Pollination and fruit-set of *Grevillea robusta* in western Kenya

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Abstract   The floral visitors of silky oak, *Grevillea robusta* A. Cunn. ex R.Br., their foraging behaviour and their effects on fruit-set were studied at Malava, western Kenya. *Grevillea robusta* is a popular tree for farm plantings in the eastern and central African highlands. Yield of seed has been disappointingly low in some areas and a lack of appropriate pollinators has been suggested as a possible cause. Investigations involved the monitoring of visitors on active inflorescences, assessment of the rewards available to potential pollinators, and exclusion experiments to establish the effects of various visitors on fruit-set. The flowers are visited mainly by birds and insects. The likely pollinators of *G. robusta* are sunbirds (*Nectarinia amethystina, N. cyanolaema, N. olivacea, N. superba* and *N. venusta*) and white-eyes (*Zosterops kikuyuensis* and *Z. senegalensis*). Very little aggressive behaviour between birds was recorded. No nocturnal pollinators were observed. Nectar was the major floral reward for pollinators, but is likely depleted by ants and honey bees, the foraging behaviour of which confirmed them to be nectar-robbers. These insects hardly ever touched stigmas during their visits. Eighty-nine per cent of bird visits were in the morning (07.00–10.00 hours) when nectar volume was highest. Inflorescences bagged to exclude birds set no fruits, and unmanipulated flowers and flowers bagged with self-pollen set no fruits, indicating a self-incompatibility mechanism. Control cross-pollinated flowers displayed greatly increased fruit-set (25.1%) compared with natural open-pollination (0.9%). All these findings confirm the importance of cross-pollen transfer to flowers and the necessity of pollinators for fruit-set. Effective seed production requires activity of pollinators for self-pollen removal and cross-pollen deposition. Seed production stands for *G. robusta* should be established where flowering is prolific and bird pollinators are abundant.

Key words: breeding system, *Grevillea*, nectar, pollination, Proteaceae, seed production, sunbirds, white-eyes.

INTRODUCTION

Over the last century, silky oak, *Grevillea robusta* A. Cunn.ex. R.Br. (family Proteaceae), a tree native to eastern Australia, has been widely planted in subtropical and tropical highland environments of eastern and central Africa, south and central America, and south Asia (Harwood 1992). *Grevillea robusta* is a popular species for farm plantings in the eastern and central African highlands. In Africa, it is grown mainly as a shade tree for tea and coffee plantations and for fuel wood and timber. The demand by farmers for *G. robusta* seedlings is increasing, but the availability of seed is often a limiting factor for current planting programs in most eastern and central African countries (Kalinganire 1994; B. M. Kamondo, pers. comm., 1996). Consequently, an understanding of the factors affecting seed production has important practical

applications, for both genetic improvement and operational seed production.

Most flowering plants set fewer than the total number of flowers produced (Stephenson 1981; Sutherland 1986; Hermanutz *et al.* 1998). Low fruit : flower ratios are typical of many hermaphroditic plant species (Stephenson 1981; Sutherland 1986) and the Proteaceae contain many species that have very low levels of fruit-set (Harriss & Whelan 1993; Hermanutz *et al.* 1998). Based on breeding system, Sutherland (1986) reported an average fruit : flower ratio of 0.2% for hermaphrodites and 0.7% for dioecious species. Reported fruit : flower ratios range from 0.1 to 7.2% for 18 different proteaceous species (Collins & Rebelo 1987) and from 0.01 to 0.09% for five *Grevillea* species (Hermanutz *et al.* 1998).

A number of explanations for low fruit-set have been proposed within the Proteacae (reviewed in Ayre & Whelan 1989). Sutherland's (1986) survey of fruit : flower ratios indicated that compatibility between and within proteaceous species was an important factor, whereas Copland and Whelan (1989) found that ineffective pollination and lack of cross-pollination limited

fruit-set for *Banksia* species. They found an increase in fruit-set with hand pollination. Similarly, in a survey of pollination experiments on 258 angiosperm species, Burd (1994) found that the majority (62%) experienced deficits in seed-set because of inadequate receipt of compatible pollen. Hermanutz *et al.* (1998) found that low fruit : flower ratios for *Grevillea* species resulted from a combination of pollen limitation, and high levels of flower and fruit predation. Collins and Rebelo (1987) also suggested fruit-set in most Proteaceae can be limited by the resources available for fruit maturation. Moreover, pollen or pollinator limitation is common in many plant species (reviewed in Bierzychudek 1981; Young & Young 1992; Burd 1994).

An understanding of floral traits can shed light on the coevolution of plants and their pollinators (Freitas & Paxton 1998). In its natural range, *G. robusta* is believed to be pollinated by nectarivorous birds such as honeyeaters (Brough 1933; Lamont *et al.* 1985; Collins & Rebelo 1987) and probably also by fruit bats (suborder Megachiroptera) (Harwood *et al.* 1997). In southern Africa, Collins and Rebelo (1987) reported sunbirds (Nectariniidae) and sugarbirds (Promeropiidae) to be the most frequent visitors to *G. robusta*. Nicolson (1993) reported the Cape white-eye, *Zosterops pallidus* (Swainson), to be active in the mornings, but found *G. robusta* to be unattractive to bees. Preliminary observations in western Kenya (Kalinganire *et al.* 1996) indicated that ants, sunbirds and white-eyes visited *G. robusta*, but their role in pollination was uncertain.

For plant species that have specialized pollinators, absence of these pollinators often appears to limit fruit-set in exotic or degraded environments where the coevolved pollinators are absent. For example, the bat-pollinated species *Calliandra calothyrsus* Meissner produces little seed when planted in some exotic environments, for example in Kenya (Boland & Owuor 1996) and Cameroon (Chamberlain & Rajaselvam 1996). Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.) failed to fruit once surrounding forest was cleared in a native environment, and successful seed orchards of this species required the planting of alternative host species for pollinators (Boshier 2000). The number of visits by bumblebee and honeybee pollinators limited fruit production of gorse (*Cytisus scoparius* Link) in an exotic environment in Oregon, USA (Parker 1997).

