American Journal of Botany 96(11): 2080–2086. 2009.

POLLINATORS, "MUSTARD OIL" VOLATILES, AND FRUIT PRODUCTION IN FLOWERS OF THE DIOECIOUS TREE *DRYPETES NATALENSIS* (PUTRANJIVACEAE)[1]

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The Putranjivaceae is an enigmatic family, notable for being the only lineage outside the Capparales to possess the glucosinolate biochemical pathway, which forms the basis of an induced chemical defense system against herbivores (the "mustard oil bomb"). We investigated the pollination biology and floral scent chemistry of *Drypetes natalensis* (Putranjivaceae), a dioecious subcanopy tree with flowers borne on the stem (cauliflory). Flowering male trees were more abundant than female ones and produced about 10-fold more flowers. Flowers of both sexes produce copious amounts of nectar on disc-like nectaries accessible to short-tongued insects. The main flower visitors observed were cetoniid beetles, bees, and vespid wasps. Pollen load analysis indicated that these insects exhibit a high degree of fidelity to *D. natalensis* flowers. Insects effectively transfer pollen from male to female plants resulting in about 31% of female flowers developing fruits with viable seeds. Cetoniid beetles showed significant orientation toward the scent of *D. natalensis* flowers in a Y-maze olfactometer. The scents of male and female flowers are similar in chemical composition and dominated by fatty acid derivatives and isothiocyanates from the glucosinolate pathway. The apparent constitutive emission of isothiocyanates raises interesting new questions about their functional role in flowers.

Key words: cauliflory; dioecy; *Drypetes*; floral scent; GCMS; glucosinolate; isothiocyanate; nectar; pollination syndrome; Putranjivaceae.

The pantropical genus *Drypetes* is of considerable interest from a phylogenetic and phytochemical perspective. Although traditionally placed in the Euphorbiaceae, most authorities now agree that it belongs in a separate clade, the Putranjivaceae (Rodman et al., 1998). Information on reproductive biology in the genus *Drypetes* is scant. Both dioecious and hermaphroditic species are known. Anecdotal reports suggest that the flowers tend to have exposed nectar and generalist pollination systems. In their broad community study, Momose et al. (1998) found that two white-flowered dioecious Asian species, *Drypetes longifolia* (Blume) Pax & K.Hoffm and *D. xanthophylloides* Airy Shaw, were visited by a wide range of insect species, especially beetles.

This study focuses on *Drypetes natalensis*, a dioecious South African species with flowers borne on the main stems (cauliflory, Fig. 1A). The flowers produce an extremely pungent sulfurous odor that can be detected by humans several hundred meters from flowering trees. Because this odor is detectable only when trees are in flower, we suspected that it arises from floral tissues. Initial analyses of samples sent to the laboratory of Roman Kaiser (Givaudan Natural Scents, Switzerland) indicated that the sulfurous floral odor of the flowers is due to isothiocyanates.

Drypetes is the only genus outside the order Capparales known to possess the glucosinolate biochemical pathway. This pathway, best known in the Brassicaceae, typically involves the action of the enzyme myrosinase to convert glucosinolates to isothiocyanates and other breakdown products. These sulfur-containing end-products (the so called "mustard oil bomb") are traditionally assumed to play a role in plant defense against herbivores (Bones and Rossiter, 1996). Many conventional floral volatiles (e.g., terpenes) are derived from biochemical pathways that produce antiherbivore compounds (Raguso, 2004). However, isothiocyanates have only very rarely been found as constituents of floral scent (Knudsen et al., 2006). We are aware of just two reported cases, both involving Capparales—a bat-pollinated *Cleome* species (Knudsen and Tollsten, 1995) and pseudoflowers of a rust fungus that infects Brassicaceae (Raguso and Roy, 1998).

The aim of this study was to document the pollination biology of *D. natalensis* with respect to its pollinators, floral morphology and nectar rewards, scent chemistry, and levels of fruit production.

MATERIALS AND METHODS

Study species—*Drypetes natalensis* is a common subcanopy tree in dune forests on the east coast of South Africa. It is dioecious and cauliflorous with flowers of both male and female trees situated on the main trunk and thicker branches (Fig. 1A, E). Flowering takes place mainly in the months of September and October.

