

Reproductive biology of *Pentadesma butyracea* (Clusiaceae), source of a valuable non timber forest product in Benin

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Background and aims – The main reproductive traits of the native African food tree species, *Pentadesma butyracea* Sabine (Clusiaceae), which is threatened in Benin and Togo, were examined in Benin to gather basic data necessary to develop conservation strategies in these countries.

Methodology – Data were collected on phenological pattern, floral morphology, pollinator assemblage, seed production and germination conditions on 77 adult individuals from three natural populations occurring in the Sudanian phytogeographical zone.

Key results – In Benin, *Pentadesma butyracea* flowers once a year during the dry season from September to December. Flowering entry displayed less variation among populations than among individuals within populations. However, a high synchrony of different floral stages between trees due to a long flowering period (c. 2 months per tree), might still facilitate pollen exchange. Pollen-ovule ratio was 577 ± 213 suggesting facultative xenogamy. The apical position of inflorescences, the yellowish to white greenish flowers and the high quantity of pollen and nectar per flower ($1042 \pm 117 \mu\text{L}$) represent floral attractants that predispose the species to animal-pollination. The main pollinators were two sunbirds (*Cyanomitra verticalis*, *Cinnyris coccinigastrus*) and three Hymenoptera (*Apis mellifera*, *Meliponula togoensis*, *Hypotrigona* sp.). Mean fruit set reached 49%, and absolute fruit production increased with tree size. Seeds were desiccation-sensitive (i.e. recalcitrant) with a maximum duration of hydrated storage of three months. Germination of seeds was most successful and rapid at 30°C (50% after nine days).

Conclusions – Our results indicate that the natural reproduction of *P. butyracea* is not limited by its ecology so that we hypothesize anthropogenic activities to be the reason for the threatened status of *P. butyracea* in Benin and Togo. However, due to its recalcitrant seeds, the conservation of its genetic resources is not feasible through *ex situ* conservation of seed banks but *in situ* strategies and/or *ex situ* conservation in orchards should be successful.

Key words – Breeding system, floral synchrony, fruit set, pollination, pollen-ovule ratio, recalcitrant seeds, conservation.

INTRODUCTION

Pentadesma butyracea Sabine (tribe Symphonieae, Clusiaceae) is a multipurpose forest tree (~20 m high) in sub-Saharan Africa distributed in (gallery) forests from Sierra Leone to Cameroun (Natta 2003, Natta et al. 2010). It is used for the production and local trade of a yellow butter extracted from its seeds' kernel (Tchobo et al. 2007, 2013, Aissi et al. 2011, Noudogbessi et al. 2013). However, due to overexploitation and habitat fragmentation it is one of the ten most threatened food tree species in Benin and Togo (Dah-Dovonon 2002,

Poidy 2002). In some areas in Benin, seed harvesting can reduce available seeds for recruitment by 70% (Avocèvou-Ayisso et al. 2009). The increasing pressure on *Pentadesma* resources results from: (i) the transfer of a subsistence product (subsistence farming by ancestors) to a commercial product (selling in all forms) (Sinsin & Sinadouwirou 2003) and (ii) its use in cosmetic trials by the French company L'OREAL (see e.g. Global patent index – EP 0180505 A1) based solely on naturally existing resources without domestication efforts (Dencausse et al. 1995). Socio-economic studies undertaken on the impact of seed harvesting on its

regeneration and the financials of its products trade in Benin indicate that the next human generation will have only half of the resources (fruits, seeds, bark, branches and timber) of *P. butyracea* available today (Avocèvou-Ayisso et al. 2009). While it is recognized as threatened and vulnerable in the IUCN Red List of Benin (Neuenschwander et al. 2011) it has not yet been added to the list of protected species in Benin and thus does not yet profit from protection from felling, pruning, limbing, mutilation, uprooting and swidden bleeding (see Neuenschwander et al. 2011).

In order to preserve *Pentadesma butyracea* as a natural resource for future generations, a sustainable conservation strategy is urgently needed. For the conservation of plant resources, except from establishing lists of threatened plants, a variety of approaches and techniques both *in* and *ex situ* have been proposed and implemented (Convention on Biological Diversity 2002, Moza & Bhatnagar 2007). In Benin, the Department of Forest and Nature Protection relies heavily on both approaches to conserve the natural plant richness (Dah-Dovonon 2002). The *ex situ* approach finds more applications for economic and domesticated plants in some private or public botanical gardens or agronomic research centers, however, without an overall management plan. The *in situ* approach focuses on wild plants.

Important factors for successful *in situ* conservation of a plant species are sexual reproduction and continuous recruitment based on a relatively large and genetically diverse population with a mixed population age structure to dampen-off the effect of epidemic diseases (Neher et al. 1987), to prevent the species from inbreeding depression (Tave 1999, Charlesworth & Willis 2009) and as pre-adaptation to environmental change (Gugerli 1997). The failure of reproductive processes is often the fundamental reason for species loss (Moza & Bhatnagar 2007).

Key factors determining a plant's reproductive biology are floral phenology and breeding system. Both shape mating possibilities between synchronous individuals, thereby influencing the transmission of genetic diversity from generation to generation and affecting the degree of genetic diversity and structure in populations (Ritland 1989, Ison et al. 2014). Patterns of floral phenology are considered to reflect evolutionary compromises in response to a set of selective forces, including the availability of water (David et al. 2012) and gene dispersers (pollinators and seed dispersers, Ranieri et al. 2012). The onset of flowering i.e. the transition from leaf to flower production, is often stimulated by a combination of internal (plant age or size) and external factors (day/night length, low temperature, fire and/or the presence of water; Erwin 2006, Elzinga et al. 2007). Thus the knowledge of phenological patterns across neighbouring populations and geographic regions in relation to local and regional environmental correlates is crucial to estimate its reproductive potential as prerequisite for species survival (Ison et al. 2014).

Plant breeding systems are primarily shaped by the plant's compatibility system (Pascarella 1997). The latter can be modified by particular floral traits preventing or allowing selfing (Ley & Claßen-Bockhoff 2012) and by the quantity and quality of pollinators' service as reduced pollination might provoke reproductive failure (Jennersten 1988, Agui-

lar et al. 2006). Reproductive failure in turn, reduces gene flow which might decrease effective population sizes, leading to inbreeding and possibly an increase in selfing (Bawa 1990, Menges 1991, Aizen & Feinsinger 1994, Gitzendanner & Soltis 2000, Mustajärvi et al. 2001, Rymer et al. 2005, Leimu et al. 2006, Coates et al. 2007). These altered reproductive patterns may cause a loss of genetic diversity and/or reduced progeny fitness due to inbreeding depression (Karron 1989, Barrett & Kohn 1991, Menges 1991, Latta & Ritland 1994, Husband & Schemske 1996) thereby endangering a species' survival.

Conservation strategies try to counteract this reproductive failure by artificially enhancing the plants' reproductive output. Besides the importance of plant mate choice to maintain genetic diversity in the species the success of potential methods depend heavily on seed characteristics. Seeds can differ greatly for example in the degree of tolerance of, or sensitivity to, desiccation. Orthodox seeds (hereafter termed desiccation-tolerant) are those which tolerate dehydration and are storable in dry conditions; they can be dried to low water contents (< 7%) with little effect on viability (Roberts 1973), while highly recalcitrant seeds (termed desiccation-sensitive; 5–10% of angiosperm species) are damaged by loss of only a small proportion of water and are unstorable for practical purposes (Berjak & Pammenter 2008). Thus the use of recalcitrant seeds in reforestation and *ex situ* conservation programs is problematic (Daws et al. 2005). Seeds can also vary in size and weight and thereby the amount of nutritive tissue to support the seedling during its early stage (Cideciyan & Malloch 1982, Giles 1990, Turnbull et al. 2012). All these different characteristics require a different handling concerning storage and germination conditions and thus have to be known and implemented in any successful conservation effort (Walters et al. 2013). Thus as a general rule, estimating the species conservation status and developing a conservation strategy requires an in-depth knowledge of the reproductive biology of the plant in focus (Bernardello et al. 1999). For *Pentadesma butyracea*, silvicultural knowledge essential for its restoration and preservation in sub-Saharan Africa is still scarce (Eyog-Matig et al. 2002). Research has so far focused only on its related genera (see Bittrich & Amaral 1996, Gill et al. 1998, Degen et al. 2001). Thus the present study aims at documenting the main reproductive traits (phenological pattern, floral morphology, pollinator assemblage, seed production and germination) of *P. butyracea* in order to gather relevant data to develop successful conservation strategies. More specifically we addressed the following questions: (1) What is the flowering and fruiting sequence over the year? Is there a high level of floral synchrony within and across individuals, populations and sites? (2) What are the floral prerequisites for breeding system and pollination and who are the pollinators? (3) What is the natural fruit and seed-set? and (4) Are seeds suitable for long term storage and what are the optimal conditions for seed germination?

