number of observations.

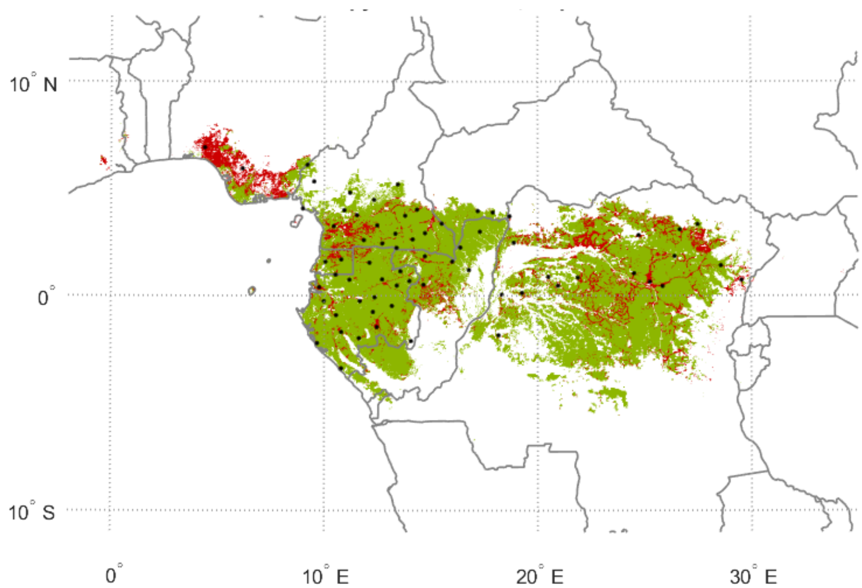
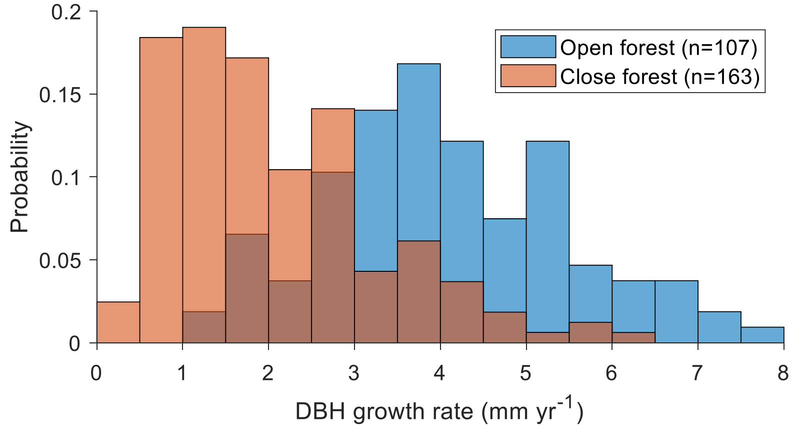


Figure 1. Binary map showing intact (green) and degraded (red) environmentally suitable area for *D. crassiflora*. Black dots represent 68 known localities used for model training.

3. Understand the basic ecology of *D. crassiflora* and test restoration approaches

Our research shows trees in a plantation that was not maintained come close to the growth rate reported for trees in natural stands. The improved access to sunlight in plantations is probably the main reason why growth rate is twice that of individuals in natural forests. Depending on the growing conditions, 0.4-0.8 cm yr^{-1} seems to be a consistent maximum rate of growth. In the forest, however, these optimal conditions are rarely encountered and most trees would grow extremely slowly under shade. The median growth rate in the closed forest of the Ekombite plantation, i.e. the conditions that best match with natural forests, was 0.18 cm yr^{-1}.

Figure 2. Distribution of diameter at breast height (DBH) annual growth rate between 1988 and August 2017 of *D. crassiflora* in Ekombitié agro-forest



3. Test alternative production technologies

To date, the project has been successful in identifying three new approaches to produce ebony saplings. Using micropropogation we achieved a germination rate of over 80% using nutrient jelly. Initial callogenisis experiments have also been successful, although further work is necessary. Finally, microcutting produced an average of 5.6 new twigs with critical leafy stems present.

PROGRESS REPORT

Background

Ebony is a generic name referring to black, hard, dense, fine grained wood sourced from several species of the tropics. These species belong to the Ebenaceae family and most of them to the Diospyros genus. *Diospyros crassiflora* Hiern (Fig. 1) was, and still is, the main source of African ebony wood. Despite its recognition, the species remains poorly understood. There is no sustainable agroforestry or plantation-based production of *D. crassiflora* currently being implemented in Central and West Africa, creating concerns both from industrial supply and ecological perspectives. Approaches for production exist, but the essential basic ecology and life history characteristics such as phenology, pollinators, seed dispersers, and environmental requirements for optimal growth of *D. crassiflora* remain largely unknown. Thus, there is an urgent need for implementing production models while testing and developing approaches that result in long-term sustainable management.

