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INSECT POLLINATION IMPROVES YIELD OF SHEA (*VITELLARIA PARADOXA* SUBSP. *PARADOXA*) IN THE AGROFORESTRY PARKLANDS OF WEST AFRICA

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Abstract—Pollinator decline, driven primarily by habitat degradation, has the potential to reduce the quantity and quality of pollinator-dependent crops produced across the world. *Vitellaria paradoxa,* a socio-economically important tree which grows across the sub-Saharan drylands of Africa, produces seeds from which shea butter is extracted. However, the habitats in which this tree grows are threatened with degradation, potentially impacting its ability to attract sufficient pollinators and to produce seeds. The flowers of *V. paradoxa* are insect-pollinated, and we investigated flower visitors in six sites in southern Burkina Faso and northern Ghana and tested whether plants were capable of fruit set in the absence of pollinators. We found that the majority of flower visitors (88%) were bees, most frequently small social stingless bees (*Hypotrigona gribodoi*), but native honey bees (*Apis mellifera adansonii*) were also common visitors to flowers early in the morning. The number of fruit produced per inflorescence was significantly lower when insects were excluded during flowering by bagging, but any fruits and seeds that were produced in bagged treatments were of similar weight to un-bagged ones. We conclude that conservation of habitat to protect social bees is important to maintain pollination services to *V. paradoxa* and other fruit-bearing trees and cultivated crops on which local livelihoods depend.

Keywords: Bees, Tree pollination, Fruit set, Livelihoods, Stingless bees, Tropical crops

INTRODUCTION

Pollinator decline, driven by agricultural intensification causing the fragmentation, degradation and loss of habitat, as well as climate change, parasites/disease and other factors (Goulson et al. 2015), can reduce pollination success and thus yield in many crop and wild plant species (Klein et al. 2007; Ollerton et al. 2011). Both the quantity and quality of yield can be affected via a reduction in the number and/or weight of fruits/seeds produced and the nutritional or commercial value of fruits, nuts and oils (Bommarco et al. 2012; Brittain et al. 2014). Conversely, an increased number and/or diversity of pollinators can improve yields (Garibaldi et al. 2016). Many studies investigating the role of pollinators in crop production have focussed on herbaceous temperate food crops (but see Klein et al. 2003; Macias-Macias et al. 2009, Kudom and Kwapong 2010 etc.), and the role of pollinators in the production of tropical tree crops has been comparatively understudied (Kwapong et al. 2014, but see Carvalheiro et al. 2010; Freitas et al. 2014), particularly in Africa (Rodger et al. 2004).

In the sub-Saharan drylands of Africa, some trees are deliberately maintained in a landscape which is also used for the cultivation of crops and/or animals in agroforestry parkland systems (Boffa 1999). These systems have been maintained by the practice of shifting cultivation, where

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cropping and grazing have alternated with fallow periods, for thousands of years (Gallagher et al. 2016). However, the semi-arid sub-Saharan drylands are a rapidly degrading habitat, under pressure from agricultural intensification, fuelwood demand and climate change (Bodart et al. 2013; Kandji et al. 2006). Reduced habitat diversity has been linked with insect pollinator decline worldwide (Kennedy et al. 2013), and degraded agroforestry parklands, which contain fewer species of tree and other vegetation due to reduced periods of fallow, may not be able to maintain the pollinator richness required for pollination of insect-dependent species (Tornyie and Kwapong 2015). Since 94% of tropical plant species are animal pollinated (Ollerton et al. 2011), this has implications for the reproduction of the majority of both crop and wild species in these habitats. Quantification and documentation of pollination deficits has been recognised as a priority issue for these areas (Gemmill-Herren et al. 2014). Although several native tree species in these agroforestry parklands produce edible or medicinal fruits, including *Parkia biglobosa* (African Locust bean), *Adansonia digitata* (Baobab) and *Tamarindus indica* (Tamarind), one of the most financially important to local communities is *Vitellaria paradoxa* (Shea/Karité).

Vitellaria paradoxa (Sapotaceae) grows in 21 sub-Saharan countries, with *V. paradoxa paradoxa* in West Africa and *V. paradoxa nilotica* in East Africa (Naughton et al. 2015), and "shea butter", extracted from the seeds of both subspecies, is the primary edible oil for 80 million people, and is growing in economic importance as a major export product, worth an estimated US$120 million annually (Naughton et al. 2015). In addition, the wood from this tree has a range of local uses (fuel as firewood or charcoal, building poles, making local utensils etc.), and the fruit pulp provides food for local communities during the "hungry season". The vast majority of shea butter production, from collection of the fruits to production of the oil, is carried out by women, and local trade in shea butter provides income to support education and diet in 18.4 million families (Pouliot 2012; Schreckenberg et al. 2006). *Vitellaria paradoxa* trees are usually not planted or sown, but naturally regenerate and, once established, certain saplings are selected and protected from damage by agricultural practices because of the value of the shea fruit. However, permanent cultivation and/or grazing, with reduced or non-existent fallow periods, prevents naturally regenerating shea seedlings attaining a size at which farmers will select for protection and recruitment into the parkland populations. In addition, a lack of tree planting, increased intensification and mechanisation of cropping, uncontrolled tree felling for fuel, and increased urbanisation greatly reduce habitat diversity and contribute to degradation of shea parklands (Boffa 2015; Elias 2013; Lovett & Haq 2000).