Thus, the pollination ecology of *G. robusta* may be a critical determinant of its seed production in exotic environments where coevolved pollinators are absent. The main aim of the present study was to identify the major flower visitors that are potential pollinators of *G. robusta* in western Kenya, and test whether avian visitors facilitated fruit-set. The availability of nectar, its volume and concentration under eastern African conditions and the likely effect of these factors on pollinator visitation were also assessed.

METHODS

Study area

The present study was conducted in a plantation of *G. robusta* at Malava, western Kenya from March to September 1996. The field site (34°51′E, 0°28′N; 1600 m a.s.l.) slopes slightly (less than 5%) and has a south-westerly aspect. The adjacent vegetation is mainly natural evergreen forest with some cleared farmland nearby. Stands of *Markhamia platycalyx* (Bark.) Sprague and *Calliandra calothyrsus*, each approximately 1 ha in extent and 4 years old, surround the studied stand of *G. robusta*.

The annual rainfall is 2413 mm (mean for 1990–1995) with 2078 mm recorded during the study year, 1996. Rain falls in every month of the year, with May and September the wettest months. At the study site, the recorded monthly means of daily temperature maxima ranged from 27°C (August) to 33°C (March) and minima ranged from 14°C (July) to 16°C (November). The monthly means of relative humidity at 07.00 hours ranged from 76% (November) to 97% (July). Matungulu (1994) classified the soil as very fine, kaolinitic, isohyperthermic, udic kandic ustalf, with a pH of 6.2.

Plant material

A provenance–progeny trial was used to test open-pollinated families of *G. robusta* raised from seed collected from a total of 90 trees across 19 natural provenances. The trial was planted in April 1991, in a design that maintained both family and provenance identity. A family refers to the progeny raised from the seed collected from a single tree. The stand covered 1.1 ha and was set out in a randomized complete block design with seven complete replicates of single-tree plots. Spacing between trees was 4 m × 4 m. At the time of the study, the mean height of the trees was 14 m ± 1.2, mean diameter at breast height was 18 cm ± 0.7, and overall survival exceeded 95%. *Grevillea robusta* in western Kenya displays a continuous flowering pattern with one major peak in September. Approximately 53% of the trees were flowering in May 1996 and 72% in August 1996. During the study period, each tree generally produced from 200 to 2500 inflorescences. Preliminary observations indicated that more than 90% of the flowers that opened during a 24-h period did so during the night hours (18.00–07.00 hours).

Inflorescences of *G. robusta* may possess up to six branches, each bearing hermaphroditic flowers grouped into racemes. In the present study, each individual branch is referred to as a raceme. Mean raceme length is approximately 105 mm ± 2.8 when fully developed, with a mean of 84 ± 2.2 (range: 15–159) early floral

green buds and 40 ± 2.3 orange coloured flowers at anthesis (Kalinganire *et al.* 2000a). The flowers, which are approximately 23 mm ± 0.2 long when fully expanded, project upward so that the raceme has the appearance of a stiff brush (Nicolson 1993). The pollen is deposited just before anthesis onto the pollen-presenter, a more or less erect oblique cone around the stigma (McGillivray 1993; Kalinganire *et al.* 2000a). The nectary, partly enclosed within the torus (Brough 1933; McGillivray 1993), secretes yellow nectar that accumulates in a large drop between the style and the perianth segment.

Identification and behaviour of visitors

Observations were made on the number of visitors to flowers, their identity to species, duration of visit, and behaviour. Observations were conducted on 4 days during March–May 1996 (28 March, 4 April, 12 April and 2 May) and five days in August–September 1996 (1 August, 8 August, 15 August, 22 August and 5 September). Although these dates were picked randomly, they fell within the peak flowering periods at Malava (Kalinganire *et al.* 2000b). On each day there were four 1-h observation periods spaced throughout the day (07.00–08.00, 09.00–10.00, 12.00–13.00 and 16.00–17.00 hours). In addition, observations were made at night (1830–2230 hours) on 14 April, 18 April, 4 August and 4 September using flashlights. Flashlights were shone discontinuously, approximately 2 min between two consecutive lightings, from a single position.

Bird foraging behaviour was assessed by observing three randomly selected trees that could be watched simultaneously. The same three trees were observed throughout the study. Observations of birds were made at a distance of 20–30 m by a team of two observers using binoculars, with one person recording data. An inflorescence was regarded as having a visit only if the bird probed among the open flowers (following Vaughton 1992). An index of visitation was calculated by dividing the total number of visits (for all bird species) during the 4-h observation period by the number of available inflorescences during this period. The index was averaged over the three observed trees, for each observation day.

The destination of different bird species was monitored after foraging. Three types of behaviour were recorded: (i) bird flies away from the stand; (ii) bird flies to inflorescence on the same tree; and (iii) bird flies to nearby *G. robusta* tree.

Insects, mainly ants and bees, were observed separately on the same three trees used for bird observations. The trees were climbed on the same days as previously listed at 08.00–08.30, 10.00–10.30, 13.00–13.30 and 17.00–17.30 hours. Insect foraging behaviour,

especially contact with the stigma, was monitored and recorded.

Floral display and nectar production

Floral displays

Trees observed for flower visitors as earlier described were assessed for floral displays (number of active inflorescences, flower colour and nectar characteristics) before commencing observations, for approximately 30 min at approximately 06.30 hours. An inflorescence was referred to as active if at the moment of observation one or more of its flowers had presented pollen.

Nectar was assessed from a total of three flowers per tree for the three trees observed for avian visitors. One flower per raceme from three different inflorescences was sampled on each of the study days for identification and behaviour of floral visitors. Racemes were identified on the preceding day (approximately 12 h before pollen presentation) and isolated by muslin bags enclosing the entire inflorescence. Nectar production was measured by removing nectar from flowers in the morning, when secretion was believed to be at its peak (A. Kalinganire, pers. obs., 1996), then flowers were exposed to floral visitors.

