Role of insects in the pollination of *Acacia nigrescens* (Fabaceae)

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Abstract

We examined pollination biology of *Acacia nigrescens* Oliver, flowering at the end of the dry season in Kruger National Park, South Africa. *A. nigrescens* produces small quantities of concentrated nectar, and has abundant pollen resources available to potential pollinators. We recorded large numbers of insect visitors and most fruit set on the tops of trees, beyond the reach of ungulate browsers such as giraffes (which consume a substantial proportion of *A. nigrescens* flowers). Wasps, flies and solitary bees were the most numerous visitors and are likely to play a significant role in pollination.

Abbreviations: ANCOVA, Analysis of Covariance; KNP, Kruger National Park; RH, relative humidity.

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1. Introduction

Acacia species are an important ecological component of the southern African bushveld (Van Wyk and Van Wyk, 1997), both in terms of species diversity and biomass. Although pollination and seed set are crucial aspects of *Acacia* biology, they remain unstudied for most species (Stone et al., 2003). Importantly, the identity of flower visitors, and their role as potential pollen vectors, is a vital aspect in developing an understanding of *Acacia* biology.

Self-incompatibility is considered an important outbreeding mechanism in *Acacia* (Kenrick and Knox, 1989), with many species being highly self-incompatible (Belovsky, 1986, Morgan et al., 2002, Raju and Rao, 2002 and Tandon et al., 2001; but see Mandal et al., 1994 and Wagner, 2000). *Acacia* pollen structure (4, 8, 16, or 32 pollen grains fuse to form polyads, Kenrick and Knox, 1982 and Knox and Kenrick, 1982) is not considered to be conducive to wind pollination (Pacini, 2000), although *Acacia* pollen may be found in aerial pollen counts (Kenrick, 2003). Flower visitors therefore play a significant role in pollination of these plants. The colour and shape of flowers are not suggestive of ornithophily (Wyatt, 1983), although birds may pollinate some *Acacia* species, particularly whilst foraging on insects or pollen (e.g. Raju and Rao, 2002 and Stone et al., 2003) or when feeding at extrafloral nectaries (Knox et al., 1985 and Vanstone and Paton, 1988). In most *Acacia* species examined to date, insects are the main pollinators (e.g. Bernhardt et al., 1984, Bernhardt and Walker, 1984, Moran et al., 1989, Raju and Rao, 2002, Stone et al., 1999, Tandon et al., 2001 and Tybirk, 1993; reviewed by Stone et al., 2003).

African *Acacia* species are divided into two subgenera. Species in subgenus *Acacia* Vassal generally have long straight thorns and cream to bright yellow capitate (pom-pom shaped) inflorescences; nectar may be absent or present in minute quantities. Species in subgenus *Aculeiferum* Vassal (such as *A. nigrescens*) have small, recurved thorns and white or cream spicate (bottle-brush shaped) inflorescences, with small amounts of nectar (Ross, 1979, Stone et al., 2003 and Stone et al., 1998).

A. nigrescens trees flower briefly for 2–3 weeks towards the end of the dry season (early September). They are the only *Acacia* away from riverine areas flowering at this time. It has been suggested that giraffes (*Giraffa camelopardalis* L.) may play a role in pollination of *A. nigrescens*, since these tall ungulates browse extensively on the flowers during the short flowering period (Du Toit, 1990 and Du Toit, 1992). We examined the potential role of giraffes by recording florivory and subsequent seed set (Fleming et al., 2006) and found that their significant browsing appeared to be detrimental to the fecundity of these trees for the year of study. As a part of that study, we recorded flowering phenology and insect visitors to these trees, and present these data here.

2. Methods

2.1. Study site and species

The study area was the central Kruger National Park (KNP), in the *Sclerocarya birrea* (A. Rich.) Hochst./*Acacia nigrescens* ('marula-knobthorn') Savanna ecotype between Tshokwane (24°47′S, 31°52′E) and Satara (24°24′S, 31°46′E). The soils supporting this vegetation type are predominantly basaltic clays (Gertenbach, 1983). The mean annual rainfall is around 530 mm, of which > 80% falls in the summer, between October and March. Field work was carried out during *A. nigrescens* flowering, between 2 and 19 September 2003; fruit set was assessed between 28 March and 2 April 2004, but is presented elsewhere (Fleming et al., 2006).