Vitellaria paradoxa flowers are hermaphrodite, predominantly outcrossing, and insect pollination has been noted to result in modest increases in fruit set (Klein et al. 2007; Okullo et al. 2003). Recent studies in a village in southern Burkina Faso have suggested that *Apis mellifera* (honey bees) are the primary pollinating species, and showed increases in pollination success when *A. mellifera* hives were nearby (Lassen et al. 2016). At least four taxa of smaller stingless and solitary bees are thought to compensate for *A. mellifera* in their absence (Lassen et al. 2016). However, there is a higher diversity of potential pollinator species in these habitats, including other bees, Diptera, Lepidoptera and Coleoptera, as well as several species of bird, and it is not clear which taxa visit shea flowers in addition to *A. mellifera* across the region. Furthermore, given geographic variation in genetic structure, microclimatic conditions, agricultural intensification and yields among sites (Boffa 2015; Gaisberger et al. 2017; Lovett & Haq 2000; Naughton et al. 2015), and the lack of applied pollination studies in this part of the world (Rodger et al. 2004), further study is required to confirm the findings of Lassen et al. (2016). In addition, it is not clear whether pollination is limiting fruit/seed set and weight, i.e. whether increased pollination could result in improved yields, particularly given the differences in yields recorded in different land-uses (Lamien et al. 2004). Pollinator limitation is common in many plant species (Burd 1994), caused by either insufficient pollinator visitation resulting in suboptimal pollen export and import, or caused by inappropriate pollen deposition (self or heterospecific).

To address these knowledge gaps, the current study tested the following hypotheses:

1. Flowers are predominantly visited by a small proportion of the available pollinating fauna, principally by honey bees and other wild social and solitary bees.
2. Shea yields (in terms of number of fruit set per inflorescence, fruit weight and seed weight) are
    i. lower when pollinators are excluded from flowers compared with open pollinated flowers i.e. flowers are dependent on animal-pollination, and
    ii. higher with pollen supplementation by hand compared with open pollinated flowers i.e. flowers are pollen limited.

MATERIALS AND METHODS

Study species

The flowers of *V. paradoxa* are produced during the dry season (December to April, depending on geographic location) in dense inflorescences at the end of usually leafless branches (Fig. 1). Inflorescences bear variable numbers of flowers (mean 31.9 ± 22.4 SD per inflorescence, $N = 330$ from 44 trees, authors' personal observations). Flowers are actinomorphic, approximately 15mm in diameter, have 8-10 creamy-white petals, and are protogynous, with the style (occasionally two styles) and fertile stigmas protruding from the buds before petals open (Hall et al. 1996) (Fig. 1). Nectar is produced at the base of the flower and is protected by petaloid staminodes which open early in the morning to allow access to flower visitors (Lassen 2016). Low volumes of nectar are produced, and small nectar standing crops have been recorded (mean 0.25 μl ± 0.58 SD per flower, $N = 20$ from 4 trees, authors' personal observations). After flowering, the ovary develops into a fruit containing one (occasionally two) seeds. Each inflorescence typically produces a small number of fruits (typically 2-3, rarely > 10, personal observations).

FIGURE 1: *V. paradoxa* inflorescence, open male-stage flowers (with dehiscing anthers), and female-stage buds (with protruding stigmas, marked with the dashed circle).

Study sites

Six sites in northern Ghana (Kanfaiyili, Damongo, Zini 1 and Zini 2) and southern Burkina Faso (Torem 1 and Torem 2) were selected opportunistically in consultation with local communities (Fig. 2, Tab. 1). In each site, 8-10 mature, flowering *V. paradoxa* subsp. *paradoxa* trees, 10-50 m apart, were selected within an area of < 0.5 km² as focal trees.

Insect surveys

In all sites, insects visiting *V. paradoxa* flowers were captured via hand-netting (Fig. 3a). Ten minutes was spent at each tree and all individuals seen to visit flowers were captured using long-handled nets. Netting was conducted early (06:00-07:30 hrs GMT), during the middle (11:30-12:30) and late (16:30-18:00) in the day in order to maximise the chances of capturing all insect species visiting flowers. Netting was conducted on eight separate days in each of the Zini sites, on two days in each of Kanfaiyili and Damongo, and Torem 1, and on four days in Torem 2.