Nectar was removed from flowers by capillarity and its volume measured in disposable glass 50-µL micropipettes. The volumes of nectar in partially filled micropipettes were determined from the column length (to the nearest 0.5 mm, equivalent to 0.2 µL) after Dafni (1992). The nectar solute concentration was measured immediately with a hand-held sugar refractometer (Bellingham & Stanley, Tunbridge Wells, England) as a percentage sucrose equivalent by weight (g sucrose per 100 g solution, abbreviated as percentage w/w). A table including temperature conversion for the refractometer with scale showing 0–50% was used to adapt the scale reading into per cent sucrose. When concentration (scale reading) was greater than 50%, nectar was extracted and diluted with a known quantity of distilled water measured with a micropipette, prior to measurement. The sucrose available per flower was estimated by multiplying nectar volume and concentration following Dafni (1992).

For each tree on each observation day, the brightness of the flowers was classified on a scale ranging from 1 to 3 where 1 is least bright (flowers green to dull orange in colour), 2 is orange-yellow flowers, and 3 is orange-yellow flowers with a shining tendency, brighter than in categories 1 and 2 above.

Nectar availability

The daily pattern of nectar production and accumulation was studied for two trees on 30 January, and nine

trees on 7 May and 1 September 1996. The sampled trees were from different families and the same trees were used across seasons. Observations were made at five times during the day: 08.00, 10.00, 12.00, 14.00 and 16.00 hours. At each observation time, three previously untouched flowers were sampled for nectar volume and concentration. Flower selection and nectar sampling were as described above.

Relative humidity (RH) and temperature were measured for each tree, at the time of each observation. The air temperature was measured by using a thermometer, and the relative humidity was recorded with a thermometer–hygrometer (Mason's wet and dry bulb hygrometer), within 1 m of the flowers. All readings were made to the nearest 0.5°C. Thermometers were not fully shielded from sunlight and this may have led to overestimates of temperature on some occasions.

Effect of visitation on fruit-set

To investigate whether the floral visitors influenced fruit-set, inflorescences at anthesis were enclosed in muslin cloth bags to exclude avian visitors from 20 July to the end of October 1996. The bags would have also excluded large insects. Six inflorescences (one inflorescence per treatment) on nine heavily flowering trees were tagged and subjected to six treatments randomly distributed on each tree. Three trees of different families were selected from each of three different provenances. Each treatment was represented once per tree, giving nine replicates per treatment. Each inflorescence and individual raceme was tagged with thread and coloured tags, a different colour for each treatment. Inflorescences included in the experiment each had one to six racemes, with 28–159 flowers per raceme. The six treatments applied were: (i) control, no treatment; (ii) unbagged 06.30–12.30 hours and bagged 12.30–06.30 hours; (iii) unbagged 12.30–18.30 hours and bagged 18.30–12.30 hours; (iv) unbagged 18.30–06.30 hours and bagged 06.30–18.30 hours (exposed to nocturnal visitors only); (v) flowers not emasculated and bagged (spontaneous autogamy); and (vi) controlled cross-pollination (xenogamy, flowers emasculated, i.e. self-pollen removed and mixed pollen from other trees of different provenances and families applied to receptive stigmas). No fruit-set was obtained from a mechanical self-pollination treatment, that is, induced self-pollination (Kalinganire *et al.* 2000a), so this treatment was excluded from this study.

The emasculation and isolation technique used was a modification of that described by Owuor and Oduol (1992) and detailed by Kalinganire *et al.* (2000a). Isolation of inflorescences from visitors was achieved using muslin cloth. For treatment (vi), flowers were emasculated before the style had been released from the perianth and before the anthers had dehisced. At this stage, pollen presenters were easily pulled out by hand, thus being separated from the perianths enclosing the anthers. Anthers were then removed. On average, 61 flowers per raceme were emasculated. All other (younger and older) flowers were removed. Cross-pollination was carried out by rubbing pollen-bearing stigmas of freshly harvested flowers on receptive stigmas 48 h after emasculation.

Isolation bags were removed at the fall of the perianths, this being an indication of initial fruit development (Kalinganire *et al.* 2000a). The number of fruit-set was counted 60 days after pollen presentation or controlled-pollination. The fruit : flower ratio (per cent fruit-set) per inflorescence was calculated from the total number of fruits at day 60 (fruits mature approximately 60 days after anthesis) and the total number of flowers per inflorescence (raceme) at anthesis or at emasculation.

Data analysis

Floral visitors

A regression analysis technique with an accumulated analysis of deviance was used to test variation among bird species in total number of bird visits, and total number of birds probing, and variation between days in number of visits and number probing. Terms fitted in the model were the effect of day and species, behaviour, interaction of behaviour and species and interaction of behaviour and day. A Poisson model was fitted with log as a link function (Mead *et al.* 1993) to test variation among bird species in their respective duration of visits, and differences between destinations after feeding in: (i) the overall number of birds; and (ii) among bird species. For destination after feeding, the model included day with species, behaviour, behaviour and species, and behaviour and days.

