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POLLINATION BIOLOGY AND SPATIO-TEMPORAL STRUCTURING OF SOME MAJOR *ACACIA* SPECIES (LEGUMINOSAE) OF THE ARABIAN PENINSULA

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Abstract

Acacias are the dominant woody plant species distributed over the vast tracts of land throughout the Arabian Peninsula. However, information on spatio-temporal structuring and pollination biology of the species is not precisely available. To determine whether any variations exist among the *Acacia* species in their temporal distribution, their flowering period was determined through monitoring the commencing, peaking and ending of flowering of each species. Moreover, if any variations exist in release of floral rewards among the different co-existing and co-flowering species as mechanisms of partitioning of pollinators, to minimize competition for pollination, the progress of their anthesis over time was recorded by scoring polyads to anthers ratio at different hours of a day. In addition, the amount and dynamics of nectar sugar per inflorescence (N =225/species) was determined following flower nectar sugar washing technique. Types and frequencies of flower visitors and their preferences were determined by recording the visitors 6 times a day. The current study revealed that the *Acacia* species of the Arabian Peninsula are spatio-temporally structured: some species co-exist yet have different flowering seasons, whereas others co-exist, flowering concurrently yet exhibit a shift in their time of peak flowering and in the time at which the peak pollen is released during the day. This study demonstrates that all *Acacia* species examined secrete a considerable amount of nectar (2.24±1.72 -10.02±4.0mg/inflorescence) which serves as a floral reward for pollinators. Insects of the Order Hymenoptera are the most prevalent visitors to *Acacia* species in the region. The variations in spatio-temporal structuring of the *Acacia* species could be due to their adaptation of reducing competition for pollinators and minimizing hetero-specific pollen transfer.

Key words: Pollinator partitioning, Nectar secretion, Floral phenology, Pollen release.

Introduction

Acacia form part of the Leguminosae (Fabaceae), the third-largest family of flowering plants. Legumes are of significant economic importance, second only to the cereals (Heywood *et al*., 2007). *Acacias* are essentially woody plants in many tropical and subtropical arid regions of the world (Ross, 1981), which accounts for their significant biomass (Wickens, 1995). These plants are an important sources of firewood, timber, forage, gum, tannins, fiber, food, folk medicine, and are also useful for environmental protection as well as soil and water conservation (Wickens, 1995; Midgely & Turnbull, 2003). *Acacias* sustain a large number of herbivorous vertebrates and invertebrates as well as many species of nectarivorous insects.

Many studies have been devoted to *Acacia* reproductive biology (Sedegley *et al.,* 1992; Tybirk, 1993; Kenrick, 2003); floral phenology (Tybirk, 1993; Stone *et al.,* 1998; Raine, 2001; Tandon *et al.,* 2001), floral rewards (Stone *et al.,* 1998; Tandon *et al*., 2001) and visitor assemblages (Tybirk, 1993; Stone *et al.,* 1998; Raine, 2001; Kenrick, 2003). Moreover, the intra and inter-specific competition among various *Acacia* species for pollinators has also been investigated (Stone *et al*., 1998; Raine *et al.,* 2002).

Competition for pollination has been considered as the main factor in the structure and timing of flowering in many plant communities (Rathcke, 1988). Sympatric species, which are unable to separate in space, may use different pollinator guilds (Rathcke, 1988), or may differ with respect to their periods of flowering (Williams, 1995) to avoid competition for pollinators. However, seasonal patterns, such as the availability of precipitation and the thermo-period, may impose constraints on flowering seasons of many sympatric species (Johnson, 1992). In such cases, further temporal deviation of pollen release throughout the day has been reported to minimize competition for pollinators (Stone *et al.,* 1996, 1998).

In this regard, several studies have been devoted to the pollination biology and partitioning of pollinators of *Acacia* species from Australia, Africa, and Latin America (Tybirk, 1993; Stone *et al.,* 1998, 2003; Tandon *et al.,* 2001). However, many*Acacia* species are widely distributed from Africa to Arabia (Ross, 1981), forming part of a widely diverse *Acacia*community (Tybirk, 1993). The lack of adequate information on ecological variation and comparative studies on *Acacia* pollination biology in other regions has been considered as an important information gap in our knowledge (Stone *et al.,* 1998; Midgley & Bond, 2001; Stone *et al.,* 2003).

Acacias are diverse and widely distributed tree genera of the Arabian Peninsula, with more than ten *Acacia* species reported to occur in this region (Aref, 2000). Different *Acacia* species, such as *Acacia origena, A. etbaica, A. tortilis, A. ehrenbergiana, A. asak, A.johnwoodii* and *A. oerfota,*are the major components of the vegetation of the coastal and inland plains of the vast areas of the region (Anon., 1977; Walter & Breckle, 1986). Several *Acacia* species have an important role in contributing vegetative biomass, ensuring ecosystem functioning, serve as excellent bee forages and source of high-quality honey which in turn improves the livelihood of people in the area. However, the pollination biology of *Acacia*, their spatio-temporal structuring, the amount of nectar secretion and dynamics, the type of pollinator guilds and partitioning of pollination niches (if any), have until now not been studied for the majority of the *Acacia* species of the region. Such information is important from a pollination ecology perspective, and for estimating the socio-economic value of the species.