2.2. Flowering biology of *A. nigrescens*

We examined the availability of *A. nigrescens* pollen and nectar as floral rewards, according to the methods described by Stone et al. (1998). Analyses were carried out for trees that were unprotected and therefore accessible to all potential pollinators; the data represent resources available to visitors at a single point in time ('standing crop' *sensu* Kearns and Inouye, 1993). Inflorescences sampled ranged from ∼ 1 m to 5 m above ground.

2.2.1. Pollen release

Three inflorescences (randomly chosen with respect to height above ground and aspect, but with 90–99% of the florets open) were collected from each of three trees approximately every 1.5 h over 1 d, from 06h00 to 17h00. Simultaneously, temperature and relative humidity (RH) were recorded at each tree using a handheld humidity/temperature meter (TES Electrical Electronic Corp, Taiwan). Pollen release was assessed by rolling each inflorescence lightly over a strip of clear adhesive tape, which was then placed on a microscope slide. Six microscope views (1.05 × 1.05 mm at 10× magnification) of each slide were randomly selected and the polyads and anthers in each view counted. The ratio of polyads to anthers (total numbers of each for each slide) was calculated and later averaged for samples from each tree; this method quantifies the pollen exposed on the surface of the *Acacia* inflorescence (Stone et al., 1998). The $\log_{10}$-transformed average ratio (transformed data did not violate the assumptions of the

analysis) was analysed by Analysis of Covariance (ANCOVA), with time as the independent factor and temperature and RH as covariates.

2.2.2. Nectar availability

After collecting pollen in the field, the same inflorescences were used for analysis of nectar volume and concentration. For each inflorescence (three inflorescences from each of the three trees at each time interval), 10–20 florets were randomly chosen and the quantity of nectar measured with 1 µl micropipettes (Drummond Scientific Co., USA). Nectar concentration (%) was measured using one of two refractometers (0–50% and 40–85%, Bellingham and Stanley, UK). ANCOVA, with time as the independent factor and temperature and RH as covariates, was carried out on $\log_{10}$-transformed volume data averaged for all inflorescences from each tree (transformed data did not violate the assumptions of the analysis).

2.2.3. Flower opening

Flower opening was recorded for marked inflorescences (no florets open at the commencement of the experiment) from each of three trees ($n = 6$, 10 and 6 because damage of some inflorescences reduced initial samples sizes of $n = 10$ for each). The percentage of florets open on each inflorescence was recorded at 6h00 and 18h00 each day. Inflorescences were classed as 'senesced' when signs of aging (e.g. browning, withering) were clearly visible. The numbers of inflorescences opening during the night (18h00–6h00) or day (6h00–18h00) were compared by χ^2 test.

2.2.4. Pollen availability with flower age

Inflorescences of known ages (1–6 days old) were opportunistically collected from the three trees analysed for nectar and pollen (at 18h00 at the end of the trials), in order to assess the correlation between pollen availability (as above) and inflorescence age. ANCOVA, with age as covariate and tree as the categorical independent factor, indicated that 'tree' was not a significant factor in determining pollen availability ($F_{2,17} = 0.42$, $P = 0.665$), and therefore pollen availability was compared with inflorescence age only, by Spearman's Rank Order Correlation.

2.2.5. Sex ratio

The sex ratio of 40 randomly selected florets was assessed for three inflorescences from each of six trees. Florets were regarded as hermaphrodite if an ovary and/or stigma was present, but as male only if neither of the above could be found upon dissection.

2.3. Pollinator visitation

Insect and bird visitation to *A. nigrescens* flowers was observed following two protocols, investigating temporal and spatial patterns of insect visitors. For each, insect visitors were recorded for 14–30 focal inflorescences, usually < 2 m from the ground for ease of

observation with binoculars. Insect visitors to the inflorescences were classified to order level, or family where possible. Visits were simply recorded rather than timed, as described by Stone et al. (1998). The *A. nigrescens* trees examined were scattered throughout the study area, < 50 m from other flowering *A. nigrescens*, and there were not more than five other flowering trees within a 200 m radius of each focal tree.