Table 1 – Characteristics of the *Pentadesma butyracea* populations studied.

Habitat types: dry semi deciduous forest surrounded by savannahs (DDF), gallery forest at foot hills (GF). Lat = latitude; long = longitude; dbh = diameter at breast height.

Populations	Geographical coordinates (UTM, zone 31N)		Mean elevation [m]	Habitat	Annual rainfall [mm]	Population size (Number of trees, dbh>10 cm)	Sample size (Number of trees sampled)	Dbh [cm] (range)	Number of individuals on dry grounds/ inundated	Number of individuals burned / non- burned
	Lat	Long								
Igbo Aladja	967239	415216	257	GF	1199	90	26	11.3–69.1	10/16	14/12
Penessoulou	1023542	336472	420	DDF	1281	27	20	13.1–90.4	5/15	17/3
Peperkou	1160673	332713	393	GF	1269	37	31	17.8–54.3	17/14	13/18
TOTAL						154	77			

MATERIAL AND METHODS

Study species, study area and sampling design

Pentadesma butyracea (yellow butter tree) belongs to the pantropical family Clusiaceae (37 genera, 1610 species; Gustafson et al. 2002). It is a native African tree species occurring from 200 to 550 m of elevation, in Guineo-Congolian evergreen forests and gallery forests from Guinea-Bissau to the Democratic Republic of Congo (Bamps 1971). In Benin, where a savanna corridor interrupts the zonal West African rainforest, it occurs in the Sudanian phytogeographical zone as defined by White (1983) (fig. 1, III), in highly aggregated stands (Sinsin & Sinadouwirou 2003), mainly in gallery forests, in savannah woodlands and at foot hills where humidity is elevated. These forest patches are probably residues of a more continuous forest occurring during the Holocene African Humid period that fragmented and degraded at the end of the Holocene due to climate changes (Salzmann & Hoelzmann 2005). More recently, these forest patches, and hence the habitat of *P. butyracea*, were additionally reduced in size due to extensive agriculture and logging (Neuenschwander et al. 2011).

In Benin, where *P. butyracea* is known under the local names Kpangnan (in Nagot), Akoto (in Anni), Yêkotchépou (in Otamari) or Sesseido (in Fulani), fully grown trees reach 10–25 m in height and a diameter at breast height (dbh) of 110 cm. The wood of *P. butyracea* is used as timber and firewood; it has very good mechanical properties similar to those of *Khaya senegalensis* and *Milicia excelsa* (Rachman et al. 1987). Flowers are whitish, fleshy, hermaphroditic, producing many stamens and some nectariferous glands around the superior ovary (Akoegninou et al. 2006). The fruit of *P. butyracea* is a berry containing seeds in a yellow pulp (Hawthorne & Jongkind 2006). *P. butyracea* often reproduces by asexual reproduction via root shoots.

All our observations were conducted on three populations separated by 90 to 200 km and situated in Central and Northern Benin (fig. 1) where the climate is Sudanian with one rainy season (March–May to October) and nearly seven months of dry season. Annual rainfall in the studied populations ranges from about 1100 mm to 1400 mm (table 1). Daily relative humidity and temperature vary between 18 and 99% and 18 and 42°C. The yearly average potential evapotranspiration (ETP) is 1550 mm for the period from 1972 to

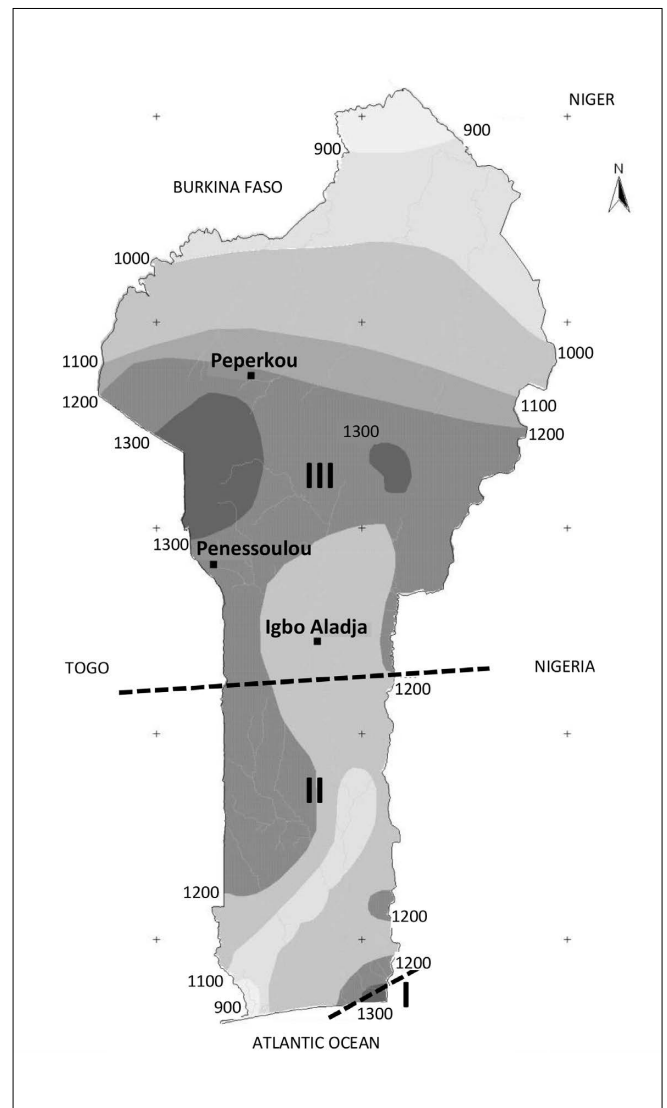


Figure 1 – Location of the three populations sampled to study the reproductive ecology of *Pentadesma butyracea* in Benin. Phytogeographical zones are adapted from White (1983): I = Guineo-Congolian, II = Guineo/Sudanian transition, III = Sudanian (hatched line separates phytogeographical zones). The different shadings show the distribution of rainfall from low (light shading) to high (dark shading; numbers refer to isohyets in mm, Akoegninou 2004).

Table 2 – Index of floral synchrony (Z) of *Pentadesma butyracea* individuals in comparison to all trees of the same population (year 2008).

N, total number of individuals (those inundated/burned or not) within a population; means followed by the same letter within a column are not significantly different at $p < 0.05$.

Populations	Trees out of water		Trees in water		Trees burned		Trees non-burned	
	N	Z ± sd	N	Z ± sd	N	Z ± sd	N	Z ± sd
Igbo Aladja (N = 26)	10	0.71 ± 0.9 ^a (45 ± 7) ^a	16	0.73 ± 0.12 ^a (39 ± 5) ^a	12	0.67 ± 0.12 ^a (44 ± 7) ^a	14	0.75 ± 0.06 ^b (34 ± 5) ^b
					Z ± sd = 0.71 ± 0.10 (36 ± 13)			
Peperkou (N = 23)	10	0.73 ± 0.13 ^a (56 ± 9) ^a	13	0.66 ± 0.13 ^a (51 ± 6) ^a	11	0.62 ± 0.16 ^a (59 ± 7) ^a	12	0.74 ± 0.08 ^b (50 ± 8) ^b
					Z ± sd = 0.70 ± 0.13 (53 ± 8)			
Penessoulou (N = 20)	5	0.84 ± 0.7 ^a (32 ± 5) ^a	15	0.84 ± 0.8 ^a (29 ± 3) ^a	3	0.80 ± 0.6 ^a (35 ± 5) ^a	17	0.84 ± 0.8 ^a (25 ± 4) ^b
					Z ± sd = 0.84 ± 0.08 (26 ± 5)			
All three populations together				Z ± sd = 0.74 ± 0.12				

2001 (Akoegninou 2004). Soils are tropical ferruginous with a breastplate of sandstone, or lateritic on a sandy subsoil.