The amount and concentration of nectar varies from plant to plant and over time (Chalcoff *et al.,* 2006). Many studies have been conducted on different plant species to quantify their nectar secretion dynamics; Petanidou & Smets, (1996); Nepi *et al,* (2001); Castellanos *et al,* (2002); Galetto and Bernardello, (2004). In general, the flowers of subgenus *Acacia*, which has spherical inflorescences, have been reported as bearing no nectar or secreting only trace amounts (Stone *et al.,*1998, 2003). However, in the study areas where these *Acacias* grow, beekeepers have been observed placing hundreds of honeybee colonies during the flowering periods of many of these *Acacia* species with the aim of harvesting high-value acacia honey (Al-Jeffri, 2009; personal observation).

With this general background, the aims of this study were to address the following questions: 1. Are sepatio-temporal variations exist as a possible means of achieving partial partitioning of pollinators among the different *Acacia* species in the region? 2. Are there variations in the dynamics and amount of nectar secretions among the different *Acacia* species? 3. Is there a difference in the timing of daily peak pollen release among co-existing and co-flowering *Acacia* species? 4. Do those *Acacias* with spherical inflorescences not produce any nectar as previously reported?

Accordingly, we investigated the pollination biology of the major *Acacia* species (flower morphology, flowering phenology and temporal distribution of floral rewards) under the typical arid climatic conditions of the Arabian Peninsula. In addition the types of pollinator guilds and their temporal association with the availability of floral rewards were also studied. The partitioning of pollen release (if any) throughout the day to minimize competition between sympatric and co-flowering species was assessed. Furthermore, the quantities and dynamics of nectar secretion, at different times of the day were recorded and inter-and intra-comparison were made among species and trees.

Materials and methods

Study site and species: This study was conducted in southwestern Saudi Arabia at three localities (Fig. 1) where the *Acacias* species used in this study were predominate. One of the sites was located at Wadi (Valley) Alkhatani, at an altitude range of 400 to 1,000 m above sea level and it comprised six *Acacia* species (*A. tortilis, A. ehrenbergiana, A. asak, A.johnwoodii*, and *A. oerfota)*. The second site was Wadi Bereha, which is located at altitude range of 1,550 - 1,750 m above sea level and the dominant plant species is *Acacia etbaica* mixed with *Ziziphus spina-christi.* The third site was located at Baljurashi, which is situated on a highland plateau, the altitude ranging between 1,900 – 2,200 m above sea level; the dominant species found were *A. origena* and *A. gerrardii* mixed with *Dodonaea angustifolia*, *Olea europaea* and *Juniperus procera.*

Floral morphology: The average size of an inflorescence was determined by measuring the diameter and length of 50 inflorescences per species. The average number of florets/inflorescence and average number of stamens/floret were determined by counting the number of florets/inflorescence (N = 50) and the number of stamens/floret (N = 50). In addition, the proportions of florets with a stigma were determined by counting the number of florets with and without a stigma in 50 inflorescences using a hand lens.

Seasonal flowering period distribution: The flowering period distribution was determined by monitoring and recording the beginning, peak and end of flowering periods of each species over a two years period. Moreover, the flowering patterns of sympatric and co-flowering *Acacia* species (*A. ehrenbergiana* and *A. tortilis)*were carefully monitored to determine whether any variation in their flowering peak periods could have occurred within a season. To accomplish this task, at the beginning of the flowering season, 20 *A. ehrenbergiana* and 20 *A. tortilis* treeswere labeled, and the flowering patterns (i.e. commencement, peak and end) were monitored and recorded. The peak flowering time was defined as the period when more than 50% of the flower buds on the labeled trees were open.

Flower phenology and time of pollen release: For the flower phenology study, 5 - 10 inflorescences per species were labeled, and their phenology monitored every 2 hours from 0500 to 1800 h. The time of flower opening, pollen release and stigma emergence, flower shading and duration of flowering were observed and recorded. To determine the peak time at which pollen is release and whether partitioning of pollen release through the day among sympatric and co-flowering species occurs, the peak time of pollen release was determined by quantifying the ratio of the relative abundance of polyads to anthers at different hours of the day (0600, 0800, 1000, 1200, and 1400 h) following the protocol described by Stone *et al.,* (1998). The progress of anthesis over time was recorded by scoring the ratio of polyads to anthers.

Relative pollen transfer efficiency: To determine the relative pollen transfer efficiency of the species, pollen-to-ovule ratios were determined by calculating pollen grains per polyad x 8 (polyads per anther) x the average number of anthers per flower/proportion of flowers with stigmas and number of ovules per ovary following the protocol as described in Baranelli *et al.* (1995). The number of pollen grains per polyad was determined through polyad reference slide preparation and microscopic examination.

Flower visitors: For the flower visitors study, three flowering trees/species were selected, and a 1 x 1 m^2 area of branches with flowers was delineated. Observations of flower visitors were made six times a day: 0600, 0800, 1000, 1200, 1400, and 1600 h. During each observation period, visitors were recorded for a period of 10 min for each tree. The observations were repeated over three consecutive days, and the visitors and frequencies of their visits were recorded for each *Acacia* species. Voucher specimens and digital photographs of flower visitors were taken and the identifications were made by expert taxonomist, the use of classification keys and reference materials. The flower visitors were classified to Order or Family level. The temporal variation at which flowers were visited and any preferences the flower visitors had to the different *Acacia* species, were recorded and analyzed.