2.3.1. Temporal patterns

Firstly, two trees were observed continuously over a day to determine temporal patterns in visitation. This entailed observation for 30 min every hour, from 6h30 until 17h00 (as per Stone et al., 1998). Data for the temporal protocol were analysed using ANCOVA, with time as independent factor, and temperature and RH as covariates.

2.3.2. Spatial patterns

Secondly, we observed trees near (< 300 m) water or far from (>1.5 km) water to determine if proximity to water affected insect visitation (Du Toit, 1992). For 26 h over 4 d, 16 individual trees scattered throughout the study site were observed for 30 min sessions (1–4, average 2, observation sessions per tree), alternating between trees near water and trees far from water. A total of 1023 insect visits were recorded for the 1106 inflorescences under observation. Temperature and relative humidity (RH) were recorded at the beginning of each session. Numbers of insect visitors to each tree (expressed per inflorescence and per half hour) were analysed by ANCOVA for differences between sites near or far from water, with RH and temperature included as covariates. These analyses were carried out for the different insect taxa separately, and subsequently for all insect visits.

Insects seen on or close to *A. nigrescens* flowers were also collected by means of a sweep net or a home-made suction device. These insects were later identified to family level (Picker et al., 2002) in order to clarify some of the 'morphotypes' recorded during observations.

In addition, any birds visiting the entire tree under observation were recorded, and their behaviour was ascertained where possible.

For all tests, the level of significance was $\alpha \leq 0.05$. Data are reported throughout as means ± 1 SD.

3. Results

3.1. Floral rewards

In addition to being edible (Fleming et al., 2006), *A. nigrescens* flowers have both nectar and pollen rewards to offer potential pollinators. Pollen was available (assessed as the pollen to anther ratio) throughout the day on *A. nigrescens* inflorescences (Fig. 1a, ANCOVA with RH and temperature as covariates: no significant relationship between

pollen availability and time of day $F_{8,16} = 0.99$, $P = 0.476$). Anthers in the process of dehiscing were also observed at all times of the day. Pollen availability declined significantly with the age of the inflorescence (measured as time since first opening, Fig. 1b, Spearman's $r = -0.822$, $t_{19} = -6.28$, $P < 0.001$).
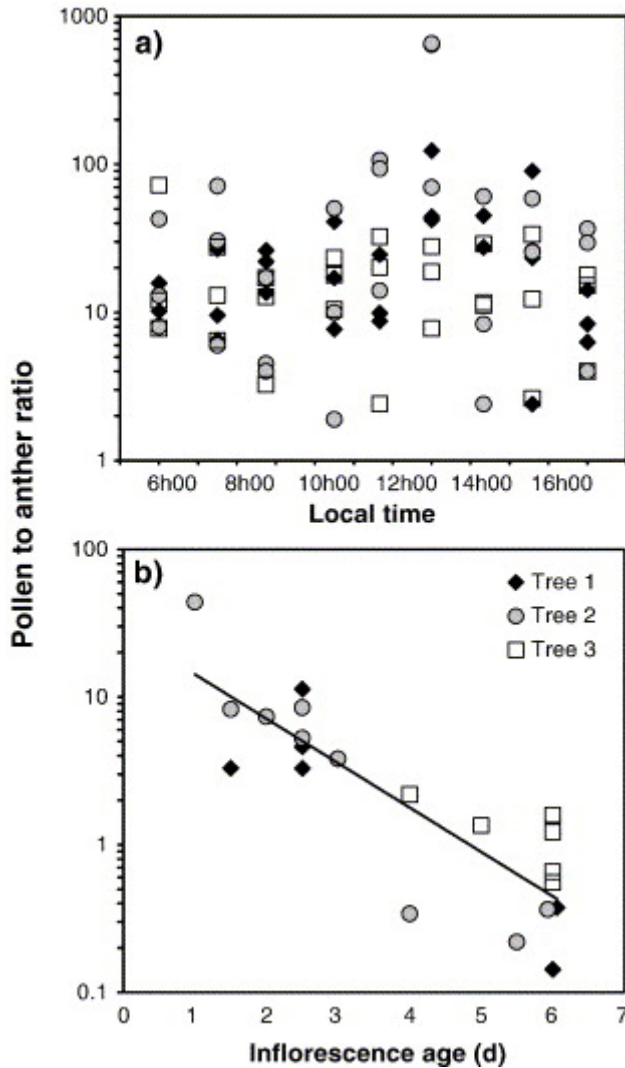