In four sites (Kanfaiyili, Damongo, Torem 1 and Torem 2), insects were also sampled using pan traps to survey the flower visitor fauna present in the sites (Westphal et al. 2008). Each trap consisted of three 1.2 m plastic pipes (60 mm diameter) driven 200mm into the soil, 1m apart from each other in a triangular pattern (Fig. 3b). Small plastic cups (35 mm deep, 70 mm diameter), painted with fluorescent white, yellow or blue paint (which have previously been shown to attract a range of insect taxa), were set into the top of each pipe and half-filled with water and a drop of detergent to break the surface tension (Droege et al. 2010). Traps were left open for 24 hours, and then the contents of each cup were strained and insects were stored in 70% alcohol until they could be identified. This was conducted four times in Kanfaiyili and Damongo and five times in each of the Torem sites.

In two sites (Zini 1 and Zini 2), direct observations of flower visitation were conducted to quantify visitation rates.

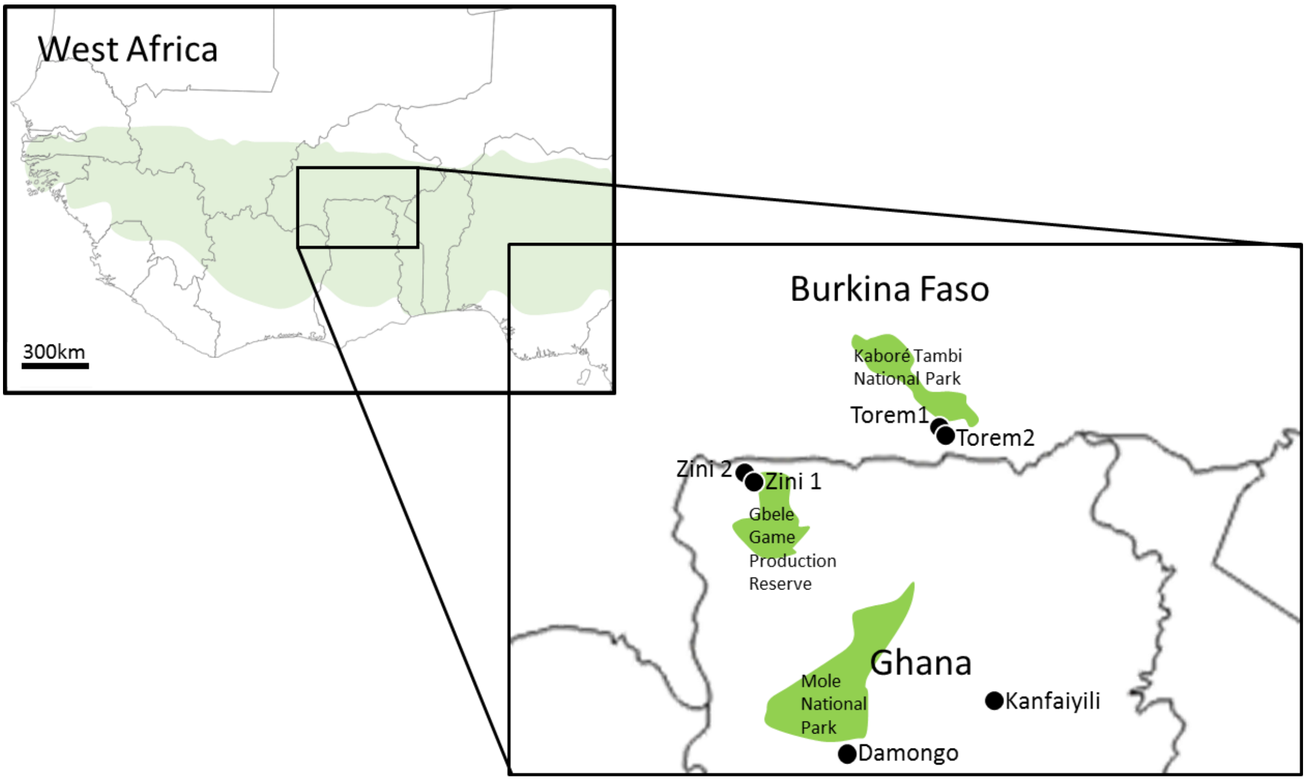

FIGURE 2: The *Vitellaria paradoxa* (shea) zone in West Africa (Naughton et al. 2015) (main map,) and sampling sites (inset). Protected areas are shaded on inset map. For details see Tab. 1.

TABLE 1: Study sites and data collected at each site.

Country	Site Name	Location (Grid Reference)	Site Description	Insect surveys			Pollination experiments		
				Pan trapping	Hand netting	Insect visitation rates	open	bagged	Supple-mental hand pollinatio n
Ghana	Kanfaiyili, Tamale	9.50688, -0.90753	Agricultural land 13km from centre of Tamale, little natural vegetation, 73km from Mole National Park	√	√		√	√	√
	Damongo	9.09127, -1.82695	Former fallow, beside school on edge of town, some natural vegetation, 8.6km from Mole National Park	√	√		√	√	√
	Zini 1	10.833333, -2.382556	Agricultural land located approx. 2 km away from dwellings, with some natural vegetation in the adjacent fallow land, 50km from Gbele Game Production Reserve		√	√	√	√	
	Zini 2	10.870583, -2.413556	Agricultural land located approx. 1 km away from dwellings with little natural vegetation, 55km from Gbele Game Production Reserve		√	√	√	√	
Burkina Faso	Torem 1	11.2170731, -1.1897331	Agricultural land within savannah landscape, 4.5km from Kaboré Tambi National Park, and 2km to Pô, chief town of Nahouri Province.	√	√		√	√	√
	Torem 2	11.206331, -1.1892506	As above, 5.5km from Kaboré Tambi National Park	√	√		√	√	√