A population was defined by a group of trees distant by less than 200 meters between adjacent trees. Populations contained from 27 to 90 individuals with a dbh > 10 cm. The dbh of trees in the three populations ranged from 11.3 to 90.4 cm (N = 77; mean ± standard deviation = 37.0 ± 13.8 cm). The distribution of the number of individuals per class of diameter is shown in electronic appendix 1. All three populations were in the proximity of a river with about one third to half of the trees growing on permanently inundated ground (table 1). Trees were also subject to man-made fires, especially in populations Peperkou and Igbo Aladja where 45.45% and 46.15% of the trees were damaged by fire (partially burned trunk). By contrast, population Penessoulou is included within a protected community forest where trees benefit protection from manmade fires since at least seven years (see table 2). Sampling design in the three populations for all activities described in the following paragraphs was summarized in electronic appendix 2. Statistical analyses were performed in R2.12.0 (Venables & Smith 2010).

Phenology and floral synchrony

To study the reproductive biology of *Pentadesma butyracea*, we randomly sampled a total of 69 flowering adult trees (Igbo Aladja: 26, Penessoulou: 20, Penessoulou: only 23 out of 31 trees, as eight trees did not flower in 2009–2010, table 1). Observations were carried out in two different phenophases (flowering and fruiting) during two successive years. The flowering was observed once a week from September to December in 2008 and in 2009. The fructification was observed once every two weeks on the same individuals from December to May in 2008–2009 and in 2009–2010.

Floral phenology – To assess the seasonal pattern of floral phenology, four successive stages and eventual changes among stages were surveyed in each phenological phase as indicated below:

- Flowering: fl1 = floral initiation; fl2 = young bud; fl3 = bud well developed; fl4 = mature flower i.e. blooming flower (see fig. 2A–E).
- Fruiting: fr1 = fruit set (= style dark-brown dehydrated, persisting ovary, stamen and calyx); fr2 = young green fruits

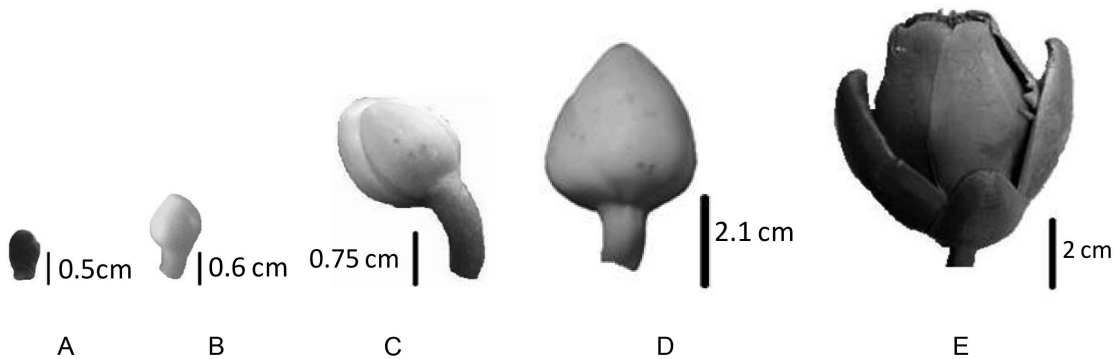


Figure 2 – Morphology and phenological stages of *Pentadesma butyracea* flowers: A, floral initiation (fl1); B–C, fl2, bud (13 to 17 days after fl1); D, fl3, developed bud (20 to 21 days after fl1); E, fl4, open flower (24 to 25 days after fl1).

in development (growing ovary with persisting calyx); fr3 = fruits brown, larger in size and well developed but not mature; fr4 = fruits mature (about 129 ± 32 mm long), brown colour, few days before falling down (see fig. 3A–D).

Daily floral phenology was followed on 75 flowers between stage fl3 and fl4 in the evening and the early morning, and the ripening of stamen and ovaries was noted to determine the timing of sexual maturity of anther and stigma (homogamous, protogynous or protandrous flower).

Floral synchrony – The quantity of flowers and fruits in a given stage per tree was quantified visually by inspecting all branches using binoculars; data were collected using the following ordinal classes: 0 = absence of flowers or fruits in a given stage; 1 = 1–25% of branches bearing organs in a given stage; 2 = 26–50%; 3 = 51–75%; 4 = 76–100%. The date of first flowering and the duration of each stage were recorded to construct the phenological diagram of each population. The floral synchrony index was computed per individual tree (X_i) and per population (Z), according to Augspurger (1983):

$$X_i = \left[\frac{n}{n-1} \right] \left[\frac{1}{f_i} \right] \sum_{j \neq i}^n e_{ij}$$

$$Z = \left[\frac{1}{n} \right] \sum_{i=1}^n X_i$$

where n = number of individuals in the population, f_i = number of flowering days of the individual i , e_{ij} = number of days on which individuals i and j flower in a synchronized way ($j \neq i$). $X_i = 1$ represents total floral synchrony where all individuals in the population are flowering during the flowering period of the individual i . $X_i = 0$ means that there is no phenological overlap between i and the other individuals. Z measures the mean flowering overlap between individuals within the population.

To compute these indices, the stage fl3 was considered instead of fl4 to prevent potential errors of flower observations on extremely tall trees where open flowers (fl4) can be confounded with wilted flowers. Whether individual floral synchrony (X_i) differed among populations and/or according to the fact that they were situated on inundated soil or were damaged by fire was tested with two-way ANOVA (i.e. two factors: site and soil hydromorphy; site and burned).

Inflorescence and flower morphological traits, pollen load and nectar reward

Seventy five inflorescences collected from fifteen trees in the three populations (five inflorescences from each tree x five trees per population) were examined and the mean number of flowers per inflorescence was determined. The difference in the average number of flowers per inflorescence between populations was tested with an ANOVA.

Sixty flowers (randomly selected from four different inflorescences from fifteen trees, five trees per populations) were dissected to determine whether all flowers were hermaphrodite, to describe the structure of the androecium and the gynoecium, and to document the number of stamens, and ovules and the presence and number of glands producing nectar per flower.

To determine the P/O-ratio pollen grains from 120 stamens from forty flowers with thecae in the state just before opening were collected and pollen grains counted under the microscope.

The number of viable pollen grains was determined by counting red pollen grains colored by acetic carmine within an anther crushed between microscope object plate and cover slide under a binocular microscope. Samples of anthers were removed from 120 stamens (initially plunged in acetic carmine) collected from forty flowers with ten flowers at each of the following four different phenological stages: stage fl3 just before anthesis, stage fl4a at anthesis, stage fl4b 24 hours after anthesis and stage fl4c 48 hours after anthesis.

The quantity of nectar produced per flower (bagged before anthesis to avoid any visits) was measured on a total of thirty flowers coming from ten trees (three flowers from each tree) of which five were non-burned trees out of the water in Igbo Aladja and five were non-burned trees situated out of the water in Peperkou. The measurement was undertaken by sequential removal of nectar present in a flower with a micropipette of 1200 μ L. Removal was done three times, each time from a different flower (at first anthesis in the morning, in the evening at 6 p.m. and 24 hours after anthesis).