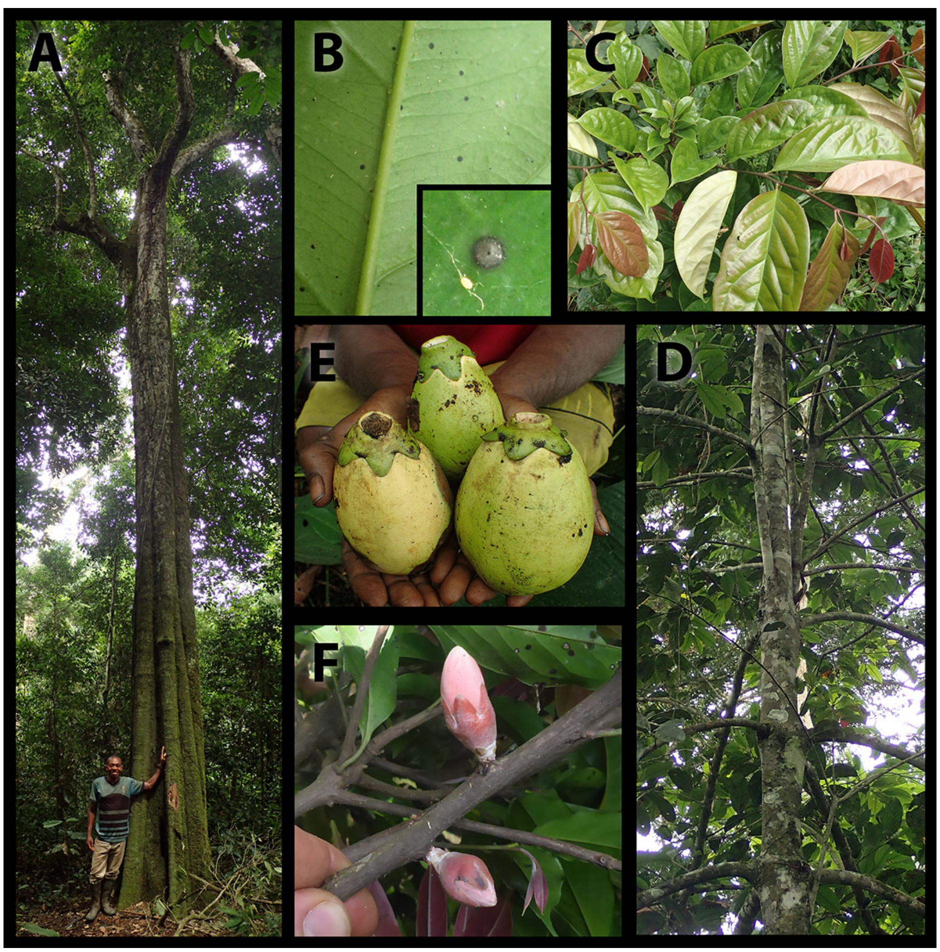


Figure 1. Several characteristic features of Diospyros crassiflora. (A). a large specimen 110 cm in diameter with fluted base; (B). abaxial surfaces of leaves are scattered with extrafloral nectaries as in most *Diospyros*; (C). young leaves have a characteristic red color, the branches are verticillate, usually by five, with much reduced leaves on the main stem; (D), growth architecture consist of successive whorls of horizontal branches on the main stem according to Massart's model (Hallé *et al.* 1978) with regular reiterations of the model (i.e. the vertical regrowth); (E). ripe fruits are obovate and yellow; (F). flowers are pink and up to 3cm long (♀in this case). Photos (A-E) Vincent Deblauwe (CBI), (F) courtesy of Nicholas Koch (Forest Solutions Inc).

Project Goals

The goal of the project is to enhance *D. crassiflora* stocks through community-driven planting efforts while collecting crucial data to identify key threats to the long-term sustainability of the species in its natural setting and refine techniques for sustainable production and management of the species in Central Africa. Finally, the Ebony Project hopes to serve as a de facto pilot project for future, larger scale restoration initiatives and public-private partnerships.

To achieve this goal, four integrated components with particular objectives are included in the project:

1. Create a scalable program for the sustainable production and stewardship of ebony seedlings in rural areas
2. Model West African ebony distribution to identify a sustainable harvesting rate and appropriate planting areas
3. Understand the basic ecology of ebony, including identification of pollinators and dispersers necessary to enhance natural reproduction and dispersal; test alternative restoration approaches to determine the most successful methods
4. Test alternative propagation approaches, including tissue culture, to identify optimal conditions for cultivating ebony toward improving the success of planting

Project implementation

The Sustainable Ebony Project was launched in 2016 with personal financial support from Bob Taylor of Taylor Guitars. Taylor Guitars is 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, Bouamir Research Camp in the Dja Biosphere Reserve and communities in the natural range of the species.

The project is coordinated at the Congo Basin Institute (CBI) in Yaoundé and implemented by CBI partners, including: 1) UCLA's Center for Tropical Research (CTR, Los Angeles), Institute of Environment and Sustainability, directed by Dr. Thomas B. Smith, 2) the International Bilingual Academy/High Institute of Environmental Science (IBAY SUP, Yaoundé), directed by Dr. Zachary Tchoundjeu, and 3) the International Institute of Tropical Agriculture – Cameroon (IITA, Yaoundé), directed by Dr. Rachid Hanna. Taylor Guitars remains the project's funder and also plays and advisory role.