Five inflorescences were observed over 10 minutes on each tree. Observations were conducted early (06:00-07:30), during the middle (11:30-12:30) and late (16:30-18:00) in the day in order to determine when visitors were most active. Observations were made on seven separate days in each site at approximately weekly intervals during February and March.

All insects were identified to order, except bees, which were identified to species.

Pollination treatments

On each tree, three pollination treatments were applied to entire inflorescences ($N = 3$ per treatment per tree) which were marked with coloured tape. These treatments were:

1. Open pollination ("open") – no manipulation of flowers
2. Pollinator exclusion ("bagged") – inflorescences were bagged whilst still in bud using bridal veil material (mesh

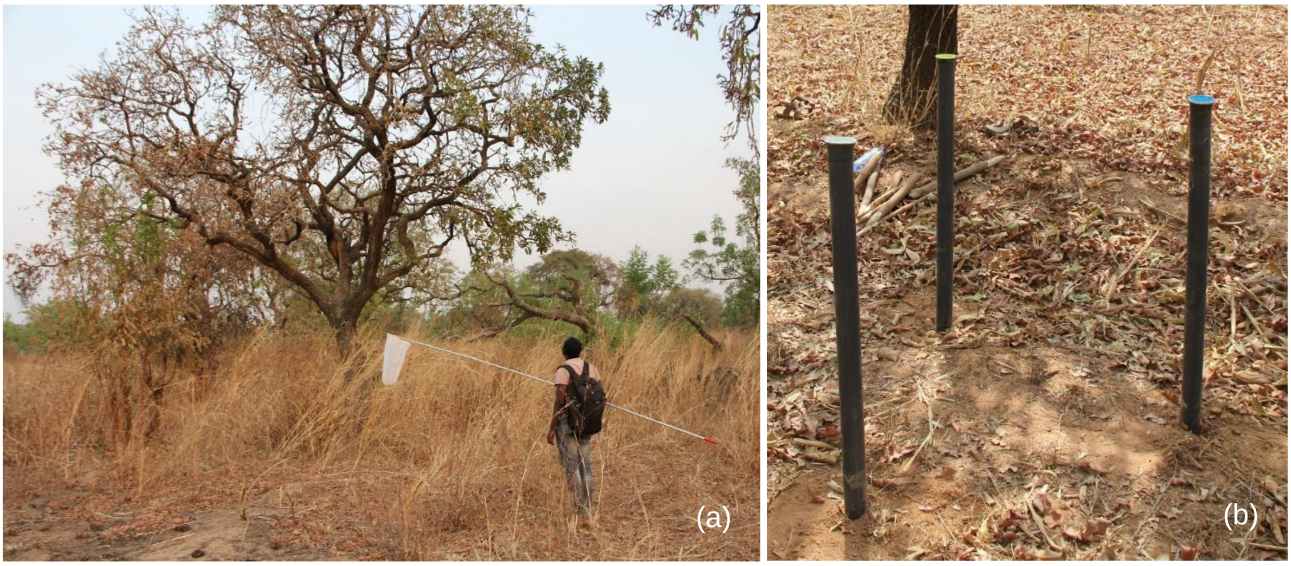

FIGURE 3: Insect trapping methods a) hand netting using long-handled nets to sample insects directly from flowers, b) pan trapping using plastic cups painted with fluorescent yellow, white and blue, half-filled with water.

size ~1 mm) – any buds with already protruding styles were removed before bagging and bags were removed when flowering was completed.

3. Supplemental hand pollination ("hand pollination") – pollen was applied to protruding stigmas directly from the anthers of flowers from different trees.

Treatments were applied during January/February 2016 when trees started to flower and fruit formation was monitored until maturity in June 2016. Due to logistical constraints, hand pollination was only performed on each inflorescence once during flowering, and any untreated flowers were removed from inflorescences. Fruits from all treatments were counted in the middle of May 2016 before fruit dehiscence, immediately weighed, and then pulp was removed and seeds counted and weighed.