Nectar sugar secretion amount and dynamics: The dynamics of nectar sugar production were determined by examining three plants/species. The nectar sugar was estimated at five periods of a day at 0600, 0900, 1200, 1500, and 1800 h. All flower buds were bagged, using fine, nylon netting, (Wyatt *et al.,* 1992), one day prior to their flowers opening. The nectar sugar was measured from five inflorescences at a time for each plant and for each sampling time, totaling 25 flower heads/day/plant. The measurements were repeated over three consecutive days; thus, a total amount of 225 inflorescences were measured for each species. One inflorescence was used for only one time measurement.

The amount of nectar sugar secretion per inflorescence was determined following the flower nectar sugar washing technique described by Mallick (2000). For this procedure, each spherical inflorescence was removed separately and placed in a small, narrow plastic vial, and washed in 1 ml of distilled water (0.5 ml in the case of *A. tortilis* flowers). Because the average diameter of flower heads differed among the species and the amount of distilled water required to submerge the flower heads varied. The flowers were then left in the distilled water for 5 minutes until the sugar had completely dissolved. In *A. asak* which has elongated inflorescences (spikes), not all florets open at the same time. For this species,20 open florets were taken from the inflorescence at each measuring period and washed using the same technique as that employed for other acacia species with spherical inflorescences. The amount of nectar sugar was then calculated per spike using the average number of florets per spike.

From the pooled solution of washed sugar, a drop of clear solution was removed using a micropipette, and the concentration was measured using a pocket refractometer (*ATAGO, No. 3840,* Japan). The mass of the sugar in the nectar secreted from each flower head was calculated from the volume and concentration of the solution measured. For the *Acacia* species with elongated (spiked) inflorescences, the mass of nectar sugar per inflorescence was calculated using the average mass of sugar per floret and multiplied by the average number of florets per inflorescence. The sucrose concentration readings (mass/total mass, g of sugar/100 g of solution) were converted to sucrose mass/volume using Weast's (1986) conversion table. The results were then compared among trees, species, and sampling time periods.

Weather data: Along with the other observations, the temperature and relative humidity (RH) of the study sites were measured at each sampling time using an Environment Meter (N09AQ, UK) and correlated with the other data recorded.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the mean amount of nectar sugar secreted per flower head per 3 h period from the trees within each species and also among the different *Acacia* species. A pairwise correlation analysis was performed between environmental factors (temperature and RH of the area) and the amount of nectar sugar secreted per inflorescence. Furthermore, the frequencies of different flower visitors and their mean temporal distribution and relative preferences for different *Acacia* species were analyzed. The analysis was performed using JMP-5 statistical software (Anon., 2002).

Results

Flower morphology: The morphological parameters of the flowers varied significantly (P = 0.000) within the studied species (Table 1). Among the species with spherical inflorescences, the smallest flower head diameter (8.4±0.63 mm) was recorded for *A. tortilis*,and the largest diameter (15.72±2.52 mm) was recorded for *A. oerfota*.These values were significantly different (F = 179.65, df = 5, P = 0.0001) (Table 1). Moreover, the numbers of florets per inflorescence, stamens per floret, stigmas per inflorescence and the proportion of florets with stigmas varied significantly among the species (Table 1). The highest values for all morphological parameters were recorded in species with elongated inflorescences (*A. asak).* However, because of the distinctive variation in the shape of the *A. asak* inflorescence, its parameters were not subjected to statistical analyses along with the other species with spherical inflorescences.

Distribution of flowering pattern: The flowering times varied among the *Acacia* species and were distributed over several months (Table 2). *A. etbaica* flowered biannually (Table 2). Among the sympatric species (*A. ehrenbergiana, A. johnwoodii* and *A. tortilis)*, it was noted that *A. johnwoodii* flowered in a completely different time compared to the other two species (Table 2). Although *A. ehrenbergiana* and *A. tortilis* co-exist and flower concurrently, the flowering peak for *A. ehrenbergiana* occurred earlier than *A. tortilis.* Differences in flowering times were also observed within species, possibly as a result of variations in their ecological distributions. Such variation was noted in species with a wide ecological distribution, such as *A. ehrenbergiana* and *A. tortilis.* These species flower relatively earlier in lowland areas and later in the highlands. *Acacia asak, A. johnwoodii and A. etbaica*flower during the same season (Table 2). However, the spatial distributions of these species vary in that *A. asak* has been observed growing mainly in steep rocky escarpments, whereas *A. johnwoodii* grows mainly in the bottoms of valleys or on gently sloping ground with deep soil structure. *Acacia etbaica* grows on steep slopes or on gently sloping ground, favoring positions on the eastern side of escarpments, and does not spatially overlap with other species which flower concurrently.

Table 1. Mean and ± sd values of the flower morphometric parameters of *Acacia* species flowers in this study.