Project components

1. Create a scalable program for the sustainable production and stewardship of ebony seedlings in rural areas.

Participating communities and CRELICAM staff are trained in vegetative propagation, multiplication, and management of *D. crassiflora* seedlings in rural areas, leveraging existing relationships with rural communities in Cameroon. The materials for construction and propagation are provided to community's members at no cost for them. Plants are given to communities at no expense and communities members will be trained to produce saplings from vegetal material available in the area and to care for and multiply plants in newly established propagation boxes and community nurseries. When ready, participating communities will plant tree saplings in their agro-forests and fields and will be paid for successful stewardship of surviving ebony trees at fixed intervals over several years until such a time when the plants are capable of surviving on their own. Ultimately, once the trees reach maturity they would be available for harvest and timber could be sold ebony mills in Cameroon, such as CRELICAM. This said, it is understood that *D. crassiflora* will not reach maturity in our lifetime.

Participating communities will also be trained to multiply fruits (e.g. *Irvingia gabonensis, Dacryodes edulis*), spices (e.g. *Ricinodendron heudelotii*) timber (e.g. *Baillonella toxisperma*) or medicinal trees (e.g. *Mondia whitei*) they use to collect in the wild, usually at long distance from the place where they live. These species will be identified by the communities themselves and incorporated in the same multiplication framework as ebony trees. Thus, in addition to ebony, these tree species will be produced and integrated in their different land-use systems. While ebony will bring early cash benefits via seedling stewardship rewards, the fruit and medicinal products of other provided species will become available for local consumption and trade prior to ebony maturation.

Deliverables:

- At least 15,000 trees of improved ebony planted and well maintained in community sites;
- At least 200 individual community participants trained to integrate the produced germplasm in their different cropping systems and manage/maintain the planted trees;
- High-value timber, fruit and medicinal species being now planted in community's agro-forest and fields instead of being sourced from pristine forests;
- Instructional nursery on CRELICAM premises and training of CRELICAM personnel in participatory tree domestication techniques using the best vegetative propagation techniques (rooting juvenile cuttings, grafting, marcotting and good botanical practices);
- Propagation plan that can be implemented to support local communities economically while providing for the sustainable production of ebony; and a steady supply of food and medicine;
- A Sylvicultural Booklet of West African Ebony (SB) that will provide guidelines that can be used by farmers and foresters;

- Forum for exchange of information among local farmers, and between farmers and other stakeholders.

2. Model West African ebony distributions to identify a sustainable harvesting rate and suitable planting areas.

A postdoctoral researcher, Dr. Vincent Deblauwe, was appointed to construct robust ecological niche maps of *D. crassiflora* in Cameroon via a three stage-modeling project. First, Global Positioning System (GPS) data collected by CRELICAM, CTR, and herbarium collections were collated and assembled. Second, Geographic Information Systems (GIS) and remote sensing was used to identify environmental variables that are significantly correlated with known populations in Cameroon. These correlations will be utilized to develop a map of ecological suitability for *D. crassiflora* across the country, including sites that have been previously visited by Taylor Guitar/CRELICAM staff or reported by botanists, as well as new sites that have not been inventoried yet, but are likely to harbor previously undiscovered stands or be suitable areas for community-based planting. Maps will include forest management units and protected areas and will indicate accessibility to enhance identification of areas suitable for inventory or planting. In the third and final stage, the map will be validated and refined through fieldwork to ground truth the predictions, and following these confirmatory efforts, the predictive models will be used to orient planting activities and be available to CRELICAM for use in prospecting, harvesting and management.

3. Understand the basic ecology of ebony, including identification of pollinators and dispersers necessary to enhance natural reproduction and dispersal; test alternative restoration approaches to determine the most successful methods.

There is a need to better understand the distribution, abundance, dynamics, and basic ecology of *D. crassiflora* to enhance enrichment/reforestation efforts. For example, its pollinators and dispersers are currently unknown. With this information, it could be possible to enhance fruit dispersal naturally or to identify areas where dispersers have disappeared and intervention for dispersal/replanting is required.

Dr. Deblauwe, is conducting ecological studies focusing on phenology, species interactions, dispersal, seed germination, seedling survival, and regeneration and will be integrated into a distribution model, which will subsequently be used to identify important areas for planting, regeneration, and future monitoring.

Deliverables:

- A thorough understanding of the ecology and distribution (including pollinators and dispersers) of *D. crassiflora*;
- Acquiring essential data that can be leveraged for increasing production and promoting natural processes that maintain and conserve the species.

4. Test alternative production approaches, including tissue culture laboratory experiments, to identify optimal conditions for cultivating ebony.

The goal of the ebony project is to build concrete foundations for the next step: to achieve a sustainable model of ebony production as well as implementing a scalable restoration approach. However, producing hundreds of thousands of saplings of a slow growing tree species from few selected source plant material is challenging. Therefore, while undertaking conventional nursery production of *D. crassiflora* via seed germination and rooting leafy stem cuttings, we are also identifying new techniques to increase the production of healthy seedlings from a single selected plant source at low cost. Despite the scarcity of feasibility studies done on slow growing tropical trees, tissue culture multiplication was identified as the best way to achieve this goal. Tissue culture experiments are now being conducted at IITA by Alvine Tchouga, a PhD student, under the supervision of Rachid Hanna, Nicolas Niemenak (University of Yaounde I) and Giovanni Forgione (Consultant, IITA).