Study sites—The main study population is located at Cape Vidal in the iSimangaliso (greater St. Lucia) Wetland Park. Here *D. natalensis* is the most

[1] Manuscript received 23 October 2008; revision accepted 11 August 2009.

The authors thank R. Kaiser (Givaudan Natural Scents) for his expert analysis of the solvent scent samples and feedback on the manuscript. R. Wethered and S. Steenhuisen provided assistance in the laboratory. Mazda Wildlife Fund provided field vehicles. The National Research Foundation (South Africa) provided financial support. This study is part of a broader investigation of dune forest ecology, and we thank the iSimangaliso (greater St. Lucia) Wetland Park authority and Ezemvelo KwaZulu-Natal Wildlife for access to Cape Vidal. A. Jürgens provided helpful discussions and comments on the manuscript.

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doi:10.3732/ajb.0800362

Fig. 1. Flowers and insect pollinators of *Drypetes natalensis*. (A) Flowering trunk of a male tree showing cauliflorous habit. (B) Cetoniid beetle *Plaesiorrhinella trivittata* on male inflorescence. (C) Cetoniid beetle *Porphyronota hebreae* feeing from female flowers. (D) African honeybee *Apis mellifera* taking nectar from male flowers. (E) Cetoniid *P. trivittata* feeding on nectar from female flowers. (F) Honeybee feeding on nectar from female flowers. (G) Fruits of *D. natalensis*. Scale bars = 10 mm.

abundant subcanopy tree, occurring at a density of 146 individuals per ha and making up 11% of all tree individuals in the forest (Nzunda et al., 2007). The second study population consisted of a remnant population of just six trees in Umhlanga, approximately 200 km to the South of the Cape Vidal site.

The study was conducted between 1999 and 2007. Surveys of pollinators were conducted during 9–10 October 1999, 23–24 September, 24 October, and 14 November 2001; 9 August and 10–11, 13–15 October 2003; and 29–30 September 2007.

Sex ratios, floral morphology, and nectar properties—Sex ratios in the study population at Cape Vidal were determined from surveys of 34 trees in 1999, 53 trees in 2000, 24 trees in 2001, and 73 trees in 2007. To assess differences in traits of male and female trees, we recorded plant trunk diameter at c. 150 cm above the base (92 trees), the number of flowers per tree (110 trees), number of flowers per inflorescence (25 trees), flower diameters and depth (24 trees), and the volume and concentration of the standing crop of nectar in flowers at 1000 hours (24 trees). We used tape measures or digital calipers, where appropriate for measures of dimensions and microcapillaries and a 0–50% refractometer for the nectar measurements. The significance of differences between sexes in mean trait values was established using *t* tests.

Pollinator observations—Information on the assemblages of flower visitors on male and female trees was recorded by walking transects through the forest in 1999, 2001, and 2007 and noting the number of individuals on a tree belonging to a particular functional group (e.g., large cetoniids, honeybees, vespid wasps). This was feasible because the flowers are mostly situated no more than a few meters above the ground. Putative pollinators were collected on male and female *D. natalensis* trees. We swabbed these insects with fuchsin gel (Beattie, 1971) to remove pollen carried on the external body parts, counted up to 100 pollen grains on the swab, and estimated the purity of the pollen load by dividing the number of *D. natalensis* pollen grains by the total number of pollen grains counted. We also kept two live cetoniid beetles in vials and examined their droppings for the presence of *D. natalensis* pollen, to establish whether they consume pollen.

Floral scent collection and GCMS analyses—Floral scent was collected using dynamic headspace extraction methods. Flowers were enclosed in polyacetate bags, and air was pumped from these bags at a realized flow rate of 50 mL/min through small cartridges containing adsorbent polymer. We obtained a total of nine samples. We initially struggled to enclose the cauliflorous flowers in bags because of their short pedicels and had to use cut stems. Later we overcame this problem by enclosing flowers on intact stems in a bag and attaching the bag to the bark by winding cotton thread around the entire stem. At the Cape Vidal site, four samples (two from inflorescences of cut branches from male and female trees, respectively, and two from inflorescences of intact branches of a male and female tree, respectively) were taken using cartridges filled with 3 mg of PorapakSQ. Sampling times were 4 h. At the Umhlanga site, additional samples were taken from inflorescences on intact branches of three male and two female trees using cartridges filled with 1 mg of tenax and 1 mg of carbotrap activated charcoal. Sampling times for these samples varied from 12 min to 120 min. Control samples were taken from stems that did not have inflorescences.

