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Short communication

Insights into the pollination requirements of the only African wild tobacco, *Nicotiana africana* (Solanaceae) from the Namib Desert

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ABSTRACT

Nicotiana species (Solanaceae) are widely distributed, mainly in the Americas and Australia. *Nicotiana africana* is the only species indigenous to Africa; its populations are confined to isolated mountains in the Namib Desert and thus little is known about this species' reproductive strategy or reliance on pollinators, if any. Plants grow in a sheltered environment among granite boulders and wind pollination is therefore unlikely. Our aim was to use a controlled hand-pollination experiment to identify the pollination requirements of *N. africana* and thereby infer the level of reliance on pollinators. One of five treatments was applied to flowers: either self- or cross-pollination, with half the flowers being emasculated and half not, and the fifth treatment consisted of unmanipulated flowers. Fruit set, seed set and seed weight were measured to determine pollinator reliance. Fruit set and seed set were similar for crossed and selfed flowers. Our findings show that *N. africana* is self-compatible but also partially dependant on pollinators for reproduction. Using floral traits, the plant's natural distribution and comparisons with other *Nicotiana* species, we predict that sunbirds (Nectariniidae) are the most likely pollinators of this species in its natural habitat.

1. Introduction

Of the about 80 recognised species of *Nicotiana*, an estimated 75% occur naturally in the Americas and 25% in Australia (Goodspeed, 1954). However, in the otherwise exclusively Australian section *Suaveolentes*, a single species is endemic to Africa (Knapp et al., 2004). *Nicotiana africana* Merxm. is found in very arid karroid shrubland in a semi-desert/desert environment in the arid Erongo, Spitzkoppe and Brandberg Mountains in the northwestern Namib Desert (Craven, 2004). It occurs in small isolated stands and grows in deep shade among granite boulders (Craven, 2004). It is not known what herbivores and/or pollinators are associated with this species in its natural habitat. Wind pollination is very unlikely in this sheltered environment.

N. africana is a sturdy perennial that can grow up to 2.5 m high. Its inflorescences (Fig. 1) are terminal panicles, each with 5–10 pale greenish white flowers that open by day (Herman, 1990). Nornicotine is the main alkaloid of the leaves and roots (Saitoh et al., 1985); nornicotine also occurs in the floral parts, but only at low concentrations, and the nectar does not contain nicotine, nornicotine or anabasine (Marlin et al., 2014). Furthermore, *N. africana* lacks a distinct floral scent, although the flowers of hummingbird- and hawkmoth-pollinated *Nicotiana* species have been shown to share floral volatiles, regardless of pollinator affinity (Raguso et al., 2003, 2006).

Three main pollination systems have been reported for *Nicotiana* species; hawkmoths, hummingbirds and sunbirds, and other pollinators such as bumblebees and small moths (Kaczorowski et al., 2005). Species of *Nicotiana* show interspecific variability in nectar volume and concentration (Kaczorowski et al., 2005) and in floral odour complexity and emission rates (Raguso et al., 2003, 2006), and this may influence pollinator preferences. Although certain species of *Nicotiana* are capable of self-pollination and others are not (Kaczorowski et al., 2005; Raguso et al., 2003; Adler et al., 2012), in many species seed production is significantly higher when the plants are exposed to pollinators (Geerts and Pauw, 2009; Schueller, 2004).

Adler et al. (2012) examined the pollinator reliance and alkaloid levels of 32 species of *Nicotiana*, excluding *N. africana*, and found that the nectar, floral and leaf nicotine concentrations were significantly lower in outcrossing than in selfing species. It has been

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Fig. 1. The inflorescence of *Nicotiana africana*.

hypothesized that the rate of outcrossing among flowers increases as nectar nicotine levels decrease, because pollinators search for nectar with low nicotine concentrations, thereby moving between flowers and transferring pollen more frequently (Kessler et al., 2012). Due to their bitter taste, nectar alkaloids such as nicotine deter pollinators (Adler, 2000) whose flower visits would presumably increase plant reproduction. However, nicotine does not deter all pollinators: sunbirds are willing to drink sucrose solutions containing moderate concentrations of nicotine, despite being less tolerant of this alkaloid than other bird species (Lerch-Henning and Nicolson, 2013).