Pollinators

On one randomly selected tree per population, four inflorescences were chosen for pollinator observations and marked

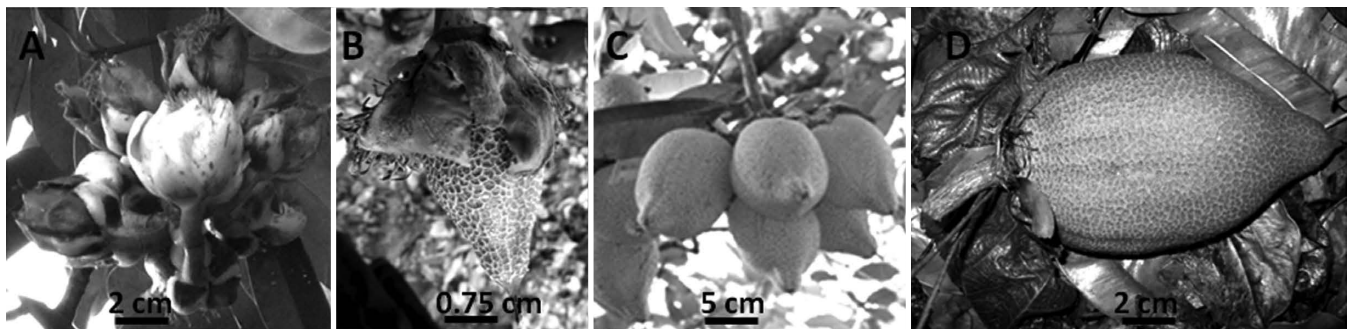


Figure 3 – Morphology and phenological stages of *Pentadesma butyracea* fructification: A, fr1, fruit set (26 to 40 days after fl4); B, fr2, young fruits in development (27 to 38 days after fr1); C, fr3, fruits well developed but not mature (43 to 62 days after fr2); D, fr4, fruits mature (21 to 35 days after fr3).

to facilitate their identification. Pollinators and their behavior were observed in November, 2008 for three days per tree concordant with the life time of the observed flowers. The choice of the days of observation was based on the flowering peak of the selected tree. The observations were conducted at 6 a.m., 9 a.m., 12 a.m., 3 p.m. and 6 p.m. for 10 minutes each time. The following data about the flower visitors were collected: species or taxonomic group, pollinator (come in contact with the sexual organs of the flower) or visitor (suck floral nectar without being in contact with the sexual floral organs), time of visitation, frequency (number of visits per minute to an inflorescence) and duration of visit, and behavior. Hereafter, pollinators – mainly insects – were captured using a ladder and a handnet. Pollinators were preserved in 70° alcohol to perform their identification in the Biological Control Center for Africa of the International Institute of Tropical Africa (IITA). After identification, a total of 25 voucher specimens belonging to three species were kept in a private collection of the first author. Birds were determined by an ornithologist (Faculty of Sciences and Agronomy, Université d'Abomey-Calavi) based on pictures taken while visiting *P. butyracea's* flowers.

Fruit and seed set

At phenological stage fr3 (fruits not yet fallen from each tree), the total number of fruits produced per tree was counted on a total of 77 trees (two repetitive counts per tree; see table 1 for number of trees sampled per population) and the respective dbh of the trees was measured. The relationship between fruit production and dbh and fruit production in 2008 versus 2009 was tested with a linear regression. The average fruit set per inflorescence ($n = 75$ inflorescences from 25 non-burned trees situated on dry ground in Peperkou (ten trees) and in Igbo Aladja (fifteen trees)) and the Seed/Ovule ratio ($n = 75$ fruits collected from 25 infructescences in the year 2008) were counted. The number of ovules was determined by the combination of viable and aborted seeds within a sampled fruit.

Seed moisture content, germination capacity and storage characteristics

To estimate moisture content, germination capacity and storage characteristics of seeds, 127 mature fruits were collected randomly under trees from the three studied populations on May 24th, 2009. All healthy seeds not attacked by insects (~ 953 seeds) were extracted from the fruits. The extraction of seeds took place eleven days after the collection of the fruits. Weight and moisture content were determined gravimetrically at the Université d'Abomey-Calavi in Benin on 100 seeds. Moisture contents were determined by weighing before and after drying seeds in an oven at 103°C for 17 h. The moisture content is the difference of these weight measurements given as the percentage of fresh weight (Willan 1992).

After collection, seeds for germination tests were sent to the Université Libre de Bruxelles (Belgium). Since their collection (2 month delay), they were regularly wetted to avoid a loss of moisture before trials. Nevertheless, seeds indicate a loss of water of about 1.5% during transportation decreas-

ing moisture content to $41 \pm 2.2\%$ ($n = 100$ seeds) before trials. In Belgium, seeds were submitted to two treatments.

Treatment 1: germination experiments – Germination capacity was determined at three different temperatures (20°, 25° and 30°C) by using five replicates of ten seeds for each temperature. The chosen temperatures (20°C and 30°C) were close to the mean minimal and maximal temperatures in the studied regions in Benin during the fruiting period. Seeds were sown in peat and incubated for 103 days in individual incubator sets (Luminincubes) with 12 h light/12 h dark. These seeds were measured (length, width and thickness) and weighted before they were placed into peat filled pots. Kruskal-Wallis tests were applied to assess whether there is a significant difference in the number of germinated and non-germinated seeds among different temperatures, and whether there is a difference in weight between germinated and non-germinated seeds.

Treatment 2: storage trials – Storage experiments were performed using 136 seeds that were stored at ambient temperature (c. 20°C), and 150 seeds stored at c. 4°C (as an usual temperature condition to explore the desiccation sensitivity, Engels & Ditlevsen 2004) in polyethylene bags to check the effect of conservation at low temperature on seed biology. Seeds were sub-imbibed only the first day of trial. Due to seed dehydration after 35 days, the moisture content of fifty seeds stored at 25°C and fifty seeds stored at 4°C was measured. From the remaining seeds five replicates of ten seeds for each storage temperature were germinated at 30°C which was the ideal germination temperature identified in treatment 1.

RESULTS

Phenology and floral synchrony

Flowering sequence – The branches that supported flowers were often born at the basis of those having ported flowers in the previous year. From thirteen to seventeen days after initiation (stage fl1) buds reached stage fl2 (fig. 2B). They were green and measured $1.4\text{--}5.7 \times 0.8\text{--}3.0$ cm. Stage fl3 occurred 20–21 days after fl1, color changed from green to green-yellowish and buds measured 8.3×4.5 cm. The stage fl4 occurred 24–25 days after fl1. Floral anthesis started in the evening at 5 p.m. At this phase, a light pressure on the anthers produced an opening of the thecae and a display of pollen grains. In contrast, the five stigma papillae of the gynoecium remained closed. Only in the morning of the following day the latter separated. At this stage, anthers and stigma extended above the level of the petals at about the same height. Each lobe of the receptive stigma projected outwards.

Anthesis was not synchronous between all flowers within an inflorescence: considering an inflorescence composed of about five cymes, it was always the terminal flower of each cyme and on overall the apical cyme that bloomed first. However, anthesis could happen at the same period for most of the overall apical flowers of the inflorescence i.e. flowering of cymes within an inflorescence was often overlapping. It took three to five days before all flowers of a cyme (and/or an inflorescence) were open. Flowers at stage fl4 lasted for about four days followed by a progressive dropping of

petals. Twenty-six to forty days after fl4, flowers entered into stage fr1. Here flowers were faded measuring $4.4\text{--}8.6 \times 3.9\text{--}4.5$ cm, their perianth had dehydrated, and corolla and the summit had become pink; sometimes numerous flowers fell down at this stage (see fig. 3A). At the stage fr2 (27–38 days after fr1, January to February), the fruits' color passed progressively from yellow to brown, perianth dehydration continued, some sepals and petals fell down from fruits but the androecium persisted; fruits measured $5.5\text{--}9.3 \times 4.6\text{--}5.8$ cm. At the stage fr3 (43–62 days after fr2 from February to April), fruits became brown, measuring $6.8\text{--}13.1 \times 4.9\text{--}7.4$ cm and kept this color until maturity (21–35 days after fr3 from April to May). The ripe fruits (measuring $8.3\text{--}17.9 \times 6.8\text{--}11.3$ cm, see fig. 2D) started to drop down.