Resource availability

The data for nectar production and floral display were analysed using residual maximum likelihood (REML) estimation (Williams & Matheson 1994). A constant, RH, temperature, and the interaction of RH and temperature were fitted as fixed effects, and family, time of the day and month were fitted as random effects. Wald statistics using a χ^2 with appropriate degree of freedom were used to test: (i) the differences between trees in nectar volume and concentration, and relative humidity and temperature effects on nectar production; and (ii) the effects of number of active inflorescences and brightness of the flowers on the total number of birds visiting and probing. Linear regressions were used to test for relationships

between the number of visits and nectar volume and concentration.
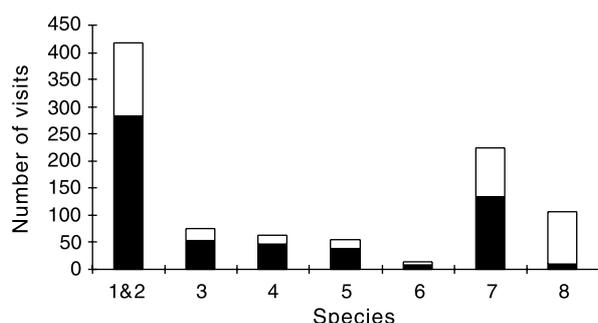
Effect of visitation on fruit-set

The significance of differences in the number of fruit-set among various treatments was tested by using a χ^2 test.
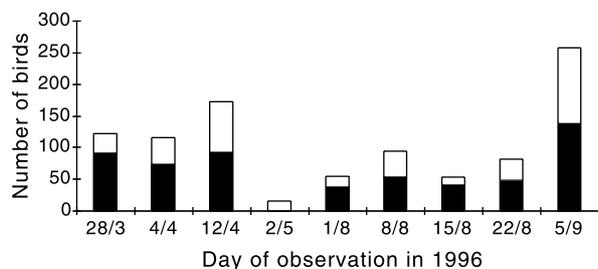
RESULTS

Floral visitors and behaviour

Type and number of birds

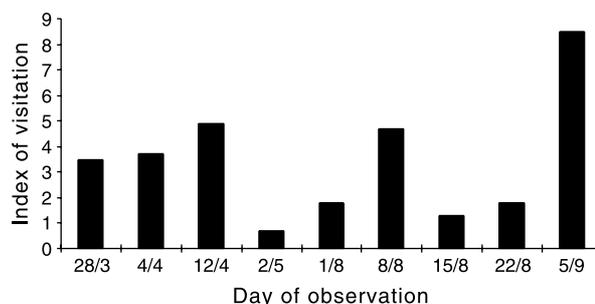The birds that visited were predominantly sunbirds and white-eyes (Fig. 1). There were two species of white-eye, *Zosterops kikuyuensis* (Sharpe) and *Z. senegalensis* (Bonaparte), but these could not be separately identified and were grouped for counting purposes. Five species of sunbirds were identified and recorded separately by species. All other visitors were grouped together; many of these were also different species of sunbirds.

Among the seven categories of bird visitors (white-eyes, five sunbird species and all other species combined), there was significant variation in the number of bird visits ($F = 14.6$, d.f. = 6, $P < 0.001$); and the number of birds probing ($F = 12.1$, d.f. = 6, $P < 0.001$; Fig. 1). The most frequent visitors were the white-eyes, together accounting for 49% of the visits. The amethyst sunbird, *Nectarinia amethystina* (Shaw), was the next most frequent visitor to inflorescences, accounting for 23% of total visits (Fig. 1). The same trend among categories was evident for the number of visitors probing.

Significant variation between days was also obtained for the total number of birds visiting ($F = 3.7$, d.f. = 7, $P < 0.001$) and probing ($F = 4.2$, d.f. = 6, $P < 0.001$; Fig. 2), and for the index of visitation ($F = 4.1$, d.f. = 8, $P < 0.001$; Fig. 3). The lowest index of visitation was 0.7 visitors per inflorescence on 2 May and the highest was 8.5 on 5 September (Fig. 3). Birds visited flowers throughout the day but numbers probing were much greater in the morning (Fig. 4). Eighty-nine per cent of total bird visits occurred between 07.00 and 10.00 hours.

Fig. 1. Total number of visits observed for each species of bird and the number probing inflorescences with open flowers of *Grevillea robusta* at Malava, Kenya. Observations made in 1996 on three inflorescences 3 of each of three different trees and for 3 inflorescences each per 4 hour-observational day at 07.00-08.00, 09.00-10.00, 12.00-13.00, 14.00, and 16.00-17.00 and 18.00 h. Species names: 1 & 2, kikuyu white-eye (*Zosterops kikuyuensis*) and yellow white-eye (*Z. senegalensis*); 3, variable sunbird (*Nectarinia venusta*); 4, superb sunbird (*N. superba*); 5, blue-throated brown sunbird (*N. cyanolaema*); 6, olive sunbird (*N. olivacea*); 7, amethyst sunbird (*N. amethystina*); 8, unidentified species mainly sunbirds. (□), No probing; (■), probing.

Feeding behaviour

Sunbirds and white-eyes perched on branches adjacent to an inflorescence. They probed with their bills into the base of open flowers through the elongated styles and stigmas to obtain nectar either from above or below. While probing flowers the birds brushed their bills and heads against stigmas, so pollen removal and deposition on stigmas was highly probable. The birds usually remained in the same position while feeding, probing within a relatively small area of open flowers. Most of the birds visited fewer than 10 flowers per raceme.

Fig. 2. Total number of birds of all species and the proportion probing inflorescences of *Grevillea robusta* per 4-h observational day at Malava, Kenya. (□), No probing; (■), probing.

Fig. 3. Index of visitation (visits per available inflorescence per 4-h observation day) per day of observation for *Grevillea robusta* at Malava, Kenya, averaged across three trees.

Some other bird species with very short bills such as bulbuls and weaver birds, mainly fruit and seed eaters, respectively, were seen bending sideways for either insects (ants) or nectar, but rarely through open flowers. It is possible, but unlikely, that these species might carry pollen on their underparts. A few other birds, mostly sunbirds, were observed at or near the inflorescences but these could not be identified.

At times, several birds were observed feeding simultaneously on the same tree, but very few aggressive interactions between individuals were detected among sunbirds and white-eyes. Bulbuls (*Pycnonotus* species) were seen chasing each other exhibiting mating behaviour.