Data analysis

Differences in 'fruit number' (number of fruit per inflorescence), 'fruit weight' (mean fruit weight per inflorescence) and 'seed weight' (mean seed weight per inflorescence) between "bagged", "open" and "hand pollinated" treatments were assessed using Generalized Linear Mixed Models (GLMMs). Thus, we compared both the quantity (fruit number) and quality of fruit (fruit and seed weight) in the presence and absence of pollinators ('open' vs. 'bagged' treatments). The comparison between 'open' and 'hand pollinated' treatments was used to assess the role of pollen limitation in determining fruit quantity and quality in the field, with differences between open and hand pollinated treatments representing the shortfall between current productivity and the potential maximum productivity with no pollen limitation. In some cases it was not possible to weigh fruits or seeds as fruit had already fallen from the trees prior to the return site visits, hence for fruit number $N = 464$, whilst for fruit weight $N = 137$ and for seed weight $N = 134$. For each response variable ('fruit number', 'fruit weight' and 'seed weight') a separate GLMM was constructed with treatment as a predictor variable (fixed factor) and 'Tree ID' nested within 'Site' as a nested random factor to account for correlation between measures both within trees and sites. GLMMs were initially fitted with a Gaussian response distribution and residuals were assessed for normality using a Shapiro-Wilk test. In all three cases, model residuals were not normally distributed, therefore models were recalculated using alternative response distributions. 'Fruit number' models were first refitted using a Poisson distribution with a log link function appropriate for count data, but model residuals showed over-dispersion and therefore the model was refitted using a negative binomial model with a log link function. 'Fruit weight' and 'seed weight' models were refitted using a 'gamma' distribution with a log link function suitable for non-negative continuous data. GLMMs were fitted using the package 'glmmADMB' in R 3.3.3 (Fournier et al. 2012; R Core Team 2017). Where GLMMs indicated significant differences between treatments, post-host analysis of the pairwise differences between treatment combinations (i.e. open vs. bagged, open vs. hand pollinated and bagged vs. hand pollinated) were calculated using the Tukey Honest Significant Difference test in the package 'multcomp' (Hothorn et al. 2008).

RESULTS

Insect surveys

Over a total of 26 ten-minute hand-netting periods, a total of 280 insects were captured whilst foraging on shea flowers (Tab. 2); 247 (88.2%) individuals were bees, from six species (*Apis mellifera adansonii*, *Ceratina moerenhouti*, *Compsomelissa nigrinervis*, *Hypotrigona gribodoi*, *Meliponula ferruginea* and *Meliponula beccari*). *Hypotrigona gribodoi* were most frequently captured (182 individuals), followed by *Apis mellifera adansonii* (48 individuals). The Zini sites had the greatest diversity of bees (five species), whilst no bees were captured on flowers at Torem I.

TABLE 2: Insects captured at each site using Pan trapping and Hand netting (K = Kanfaiyili, D = Damongo, T1 = Torem 1, T2 = Torem 2, Z = Zini – both sites combined).

	Pan trapping				Hand netting				
Site	K	D	T1	T2	K	D	T1	T2	Z
Number of trapping sessions	4	4	5	5	2	2	2	4	16
Bees									
Apis mellifera adansonii	0	1	0	0	11	6	0	1	26
Hypotrigona gribodoi	2	7	8	3	6	2	0	1	173
Meliponula ferruginea	0	0	0	0	2	2	0	0	2
Meliponula beccari	0	0	0	0	0	0	0	1	4
Compsomelissa	0	0	1	3	0	0	0	0	2
Xylocopa olivacea	1	0	0	0	0	0	0	0	0
Amegilla calens	2	2	8	4	0	0	0	0	0
Lassioglossum duponti	0	4	1	1	0	0	0	0	0
Lipotriches natalensis	0	0	3	3	0	0	0	0	0
Pseudoanthidium truncatum	0	0	0	1	0	0	0	0	0
Ceratina moerenhouti	0	0	0	0	0	0	0	1	0
Bees total	**9**	**22**	**27**	**16**	**19**	**10**	**3**	**4**	**207**
Wasps	5	13	7	5	0	1	1	3	3
Flies	24	65	26	8	1	0	0	6	0
Beetles	4	12	10	10	0	2	1	0	0
Ants	0	5	2	1	0	0	0	0	4
Bugs	0	8	1	3	2	0	0	1	0
others	11	15	14	18	1	1	4	2	0
All insects total	**53**	**140**	**87**	**61**	**23**	**14**	**9**	**16**	**214**
Insects per trapping session	13	35	17	12	11.5	7	4.5	4	13.4
number of bee spp	3	4	5	6	3	3	0	4	5
% bees	17	16	31	26	83	71	33	25	97

TABLE 3: Shea flower visitation in the two sites in Zini by *Apis mellifera adansonii* and other bees early (06.00-07.30), during the middle (11.30-12.30) and late (16.30-18.00) in the day.

		Total number of bees observed		Average number of visits per inflorescence per hour
		Apis mellifera	Other bees	
Zini1				
	Early	79	223	0.014
	Middle	0	37	0.0017
	Late	8	122	0.0060
Zini2				
	Early	56	151	0.0096
	Middle	0	21	0.00097
	Late	5	82	0.0041

A total of 341 insects were captured in pan traps over the four sites (Tab. 2). Seventy-four (21.7%) individuals were bees, from eight species (*Amegilla calens, Apis mellifera adansonii, Compsomelissa nigrinervis, Hypotrigona gribodoi, Lassioglossum duponti, Lipotriches natalensis, Pseudoanthidium truncatum* and *Xylocopa olivacea*). Other insects were only identified to Order, including Hymenoptera (wasps and ants), Diptera (flies), Coleoptera (beetles), Hemiptera (bugs) and other unidentified specimens (Tab. 2). The highest abundance of insects was captured at Damongo, but Torem 2 had the greatest species richness of bees (six species), whilst Kanfaiyili had only three bee species.