The main objective is to identify optimal conditions for cultivation and implement bioreactors, a tissue culture technology to reproduce *D. crassiflora* rapidly without having to wait decades for trees to mature to provide culture material. The material thus produced is free of germs and contains the exact genetic quality of the original material. These tissue culture saplings will be used in planting trials in instructional nurseries on the CRELICAM premises and community nurseries in rural villages. These nurseries will be used to refine different production techniques and to train community members in germination and rooting of cuttings.

Deliverable:

- Tissue culture experiments to identify optimal conditions for cultivating *D. crassiflora* efficiently and maximize production;

Results

1. Create a scalable program for the sustainable production and stewardship of ebony seedlings in rural areas.

1.1. To set-up an instructional nursery in Crelicam and train its personnel on rooting of cuttings

Eight Crelicam employees were trained on building non-mist-propagators and humidity chambers, and rooting of leafy stem cuttings. Eight non-mist propagators were set-up. Cuttings from timber species (*Diospyros classiflora* or ebony, *Triplochiton scleroxylon* or ayous, *Pterocarpus soyauxii* or padauk, *Baillonella toxisperma* or moabi and *Guibourtia demeusei* or bubinga), fruit species (*Irvingia gabonensis* or bush mango) and vegetable (*Gnetum africanum* or eru) were set-up. So far, respectively 300 and 30 rooted cuttings of ebony and padauk, have been transplanted in plastic pots and are under acclimatization.

1.2. To train at least 200 local farmers on production and integration ebony in different cropping systems

For this objective, with the appreciable contribution of Crelicam, two communities were targeted: Ekombite (Dja-et-Lobo, South Cameroon) and Somalomo, Haut-Nyong, East, Cameroun.

On April 12th, 2017, an agreement was signed between two representatives of Ekombite, Dja-et-Lobo, South Cameroon, community and Crelicam/IBAY. Soon after, Two MASTERS students from the Higher Institute of Environmental Science (IBAY), Eloumou Donald Nazer and Tagne Mambou marc-Aurel, with the help of Mr Thaddee Sado, researcher, implemented the construction of the shade house (4m x 6 m) and a non-mist propagator (3m x 1m) in the community. Since then, the evaluation and monitoring of the acclimatization of rooted cuttings has been done monthly.

To diversify the sources of income and food of the beneficiaries, priority species to plant in addition to D. crassiflora were defined with the community. They are *Irvingia gabonensis* (bush mango), *Dacryodes edulis* (safu), *Gnetum africanum* (eru), *Triplochiton scleroxylon* (ayous), *Persea Americana* (avocado) and *Garcinia kola* (bitter kola). At the time of writing, about 300 cuttings of ebony, 200 seeds of bush mango and 150 avocados, 35 cuttings of ayous and 4 cuttings of safu have been nursed.

20 members of the community were trained on vegetative propagation techniques:

- Preparation of the non-mist propagator and composition of the most suitable substrate;
- Collection and preparation of leafy stem cuttings to be introduced inside the non-mist propagator;
- Preparation of seeds for germination.

Figure 2. Training of propagation techniques in Ekombite, May 2017. Ekombite community representative, IBAY students and researchers, and Crelicam staff were present during the event.

2. Model West African ebony distributions to identify suitable harvesting and planting areas.

The distribution of *Diospyros crassiflora* Hiern, as it is currently circumscribed, was classified by White (1978) as "Eastern wide", i.e. a relatively widespread species from Guineo-Congolian region excluding Upper Guinea. It is found in South Nigeria, Cameroon, RCA, Gabon, Congo and RDC. In its Congolian extension, it is found in a relatively narrow band between the equator and 4°N to the eastern rim of the Congo basin. It appears to be absent from the wettest forests (Northern Gabon and Equatorial Guinea) or at least is very rare there. It is not a characteristic species of the semi-evergreen forest but occurs in islands of evergreen forest within the latter (White, 1978).

We collated geographical coordinates of known populations of *D. crassiflora* from different sources: (i) Data from Crelicam and other private timber companies; (ii) herbarium samples from our own collections and the Rainbio dataset, a comprehensive database of georeferenced records of vascular plants (Dauby *et al.* 2016); (iii) tree inventory data from old-growth forest without signs of recent human disturbance collated by Gilles Dauby and Ferry Slick (Slik *et al.* 2015) and sampled in the Dja Reserve by ourselves. The herbarium data acquired from several herbaria institutes (BR, BRLU, K, LISC, MO, P, and WAG (incl. AMD, L & U as well)) goes back to the 19th century, include trees of all diameter and give the highest possible level of identification accuracy because voucher specimens are freely available. 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 distribution of known occurrences show that the species thrives in most of the eco-regions of the rainforest biome of Central Africa, from see level up to 1000 m in elevation (Fig. 3).