Duration of visits

The duration of visits to inflorescences varied significantly among species ($\chi^2 = 23.1$, d.f. $= 6$, $P < 0.001$; Fig. 5). The olive sunbird *N. olivacea* stayed longest on inflorescences with a mean of 37 s (range: 25–40 s) and the variable sunbird *N. venusta* stayed for the shortest time with 9.6 s (range: 1.4–21.7 s). Some individual white-eyes remained at an inflorescence for up to 60 s.

### Destination of birds after feeding

After feeding, most of the birds moved horizontally rather than vertically to higher or lower inflorescences on the same or adjacent branches. Sixty-six per cent of all bird visitors went to a nearby *G. robusta* tree, significantly more ($\chi^2 = 22.9$, d.f. $= 2$, $P < 0.001$) than to the alternative destinations. Twenty-eight per cent moved to another inflorescence of the same tree. Very few birds (6%) flew away from the *G. robusta* stand after visiting the observed trees. The proportions of birds displaying different types of behaviour differed significantly among species ($\chi^2 = 41.3$, d.f. $= 5$, $P < 0.001$; Fig. 6).
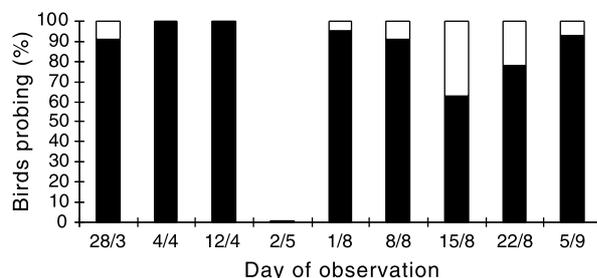
Fig. 4. Proportion of all birds observed probing in the morning (07.00–08.00 and 09.00–10.00 hours) and in the afternoon (12.00–13.00 and 16.00–17.00 hours 14.00, 16.00 and 18.00) for each 4-h observational day on *Grevillea robusta* flowers at Malava, Kenya. Each day's observations made on three inflorescences of each of three different trees. On 2 May no birds were observed probing flowers. (□), Probing in the morning; (■), probing in the afternoon.

Ants, bees and other visitors

The observed trees were also visited by grey-black ants (*Messor barbarus* Andre) and brown ants (*Formica sanghinea* Emery) throughout the day, and by honeybees (*Apis mellifera* L.), with most of the few honeybee visits recorded in the afternoons. Although the ants and honeybees visited the flowers, they did not appear to be potential pollinators. Their approach to the flowers commenced from the base of the raceme and they walked through the flowers for nectar feeding, making little or no contact with stigmas. During the day, flies and aphids were observed around active flowers but did not touch the stigmas. At night, white moths were seen occasionally flying around the trees, but they were not
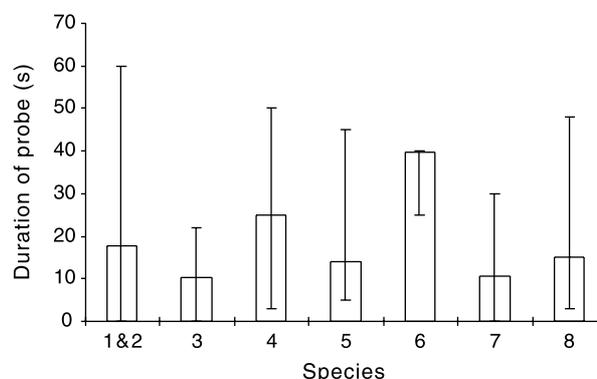
Fig. 5. Mean duration of visit per species of bird on inflorescences of *Grevillea robusta* at Malava, Kenya. Bars are the mean duration per species (mean over all 81 inflorescences for all time periods observed for 9 days over 27 trees) and vertical lines are ranges. Species names and numbers as in Fig 1.
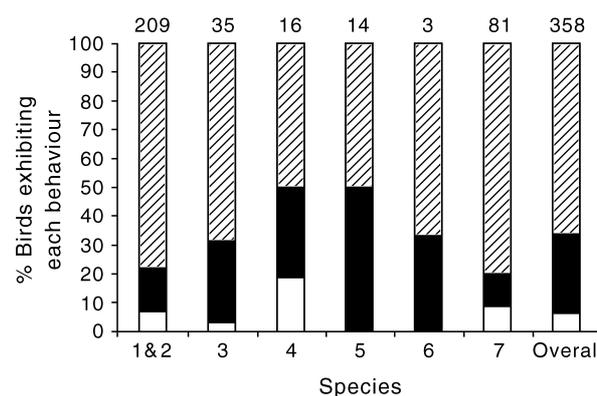
Fig. 6. Behaviour of avian visitors of each species after probing *Grevillea robusta* flowers at Malava, Kenya, showing three behavioural categories: (i) visitor flies away from the stand; (ii) visitor flies to inflorescence on same tree; and (iii) visitor flies to nearby *G. robusta* tree, measures as percentage of birds exhibiting each behaviour. The total number of birds observed for each species is given. Species names and numbers as in Fig 1. (▨), Nearby tree; (■), same tree; (□), away from stand.

observed on open flowers. No other nocturnal floral visitors were observed.
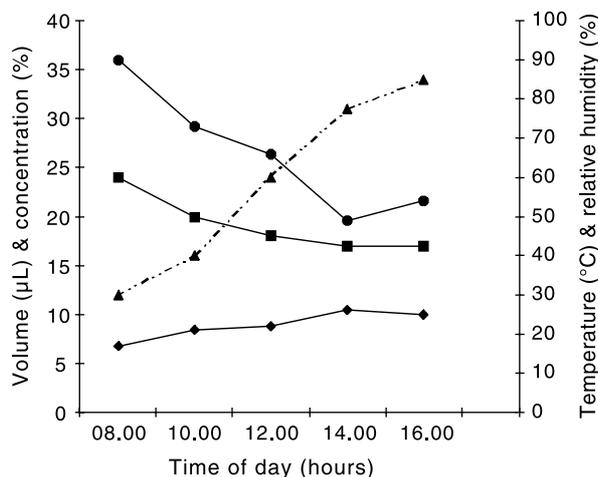
Floral display and nectar production

Floral displays and avian visits

There was a significant relationship between the brightness of the flowers and the number of birds visiting per tree (Wald test $\chi^2 = 9.7$, d.f. = 2, $P < 0.01$) and on the number of birds probing per tree ($\chi^2 = 14.7$, d.f. = 2, $P < 0.001$). Trees with brighter flowers attracted more birds. For a total of 976 birds observed, the overall visitation rate was 1.6, 2.7 and 6.6 visitors per inflorescence, respectively, for green to dull orange inflorescences (category 1), orange-yellow inflorescences (category 2) and bright orange-yellow inflorescences with a shining tendency (category 3).