Fig. 1. Pollen to anther ratio for inflorescences from three *Acacia nigrescens* trees (three inflorescences sampled at each time point). There was no effect of time of day (a) on pollen availability, but age of inflorescences (b) did have a strong effect (note log scale). Age was calculated as the number of 24-h periods that had elapsed since the first open floret was found.

A. nigrescens inflorescences produce an average of 0.011 µl nectar per floret (averaged over the day, maximum 0.035 µl per floret). Greater volumes (Fig. 2a) of less

concentrated (Fig. 2b) nectar were available early in the day compared to the afternoon. However, for both nectar volume ($F_{8,16} = 0.85$, $P = 0.578$) and nectar concentration ($F_{8,16} = 1.84$, $P = 0.143$), there was no significant effect of time of day once temperature and relative humidity were taken into account as covariates (in ANCOVA). Nectar concentration ranged between 41.5% and 74% (w/w) and was strongly correlated with changes in both temperature and RH (Fig. 2c and d).
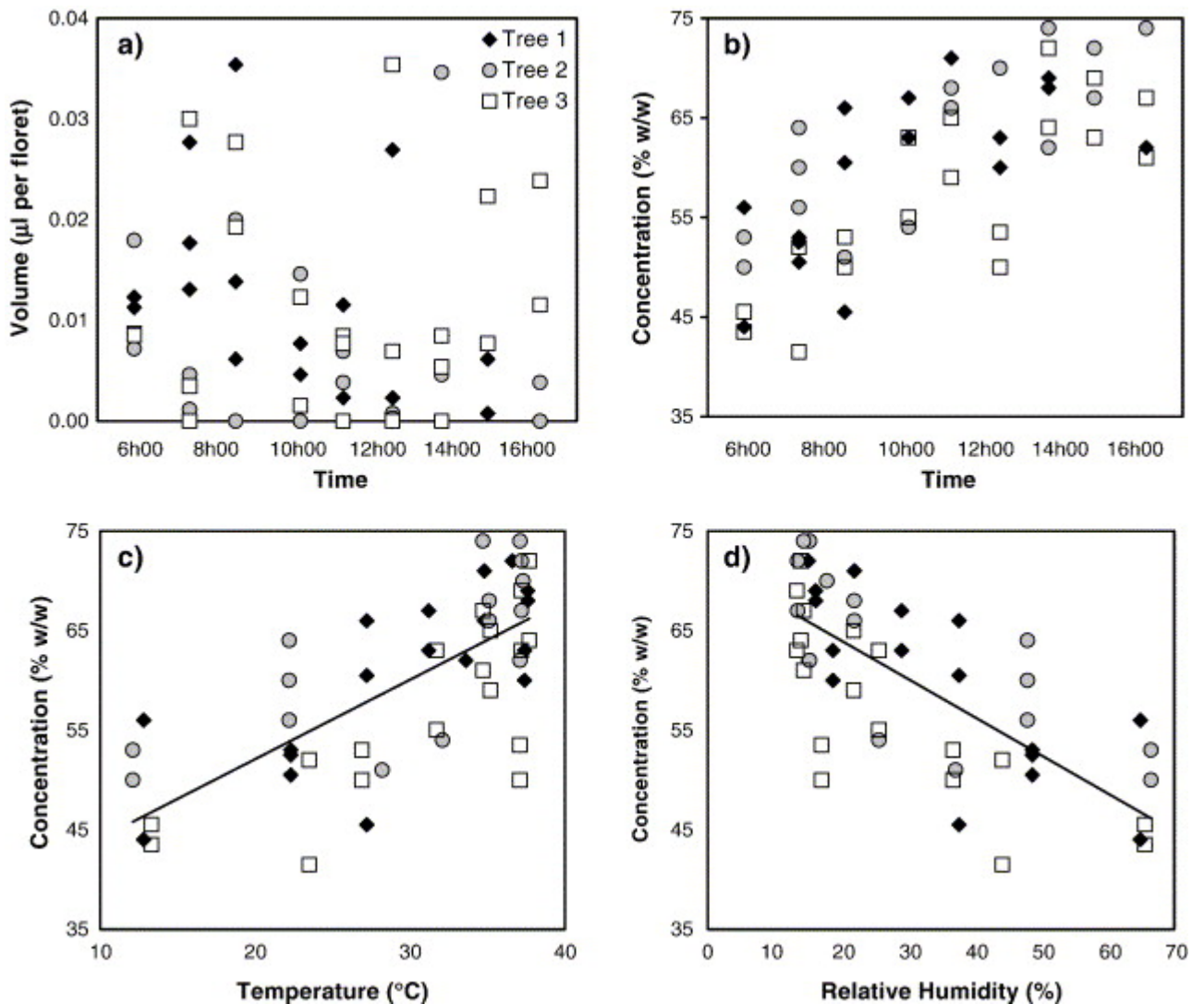