Seasonal pattern of phenology – The flowering (stages fl1 to fl4) occurred in a single yearly cycle, generally at the end of the rainy season and the first half of the dry season from September to December, sometimes until January for the late trees (fig. 4). The onset of flowering varied between populations: population Penessoulou always started and ended

flowering first and population Peperkou flowered last (ten to fourteen days delay between the mean dates of flowering initiation) (fig. 4). Finally, there was a variation between years: in all three populations the flowering period was shorter in 2008 than in 2009 and flowering started three weeks later in 2008 (22 September) than in 2009 (1 September; cf. fig. 4). Within a tree, the onset of flowering was not uniform in all branches and could reach a difference of up to 28 days between the first and last branches bearing flowers. Between individuals of the same population, the onset of flowering could be delayed (0-18 days shift): individuals standing in water and those on land showed the same temporal patterns ($p = 0.52$, $F = 0.41$) but non-burned individuals started flowering earlier than burned trees ($p = 0.007$, $F = 7.65$). No interaction was found between both factors used in the two-way ANOVA test: site and soil hydromorphy ($p = 0.50$, $F = 0.69$), site and burned ($p = 0.86$, $F = 0.14$). Fructification could be observed during the second half of the rainy season from December to April with fruits starting to drop down in April

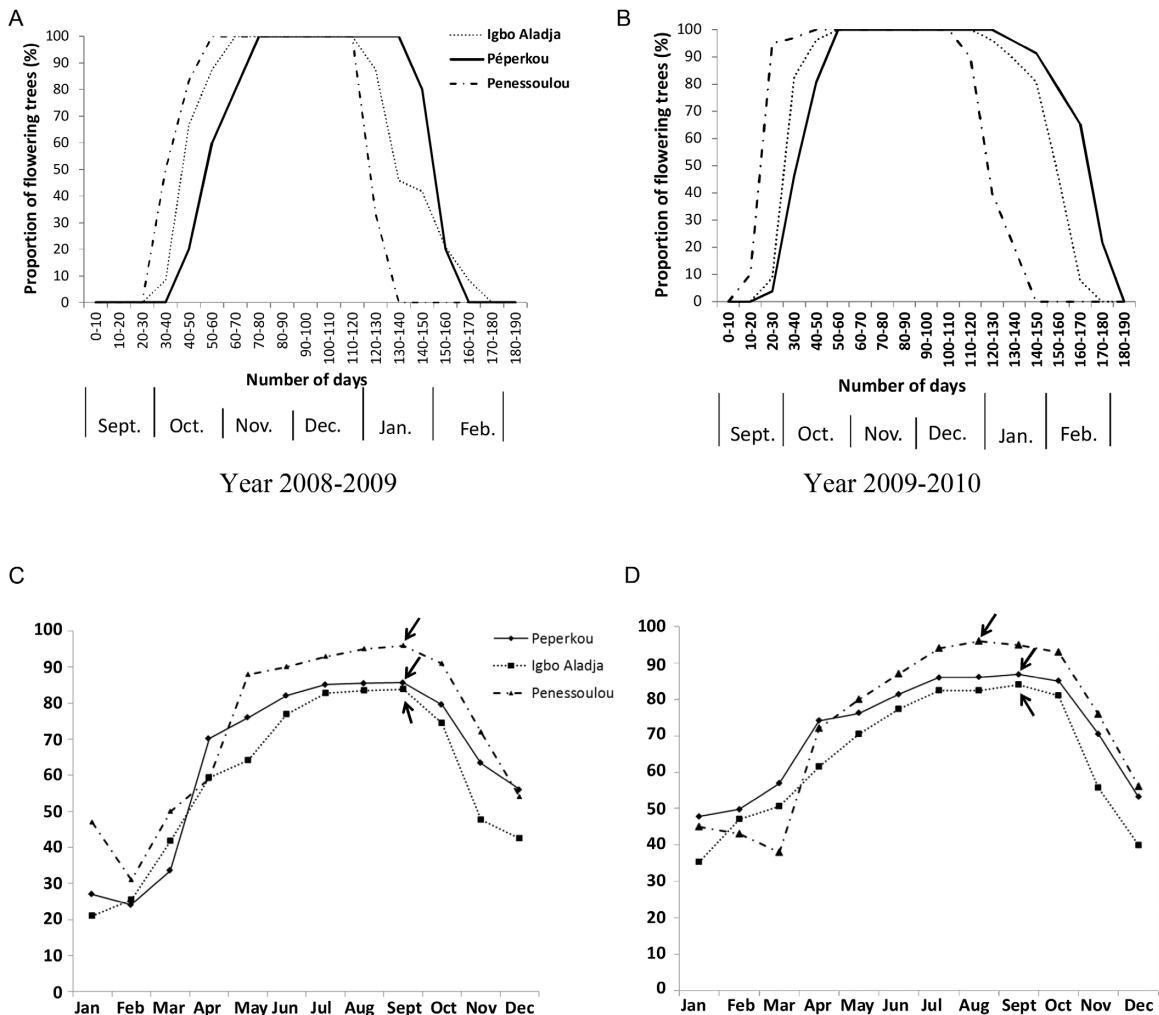


Figure 4 – Phenological diagram of the three populations of *Pentadesma butyracea* in Benin in: A, 2008–2009; and B, 2009–2010 (percentage of trees with > 25% of flowers in stage fl1). Graphs C & D show monthly relative humidity for the same populations in each of both years; arrows indicate months of the higher relative humidity (data collected from <http://www.tutiempo.net/en/Climate>).

with the last once dropping in July and harvesting by humans starting in May.

Floral synchrony within trees and populations – The synchrony index of an individual tree with respect to all others varied between 0.42 and 0.93. The mean values per population ($Z \pm sd$) were not significantly different between populations (see table 2). The mean synchrony index per population was only slightly but not significantly higher (0.84 ± 0.08) in Penessoulou than in Igbo Aladja and in Peperkou (0.71 ± 0.10 and 0.70 ± 0.13 respectively). Trees that were burned by man-made fires in the previous year flowered on average later (18 ± 7 days in Igbo Aladja, 17 ± 6 days in Peperkou and 7 ± 5 days) and significantly longer (see table 2) than non-burned trees and displayed in all populations a lower synchrony index than non-burned trees (significant only in populations Igbo Aladja and Peperkou with many burned trees, table 2). However, concerning soil hydromorphy, none of the three populations displayed a significant difference in synchrony between trees growing in water and those standing on dry ground (table 2).

Inflorescence and floral traits

Morphology of inflorescence and flower – Inflorescences of *Pentadesma butyracea* were situated at the top of flower-

ing branches and composed each by a cyme of three to seven biparous cymes carrying three to fifteen flowers (Penessoulou: 8 ± 3 flowers, Peperkou: 7 ± 3 flowers, Igbo Aladja: 7 ± 3 flowers), showing similar values between populations (ANOVA: $df = 72$, $F = 0.182$, $p = 0.83$). All flowers were very large (6–7 cm long and 5–6.5 cm in diameter), hermaphrodite, actinomorphic and yellowish or white greenish in color. The calyx was greenish, composed of five (pentamerous) free, nested, unequal (two small sepals were external), oval sepals. The corolla was green-whitish, composed of five equal and free petals egg-shaped, oblong to oval, alternate with sepals, very concave, aestivation was twisted sinistrorse (fig. 5). The androecium was constituted of five phalanges of epipetalous thread stamens fused at the base. Every phalange was composed of 49 ± 6 stamens and 0–5 staminodes. Thus every flower had on average 245 ± 30 stamens. Every stamen was composed of a filament (long of 4–6.5 cm) and a basifixed anther (1.3–1.6 cm in length) which dehiscence introrsely (fig. 5). The androecium showed a low spatial separation from the stigma. The inner anthers were at the same height as the stigma and even touching the latter while more distant stamens were shorter. On 20% of phalange and 46% of the flowers examined there were 1–5 highly variable distorted stamens found within a phalange (curved leaf, anther absent, etc., fig. 5). The ovary was superior (see fig. 3), usually