There was no significant relationship between the number of active inflorescences (Wald test $\chi^2 = 2.1$, d.f. = 2, $P > 0.05$), nectar concentration ($\chi^2 = 0.5$, d.f. = 2, $P > 0.05$) or nectar volume ($\chi^2 = 2.3$, d.f. = 2, $P > 0.05$) and the number of birds probing on *G. robusta* flowers.

Nectar availability

Nectar volume and concentration changed significantly over the course of the day. Significant variation was obtained for nectar volume (REML Variance Components Analysis, $P < 0.001$; Fig. 7), nectar concentration ($P < 0.001$; Fig. 7) and nectar reward ($P < 0.001$; Fig. 8) between observed times during the day.

Generally, nectar volume was at a maximum in the morning. It declined towards midday and there was a slight rise again toward evening depending on prevailing climatic conditions. Relative humidity was negatively and significantly related to nectar concentration ($r^2 = 0.24$, $n = 60$ in January; $r^2 = 0.36$, $n = 45$ in May; 0.62, $n = 45$ in September, $P < 0.001$) on all days. For temperature, a significant relationship with concentration was obtained in January ($r^2 = 0.61$, $n = 60$, $P < 0.001$) and in September ($r^2 = 0.61$, $n = 45$, $P < 0.001$); and a significant negative relationship with volume only in January ($r^2 = 0.25$, $n = 60$, $P < 0.001$).

Individual flowers produced mean nectar volumes of 13.1 μL ± 0.9, 12.5 μL ± 1.0 and 19.1 μL ± 1.0 on 30 January, 7 May and 1 September, respectively. For nectar concentration, corresponding flower mean values were, respectively, 41.9, 21.6 and 23.5%. The highest mean nectar volumes were recorded at 08.00 hours in the mornings and the lowest in the afternoons at 16.00 hours. Nectar concentration was lowest in the mornings and highest in the afternoons for all observed periods. The lowest reward per flower recorded was in the mornings and the highest after midday (Fig. 8). No empty flowers were observed during the present study. The absolute highest nectar concentration (64%) recorded was on 30 January, a day of hot and dry weather. This day was hotter and drier than 7 May and 1 September, with a maximum temperature of 32°C and corresponding RH of 55%. The maximum temperature and RH for 7 May and 1 September were 26°C and 61% RH, and 26°C and 66%, respectively.

Effect of visitation on fruit-set

The number of fruits increased greatly ($\chi^2 = 2492$, d.f. = 5, $P < 0.001$; Table 1) following cross-pollination

Fig. 7. Mean nectar volume (μL) and concentration (% sucrose equivalents) per flower at different times of the day with corresponding values of temperature and relative humidity (RH) for *Grevillea robusta* on 1 September 1996 at Malava, Kenya. Nectar collected from 9 trees from 3 different inflorescences for a total of 27 flowers per tree (1 flower per inflorescence) per time of day. Mean nectar values are 19.1 μL ± 1.0 (range: 9-37 μL) for mean nectar volume and 23.5 % ± 1.5 (range: 7-49) for mean nectar concentration. The pattern of variation was similar for January and May observations. (■), Volume; (▲), concentration; (◆), temperature; (●), relative humidity.
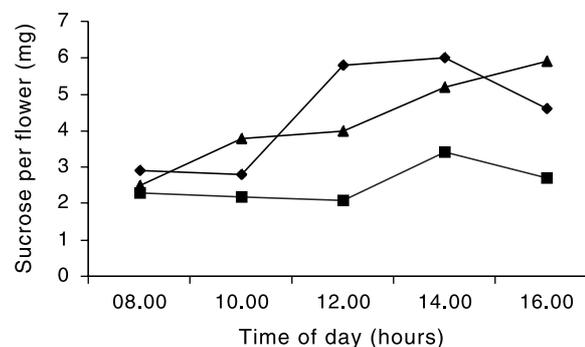

Fig. 8. Mean sucrose reward per flower at different times of the day for *Grevillea robusta* on (◆) 30 January, (■) 7 May and (▲) 1 September 1996 at Malava, Kenya.

Table 1. Effect of bagging and pollination treatments on fruit-set in *Grevillea robusta* at Malava, Kenya

	Treatments					
	1	2	3	4	5	6
Total flowers	1855	2202	2452	2702	2613	546
Total fruit-set	17	2	7	0	0	137
% Fruit-set	0.9	0.1	0.3	0.0	0.0	25.1
Variation among treatments (χ^2)	2492***					

Six inflorescences, i.e. one inflorescence per treatment per tree (nine trees) were used. An inflorescence had 4–6 racemes and the total number of flowers per inflorescence varied from 28 to 562.