Samples taken using Porapak cartridges were eluted with c. 30 μL of 9 : 1 hexane : acetone solvent, as described by Kaiser and Tollsten (1995). Gas chromatograph mass spectrometer (GCMS) analyses of the Poropak-based samples were carried out in the laboratory of Roman Kaiser (Givaudan Natural Scents, Dübendorf, Switzerland) using a DB-WAX column (J & W Scientific, Folsom, California, USA) and the instrumentation and temperature programs described in detail by Kaiser and Tollsten (1995). All compounds were identified by comparison of their mass spectra and retention times with authentic reference compounds available in the Givaudan collection.

Samples taken using tenax : carbotrap cartidges were thermally desorbed and analyzed using a Varian CP-3800 GC (Varian, Palo Alto, California, USA) with a 30 m × 0.25 mm internal diameter (film thickness 0.25 μm) Alltech EC-WAX column coupled to a Varian 1200 quadrupole mass spectrometer in electron-impact ionization mode. Cartridges were placed in a Varian 1079 injector equipped with a "Chromatoprobe" thermal desorbtion device. Helium at a flow rate of 1 mL·min^{-1} was used as the carrier gas. The injector was held at 40°C for 2 min with a 20 : 1 split, increased to 200°C at 200°C·min^{-1} in splitless mode for thermal desorbtion, held for 10 min. After a 3-min hold at 40°C, the GC oven was ramped up to 240°C at 10°C·min^{-1} and held there for 12 min. Compounds were identified using Varian Workstation software with the NIST05 mass spectral library and verified, where possible, using retention times of authentic standards and published Kovats indices. Compounds present at similar abundance in the controls were considered to be contaminants and excluded from further analysis. Emission rates for flowers sampled using the tenax : carbotrap cartridges were determined from the peak area of compounds relative to that of known amounts of standards, sampling time and number of flowers sampled. Standards were injected into cartridges and thermally desorbed under identical conditions to the samples. Emission rates for compounds for which standards were not obtainable were estimated using standards with similar functional groups and retention times.

Responses of beetles to floral volatiles—Response of *Porphyronota hebreae* cetoniid beetles to the odor of *D. natalensis* flowers was tested in a Y-maze olfactometer from 13 to 15 October 2003. The run was composed of three sections of clear Perspex pipe, one central tube and two tubes forming the arms of the "Y" with metal box compartments and fans fitted to their ends. The fans drew air into the chamber from both ends simultaneously. During choice trials, freshly collected male flowers of *D. natalensis* were placed in one of the two compartments. In these trials, the fan drew air over the flowers in the filled compartment and provided a scent cue from that arm of the "Y". Cetoniid beetles collected from flowering *D. natalensis* tree in the field were individually released into the mouth of the central tube, and their behavior was timed until they made a choice of direction at the "Y".

To ensure that a beetle's choice was not influenced by other variables besides scent, we first ran 45 trials (three replicates each for 15 cetoniid beetles) testing for random choice. No flowers were present in the compartments, and the Y-maze was positioned to face the direction of the sun. The airflow from the fans was regulated to ensure equal flow down both arms of the maze. In each trial, the beetles were allowed to choose which arm of the maze they would enter. We found that under these conditions there was no preference for a single direction.

In the choice experiment, 100 trials were run using 15 cetoniid beetles. Each beetle was run in up to 10 trials with the flowers in either the right or the left arm of the "Y". A beetle was considered to have made a choice once it had walked at least half way down one of the arms. The positions of the flowers were swapped periodically. The results were analyzed using the mean proportion of choices made by individual beetles as replicates. The significance of the difference between the angular transformed grand mean for these choices, and a proportion of 0.5 was tested using a one-tailed *t* test.