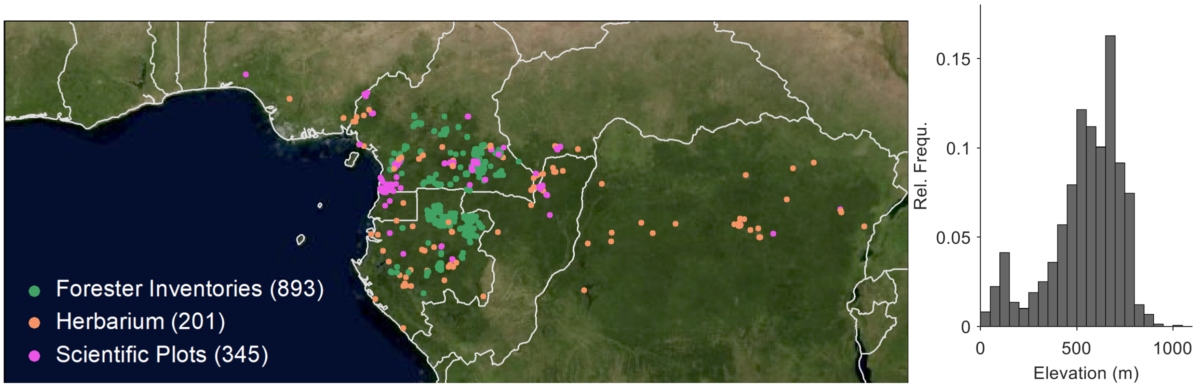


Figure 3. (Left) Geographical distribution of known localities of Diospyros crassiflora. Color of dots indicate the origin of data. Counts are shown in brackets. (Right) Distribution of elevation a.s.l.

We used a maximum entropy (MaxEnt software version 3.3.3k) modelling approach to delineate the environmental envelope encompassing all known localities. MaxEnt is a machine-learning algorithm (Phillips *et al.* 2006) that has been shown to be particularly efficient for modelling species distributions (Elith *et al.* 2006). It is a nonparametric method offering a good trade-off between interpretability and ability to account for complex response curves. The algorithm iteratively computes the probability distribution of the response that maximizes entropy (i.e. is closest to a uniform distribution) while meeting the constraint that the expected value of each environmental predictor (or its transform) under this estimated distribution matches its empirical average (Phillips *et al.* 2006).

Pedological factors are not available at an adequate resolution and were thus not considered here although they might actually influence survival and reproduction. Given the scarce coverage of weather stations in Central Africa, we used climatic data derived from remote sensing observations, namely MODIS for temperature and cloud cover and CHIRPS for rainfall (Deblauwe *et al.* 2016). The selected variables were average cloud frequency (Wilson & Jetz 2016), annual mean radiation (W m^{-2}) (Kriticos *et al.* 2012) climatic water balance, annual mean temperature, temperature seasonality, minimum temperature of the coldest month and seasonality of precipitation (Deblauwe *et al.* 2016). The spatial resolution of the analysis is determined by that of the coarsest data set, i.e. the MODIS data, with a 0.05-degree pixel size (ca. 5 km on the equator). All other layers were upscaled (via averaging) to match this resolution. At this resolution, we found 488 grid cells with at least one reported *D. crassiflora*, i.e. unique occurrences.

The strong sampling bias in favor of Cameroon and Gabon, visible in Fig. 3., was decreased by randomly selecting locations at least 0.6 degrees (ca. 70 km) apart. The 68 locations thus selected are visible as black dots on the suitability map in Fig. 4. The area of analysis considered in this study is the convex hull around all known localities extended further by a 5 degrees buffer zone.

This delimitation avoids including areas that might be suitable for *D. crassiflora* but are inaccessible to the species due to dispersal limitation.

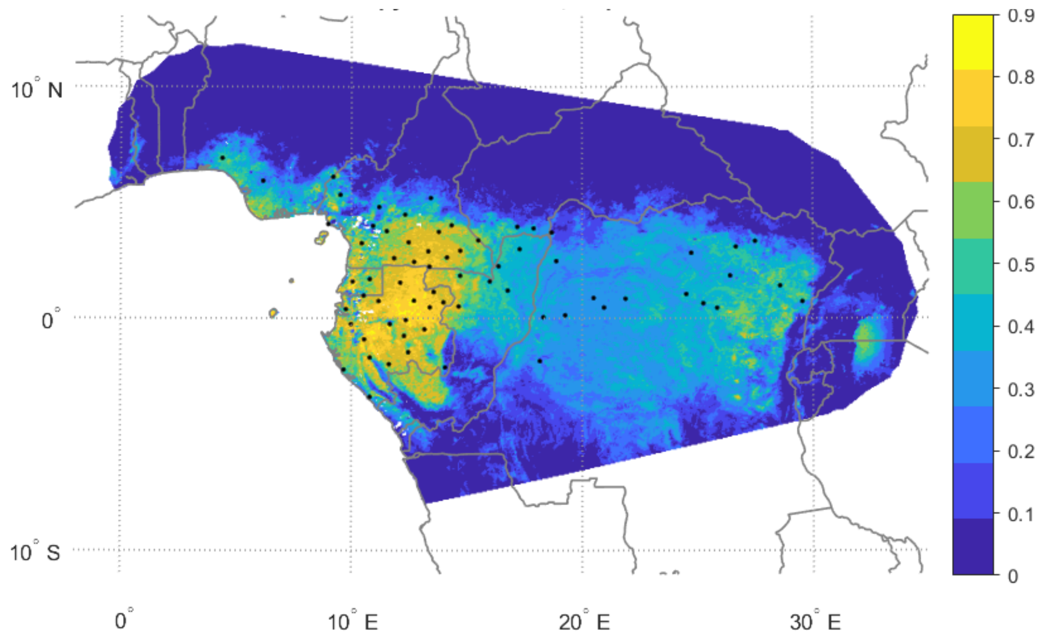


Figure 4. *D. crassiflora* suitability map built using MaxEnt algorithm. Black dots represent 68 known localities used for model training.