TABLE 4: Fruit set (number of fruits per inflorescence), fruit weight (mean per inflorescence) and seed weight (mean per inflorescence) following pollination treatments ("Bagged" = pollinator exclusion; "Open"= no manipulation; "Hand pollinated" = supplemental hand pollination; *N* = number of inflorescences, fruits and seeds respectively).

Site	Treatment	Fruit number			Fruit weight (g)			Seed weight (g)		
		N	Mean	Range	*N*	Mean	Range	*N*	Mean	Range
Damongo	Bagged	30	0.27	0 - 2	1	9.96	9.96 - 9.96	1	0.32	0.32 - 0.32
	Open	29	1.21	0 - 6	6	21.31	10.66 - 28.24	6	7.74	3.87 - 9.86
	Hand pollinated	30	1.40	0 - 6	9	16.63	8.17 - 25.64	9	6.56	3.38 - 10.37
Kanfiayili	Bagged	30	0.93	0 - 4	8	11.27	3.93 - 17.32	8	5.09	1.04 - 8.43
	Open	30	3.17	0 - 16	9	10.47	5.24 - 16.22	9	5.32	2.72 - 8.16
	Hand pollinated	28	2.25	0 - 12	10	9.37	3.37 - 23.98	10	5.63	2.31 - 16.90
Torem 1	Bagged	30	0.50	0 - 3	5	23.2	14.75 - 36.82	5	7.56	5.77 - 8.98
	Open	30	1.37	0 - 11	3	20.32	16.40 - 22.47	3	8.2	7.83 - 8.68
	Hand pollinated	30	2.53	0 - 10	8	20.85	14.54 - 40.71	8	7.82	4.25 - 14.60
Torem 2	Bagged	30	2.20	0 - 6	3	13.41	10.96 - 16.78	3	6.55	6.31 - 6.93
	Open	30	2.47	0 - 6	12	18.02	11.04 - 34.46	12	7.36	4.56 - 11.35
	Hand pollinated	29	3.72	0 - 14	14	19.24	9.29 - 27.19	14	7.13	4.07 - 10.55
Zini1	Bagged	27	0.48	0 - 3	9	16.58	0.70 - 31.75	6	8.13	5.90 - 9.70
	Open	27	2.00	0 - 7	20	25.56	16.10 - 40.00	20	8.15	2.80 - 11.60
Zini2	Bagged	27	0.19	0 - 1	5	21.5	14.30 - 27.20	5	5.98	2.30 - 8.10
	Open	27	0.85	0 - 3	15	24.24	13.90 - 38.00	15	8.42	2.30 - 14.10

Observations of visitation rates at the two Zini sites confirmed that bees were the most frequent visitors to flowers. During a total of 71.5 hours of observations, 784 flower visitors were recorded: 148 *A. mellifera* and 636 other bees (Tab. 3). *A. mellifera* were mainly active early in the day (06:00-07:30), were never seen on flowers in the middle of the day, and rarely seen later in the day. Other bees were also most active early in the day, less active in the middle of the day, but were also reasonably active later in the day. Total visitation rates (bees per inflorescence per hour) were more than twice as frequent in the morning compared to the afternoon (0.059 visitors per hour in the morning vs. 0.025 visitors per hour in the afternoon).

Pollination treatments

On average, across all sites, open pollinated inflorescences produced 1.86 ± 0.18 (mean ± s.e.) fruits per inflorescence, whilst pollinator exclusion (bagged inflorescences) produced 0.78 ± 0.09 fruits. Fruit set was marginally higher with supplemental hand pollination, with 2.47 ± 0.26 fruits per inflorescence (Tab. 4).

Fruit number was significantly higher in 'open' and 'hand pollinated' treatments than in 'bagged' treatments ($\beta = 0.936 \pm 0.129$, $P < 0.001$; and $\beta = 1.107 \pm 0.139$, $P < 0.001$ respectively). There was no significant difference in fruit number between 'open' and 'hand pollinated' treatments ($\beta = -0.135 \pm 0.176$, $P = 0.717$) (Fig. 4a). Fruit weight and seed weight did not differ significantly between treatments ($\chi = 3.864$, $P = 0.145$ and $\chi = 1.144$, $P = 0.564$) (Fig. 4b & 4c). Some variation was observed among sites, with bagged flowers at Torem 1 and open flowers at Kanfiayili producing relatively more fruit than the same treatments in other sites (Appendix 1).