Treatment codes: 1, left unbagged (natural open-pollination); 2, exposed to visitors from 06.30 to 12.30 hours; 3, exposed from 12.30 to 18.30 hours; 4, exposed from 18.30 to 06.30 hours; 5, bagged continuously (autogamous self-pollination); 6, cross-pollination, self-pollen removed and pollen from different families applied to stigmas. ***Significant difference at $P < 0.001$.

by hand, with 25.1% fruit-set compared with a fruit-set of 1, 0.1 and 0.3% obtained, respectively, for natural open-pollinated flowers, flowers exposed to visitors from 06.30 to 12.30 hours, and exposed from 12.30 to 18.30 hours. The flower : fruit ratio for open pollinated flowers, although very low, was significantly ($\chi^2 = 18.3$, d.f. = 2, $P < 0.001$) greater than for the two treatments where birds were partially excluded, suggesting that the presence of birds increased seed production. No fruits were set from flowers exposed to nocturnal visitors, or flowers that were bagged continuously excluding avian visitors.

DISCUSSION

Floral visitors and pollinators

The likely pollinators of *Grevillea robusta* at Malava, Kenya are sunbirds (*Nectarinia amethystina, N. cyanolaema, N. olivacea, N. superba* and *N. venusta*) and white-eyes (*Zosterops kikuyuensis* and *Z. senegalensis*). The effective removal and transfer of pollen by sunbirds and white-eyes was supported by the absence of fruit-set in inflorescences bagged to exclude birds. The present study confirms earlier observations that the most frequent visitors on *G. robusta* flowers were sunbirds and white-eyes at Malava (Kalinganire *et al.* 1996), and sunbirds and the Cape white-eye in South Africa (Collins & Rebelo 1987; Nicolson 1993). In its natural range, *G. robusta* is believed to be mainly pollinated by nectarivorous birds (Brough 1933; Collins & Rebelo 1987). Ants, bees and other species of birds that visited flowers at Malava were judged not to be effective pollinators. We also found that there were no likely nocturnal pollinators.

Hundreds of ants visited flowers at all times throughout the day for nectar whereas honeybees rarely visited the flowers during the present study. Although Brough (1933) reported that bees were abundant on inflorescences of *G. robusta* in Sydney, Australia, they were less

attracted to *G. robusta* flowers at Malava, whereas at Cape Town, South Africa, honeybees did not visit *G. robusta* flowers (Nicolson 1993). The few bees observed made contact with the stigmas only if disturbed by the observer while feeding on nectar.

Contact with the stigma by visitors is necessary for pollination (Vaughton 1992). Thus, the foraging behaviour of ants and bees on *G. robusta* flowers confirmed that they are nectar-robbers, and are unlikely to act as pollinators. Brough (1933) reported similar behaviour, as did Taylor and Whelan (1988) on *Grevillea* × *gaudichaudii* R.Br. ex Gaudich., where bees harvested nectar without effecting pollination. Sunbirds and white-eyes made consistent contact with the stigma of *G. robusta* while feeding on nectar and would have collected pollen from the pollen presenter adjacent to the stigma. Other birds (e.g. bulbuls, weaver birds) present on flowers had short bills and were unable to feed through the open flowers. Instead they fed sideways and were confined mostly to the edges of the raceme. They made very little contact and in most instances they did not touch the stigmas.

Bats and other mammals were not observed on *G. robusta* in western Kenya. Bats were reported foraging in a *C. calothyrsus* stand under similar environmental conditions in Kenya (Boland & Owuor 1996), but did not visit *G. robusta* flowers during the present study.

Resource availability and avian visits

Nectar is most abundant in *Grevillea robusta* flowers in the early morning. Nectar volumes had dropped substantially by late morning, presumably as a result of evaporation and/or harvesting by birds and insects. Active regulation of nectar sugar content and maintenance of constant nectar volume by the flowers, as reported by Nicolson (1993, 1995) in South Africa, was not observed in the present study. In contrast, there usually was a steady decrease in volume and an

increase in nectar concentration throughout the day as temperature increased and relative humidity decreased. The overall trends show low values for nectar concentration and high volumes between 08.00 and 10.00 hours, corresponding to the period of high frequency of avian floral visitation. In the afternoon, lower nectar volume, higher sugar concentration and higher rewards corresponded to a low avian visitation.

Opening of individual flowers in a raceme is staggered over 2–3 days (Kalinganire *et al.* 2000a) and nectar secretion on a raceme may extend for up to 7 days (Kalinganire 1999). This increases the chance of multiple bird visits to a raceme over several days, leading to pollen removal from, and subsequent pollen deposition on, open flowers.

There were significant differences between observation days in bird species visiting and probing *G. robusta* flowers. Relatively few trees were flowering throughout the stand in March – April, with few flowers per tree (Kalinganire *et al.* 2000b). This might be one of the reasons fewer birds were observed on the site during this period than in the later observations. Studies of other species with avian visitors have shown that low flower density resulted in low visitation rate of pollinators (e.g. Augspurger 1980). However, the reason why there were fewer avian visits and no probing on 2 May (Figs 2,4) could not be established. Indigenous tree species growing naturally in natural forest within a few hundred meters of the study site provided alternative nectar resources, which may have varied seasonally. The flowering of some of these indigenous tree and shrub species is prolific (A. Kalinganire, pers. obs., 1996) and the area of forest is much larger than that of the *G. robusta* stand. Observations over a 2-day period in September 1996 on older, more heavily flowering stands of *G. robusta* near Embu, Eastern Kenya with a similar indigenous flora showed that there were few avian visitors to *G. robusta* and that other tree species were more attractive to nectarivorous birds (A. Kalinganire, unpubl. data).

In its natural range (latitude 26–30°S) the flowering of *G. robusta* is heavy and synchronized during a period of approximately 1–2 months in spring (Harwood 1992), when it attracts abundant avian flower visitors. In Canberra, Australia (latitude 35°S, outside the species natural range), Kalinganire (1999) reported a synchronized flowering period in November–December, with more abundant and relatively dilute nectar (22 µL nectar volume and 18.5% concentration) than in western Kenya. The number of avian visitors was higher in Canberra and fruit-set of open-pollinated flowers (at least 4.4%) higher than in Kenya (Kalinganire 1999). At Malava, the species flowered throughout the year, with flowering peaks occurring in May and October (Kalinganire *et al.* 2000b). This non-synchronized flowering might make the stand less attractive to avian visitors.