The suitability values were then converted into binary suitable/unsuitable values using the maximum training sensitivity plus specificity threshold as recommended by Liu *et al.* (2013). The species is shade tolerant (Bongjoh & Nsangou 2001) and only occurs in mature evergreen to semi evergreen forests (Letouzey & White 1970). Therefore we removed from the area predicted to be climatically suitable, all cells corresponding to degraded forests, flooded forests and montane forests in the land use map of Mayaux *et al.* (2004). The final map shows the intact and degraded forests that are climatically suitable D. crassiflora (Fig. 5).

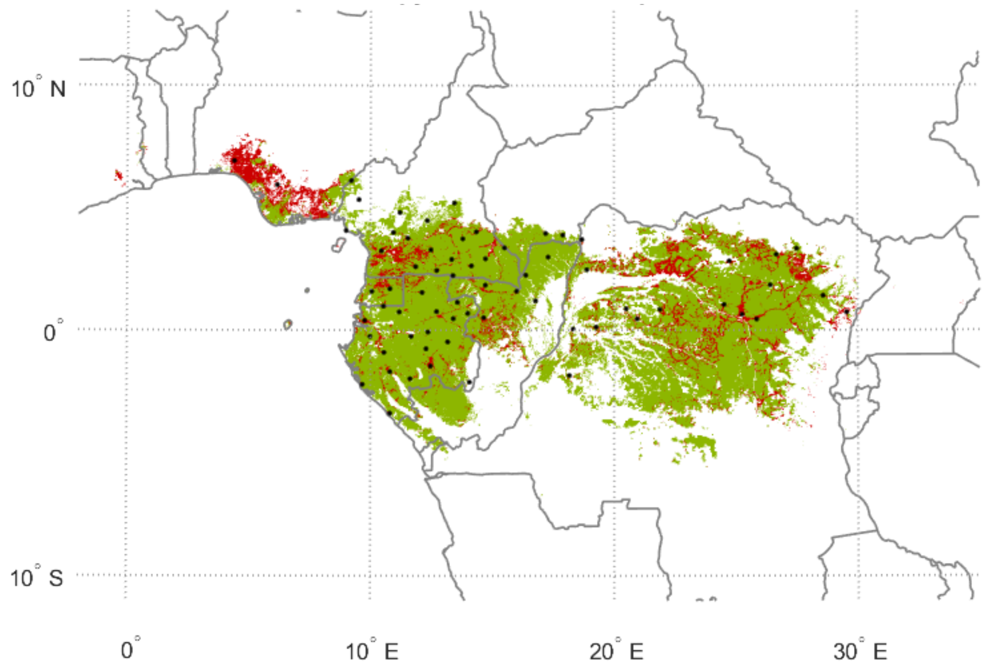


Figure 5. Binary map showing intact (green) and degraded (red) environmentally suitable area for *D. crassiflora*. Black dots represent 68 known localities used for model training.

The area environmentally suitable for *D. crassiflora* covers 1.36 million square kilometers. However, in 2000, 14% of this area comprises degraded forests or cropland (Mayaux *et al.* 2004), leaving 1.16 million square kilometers of intact forests where *D. crassiflora* are likely to be found. The 448 forest inventories located within this area have an average *D. crassiflora* density of 22.1 trees with DBH above 10cm (i.e. ca. the minimum diameter of fertility, see below) and 0.769 trees with DBH above 60 cm (i.e. the minimum diameter for exploitation in Cameroon (Sepulchre *et al.* 2008)) per square kilometer. The extant population of potentially fertile trees can therefore be estimated to be 30.9 million at the regional level. Area and estimated size of populations at the country level are shown in Table 1. These numbers should be taken with caution as our current knowledge of D. crassiflora density and distribution is only based on a limited and geographically biased number of observations. Also, due to the lack of adequate data, we were unable to remove permanently (*Raphia* sp. forests) and periodically (e.g. *Uapaca* sp. forests) flooded forests from the suitable area. The largest patches of flooded forests, e.g. at the core of the Congo basin, were considered as unsuitable using the map of Mayaux. However, at smaller scale, these forests are present along every stream and constitute a substantial part of the area.

Due to the relative low market demand for ebony and the logistical and processing challenges associated with the species, logging for ebony itself is not seen as a major threat. However, this does not mean that the long-term sustainability of the species is guaranteed as other factors such as land conversion, habitat loss due to the logging of other species, and the possible loss of seed dispersers may all prove significant to the long-term sustainability of the species.

Table 1. Estimated suitable area and population size for *D. crassiflora*. Areas were modeled using MaxEnt algorithm. Degraded forest area in year 2000 was estimated following Mayaux *et al.* (2004). Population size was deduced from MaxEnt model and observed densities.