Species	N	Flower morphometric parameters (mean ± sd)				
		Diameter (in mm)	Florets/ inflorescence	Stamens/ floret	Stigmas/ inflorescence	% Of stigma to florets
A. asak	50	44.84 ± 4.81	141.04 ± 35.46	162.04 ± 16.91	141.04 ± 35.46	100.00 ± 0.00
A. origena	50	11.52 ± 0.97^{c}	50.52 ± 6.47^{b}	41.34 ± 7.48^{d}	10.78 ± 3.21^{e}	21.87 ± 7.97^{e}
A. oerfota	50	15.72 ± 2.52^{a}	63.98 ± 6.69^{a}	57.04 ± 7.69^{a}	54.19 ± 9.85^{a}	85.63 ± 18.11^{b}
A. etbaica	50	14.34 ± 0.94^{b}	27.70 ± 4.88^{d}	52.84 ± 5.16^{b}	19.82 ± 4.88^{d}	73.33 ± 20.78^{c}
A. tortilis	50	8.40 ± 0.6^{3d}	37.98 ± 5.24^{c}	45.90 ± 6.63^{c}	37.98 ± 5.245^{c}	100.00 ± 0.00^{a}
A.ehrenbergiana	50	12.20 ± 1.14^{c}	51.94 ± 6.77^{b}	46.22 ± 5.57^{c}	44.88 ± 14.57^{b}	86.31 ± 25.28^{b}
A. johnwoodii	50	12.18 ± 0.83^{c}	38.48 ± 4.06^{c}	34.14 ± 3.39^{e}	13.21 ± 6.77^{e}	34.83 ± 17.91^{d}
DF		5	5	5	5	5
F-value		179.649	248.557	86.997	234.097	165.607
P-value		0.000	0.000	0.000	0.000	0.000

* Values followed by different letters are significantly different

§For A. asak, lengths were used to determine its inflorescence dimensions

Table 2. Distribution of the flowering period of the *Acacia* species in this study.

Species	Jan.	Feb.	Mar.	Apr.	May	June	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
A. asak								x	x	x		
A. ehrenbergiana		x	x	x	x							
A.etbaica					x	x		x	x	x		
A.johnwoodii									x	x	x	
A.oerfota	x	x										x
A. origena					x	x						
A.tortilis			x	x	x							

Flower phenology and the time of pollen release: In all *Acacia* species with spherical inflorescences, the majority of the inflorescences open early in the morning before 0600 h and last for a single day, wilting between 1500 and 1800 h. However, in *A. oerfota*, the inflorescences did not open at a specific time of day. In *A. asak*, which has an elongated inflorescence, half of the florets open on one day, and the remaining half opened on the following day *A. johnwoodii*, unlike the other *Acacia* species with spherical inflorescences, few inflorescences opened half of their florets on one day, and the remaining half the following day. This phenomenon is similar to that observed in other *Acacia* species in the subgenus *Aculeiferum*, which have elongated inflorescences. Based on the average polyad-to-anther ratio, the peak time of pollen release varied from species to species (Fig. 1). The peak time for *A. tortilis* and*A. origena*,was extremely early(approximately 0600 h), at an average temperature of 25°C and a RH of 40.01%.The time of peak pollen release for *A. ehrenbergiana* and *A. johnwoodii* was approximately 0900 h, the average temperature was 32.1°C and RH 28.8%. Peak pollen release times for *A. asak* and *A. etbaica* were recorded relatively later at 12:00 h, at an average temperature of 36°C,and RH of 20.12%.

The peak pollen release time for *A. oerfota*, was not as distinct as that of other *Acacia* species, (Fig. 1) this was possibly could be due to the continuous opening of inflorescences throughout the day. In the co-existing, co-flowering *Acacia* species of *A. tortilis* and *A. ehrenbergiana*,the peak pollen release time shifted slightly; *A. tortilis* released its pollen earlier, at 0600 h, and *A. ehrenbergiana* released its pollen at 0900 h. In most of the studied *Acacia* species, (*A. origena*, *A. ehrenbergiana, A. tortilis, A. johnwoodii, A. asak*and*A. etbaica)*, the stigmas began to elongate above the stamens between 1200 and 1500 h. However, insome *A. etbaica*inflorescences, the stigmas appeared early (0600 h), which may indicate the protogynous nature of some individual trees. In *A. oerfota,* the stigmas appeared at different times, depending on the time the inflorescences opened.

Relative pollen transfer efficiency of the *Acacia* species studied: Based on the pollen grain-to-ovule ratio estimation, *A. origena* and *A. asak* had the highest number of pollen grains for a single ovule, with ratios of 4649.30 and 3445.37 respectively(Table 3), indicating the species ability to transfer pollen are less efficacious. Relatively lower ratios were recorded for *A. ehrenbergiana* (960.79) and *A. tortilis (*944.57), indicating that the pollen transfer efficiency of the species is more than that of the *A. origena* and *A. asak*. .

Nectar sugar secretion amounts and dynamics: The nectar sugar amounts and dynamics are varied from species to species (Table, 4). The highest average accumulated nectar sugar (7.65±4.17 mg/inflorescence) was recorded for *A. oerfota*, and the minimum recorded for *A. etbaica* (1.60±1.58 mg/inflorescence). Results of the ANOVA analysis for the mean accumulated nectar sugar per inflorescence was significantly varied ($N = 225$, $F = 154.97$, $df = 6$, $P = 0.000$). In addition, the average nectar sugar amount per inflorescence, varied significantly (P = 0.000) among the different recording periods across all species (Table 4). Regarding the dynamics of nectar sugar secretion, most of the species began secreting nectar early in the morning (approximately 0600 h) and continued to increase their production of nectar throughout the day, reaching a peak at 1500 h (Table 4). However, *A. johnwoodii* and *A. ehrenbergiana* reached their peak nectar productions at 1200 h and 1800 h, respectively.

Table 3.Pollen transfer efficiency of the *Acacia* species in this study. Calculations based on the pollen grain-to-ovule ratio.