Fig. 2. Volume and concentration of nectar available from three *Acacia nigrescens* trees (three inflorescences sampled at each time point). There was no significant effect of time of day on the volume available (a). Concentration increased over the day (b) in response to temperature (c) and relative humidity (d). Regression lines (c and d) are fitted to the average data for each tree ($n = 3$ for each time point).

A. nigrescens inflorescences were generally slow and highly variable in anthesis rates. For the majority of inflorescences (17 of 22 observed) $\geq$ 50% of the florets opened in under 12 h, but the florets on the other five inflorescences opened gradually, over up to 48 h. The average time for $\geq$ 50% opening was 16.4 ± 9.5 h, and this took place either during the night ($n = 11$) or the day ($n = 6$), which was not significantly different from parity ($\chi_1^2 = 1.33$, $P = 0.250$). Finally, there was also considerable variation in the length of time between 100% opening and the beginning of senescence (average 0.86 ± 0.83 d, range 0–3 d).

Hermaphrodite florets comprised 95.4 ± 8.3% (range 75–100%) of those sampled (40 florets from $n = 18$ inflorescences from six trees). The average number of florets per inflorescence (counted from inflorescences collected to measure pollen release) was 130.0 ± 25.4 ($n = 22$).

3.2. Visitors to *A. nigrescens* flowers

A diverse array of insect visitors was caught on and around flowering *A. nigrescens*. In addition, there were numerous vertebrate visitors. Thirteen species of birds were recorded visiting trees in bloom during observations: rattling cisticola (Cisticolidae: *Cisticola chinianus*), green-winged pytilia (Estrildidae: *Pytilia melba*), black-backed puffback (Malaconotidae: *Dryoscopus cubla*), grey go-away-bird (Musophagidae: *Corythaixoides concolor*), sunbird species (Nectariniidae), black-headed oriole (Oriolidae: *Oriolus larvatus*), chin-spot batis (Platysteiridae: *Batis molitor*), weaver (Ploceidae: *Ploceus* sp.), dark-capped bulbul (Pycnonotidae: *Pycnonotus tricolor*), greater blue-eared and Cape glossy starlings (Sturnidae: *Lamprotornis chalybaeus* and *L. nitens*), long-billed crombec (Sylviidae: *Sylvietta rufescens*), and white-browed scrub-robin (Turdidae: *Cercotrichas leucophrys*). The majority of birds demonstrated behaviours that were not likely to bring about pollination (mostly using the trees as perch sites), however the sunbird and chin-spot batis were observed catching insects among flowers, whilst the grey go-away-bird was observed eating flowers. Vervet monkeys *Cercopithecus aethiops* L. (S. Whitfield, KNP Ranger, pers. comm.) and giraffes (Fleming et al., 2006) were also observed eating *A. nigrescens* flowers during the period of this study. It is not possible to make direct comparisons between these visitors due to vast differences in numbers, body sizes and visibility or approachability.