Fruit production—*Drypetes natalensis* fruits were collected from trees in October 2003. Because the ovaries of virtually all flowers become swollen, it was necessary to determine the proportion of flowers that develop fruits with viable seeds. All seeds were removed from the fruits, and 815 seeds were randomly selected for use in a tetrazolium assay (Kearns and Inouye, 1993) to determine the percentage of viable seeds. To ensure that seeds were fully hydrated, we first soaked them in water for 24 h and then cut the seeds in half, bisecting the embryo. The seeds were then placed in Petri dishes with a 0.1% 2,3,5-triphenyl-2H-tetrazolium chloride solution and maintained at 22°C for 24 h. After the staining period, we evaluated the staining pattern and intensity. Seeds showing the development of dark red staining in the embryo were considered to be viable.

RESULTS

Sex ratio, floral morphology, and nectar properties—The male to female ratios for flowering trees, represented as relative counts, were 22 : 12 (1999), 31 : 22 (2000), 23 : 1 (2003), and 39 : 34 (2007). This variation among years is significant (G = 17.8, $P < 0.0001$). The overall ratio of 115 : 69 is significantly male-biased (goodness of fit test, $G = 11.5$, $P = 0.006$).

There were no significant differences in trunk diameter between male and female trees (Table 1). However, male trees had a 10-fold greater number of flowers (Table 1). The mean number of flowers per inflorescence was also significantly greater on male than on female trees (12.1 vs. 4.6, Table 1). Flower diameter, nectary width, and flower depth did not differ significantly between the sexes (Table 1). There were also no significant differences in the mean standing crop (13.8 μL for male flowers and 8.8 μL for female flowers, Table 1), or mean

TABLE 1. Nectar properties and morphological traits of male and female trees of *Drypetes natalensis*

Trait	Males ± SD (*N*)	Females ± SD (*N*)	*t*	*P*
Nectar volume (μL)	13.8 ± 6.2 (11)	8.8 ± 5.7 (12)	1.98	0.06
Nectar sugar concentration (g/100 × *g*)	16.2 ± 6.7 (12)	13.3 ± 11.7 (12)	0.71	0.48
Diameter at breast height (cm)	21.5 ± 16.8 (62)	17.3 ± 13.7 (30)	1.21	0.22
Number of flowers per tree	4856 ± 5396 (75)	440 ± 640 (35)	6.98	<0.001
Number of flowers per inflorescence	12.1 ± 6.4 (16)	4.6 ± 2.7 (9)	3.30	0.003
Flower diameter (mm)	18.3 ± 1.7 (14)	18.1 ± 1.8 (10)	0.33	0.7
Nectary width (mm)	8.5 ± 0.9 (14)	8.9 ± 0.9 (10)	1.2	0.22
Flower depth (mm)	7.8 ± 1.1 (14)	7.7 ± 1.0 (10)	0.13	0.89

concentrations of nectar (16.2% for males and 13.3% for females, Table 1).

Pollinator observations—The main flower visitors observed were African honeybees (*Apis mellifera adansonii*), cetoniid beetles (*Plaesiorrhinella trivittata* and *Porphyronota hebreae*), and vespid wasps (*Belonogaster*) (Fig. 1, Table 2). There were significant differences between tree sexes in the composition of their flower visitors, with cetoniids particularly overrepresented on male trees ($G = 7.67$, $P = 0.02$, Table 1). All the individuals captured carried visible loads of *D. natalensis* pollen (Fig. 1B, C), and 48% of the honeybees, 100% of the cetoniid beetles and 0% of the wasps had more than 100 pollen grains on their body. Overall, 65.4% of the insect flower visitors had more than 100 pollen grains on their body. The pollen loads of captured insects consisted almost entirely of *D. natalensis* pollen (Table 2). Insects captured on female trees carried significant loads of *D. natalensis* pollen (Table 2) indicating that pollen transfer from male trees was taking place. Effective contact between pollen-laden parts of the insects' bodies and stigmas was observed for all groups (cf. Fig. 1F). Honeybees and wasps visited flowers mainly for the purpose of feeding on nectar, but cetoniids fed on both nectar and pollen. The latter was confirmed by high numbers of *D. natalensis* pollen grains in the droppings of two beetles.