Species	Florets/ inflorescence	Stamens /floret	polyads/ anther	Pg/ polyad	Pg/ florets	% Of florets with stigmas	Ratio of Pg to stigmas	Average number of ovules	Ratio of Pg to ovules
A. origena	50.52	41.34	8.0	24.0	7937.28	0.22	36078.55	7.76	4649.30
A. etbaica	27.71	52.84	8.0	16.0	6763.52	0.73	9265.10	6.40	1447.67
A. asak	141.04	162.04	8.0	16.0	20741.12	1.00	20741.12	6.02	3445.37
A. tortilis	37.98	45.90	8.0	16.0	5875.20	1.00	5875.20	6.22	944.57
A. ehrenbergian	51.94	46.22	8.0	16.0	5916.16	0.86	6879.26	7.16	960.79
A. johnwoodii	38.48	34.14	8.0	16.0	4369.92	0.35	12485.49	8.52	1465.43
A. oerfota	63.98	57.04	8.0	16.0	7301.12	0.86	8489.67	6.06	1,400.94

Pg = Pollen grain

Table 4. Comparison of the average amount of nectar sugar (mg)/inflorescence in seven *Acacia* species at different times of the day.

Species	N	Nectar sugar (mean ± sd) in mg/inflorescence at different times of the day						F-value	P -value
		0600 AM	0900 AM	1200 PM	1500 PM	1800 PM	DF		
A. origena	45	3.04±2.04^{a}	2.64±2.18^{a}	3.20±2.61^{a}	3.33±2.32^{a}	1.42±0.94^{b}	4	6.15	0.000
A. asak	45	1.31±2.34^{c}	3.04±2.84^{bc}	4.66±2.19^{ab}	5.03±4.22^{a}	5.03±3.46^{a}	4	12.34	0.000
A. oerfota	45	2.80±1.60^{d}	6.18±2.61^{c}	8.62±2.73^{b}	10.62±3.71^{a}	10.02±4.01^{ab}	4	49.52	0.000
A. etbaica	45	0.82±0.94^{b}	1.80±1.98^{a}	1.38±1.30^{ab}	2.24±1.72^{a}	1.73±1.45^{a}	4	5.47	0.000
A. tortilis	45	0.96±0.62^{c}	1.48±1.04^{bc}	2.28±1.83^{ab}	2.98±2.39^{a}	2.40±1.97^{ab}	4	9.97	0.000
A.ehrebergiana	45	4.22±2.39^{c}	4.80±2.72^{c}	5.62±2.77^{c}	7.29±2.61^{b}	8.98±2.25^{a}	4	26.00	0.000
A. johnwoodii	45	1.47±1.25^{b}	1.98±1.56^{b}	4.02±1.94^{a}	3.38±2.11^{a}	2.22±1.18^{b}	4	18.40	0.000

Values followed by different letters are significantly different

The nectar sugar secretion dynamics of the species, which were calculated as the difference in the amount of nectar sugar between two consecutive measuring times, revealed that a considerable amount of nectar sugar was available for flower visitors at different times for most of the species up to 1500 h apart from *A. johnwoodii* that ceases around 1200 h. After 1500 h the nectar sugar amount decreased to zero or became negative for all species except *A. ehrenbergiana*,which continued to secrete nectar up to 1800 h (Fig. 2). These results indicate that nectar secretion of most of species ceases after 1500 h. Temporary decreases in nectar sugar secretions were recorded for *A. origena* (at approximately 0900 h) and *A. etbaica* (1200 h), after which time slight increase in the amount of secretions were observed (Fig. 2).

In general, there was no significant variation in the amount of nectar sugar secreted by different trees within the same species except, in *A. etbaica* trees which were significantly varied (p<0.0001). No significant variation occurred in the amount of nectar sugar produced on different days although *A. oerfota* and *A. ehrenbergiana* which showed slight variations.

Effect of weather conditions on the amount of nectar sugar secretion: Temperatures throughout the study period were high; however, relatively low temperatures were recorded in the morning, rising gradually toward midday, after which the temperatures decrease (Fig. 3). The RH during the study period was generally low, relatively high in the morning, decline at midday and gradually rising again in the afternoon (Fig. 3). A significantly positive correlation (r = 0.26 to 0.49) was shown between the amount of nectar sugar secreted and ambient temperatures of the study sites. However, the amount of nectar sugar secreted and RH were found to be significantly negatively correlated (r = - 0.27 to - 0.48).

Insect visitors: A total of 2,722 individual insects were observed visiting the flowers representing four Orders of insects; (Hymenoptera, Diptera, Coleoptera and Lepidoptera). Among these, insects from Order Hymenoptera (honeybees, wasps wild bees and ants) were the most dominant, accounting for 83.95% of the total number of flower visitors, followed by Diptera (flies) which accounted for 7.57% of the total visitors. The frequencies of visitors and their distribution across the Acacia species studied are shown in Table 5. Honeybees and wild bees were the most abundant of Hymenoptera accounting for 48% and 28% of this Order respectively.

Temporal distribution of flower visitors: The patterns of flower visitations by insects varied significantly at different times of the day (F = 6.08, df = (5,114), P < 0.001). The Hymenoptera (honeybees, wild bees, ants and wasps) and Lepidopterans (butterflies and moths) showed significant temporal variations (P = 0.000) in the pattern of their daily visits to the flowers (Table 6). Hymenoptera visits were associated to the times at which both pollen and nectar were available, whereas the Lepidoptera visit was associated with the peak in nectar availability. The Diptera did not show any significant temporal variation (P = 0.155) in their flower visiting pattern during the day.