3.2.1. Temporal pattern of insect visitation (Fig. 3)

There was no significant pattern in total insect visits with time of day (ANCOVA: $F_{10,9} = 1.35$, $P = 0.330$; neither temperature, $P = 0.064$, nor RH, $P = 0.103$, was a significant covariate). There were also no significant visitation patterns for individual insect families or orders (ANCOVA results indicated below). Insect taxa visiting *A. nigrescens* inflorescences included Lepidoptera ($F_{10,9} = 0.85$, $P = 0.604$; Danaidae, Gelechidae, Lycaenidae, Nymphalidae), Coleoptera ($F_{10,9} = 0.64$, $P = 0.749$; Bruchidae, Chrysomelidae: common subfamily *Alticinae*, Cucujiidae, Curculionidae, Dermestidae, Meloidae, Melyridae, Mordellidae), Hymenoptera ($F_{10,9} = 0.70$, $P = 0.704$; Anthophoridae, Apidae, Colletidae, Halictidae, Megachilidae, Scoliidae, Sphecidae,

Tiphiidae, Vespidae, and two unidentified families), and Diptera ($F_{10,9} = 1.11$, $P = 0.442$; Bombyliidae, Calliphoridae, Syrphidae, Tachinidae). Fly visitors to *A. nigrescens* inflorescences were present throughout the day (Fig. 3).
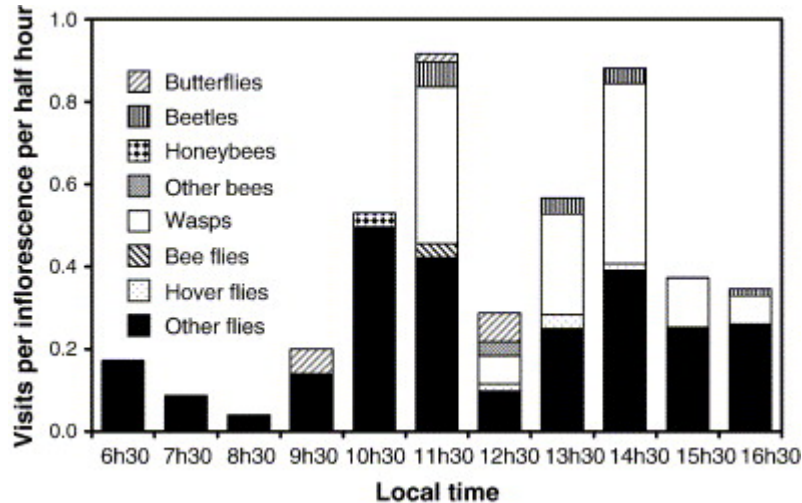

Fig. 3. Temporal pattern of insect visitation data for two flowering *Acacia nigrescens* trees observed for a whole day each. Each time division represents a half hour of observation starting at that time. Only those insects thought to be possible or likely pollinators have been included; ants (Hymenoptera: Formicidae), spiders (Arachnidae) and bugs (Hemiptera) were excluded. Taxa shown are as follows: butterflies (all Lepidoptera), beetles (all Coleoptera); Hymenoptera are divided into honeybees (Apidae), other bees (Colletidae, Halictidae and Megachilidae), and wasps (all other Hymenoptera); Diptera are divided into bee flies (Bombyliidae), hover flies (Syrphidae), and other flies (Calliphoridae and Tachinidae).