Sunbirds and white-eyes patterned their foraging mainly over short (1–2 h) time spans with more visits in the morning than in the afternoon, probably because of significantly higher nectar volume present per flower in the morning. The birds concentrated their visits during a period of low rewards but with higher volumes per flower. Gill and Wolf (1977) reported the same feeding behaviour for sunbirds feeding mainly on mistletoes in the Rift Valley, central Kenya, and Nicolson (1995) for the Cape white-eye feeding on *G. robusta* in South Africa. Moreover, the daily variation in timing and duration of visits is likely affected by the changing weather conditions among observation days and the availability of other food sources around the study area.

Sunbirds are specialized nectar feeders (Percival 1965; Gill & Wolf 1975a,b; Burd 1994) and rely solely on nectar for their energy requirements (Wolf 1975; Wolf & Gill 1986). White-eyes rely on insects and nectar as their main food sources (Percival 1965; Wolf & Gill 1986) and are reported to be most active in the mornings by these authors. Sunbirds increase their foraging efficiency by avoiding recently visited, empty flowers and by visiting flowers with greater than average nectar volumes (Gill & Wolf 1975b). Nectar that is present in large amounts in the early morning is readily accessible to other visitors, mainly ants. Generally sunbirds tend to move away from areas of low resource density (Gill & Wolf 1977). Consequently, nectar depletion by high levels of ant activity and gradual nectar evaporation, leading to lower volumes during the day, may force the birds to depart from the *Grevillea* stand for alternative tree species with higher rewards. Similar findings were reported by Roubik (1982) for *Pavonia dasypetala* (Turcz.) in central Panama. Burd (1995) reported that enhanced rewards increased the visitation rate of the scarlet-tufted malachite sunbird, *Nectarinia johnstoni* (Shelley), on *Lobelia deckenii* (Knox). The effect of sugar concentration of *G. robusta* on the likely pollinators' densities, their foraging times and number of probes is not clear, as nectar volume and concentration are inversely related. However, Wolf (1975), De Benedictis *et al.* (1978) and Burd (1995) reported a stronger effect of volume than of concentration on probing behaviour, the decision by a bird to leave an inflorescence being based in part on the volume of the nectar load ingested and not solely on energy intake. The number of available flowers per raceme was also important for the probing behaviour in these studies.

Our study suggested that nectar volumes may be important in relation to the variation in the visitation rates and foraging times of white-eyes and sunbirds probing *G. robusta* flowers at Malava. The foraging time for sunbirds and white-eyes is presumably regulated primarily by nectar availability and concentration per flower. However, flower colour was found to have a

strong relationship with bird visitation, with a tendency for more visits to brighter than normal inflorescences. Although flower colour is a major signalling mode that acts to allow pollinators to differentiate one species from another, it is not sufficient to prevent straying if other flower parameters are equal (Heinrich 1975).

Pollinators and their effect on fruit-set

After feeding, the majority (66%) of birds went to a nearby *G. robusta* tree, an ideal behaviour for effecting cross-pollination. Fewer (28%) birds moved to another inflorescence on the same tree, potentially facilitating pollen movement between flowers of the same tree and geitonogamous selfing (Lloyd & Schoen 1992; Harder & Barrett 1996). In general, the foraging behaviour of pollinators provided opportunities for xenogamous pollen movement, thus promoting outcrossing with a subsequent potential high fruit-set. Pollinators with high visitation rates (white-eyes and *Nectarinia amethystina*) made short visits to individual inflorescences, conducive to outcrossing. The less frequent visitors (e.g. *Nectarinia olivacea*) tended to spend a longer time probing a single raceme. This behaviour would favour self-pollination.

Although their fruit : flower ratio was low, open-pollinated flowers, exposed and undisturbed, set significantly more fruits than the flowers restricted to visitors in the morning and afternoon periods. Controlled pollination with outcross pollen produced much higher fruit-set than the natural-pollinated flowers. Therefore, the absence of cross pollen will reduce seed production, as control self-pollinated flowers (Kalinganire *et al.* 2000a), and spontaneous self-pollinated flowers produced no fruits. The results suggest that low levels of activity by bird visitors at Malava reduced considerably the seed production of *G. robusta,* a result of low cross-pollen transfer. As stated by Bierzychudek (1981), if hand-pollinated plants produce more seeds than naturally pollinated controls, then reproduction is limited by visitor activity.

Grevillea robusta has been introduced to western Kenya, but its main coevolved pollinators have not. The main coevolved pollinators are nectar feeding birds such as the red wattle bird, *Anthochaera carunculata* (Shaw) (Brough 1933; Kalinganire 1999). This species is large (approximately 350 mm in length) compared with sunbirds and white-eyes (approximately 90–120 mm). Wattle birds, being large, cover the inflorescence when feeding and soon become covered in pollen with a subsequent higher rate of seed-set in planted stands in Canberra, Australia. Although sunbirds and white-eyes are the likely pollinators of the species in Kenya, their size as well as their frequency may well be a limitation to the maximum level of seed-set possible.

In summary, a shortage of suitable pollinators, leading to inadequate cross-pollen transfer, seems likely to be a major factor limiting fruit production of *G. robusta* at Malava. Other limiting factors may include heavy rain during peak flowering times at Malava, which as well as deterring bird visitors may wash away pollen from flowers before they are visited. Resource limitation can probably be excluded as a factor limiting fruit-set, because the cross-pollination treatment set many more fruits per inflorescence than the open-pollinated treatments. High levels of flower and fruit predation reported by Hermanutz *et al.* (1998) for other *Grevillea* species were not observed in the present study and could not account for the low fruit-set.

The importance of pollinators needs to be recognized when considering the establishment of seed production stands of *G. robusta* in the central and east African highlands. Effective seed production will depend on the abundance of appropriate pollinators, mainly sunbirds and white-eyes at least in western Kenya.

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