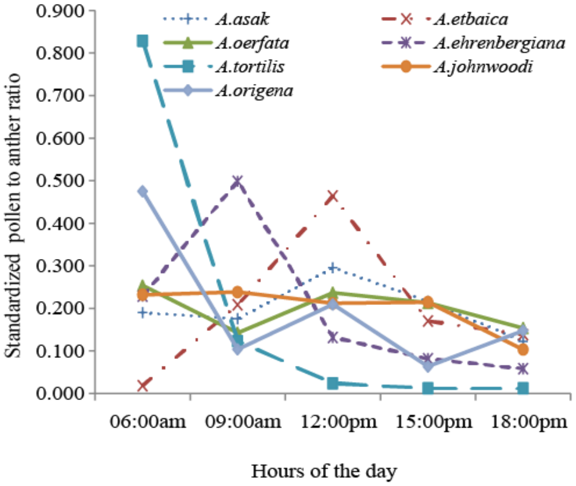

Fig. 1. Polyad-to-anther ratio of *Acacia* species at different times of a day as an indicator of their peak pollen release time.

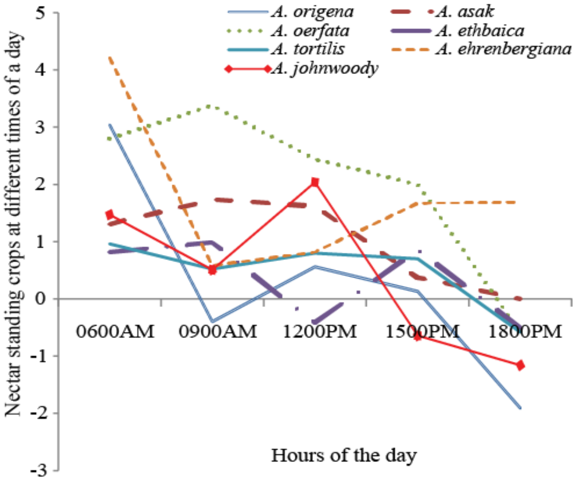

Fig. 2. Calculated nectar sugar standing crops of species (in mg/ inflorescence) at different times of day.

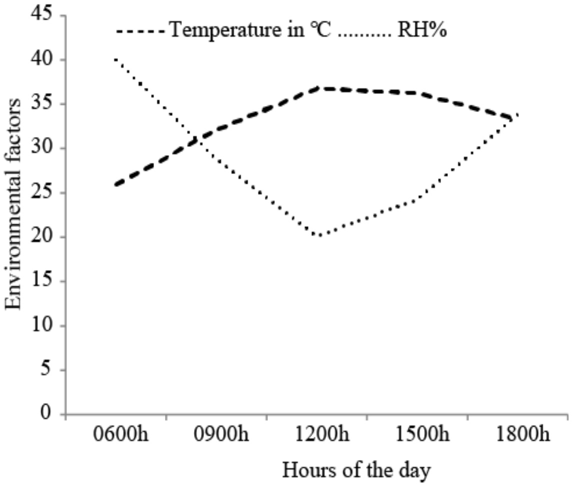

Fig. 3. Average temperature and humidity trends at different hours of the day during the study periods.

Preferences of flower visitors for different *Acacia* species: Significant variations (p=0.000) in the preference of flower visitors were recorded for different *Acacia* species at different times of day. Hymenoptera were observed as having preference for *A. origena, A. asak,* and *A. etbaica* compared to the other species in this study. Since, the availability of flower resources vary, both on a spatial and temporal level among the *Acacia* species, it seems difficult to critically compare the insect visitors preferences towards different *Acacia* species.

Discussion

Flower morphology of the *Acacia* species studied varied significantly with respect to their size, number of florets, number of stamens and sex distribution. The significant size variation within flower morphology may influence the size of the insect visitors which have contributed to the partitioning of pollinators in that insects with a larger body size such as, nectar-feeding wasps (*Vespa orientalis*), medium-to-large beetles, and wild bees (*Xylocopa* sp.) were attracted to *Acacia* species with larger flower heads (*A. ehrenbergiana, A. origena* and *A. oerfota*). This could be attributed to the fact that smaller flower heads would not be able to support the landing of these larger insects. The presence of variations in pollinator guilds as a result of variations in flower head size has been well documented (Kenrick *et al.,* 1987; Rathcke, 1988; Stone *et al.,* 2003).