3.2.2. Spatial pattern of insect visitation (Fig. 4)

Almost three times as many insect visits were recorded for trees far from water compared with those near water (Fig. 4b, ANCOVA: $F_{1,48} = 12.03$, $P = 0.001$; as covariates, RH significantly affected numbers of insects recorded $P = 0.010$, whilst temperature did not have a significant effect $P = 0.125$). When insect taxa were considered separately, honeybees (*Apis mellifera* L.) were more commonly recorded as visitors to trees near water, whilst flies (Calliphoridae and Tachinidae), other bees (Colletidae, Halictidae and Megachilidae) and wasps (all other Hymenoptera) were all more common on trees further from surface water (Fig. 4a).
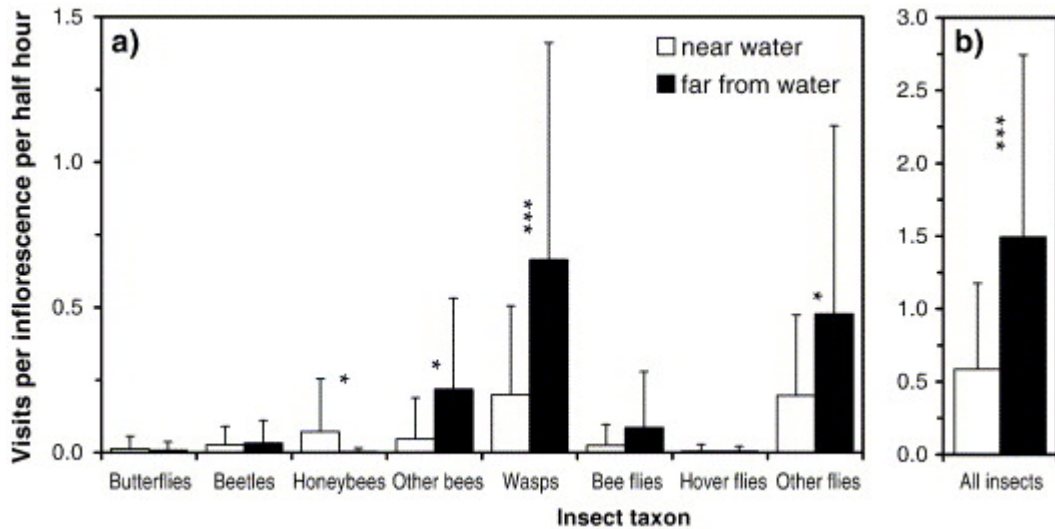
Fig. 4. Spatial comparison of insect visitation for 16 individual trees located near (< 300 m) water or far from (> 1.5 km) water, carried out for half-hour intervals totalling 26 h over 4 d. Data are (a) for individual taxa divided as per Fig. 3, and (b) for all taxa together. Values are means ± 1 SD; ANCOVA results for differences in number of visitors to trees near or far from water: $^*P < 0.05$, $^{***}P < 0.001$.

4. Discussion

Insects are likely to play a significant role in the pollination of *A. nigrescens*, as has been demonstrated in closely related species (see review by Stone et al., 2003). *A. nigrescens* flowers produce highly concentrated nectar, albeit in tiny amounts, suggestive of entomophily (Wyatt, 1983). Nectar concentration and volume clearly reflected the effect of evaporation (and possibly nectar harvesting) during the day, as also noted for *A. senegal* (L.) Willd. (data cited by Stone et al., 2003). Available nectar volumes seem too small to attract birds, and nectar feeding by birds was never obvious. The most frequent insect visitors were flies (which show limited movement between trees), bee flies, wasps, solitary bees, and honeybees. Solitary bees are thought to be important pollinators of *Acacia* species 'wherever their pollination has been studied in depth' (Stone et al., 2003). Solitary bees were less common visitors to *A. nigrescens* than they were to five *Acacia* species studied by Stone et al. (1999), however these authors did not find high numbers of wasps as we did for *A. nigrescens*. Tybirk (1993) recorded insect visitors to *Acacia* species at different times of day and found much higher rates of visitation for *A. senegal* than in our study, but comparable rates for *A. tortilis* (Forsk.) Hayne and *A. albida* Del. Those *Acacia* species which provide floral nectar apparently attract much more diverse assemblages of flower visitors than species that have only pollen rewards (Stone et al., 2003). Since only *A. nigrescens* flowers were observed during this study, and leaves and flowers appeared to be spatially separated (Fleming et al., 2006; as has been shown for a

Mexican ant-acacia, *Acacia hindsii*, Raine et al., 2002), extrafloral nectaries on the leaves were likely to play a minimal role in attracting pollinators.