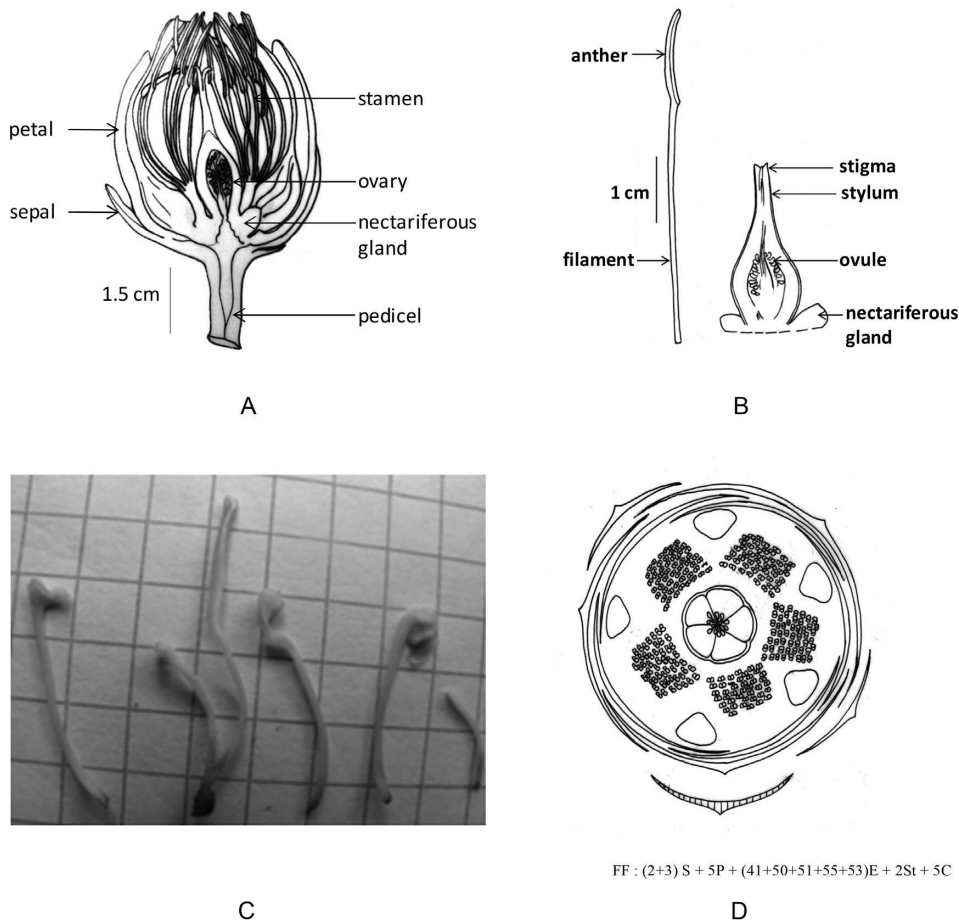


Figure 5 – Some morphological characters of *Pentadesma butyracea* flower: A, longitudinal section of flower; B, detail of a stamen (left) and gynoecium (right); C, five forms of staminodes surrounding a normal stamen; D, floral diagram and formula of *Pentadesma butyracea*.

composed of five carpels forming five locules. The style was short and ended in the stigma composed of five stigmatic papillae. The ovary was surrounded by five glands producing nectar (alternate with phalanges). Placentation was axile in two to three rows of ovules per locule. The number of ovules varied between 53 and 188 ovules per ovary (121 ± 22 , $n = 75$ ovaries on 75 flowers). Ovules were anatropous.

Pollen load and viability – Viable pollen grains in the anthers were often in packages of 2–17. They were circular and of variable sizes. Their mean number counted at stage fl3 was 287 ± 156 per anther. This number decreased with the age of the flower to 21 ± 19 , 48 h after anthesis. The data was pooled across populations. For an average number of 245 \pm 30 stamens per flower, a flower produced on average 70,000 \pm 4,700 viable pollen grains. Staminodes did not contain any pollen grains. Based on fresh anthers that had not yet lost any pollen grains (stage fl3), the P/O-ratio was 577 ± 213 .

Nectar reward – Every flower presented five cone-shaped nectariferous glands alternating with petal and stamen phalange. Each gland measured 0.8×0.5 cm with nectar seeping from its top. The data was pooled across populations; the mean total nectar volume measured was 1042 ± 117 μ L per flower. Nectar was produced continuously during floral anthesis. The quantity decreased with flower age from 1042 ± 117 μ L at first anthesis in the morning at 06:30 a.m. to 694 ± 116 μ L in the evening at 6:00 p.m. and to 447 ± 73 μ L after 24 hours. It was observed that with the continuous opening of the flower, nectar flew out of the flower through openings appearing in between the petals.

Pollinators

The flower visitors were birds and insects (electronic appendix 3), identical across populations. Birds were male and female sunbirds of the Nectariniidae: *Cyanomitra verticalis* and *Cinnyris coccinigastrus*. When anthesis started (fl4), sunbirds were the first animals to arrive and to gain access to floral rewards. They landed on the inflorescence axis, held onto these axes and pecked the anthers of opened flowers, thereby harvesting pollen on their beak. They also actively sucked the nectar at the bottom of flowers usually by perforating the petal with their long and pointed beak. Here, they chose with high precision always the center of each petal that covered the nectariferous gland (cf. electronic appendix 4). There was never more than one bird at a time on an inflorescence. The duration of their visit on an inflorescence varied between three and seven seconds, harvesting a maximum of three flowers per inflorescence. When they left an inflorescence, they often returned, occasionally even within a minute.

Insects visiting *P. butyracea* included three species of Hymenoptera belonging to three different genera of the family Apidae: *Apis mellifera*, *Meliponula togoensis* and *Hypotrigona* sp. They collected either nectar or pollen or both in the flowers of *P. butyracea*. Similarly to birds, bees moved from one flower to another within the same inflorescence, however, they spent more time per flower (two to three seconds), visited more flowers per inflorescence (three to five flowers) and thus stayed in total longer within the same inflorescence (12–23 seconds). After the visitation to the anthers

of a flower they showed pollen pincushions on their legs. To reach the nectar, these Hymenoptera took advantage of the openings at the base of the petals arranged by the birds (see online supplementary material 4); one to six bees would enter the same flower at a time to suck the nectar emitted by the nectar glands at the bottom of the flower.

Fruit and seed set

While the mean number of flowers per inflorescence was 7 ± 3 (ranging from 3 to 15, $n = 75$ inflorescences), the mean number of fruits per infructescence was only 3 ± 2 (ranging from 0 to 9 fruits, $n = 75$ infructescences) resulting in a mean rate of free fertilization or fruit set of $49 \pm 19\%$.

The fruit of *P. butyracea* was pear-shaped, measuring 7.0–29.0 cm in length (12.9 ± 3.2 cm) and 2.8–15.0 cm in diameter (8.6 ± 2.3 cm). Its fresh weight varied between 263 and 1705 g (820 ± 333 g). Every fruit contained 1–34 seeds (8 ± 4 seeds, $n = 70$ fruits). The Seed/Ovule-ratio was 0.06 indicating a mean rate of $94 \pm 4\%$ ovule abortion per flower.

Seeds were dark brown, measuring 26–55 mm in length (38.2 ± 5.3 mm), 20–39 mm (29.0 ± 2.3 mm) in width and 16–33 mm (23 ± 3.2 mm) in diameter. Fruit and seed shapes were very polymorphic within and between individuals.

Across populations, trees produced 0–840 fruits (106 ± 135 ; $n = 73$ trees). Average numbers of fruits produced per tree were similar in 2008 (92 ± 131) and in 2009 (89 ± 117). The number of fruits correlated with tree diameter (Pearson: 2008 $r = 0.45$, $p < 0.001$, 2009 $r = 0.41$; $p < 0.001$) with a peak of 346 ± 392 (70–80 cm) and 240 ± 247 (60–70 cm) fruits in the years 2008 and 2009, respectively (see electronic appendix 5), however, there was no significant correlation on individual tree level (Pearson: $r = 0.22$, $p = 0.05$), i.e. individual trees did not produce the same amount of fruits in both years.

Seed moisture content, optimal germination conditions, long term storage

Moisture content – The seeds sampled measured on average 41 ± 5.1 mm in length, 30 ± 2.7 mm in width and 24 ± 3.1 mm in diameter ($n = 100$). Their fresh weight was 17.1 ± 3.0 g and their dry weight 9.8 ± 1.7 g. The moisture content was $MC = 42.5 \pm 2.9\%$ (see details about seed characteristics in electronic appendix 6).