The phenological patterns of the *Acacia* species in this study varied both temporally and spatially, which may be due to an adaptation of the species to avoid competition for pollination. The roles of both biotic and abiotic factors in the temporal and spatial structuring of plant species have been well documented (Levine *et al.,* 2010; Reckinger *et al.,* 2010). Competition for pollination has been reported as an important factor in the structuring of many plant communities (Rathcke, 1988), and is also responsible for phenological patterns (Sakai *et al.,* 1999). Therefore, the temporal separation observed in the *Acacia* species in this study may be associated with competition for pollination. In this regard, separation of flowering seasons has been considered as a means of achieving partial partitioning of pollinators among the different sympatric *Acacia* species (Williams, 1995; Stone *et al.,* 1998). In this study it was also noted that variations occurred during peak flowering periods, within the same season, between the two co-existing and concurrently flowering *Acacia* species, *A. tortilis* and *A. ehrenbergiana*. This could be a further adaptation of these species to attempt to minimize competition for pollinators. Because the *Acacia* flowers are not morphologically specialized for specific flower visitors hence, the species are expected to share pollinators of the same species; it is presumed that this has forced the adaptation and development of separate flowering seasons in order to avoid competition for pollinators (Ashton *et al.,* 1988); which agrees with the observations made in the current study. The spatial variation of the *Acacia* species in this study could also be partially attributed to avoiding competition for pollinators, as reported by Rathcke (1988). In this regard, environmental factors, such as variations in the amount of water stored by plants (Borchert, 1994), changes in temperature (Williams-Linera, 1997) and changes in photoperiod are regarded as important factors involved in triggering of phenological events in tropical plants.

Most of the *Acacia* species examined in this study typically release their pollen before the appearance of the stigma, indicating the protandrous nature of the species which is in agreement with the findings by Tybirk (1993) and Stone *et al.* (1996). In this study the observed variation in the peak pollen release times within a day among the different studied acacia species (Fig. 1) could be due to their adaptive mechanism to minimize competition for pollinators. Such separation is particularly important for co-existing and co-flowering species (*A. tortilis* and *A. ehrenbergiana).* The variation in peak pollen release times within a day among co-existing and co-flowering acacia species and its role in partitioning of pollinators have been well documented (Stone *et al.,* 1996, 1998).

The peak pollen release times of the different species were associated with specific temperature and humidity conditions, which may indicate that each species has adapted to response to the environmental factors differently. The association of the peak pollen release time with specific weather conditions has been indicated as an adaptation of the species to partition pollinators (Stone *et al.,* 1998). Unlike other *Acacia* species, in this study a distinct peak pollen release time was not observed for *A. oerfota*(Fig. 1). This result could be due to the absence of any competition for pollinators as the flowering period of *A. oerfota*temporally differs from that of other species occurring in the area. Thus, there is no reason for *A. oerfota*to adapt in order to have a specific pollen release time. Similarly, the absence of a synchronized peak pollen release time in a day was observed in African *Acacia* species which flower separately and do not compete with other *Acacias* for pollinators (Stone *et al.,* 1998).

Relatively low pollen-to-ovule ratios were recorded for *A. tortilis* (944.57) and *A. ehrenbergiana* (960.79), indicating that the pollen transfer efficiencies of these two species were higher than those of other *Acacia* species from the same region (Table 3). The pollen-to-ovule ratios of these species were also considerably lower than those of the two African Acacia species, *A. nilotica* and *A. senegal*, which were estimated to be 4,229.69 and 1,212.44, respectively, based on published data of Anon., (1983), Kordofani and Ingrouille (1992), Stone *et al.* (2003) and Tantawy *et al.* (2005). Because a low pollen-to-ovule ratio is associated with a high pollination efficiency of a species (Harder & Johanson, 2008); these two *Acacia* species might be considered more efficient than the other species in their pollen transfer ability and reproductive success. This could be due to their increased ability to cope with harsh environmental conditions via a more efficient pollen dispersal mechanism.

The nectar sugar amount which was calculated as the difference between two consecutive measuring periods indicated that the species were continuously secreting nectar. This could be important for attracting pollinators over an extended period of time. Moreover, different *Acacia* species were observed to attain their peak nectar secretion at different time points during the day (Table 4), which is important for the partitioning of pollinators to different times of the day. Similarly, continuous nectar secretion and variation in the peak nectar secretion time, as well as its role in attracting pollinators, have been reported in different plant species (Schmidt *et al.,* 2012).

Nectar sugar secretion was observed to positively correlate to temperature, indicating that temperature may stimulate nectar secretion of *Acacia* species, which may also indicate their better adaptation of the species to hot climatic conditions. Similarly, positive correlations between the nectar secretion amount and temperature have been reported for many other plant species (Petanidou & Smets, 1996; Nuru *et al.,* 2012). Although Deppe *et al.* (2000) reported a positive correlation between nectar sugar amount and RH, in the current study, a negative correlation was recorded over most of the study period. This discrepancy could have occurred because the RH of the air in the study area declined sharply between 0900 h and 1200 h (Fig. 3) at which time the flowers became physiologically mature and secreted more nectar.

Unlike the previous studies by Stone *et al.* (1998, 2003), which reported the absence of nectar in the genus *Acacia's* with spherical inflorescences, in the current study all the *Acacia* species withspherical inflorescence secreted a significant amount of nectar.The discrepancy in the study results may be attributed to the variance in the species of interest, ecological factors and the type of estimation techniques or equipment used (e.g., micropipettes) to extract the nectar. Indeed, it is difficult to properly extract highly viscous nectar using a micropipette. Similarly, Ettershank and Ettershank (1993) concluded that *Eucryphia lucida* (Eucryphiaceae) flowers did not secrete nectar after 1000 h; however, this conclusion was due to difficulties in removing dehydrated sugar (>70% concentration) using capillary tubes. By using the flower nectar washing techniques, Mallick (2000) was able to demonstrate that *Eucryphia lucida* secretes nectar throughout the day, and most of the nectar was secreted after 1000 h.