Country	Proportion of environmentally suitable area degraded in 2000 (%)	Intact suitable area in km², thousands (% of country)	Estimated number of trees with DBH > 10cm (millions)	Estimated number of trees with DBH > 60cm (thousands)
Nigeria	64.5	24.8 (2.9)	0.66	19.05
Cameroon	11.7	155 (33.4)	4.10	119.36
Gabon	5.8	216 (82.5)	5.72	166.20
Equatorial Guinea	17.7	19.2 (72.0)	0.51	14.75
Republic of the Congo	8.4	154 (44.8)	4.08	118.56
Democratic Republic of the Congo	14.3	57 (24.5)	15.09	438.92
Central African Republic	2.2	28.1 (4.5)	0.74	21.61
TOTAL	14.2	1167	30.90	898.44

3. Understand the basic ecology of *D. crassiflora*, including identification of pollinators and dispersers necessary to enhance natural reproduction and dispersal; test alternative restoration approaches to determine the most successful methods.

Diameter distribution

Diameter data reported from tree inventories in old-growth forest without signs of recent human disturbance were provided for the whole range of the species by Slik et al. (2015) and collected in the Dja Reserve, Cameroon, by ourselves. The negative exponential distributions of diameter in these forests (Fig. 6) is characteristic of a shade-tolerant species and suggest a high regeneration rate. The same exponentially decreasing trend was reported by Sepulchre *et al.* (2008) for both evergreen and semi-evergreen forests over millions of inventoried hectares but not taking into account trees below 20 cm in diameter. From an analysis of disturbance regimes and regeneration dynamics in Southern Cameroon (Bongjoh & Nsangou 2001) and our own observations in plantation and in the Dja Reserve, *D. crassiflora* is able to germinate, persist, and grow under a completely closed canopy. However, it will thrive if a direct access to sunlight is provided (Fig. 7), even to saplings. All together our observations and previous reports suggest a shade tolerant (but not strict) temperament.

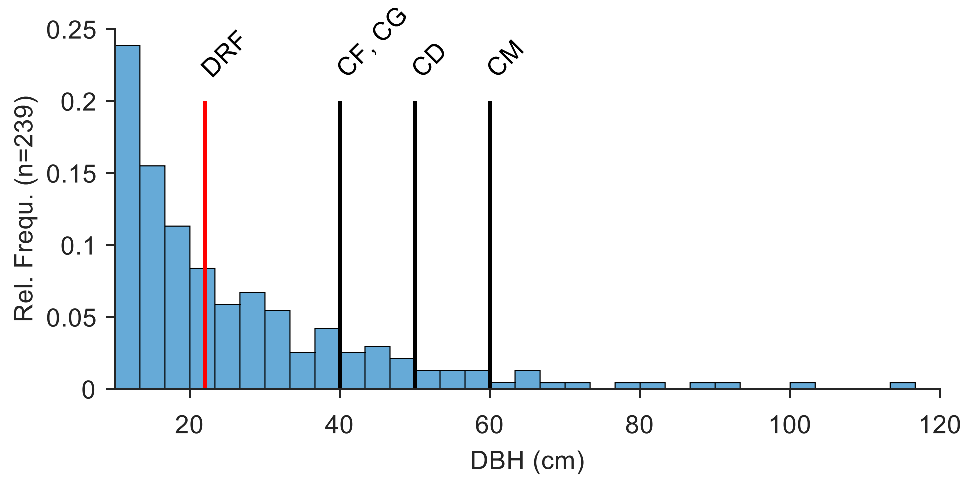


Figure 6. Distribution of 239 *D. crassiflora* diameter at breast height (DBH) observed in tree inventory data from old-growth forest without signs of recent human disturbance. Vertical lines indicate diameter for regular fructification (DRF) and minimum diameter for felling according to regulation in Central African Republic (CF), Republic of the Congo (CG), Democratic Republic of the Congo (CD) and Cameroon (CM) (Sepulchre *et al.* 2008).

Growth rate

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, Dja-et-Lobo, Cameroun, *D. crassiflora* trees were planted in a 4ha cocoa field in 1988. About half of the plantation was maintained by periodically removing regrowth and small trees while the other half was left as a closed forest. In July 2017, we measured 300 trees in the plantation among which 270 had no malformation or injuries on the main stem. We found that the trees in the cleared agro-forest had an annual diameter growth rate of 0.40 cm yr^{-1} on average and reaching up to 0.65 cm yr^{-1} (95th percentile). The trees in the closed 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) (Fig. 7).

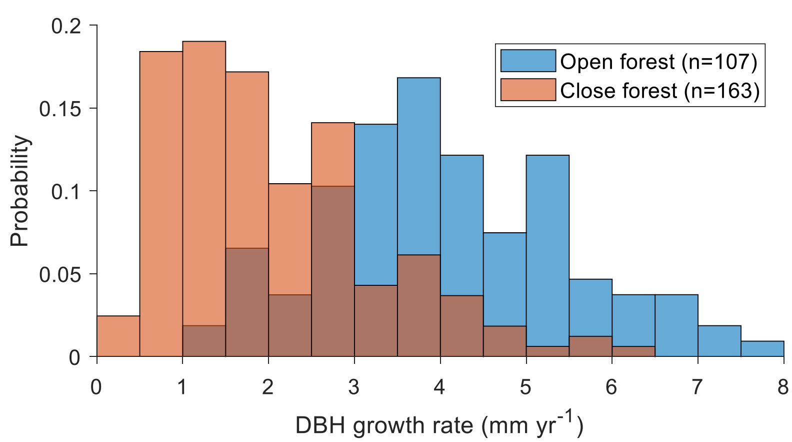


Figure 7. Distribution of *D. crassiflora* diameter at breast height (DBH) annual growth rate between 1988 and August 2017 in Ekombitié agro-forest.