Initial germination – Figure 6 shows that germination occurred at different rates depending on the incubation temperatures (20°C, 25°C, 30°C) and conservation treatments (storage at 4°C and 20°C). Germination occurred earlier at 30°C (first seeds germinated nine days after incubation) than at 25°C and 20°C (first germination after 19 and 22 days, respectively) (fig. 6). However, at the end of the study i.e. after 103 days of incubation, the same cumulated germination rate was found (50%) except at 25°C (40%). Non-germinated seeds ($n = 85$) were hardened and dead, and originally heavier and significantly bigger than those ($n = 65$) that germinated (Wilcoxon-Mann-Whitney, bilateral test, $W = 1661$, $p < 0.005$) (see details on seeds characteristics in electronic appendix 6).

Storage trial – None of the seeds stored at 4°C germinated: neither during the storage trial nor during the subsequent germination trial. Of the seeds stored at 20°C, 41% (corresponding to 56 seeds from a total number of 136 seeds) started to germinate already before the end of the five weeks storage trial. Of the fifty seeds used for the subsequent germination trial under ideal conditions determined in the initial germination trials (86 days of incubation at 30°C, see fig. 6) sixteen seeds (32%) germinated after 86 days. The MC was $22.7 \pm 8.1\%$ (N = 50) for seeds stored at 4°C and $34.3 \pm 6.2\%$ (N = 50) for those stored at 20°C. In total, we found a potential minimum storage duration of 3.5 months at 20°C (35 days of storage trials + 60 days of delay between collection in the field and start of storage trials).

DISCUSSION

Floral traits associated to breeding system

The arrangement of numerous stamens in five phalanges is a monotypic floral trait of the six genera belonging to Symphoniae except *Symphonia globulifera* (Gustafsson et al. 2002, Stevens 2007). Flowers in *P. butyracea* were slightly proterandrous (anthers were earlier ripe than the stigma); however this short delay of stigma versus anther ripening overnight in the absence of pollinators is not regarded as a factor influencing the breeding system. Instead the parallel maturation of male and female organs and their close position within the flower predispose the latter for selfing. Indeed, *P. butyracea* seems to be partially self-compatible as shown by a tree planted out of its range in the south of Benin nine years ago and some other isolated trees (resulting from fragmentation of gallery forests) which commonly produce small fruits (B. Sinsin, University of Abomey-Calavi, pers. comm.). A germination test of all seeds collected from the

single tree growing in south of Benin, showed that all fruits produce viable offspring (Ewédjè, unpublished data). Still, the high number of pollen grains per anther (287 ± 156) and the relatively high P/O-ratio ($P/O = 577$) indicate a high allocation to male function, as generally found in predominantly out-crossing species (*Trichostema* spp., Spira 1980). The P/O-ratios of most xenogamous, animal-pollinated plants are between 1200 and 8000 (Cruden 2000) suggesting that the yellow butter tree is facultative xenogamous. Also preliminary data using genetic markers confirm that the species is mostly allogamous (Ewédjè 2012) just as many self-compatible tropical trees (see e.g. Gribel & Gibbs 2002, Hufford & Hamrick 2003, Naito et al. 2005, Del Castillo & Trujillo 2007) including its close relative *Symphonia globulifera* (Carneiro da Silva et al. 2009).

The atypical flowering/fruiting cycle of *Pentadesma* – a keystone species in the Sudanian region?

P. butyracea flowers once a year at the end of the rainy season and during the first two months of the dry season. The onset of flowering in September/October corresponds to the months where humidity, which is indirectly related to rainfall, reaches its maximum mean value (see fig. 4C & D) and which is a little earlier in Penessoulou than in the other two populations. This indicates that a maximum amount of water could be a required signal which induces flowering in *P. butyracea*. This is congruent with the general assumption that the main determinants of phenology of tropical tree species are temperature, light/insolation, rainfall and relative humidity (Frankie et al. 1974, Borchert 1994, Piechowski 2007).

This flowering and fruiting pattern of *Pentadesma butyracea* in the Sudanian zone is typical for equivalent data taken on other woody species in this region (Seghier et al. 2009, Djossa et al. 2012). In the Sudanian zone flowering occurs generally during the dry season (January to May) and

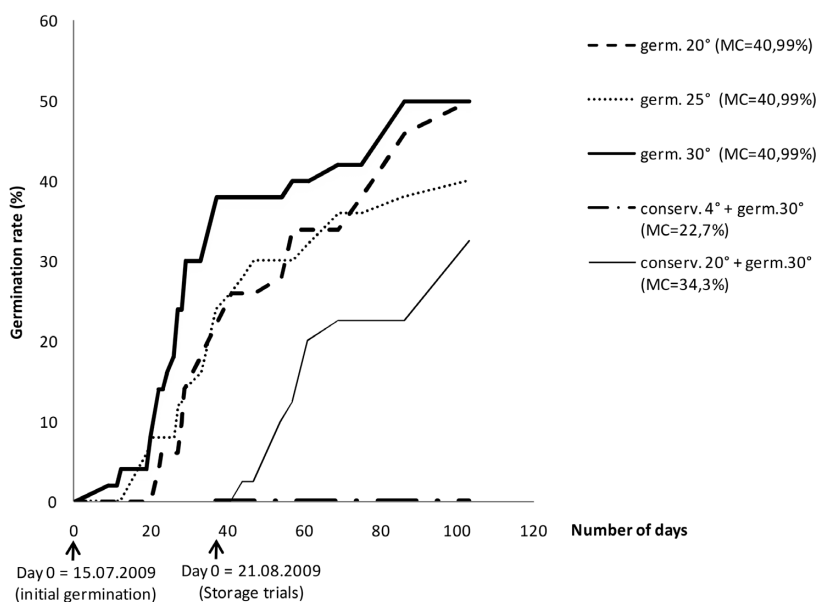


Figure 6 – Evolution of *Pentadesma butyracea* seed germination (germ.) rate according to different incubation temperatures (20°C, 25°C, 30°C) and conservation (conserv.) treatments (storage at 4°C and 20°C). MC, moisture content.

fruiting during the rainy season (April to December) reducing the risk of seedling germinating during the unfavorable period, i.e. dry season. Being a typical species of moist forest *Pentadesma butyracea* occurs in Benin in gallery forest. It thereby has conserved its fruiting pattern during the rainy season from the moist forests (see White & Abernethy 1996).

In central Africa gorillas and elephants are the main seed dispersers taking advantage of the fleshy fruit of *P. butyracea* (White & Abernethy 1996). These animals were however, absent in the studied habitat in Benin because of hunting leaving an open question about the current dispersal of this species. Limited dispersal between populations might have a negative effect on genetic diversity and increase population differentiation. These trends might be further reinforced by a reduced pollinator mediated gene flow between populations due to an increased distance between fragmented population residuals (Calvino-Cancela et al. 2012).

How floral synchrony pattern might favor out-crossing

In *P. butyracea*, there was a minimum flowering period of two months for every tree as individual branches came into flowering consecutively. Consequently, synchrony of flowering within trees was low but between individual trees within populations often very high ($Z = 0.70$) (table 2), despite a difference in the onset of flowering that can reach two weeks. This value of flowering overlap is common in many other cross-pollinated plant species (Augspurger 1983, Guitián & Sánchez 1992, Sina 2006, Michalski & Durka 2007), increases the attraction of pollinators (Piechowski 2007) and facilitates pollen exchange among trees within and among populations. Thus in the case of an efficient pollination (estimated pollen dispersal distance: 450 m, Ewédjè 2012), a low level of genetic differentiation could be expected within populations of this tree species. The delayed or canceled flowering of burned trees suggests that wildfires are creating significant biological stress as found in grasses and trees (White et al. 1991, Erwin 2006).