We determined the potential breeding system of *N. africana* as selfing or outcrossing, to ascertain whether this species is reliant on a pollen vector. We hypothesized that, due to the plant's highly isolated natural distribution and the harsh environment where it occurs, *N. africana* will show little reliance on pollinators and is likely to be self-compatible. Considering the rarity and remote desert location of this species, field work was not a practical option and we opted for the controlled environment of a glasshouse.

2. Materials and methods

Seeds were originally obtained from T. Doroszewska (Institute of Soil Science and Plant Cultivation, State Research Institute, Pulawy, Poland). The methods for propagating and maintaining *N. africana* plants were described in Marlin et al. (2014). For the present study, the pollination experiment began in January 2013 when the first plants started flowering and continued until September 2013 in order to obtain a sufficient number of replicates. The glasshouse was free of natural pollinators. Plants were maintained at 20.05 ± 0.42 °C (mean ± SE) and a relative humidity of 52 ± 1% RH.

Flowers were allocated the following hand-pollination treatments: 1) unmanipulated (no emasculation, no pollination by hand), 2) emasculated untreated (emasculated but not pollinated), 3) crossed, 4) emasculated crossed, 5) selfed and 6) emasculated selfed. Because flowers did not mature simultaneously, each experimental plant was used for 2–4 treatments, assigned randomly, as flowers became available. Treatments within plant combinations therefore differed between plants (12 in total). Treatments were chosen to ensure that all possible scenarios were accounted for. Each treatment was replicated five times, and a minimum of 10 flowers was sampled per replicate.

Emasculation was carried out shortly after anthesis, but before the anthers had dehisced, to eliminate the possibility of natural self-pollination. As soon as the flowers opened, the still intact anthers were carefully removed by hand and kept in marked glass vials where they were allowed to mature until they had dehisced and released pollen; vials were stored in a dark and cool room. This pollen was then used for pollinating flowers, either on the same plant (selfed and emasculated selfed treatments), or on different plants (crossed and emasculated crossed treatments). We used a fine camel hair paintbrush, washed with distilled water between flowers and dried, to transfer pollen to receptive stigmas, usually between 0900 h and 1100 h. Flowers in the selfed and outcross treatments were pollinated before anthers started to release pollen, to ensure that either pollen from the same plant, in the case of selfed flowers, or pollen from a different plant, in the case of outcrossed flowers, was the first pollen to come into contact with the stigma. Each flower was pollinated once only.

Mature fruits (capsules) were collected before they released their seeds, approximately 30 days after the flower first opened, and dried at 50 °C for at least 48 h. Data for fruit set are based on the number of capsules that reached maturity, *i.e.* produced seeds, compared with the original number of flowers collected, and are presented as percentages. We recorded the number of flowers aborted in each treatment during the study period. Immature fruit abortion was high in the emasculated unmanipulated treatment (only seven flowers in this treatment were not aborted), and therefore this treatment was excluded from further analyses. Aborted flowers did not produce seeds. For each mature fruit we measured seed set (number of seeds produced per fruit), and thereafter the mean seed weight was calculated for each treatment on each plant. Seed set data were used to calculate the self-compatibility index (SCI = emasculated selfed/emasculated crossed) and self-fertility index (SFI = unmanipulated/emasculated crossed) (Lloyd and Schoen, 1992; Schoen and Lloyd, 1992).

The percentage fruit set data were arcsine transformed to meet the requirements of normality and homogeneity, and thereafter a one-way ANOVA was used to determine whether there were differences in fruit set between pollination treatments. Seed set was analysed with a generalized linear mixed model (Schall, 1991) with a Poisson distribution and a log link function. The mean seed weight between the pollination treatments was analysed with linear mixed model analysis (REML) (Payne et al., 2012). For both tests pollination treatment was included as fixed and plant as random effect. Fisher's protected LSD test was used to separate means at the 5% level of significance ($P < 0.05$). Data were analysed with GenStat® 2012.

To predict the pollination mode of *N. africana*, we determined which treatment had the highest reproductive fitness, *i.e.* highest fruit set, seed set and mean seed weight.

3. Results and discussion

Abortion of immature fruit was high throughout the study and the percentage fruit set (percentage of flowers that produced seeds) differed significantly among treatments ($F_{4, 20} = 2.966$, $P = 0.045$). It was significantly lower in the unmanipulated flowers compared to the other treatments (Fig. 2A).