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).

Overall, these results show an extremely slow rate of DBH growth either in forests or plantations. D. crassiflora may have slowest growing rate of all species of the Central African rainforest. The improved access to sunlight in plantations is probably the main reason why growth rate in plantations is twice that of individuals in natural forests. Considering the observed 0.4 cm yr^{-1} mean DBH growth rate in open conditions, and the diameter distribution found in the tree inventory data, we can calculate that sexually mature trees (considering a minimum diameter of fecundity of 10 cm, see phenology section) are at least 65 years old on average. Similarly, harvestable trees (DBH > 60 cm) are at least 200 years old on average. Depending on light availability, 0.4-0.8 cm yr^{-1} seems to be a consistent maximum rate of growth. In the forest, however, these optimal conditions are rarely encountered in the wild and most trees would grow much slowly under shade. The median growth rate in the closed forest of the Ekombite plantation, i.e. the conditions that best match with natural forests, was 0.18 cm yr^{-1}.

Phenology

Until field data on fruiting and flowering phenology of *D. crassiflora* is available, we focused on obtaining this information from herbarium specimens. For this we examined fertile specimens in Wageningen's Naturalis Biodiversity Center (WAG), National Museum of Natural History, Paris (P) and Botanical Garden Meise (BR). Monthly availability of fruits and flowers is shown in Fig. 8. Flowers are produced at the onset of the rainy season, which differs by country. Fruits (ripe or unripe) are found two to six months later.

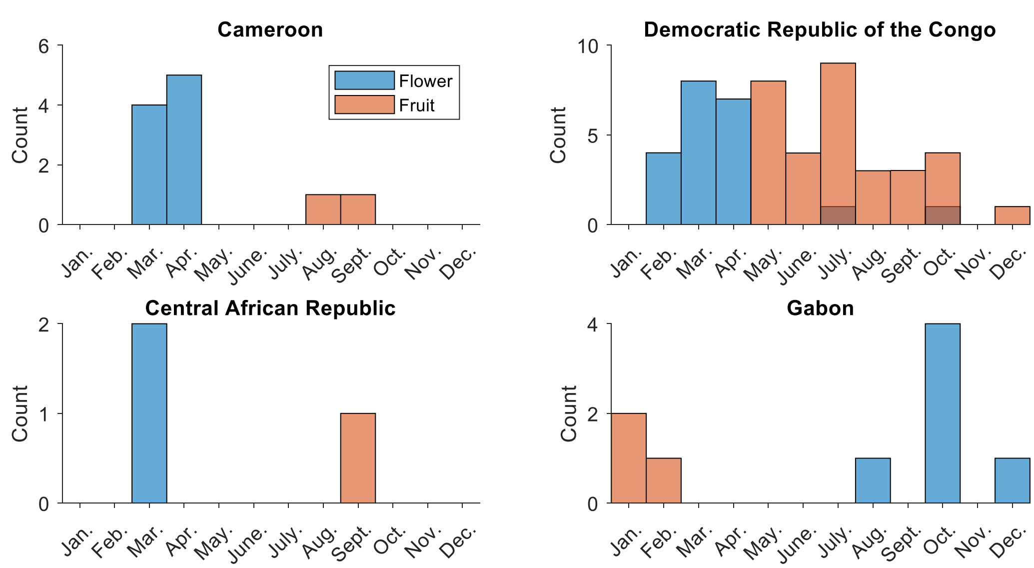


Figure 8. Count of herbarium specimens of *D. crassiflora* found with fruits or flowers in Muséum National d'Histoire Naturelle (Paris, France), Naturalis Biodiversity Center (Leiden, Netherlands) and National Botanic Garden of Belgium (Meise, Belgium).

In the Ekombitié plantation described above, we found that the minimum diameter of fecundity, the threshold above which fructification may happen, even if the diaspore production is low, was 8 cm. The diameter for regular fructification (>70% of fertile tree), was reported to be 22 cm from three observations in old growth forests by Doucet (2003). We were not able to estimate this parameter by ourselves because flowering rate was exceptionally low in 2017.

4. Test alternative production approaches, including tissue culture laboratory experiments, to identify optimal conditions for cultivating ebony.

Micropropagation is a technique by which a tiny piece of parent plant is multiplied on nutrient jelly in the laboratory. The first step was to secure the production of parent material from seeds. During the preliminary experiments, as germination success rate higher than 80% was obtained. Callogenesis trials were performed from leave tissues. An optimal mixture of hormone was identified to generate the production of callus, a mass of undifferentiated parenchymatous cells. However, the induction of new plantlets has not been observed yet. Further experiments using different plant organs will be conducted in the future.

An optimal mix of hormones was identified to produce 5.6 new twigs on average from each microcutting sampled from parent plants. An optimal rooting medium was designed, giving rooting rate around 60%. During rooting, an important development of the leafy stem was observed.

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