The significant temporal variation (within a day) of the flower visitation pattern of Hymenoptera (honeybees and wild bees) could be associated with variations in the peak times of floral rewards in different *Acacia* species. Temporal variations in the visitation patterns of insects as a result of variations in the peak nectar secretion and pollen release times among different *Acacia* species are well documented (Stone, 1998). The absence of visitation of Lepidoptera (butterflies) early in the morning and their more frequent visitation in the afternoons could indicate strong association with the time where nectar is available. The absence of temporal variation in the flower visitation patterns of Diptera and Coleoptera, could be due to the feeding habits of these Orders, as Coleopteras consume pollen and parts of flowers. The significant variations in the preferences of flower visitors for different species at different hours of the day could be due to the presence of significant variations in the peak nectar secretion and pollen release times among the different *Acacia* species, which agrees with the observations made by Stone *et al.* (1998).

However, in the current study, it was difficult to compare the preferences of flower visitors to the different *Acacia* species as the availability of floral rewards in most of the species showed spatial and temporal variation. Nevertheless, insects with a larger body size (nectar-feeding wasps (*Vespa orientalis)*, medium-to-large sized beetles andwild bees (*Xylocopa sp*.)) were observed on *Acacias* with relatively larger flower heads (*A. ehrenbergiana, A. origena,* and *A. oerfota*). These larger insects cannot easily alight on small flower heads (i.e. *A. tortilis*) or flowers with sparse florets (i.e. *A. etbaica*),which are structurally not strong enough to support insects with larger body sizes.The association of larger flower visitors with relatively large sized flowers has been previously recorded by Stone *et al. (*1998, 2003). The occurrence of variations of pollinators recruited as a result of floral dimension variations (Kenrick *et al.,* 1987), and the use of different pollinator guilds among sympatric species have also previously been reported (Rathcke, 1988). Unlike the previous report by Stone (1998), who reported that honeybees only collect pollen from *A. tortilis*, this was not the case in the current study; honeybees collected both nectar and pollen from the *A. tortilis* at different times of day.

Table 5. Frequency of flower visitors across different *Acacia* species.

Flower source	Frequency and proportion of flower visiting insects across different species, N (%)				
	Hymenoptera	**Diptera**	**Coleoptera**	**Lepidoptera**	**Total**
A. origena	378(94.74)	20(5.01)	1(0.25)	0(0.00)	399
A. asak	604(98.37)	5(0.81)	0(0.00)	5(0.81)	614
A. oerfota	300(74.63)	85(21.14)	12(2.99)	5(1.24)	402
A. etbaica	432(95.36)	4(0.88)	3(0.66)	14(3.09)	453
A. tortilis	370(82.22)	16(3.56)	44(9.98)	20(4.44)	450
A. ehrenbergiana	153(87.93)	1(0.57)	10(5.75)	10(5.75)	174
A. johnwoodii	48(20.87)	75(32.61)	6(2.61)	101(43.91)	230
Total	2285 (83.95)	206 (7.57)	76(2.79)	155(5.69)	**2722**

***** Values outside the parenthesis are frequencies; values in parenthesis are proportions

* Hymenoptera (honeybees, wild bees, ants and wasps); Diptera (flies); Coleoptera (beetles); and Lepidoptera (butterflies and moths)

Table 6. Mean temporal distribution of flower visitors during a day.

Time	N	Order of Insects (mean±sd)			
		Hymenoptera	**Diptera**	**Coleoptera**	**Lepidoptera**
0600	45	3.53 ± 4.19b^{c}	0.69 ± 1.95^{a}	0.31 ± 0.67^{ab}	0.00 ± 0.00^{b}
0900	60	13.18 ± 11.12^{a}	0.85 ± 1.55^{a}	0.73 ± 2.02^{a}	0.53 ± 1.50^{ab}
1200	60	12.68 ± 10.05^{a}	0.83 ± 1.24^{a}	0.73 ± 0.65^{b}	0.95 ± 1.89^{a}
1500	60	6.83 ± 6.81^{b}	0.81 ± 1.65^{a}	0.18 ± 0.37^{b}	1.07 ± 2.08^{a}
1800	60	2.73 ± 4.73^{c}	0.32 ± 0.79^{a}	0.00 ± 0.00^{b}	0.03 ± 0.18^{b}
DF		4	4	4	4
F-value		21.57	1.68	4.59	6.43
P-value		0.000	0.155	0.001	0.000

Values followed by different letters are significantly different

Hymenoptera (honeybees, wild bees, ants and wasps); Diptera (flies); Coleoptera (beetles); Lepidoptera (butterflies and moths)

Conclusions

Generally, the *Acacia* species in the areas studied are spatio-temporally structured in such a way that some species co-exist spatially but have different flowering seasons, whereas others co-exist and flower concurrently but vary with respect to the time of peak flowering within a season, and peak pollen release period within a day. Furthermore, other *Acacia* species are spatially separated but share the same flowering season. All of these phenomena may be considered as adaptations by the species to avoid competition for pollinators and minimize heterospecific pollen transfer. Similarly, Stone *et al.* (1998) recognized similar mechanisms as major contributing factors to the structuring of the flowering of sympatric *Acacia* species in Africa. A mechanism for the minimization of competition for pollinators is believed to be an important force in the structuring of plant communities. One of the major reasons for the partitioning of pollinators among *Acacia* species could be to avoid the detrimental effect of heterospecific pollen transfer, which has the potential to reduce the reproductive success of a species (Fishbein & Venable, 1996).

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