Raju and Rao (2002) note that *Acacia sinuata* (Lour.) Merr. (which flowers in the dry season) has fewer insect visitors than an allied, wet season-flowering species. Similarly, Du Toit (1992) suggested that *A. nigrescens* trees might be disadvantaged since insects may not be common visitors to these trees due to distance from surface water in the late dry season. Honeybees were more common visitors to trees close to water, although bees may in fact fly considerable distances to obtain water (for example, honeybee species have been recorded foraging 10–20 km from their nest, Beekman and Ratnieks, 2000 and Dyer and Seeley, 1991). In contrast, other bees, wasps and flies were actually more common visitors to trees far from water, and the total number of insect visitors was higher for trees away from surface water compared with those near water. It is possible that simultaneously flowering riverine species (*Acacia robusta* Burchell, *Acacia xanthophloea* Benth., *Combretum hereroense* Schinz and *Combretum microphyllum* Klotzsch) competed for these insect pollinators.

Where several *Acacia* species co-occur and flower synchronously, competition for pollinators has apparently led to the partitioning of available floral resources throughout the day (Stone et al., 1996, Stone et al., 1998 and Stone et al., 2003). Five sympatric and synchronously flowering Tanzanian *Acacia* species release their pollen with precise non-overlapping windows of 2–4 h each (Stone et al., 1998). This has reduced competition for pollinators and may have increased their fidelity. We did not find any diurnal patterns of peak pollen availability in *A. nigrescens*. This finding may not be surprising, since *A. nigrescens* is the only *Acacia* species (away from riverine areas) flowering at this time and therefore does not compete with other species for pollinators (discussed extensively by Stone et al., 2003). For *A. nigrescens*, September flowering exposes flowers to intense predation given that there is limited other browse for ungulates (Fleming et al., 2006), but this cost may be outweighed by the advantages of flowering when few other floral resources are available.

Although we noted a significant decline in pollen availability after 3 d (note log scale in Fig. 1b), *A. nigrescens* inflorescences have pollen available for up to 6 d. Individual florets on an inflorescence also showed evidence of senescence 0.86 ± 0.83 d from anthesis (range 0–3 d), although we did not test pollen viability or stigma receptivity. The majority of species in the subgenera *Acacia* and *Aculeiferum* open and senesce within 1 d, their entire sexually active life lasting not more than 16 h (Stone et al., 2003), although the flowers of two other *Acacia* species (*Acacia farnesiana* (L.) Willd. and *Acacia karroo* Hayne), are known to remain receptive to pollen for a number of days (4 and 5 d respectively, Stone et al., 2003).

The high proportion of hermaphrodite florets in *A. nigrescens* (95%; see also Raine, 2001 and Stone et al., 2003 for other examples) does not support the suggestion that a large proportion of the flowers are the equivalent of petals and 'just for show' (Ross, 1979). Failure to develop fruit may be linked to other factors such as resource allocation. The ratio of hermaphrodite flowers 'would seem to be, universally, a highly variable and

plastic character that allows individual plants to respond to environmental conditions and resource availability during floral development' (see review by Kenrick, 2003; p 122). A high proportion of hermaphrodite flowers in *A. nigrescens* would be of benefit considering the destruction of many of their flowers (by invertebrate flower predators, or giraffes, Fleming et al., 2006), since it maximises the probability that flowers remaining on the tree are fertile. Finally, *A. nigrescens* florets become detached from the inflorescence very easily, enabling whole florets to be moved from tree to tree, promoting pollination by animals such as giraffes, which carry whole florets on their faces (Fleming et al., 2006). It remains to be established whether male flowers detach more readily than hermaphrodite flowers, thus not compromising female fitness. We have not come across any record in the literature that mentions high levels of floret detachment in other *Acacia* species, although Stone et al. (2003) noted whole *Acacia* anthers transported by hoverflies.

In summary, we have recorded large numbers of visitors to *A. nigrescens*. Giraffes browse on flowers and appear to be detrimental to overall fitness of the trees (Fleming et al., 2006), whilst bird visitors were not observed to forage at inflorescences specifically for nectar or pollen. The large numbers of insect visitors to *A. nigrescens* suggest flies, wasps or bees as the most likely pollinators of these trees, as has been recorded for other *Acacia* species. Both pollen and nectar resources are available as rewards for these visitors.

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