DISCUSSION

Visitors to *V. paradoxa* flowers during our study were primarily bees, despite the presence of other flower-visiting insects in the shea parklands during the flowering season. Unlike Lassen et al. (2016), we did not observe sunbirds visiting shea flowers, nor the flowers of the hemi-parasitic plants that grow on shea trees (Zwarts 2015), during observation periods. It is possible that the phenology, nectar chemistry or structure of the flowers place constraints on which insects can forage on *V. paradoxa* flowers. Those insects active later in the day, or with a proboscis which is too short to access the nectaries, may be prevented from utilising shea as a forage resource, and thus not be frequent visitors or effective pollinators (Nienhuis & Stout 2009; Stang et al. 2006). In addition, the nectar chemistry, and secondary compounds present in the nectar (Meda et al. 2005), may also affect which species visit flowers (Adler 2000; Tiedeken et al. 2016).

Honey bees (*Apis mellifera adansonii*) and six species of stingless bee were recorded as the most frequent visitors, and like Lassen et al. (2016), we found that honey bees preferred to visit early in the morning, whilst temperatures were lower. Very little nectar was found in flowers, even though we sampled shortly after dawn. Later in the day, the very low nectar volumes available in flowers, as well as higher temperatures, might explain the absence of honey bees. However, without further understanding of patterns of nectar secretion, visual and olfactory floral traits, or quality of nectar

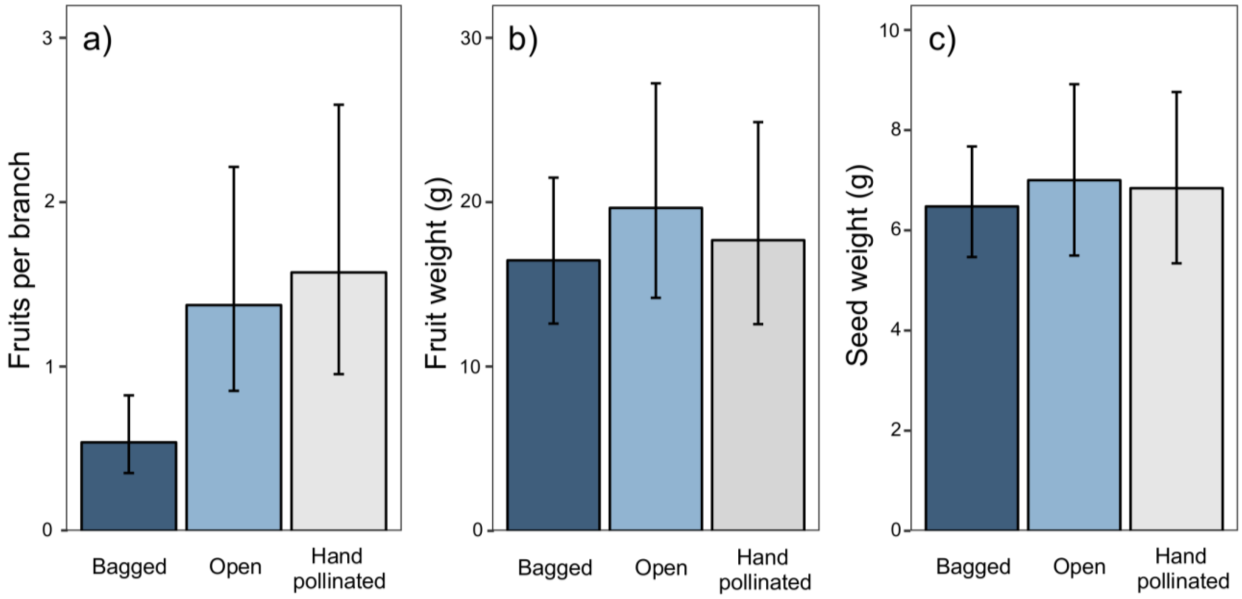

FIGURE 4: Fitted mean ± 95% of confidence intervals based on GLMM outputs for a) fruit set, b) fruit weight and c) seed weight from bagged, open and hand-pollinated shea flowers.

and pollen rewards, in both shea and other co-flowering species, it is not possible to speculate on what is driving visitor behaviour. We did not assess the behaviour or relative pollination efficiency of the different bee visitors in this study. However, some assessment of the ability of different species to pick-up and deposit pollen appropriately (spatially and temporally), in addition to the frequency of their visits, would be important to determine which pollinators are the most effective (Kasina et al. 2013). It is possible that the stingless *Hypotrigona gribodoi* bees, whilst more frequent visitors than honey bees, are less efficient as pollinators due to their smaller body size. Furthermore, observations of behaviour of different species would held to determine how pollination service varies spatially within trees and within populations (Kwapong et al. 2014).