A mixed pollination system and its implications for gene flow

The animal pollination system of *P. butyracea* was of a general nature and uniform across populations with two different bird species and three different insect species attracted by a large open flower morphology and the offer of an enormous quantity of nectar ($1042 \pm 117 \mu\text{L}$) and pollen grains (69825 ± 4680) per flower. Sunbird pollination of *P. butyracea* was also recorded in Gabon (White & Abernethy 1996). Particular adaptations to bird pollination include the apical position and sturdiness of inflorescences on branches which allow an easy open access and a landing on the latter and the color (yellowish or white greenish) (Primack 1987). The concave corolla, however, seems to be more adapted to bees than to birds. Birds usually prefer a tubular corolla (Lange & Scott 1999, Freitas et al. 2006). Pollen transport in *P. butyracea* via birds is probably accomplished on the bird's beak (see also Frost & Frost 1981) or head (see also Wester & Claßen-Bockhoff 2007) where pollen adheres to when the bird pecks the anthers of open flowers. Regarding pollination behavior, the birds were faster during their visits than the insect

pollinators and visited less flowers per inflorescence. Thus birds might facilitate less geitonogamy than insects. Also pollen dispersal distances are potentially different between birds and insects. Sunbirds are thought to disperse outcross pollen over longer distances (Degen et al. 2001) than Hymenoptera. However, insects display large variations in their potential flight distances (Roubik 1989). Given a distance between neighboring populations within the flight range of the observed pollinators and an efficient pollination, the diverse pollinator spectrum (also primates and bats, see Gautier-Hion & Maisels 1994, White & Abernethy 1996, Pettersson et al. 2004) combined with the high level of flowering synchrony within and between populations ($X_i = 0.62-1$ and $Z = 0.62-0.89$) could lead to substantial gene flow and a low genetic differentiation between populations.

Fruit and seed set

P. butyracea plants present a higher mean fruit set ($49 \pm 19\%$) from open-pollination than other tropical tree species (Bawa & Webb 1984). A positive correlation can be observed between tree, fruit and seed size. Taller trees (except old trees of dbh = 90 cm) present a higher fruit and seed set than smaller trees (see also Moupela et al. 2014). They have an advantage over smaller trees by intercepting more light for photosynthesis due to a higher and larger canopy and might therefore be able to produce large seeds and fruits (Fandohan et al. 2011, Ewédjè et al. 2012).

Regarding the high number of pollen grains per ovule and flower, and the pollination by animals a high fertilization rate of ovules per fruit might be expected in this hermaphrodite plant (Sutherland 1986). However, we detected a high rate of unfertilized ovules per fruit (94%). Similar results have been described in other plant species (Schemske & Horvitz 1988, Ley 2008). Our findings could be explained by:

- (1) pollen limitation due to an insufficient pollinator visitation rate, a pollen loss during transport by pollinators, and/or a short pollen viability. To test this, further hand-pollination experiments should be conducted.
- (2) a high level of geitonogamy due to clonal populations and phenological synchrony within trees paired with a low acceptance of self-pollen (= partial self-incompatibility), which could also be tested with hand-pollination experiments.
- (3) a selective abortion, resulting from resource limitation, to favor high quality (i.e. out-crossed) fruits and seeds (Kay 2006, Sun et al. 2007).

The longevity, storage and germination of *P. butyracea* seeds

The seeds of *P. butyracea* are recalcitrant. In recalcitrant seeds the embryo maintains a high tissue moisture content sustaining metabolic activity throughout ontogeny but bursts the seed tissues shortly after dispersal (Farnsworth 2000). Thus seeds are typically large (Hong & Ellis 1996) to reduce the rate of seed desiccation. Still they are generally short-lived and die rapidly when they are dried or chilled (Pritchard 2004). The moisture content in seeds of *P. butyracea* ($MC = 42.5 \pm 2.9\%$) was similar to sheas (*Vitellaria paradoxa* $MC =$

41–48%, Gaméné et al. 2004) lower than the MC estimated in the recalcitrant seeds of neem (*Azadirachta indica* 70%, Neya et al. 2004) but much higher than in many other recalcitrant seeds (e.g. *Khaya senegalensis* MC = 3.41%, Gaméné & Eriksen 2004; *Zanthoxylum zanthoxyloides* MC = 13.85%, *Parinari curatellifolia* MC = 17.49%, *Lophira lanceolata* MC = 21.30% and *Kigelia africana* MC = 26.98%, Sanon et al. 2004). Recalcitrance is a trait commonly found in the family Clusiaceae (Carvalho et al. 1998, Daws et al. 2005, Orwa et al. 2009) rendering these seeds unsuited for long-term storage (see also *Kigelia africana*: max. storage three months in Pritchard et al. 2004, *Vitellaria paradoxa*: max. storage: nine months in Gaméné et al. 2004) apart from costly Cryogenic technologies (Walters et al. 2013). For *P. butyracea* only a short term storage of up to 3.5 months could be successfully tested under sub-imbibitions around 20°C. Clearly further work on the effect of partial drying on recalcitrant seed viability and storability is required.

Germination of *P. butyracea* was most rapid at 30°C which corresponds to the soil temperatures that the dispersed seeds might experience in their natural environment (Daws et al. 2002) reflecting a genetically fixed adaptation to the local environment. Still the germination rate obtained here at 30°C is significantly lower than the one observed by Ouattara (1999) in Ivory Coast (germination rate of 95% of incubation at room temperature). Our result was potentially due to the fluctuations of temperature and the loss of seed moisture content in the time between seed collection in the field at the start of the trials in Belgium. For the further treatments at lower temperatures we would have expected a near linear relationship between temperature and germination. However, germination rate was lowest at 25°C and not at 20°C. That our results deviate from the expectation might be due to an inconstancy of temperature in the specific incubator we used for the trials at 25°C.

IMPLICATIONS FOR CONSERVATION

On the basis of the assumption that tree diameter is proportional to age, our survey on the 77 individual trees revealed a continuous population age structure in the three investigated populations (electronic appendix 1). This indicates an undisturbed recruitment in the past, however, Avocèvou-Ayisso et al. (2009) observed a reduction in seed availability for recruitment by 70% which might set the survival of populations at risk in the future. Avocèvou-Ayisso et al. (2009) and Djossa et al. (2007) documented a low number of seedlings (dbh < 5 cm) and saplings (dbh 5–10 cm) especially in unprotected and harvested stands. Still, *P. butyracea* can partly compensate reduced seed-set by asexual reproduction via root shoots (more than 84 juveniles firmly attached to the roots could be counted under a tree crown and logged trees might regenerated with six to eleven sprouts from its stump, Ewédjè, pers. obs.). Vegetative propagation might thus be an alternative silvicultural strategy for conservation. Thus in the future overall recruitment should be followed up and the relative contribution of sexual versus vegetative offspring to the formation of a population be determined. Thereby it would be of prime importance to assess their potential difference in fitness as prerequisite for successful species survival. In the

closely related *Symphonia globulifera* regeneration via seeds or vegetative reproduction was dependent on water regime and showed a higher mortality of saplings from vegetative reproduction (see dos Santos & Nascimento 2012).

A further potentially important threat to the species' survival might be population fragmentation (Dah-Dovonon 2002, Poidy 2002). It is not known to which extend the observed pollinators are still able to maintain a regular gene flow between the extant distant populations. Currently, only a moderate genetic differentiation is estimated between populations ($F_{ST} = 0.13$, Ewédjè 2012). However, the maintenance of genetic diversity of the species as a preadaptation for environmental change is crucial for its survival and should be tested by further genetic work. Here especially the presence/absence of inbreeding effects should be investigated to estimate the potential for the fixation of detrimental mutations especially frequent in small populations (Robledo et al. 2004).

The data has shown that only *in situ* conservation and/or *ex situ* conservation through orchard plantations are promising due to the recalcitrant nature of seeds unsuitable for long-term storage. The easy germination of seeds and the generalist pollination characteristics of the tree species (general pollination system, high pollen production, facultative outcrossing) will certainly facilitate any conservation effort. In the case of Benin, an orchard for the conservation of *Pentadesma butyracea* accessions has been recently established within the coastal forest at Ahozon (02°22'59.0"N, 02°09'38.7"E of geographical coordinates).

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (<http://www.ingentaconnect.com/content/botbel/plecevo/supp-data>) and consist of: (1) frequency distribution of trees per class of tree diameter in the population Igbo Aladja; (2) summary of data sampling strategy in three populations of *Pentadesma butyracea* from Benin; (3) complete list of pollinators identified on *Pentadesma butyracea* and kept as private collection; (4) petals of *Pentadesma butyracea* flowers perforated by sunbirds; (5) average fruit set of *Pentadesma butyracea* according to tree diameter class; and (6) seed characteristics of randomly sampled *Pentadesma butyracea* seeds used for measurements of moisture content and of seeds used for germination trials separated by germinated and non-germinated seeds.

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