We confirmed that *V. paradoxa* flowers require insect visits for fruit production (Lassen et al. 2016; Yidana 2004). Fruit set per inflorescence more than doubled in the open compared with bagged treatments. Fruit can fall to the ground or be taken by birds/bats and so it is possible that our fruit counts for this treatment are under-representative of actual fruit set. Some fruit were produced from the bagged treatments, particularly in Torem 1, and we assume that this was due to within-bag pollen movement facilitated by wind or animals, pollen entering through the mesh of the bags, stigmas extruding outside of bags, or due to incomplete protogyny (Silva & Goring 2001). Flowers do appear to be strongly protogynous though, with the stigma becoming receptive before anthers dehisce, making within-flower self-fertilization unlikely. However, on a single inflorescence there can be many closely-packed flowers at different stages of maturity (Fig. 1). Thus within-inflorescence selfing can occur. Indeed, within a single tree, insect pollination can facilitate geitonogamy (within-plant selfing) – this might be particularly common on trees with an abundance of flowers, or on trees isolated from neighbours.

Although hand pollination was associated with higher mean fruit set in the Torem sites and at Damongo, the same was not true at Kanfaiyili where open flowers produced more fruit per inflorescence than at the other sites, and overall there was no statistical difference in fruit set between and hand and open pollinated inflorescences. This could be an artefact of the methods used: hand pollination was performed on each tree only once during flowering and we removed non-treated flowers, thus potentially reducing the total number of flowers on hand-pollinated inflorescences. It may have been better to mark treated flowers and repeat treatments throughout the flowering season so that all flowers on an inflorescence were treated with outcross pollen. Also it is possible that we treated non-receptive stigmas, as 27.5% of hand-pollinated inflorescences produced zero fruit (data not shown). Alternatively, we may have just failed to detect pollen limitation because hand pollinations were not done at the scale of the whole tree (Wesselingh 2007). Furthermore, fruit set may be limited by other biotic and abiotic resources, which vary greatly from site to site, including soil, nutrients, water availability, pesticide use, and pest and pollinator density. Given the variation between sites and the methodological limitations, and potential other constraints on fruit production, it would be worth repeating these tests for pollen limitation.

Since we found no evidence for reduced fruit or seed weight as a result of pollinator exclusion, we could also be tempted to conclude that whilst pollinator visitation influences yield quantity, it has no impact on quality. However, we did not perform germination tests to determine biological "quality", nor did we examine the oil content of the seeds to determine economic quality according to treatment. These are questions that require further investigation.

The lack of bees captured on flowers at Torem 1 may simply have been due to low sampling effort (only two netting sessions were conducted). Bees were captured at this site in pan traps, and given fruits were produced from the open pollination treatments at this site, we can assume that flowers were visited at some point. More comprehensive surveying of visitors, including potential nocturnal pollinators, would be beneficial, although logistically difficult.

All six sites used in this study were within actively cultivated systems, except the one on the edge of the small town of Damongo, which was an old fallow - the current shea tree distribution at this site shows that these were once-farmed parklands. This site was also within 10km of the largest protected area in Ghana (Mole National Park), and in an area which has experienced a decline in farming due to recent economic focus on timber extraction. These factors may explain the elevated number of insects captured in pan-traps at this site. However, we did not see an increase in the number of flower visitors, or in fruit set, at Damongo. Although previous studies have shown that proximity to protected areas can increase pollination services to tropical trees (e.g. Freitas et al. 2014), our study design did not allow us to test this here. While three sites (Damongo, and the two Zini sites) were located relatively near to protected areas, and these areas may provide resources for bees, small stingless bees are unlikely to travel this far to forage (Araújo et al. 2004). Honey bees can travel over large distances to forage, and so the spatial pattern of resources at a landscape scale may influence visitation and pollination of shea. The proximity to urban centres, habitat structure and land- use (including fallow periods, ploughing methods, tree density, pesticide use etc.) could all influence bee abundance and deserve further consideration. Furthermore, how the pollinators of *V. paradoxa* respond to other flowering plants in the parklands, and what limits their populations (natural enemies and response to environmental fluctuations, as well as anthropogenic activity) should all be addressed in order to improve management recommendations for maximising pollination services in the shea parklands.

In conclusion, we have demonstrated that bees are very important for the pollination and fruit set of *V. paradoxa* across several sites in the agroforestry parklands of West Africa. Continued clearing of natural habitat and less regeneration via fallows could potentially damage bee populations (Tornyie & Kwapong 2015). Beekeeping, with both honey bees and stingless bees, as well as enhancing plant diversity in the parkland as a food source for bees, could therefore present a win-win opportunity for local communities, enhancing both pollination services to *V. paradoxa* and other useful fruit-bearing trees and other crops (Kudom & Kwapong 2010; Kasina et al. 2013; Kiatoko et al. 2014), and providing honey to supplement household incomes. Furthermore, increased semi-natural habitat may have wider biodiversity benefits, for example in supporting nesting and foraging sites for resident and migrant birds (Zwarts 2015). However, more research is needed to understand the pollination ecology of these sub-Saharan ecosystems, including both cultivated and wild plant species, as well as community-level interactions (Rodger et al. 2004; Gemmill-Herren et al. 2014).

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APPENDICES

Additional supporting information may be found in the online version of this article:

APPENDIX I. Fruit set per treatment per site

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