Floral scent—We identified c. 90 compounds in the scent of *D. natalensis* flowers (Table 3). Most of the identified compounds were alcohols, esters, and ketones derived from fatty acids. We also found several monoterpenes and aromatic esters and alcohols. The scent contained three isothiocyanates (isopropyl isothiocyanate, isobutyl isothiocyanate, and 2-methylbutyl isothiocyanate) and several related nitrogenous compounds, such as aldoximes.

Volatile emission rates of female flowers (131–249 ng/h) were much lower than those of male flowers (2120–3765 ng/h) (Table 3). We recorded fewer compounds in samples from females than in those from males, but the main compounds were similar for both sexes (Table 3).

Responses of beetles to floral volatiles—We carried out a total of 100 choice trials involving 15 *P. hebreae* beetles. In an analysis in which the mean angular transformed proportions of choices made by each beetle were considered replicates, the grand mean proportion of choices in favor of the *D. natalensis* flower odor in the Y-maze olfactometer was found to significantly exceed 0.5 (back-transformed mean proportion of choices in favor of the scented arm = 0.73, $t = 2.1$, $P = 0.023$, $N = 15$).

Fruit production—We sampled 168 fruits from 12 *D. natalensis* individuals. Using these fruits to assess natural levels of seed viability in *D. natalensis*, we found that 30.95% of fruits had seeds with viable embryos. The removal of stigmas from flowers prior to pollination resulted in 0% viability, indicating that agamospermy does not occur in this species.

DISCUSSION

The evolution of dimorphism in floral cues and rewards of dioecious plants must ultimately be restrained by selection if pollinators show sex-specific foraging (Ashman et al., 2005). In this study, male and female trees were visited by the same pollinator assemblages, albeit at significantly different frequencies, and insects captured on female trees carried large loads of *D. natalensis* pollen, indicating that foraging bouts involve both sexes. Nevertheless, scent emission per male flower is about 10-fold greater than per female flower (Table 3), which, in combination with the greater number of flowers on male trees, probably makes male trees more attractive to pollinators. Indeed, the number of insects observed on males trees exceeded those observed on female trees by a factor of three (Table 2). However, these data do not reveal the actual rates of pollinator visitation. We cannot exclude the possibility that we observed more insects on male trees simply because they have longer residency times on these trees.

Fruit set in *D. natalensis* at c. 31% of flowers is similar to previous estimated means of 26% of flowers for dioecious for-

TABLE 2. Functional groups of insects observed to visit male and female flowers of *Drypetes natalensis* and composition of their pollen loads.

	Insects observed		Insects captured for pollen load analysis		Pollen loads (percentage *Drypetes* pollen) ± SD	
Group	Female flowers	Male flowers	Female flowers	Male flowers	Female flowers	Male flowers
Honeybees	37	94	20	32	84.7 ± 15.8	97.8 ± 5.5
Cetoniids	6	40	1	23	99.0 ± 0.0	95.0 ± 8.2
Vespid wasps	7	8	1	0	100.0 ± 0.0	—
Hemiptera	0	1	0	1	—	88.1 ± 0.0
Longhorn beetles	0	1	0	1	—	93.0 ± 0.0
Small beetles	0	1	0	1	—	83.3 ± 0.0
Coccinellids	1	0	1	0	100.0 ± 0.0	—

TABLE 3. Floral scent composition for 10 plants of *Drypetes natalensis*. Compounds are arranged by relative retention time within each compound class. Values are percentages of total peak area (excluding contaminants), except for those in the last line which are emission rates per flower. TD = thermal desorbtion, SOL = solvent elution, Intact = flowers sampled in-situ, Cut = flowers sampled from cut stems. tr = compounds making up < 0.1% of peak area.

	Male					Female			
	TD			SOL		TD		SOL	
Compounds	Intact	Intact	Intact	Cut	Intact	Intact	Intact	Cut	Intact
Aliphatic acids									
Acetic acid	6.1	1.5	tr	—	—	1.1	2.5	—	—
Caproic acid	0.1	tr	tr	1.1	—	—	0.1	1.0	—
Heptanoic acid	—	—	—	0.2	—	—	—	0.1	—
Caprylic acid	—	—	—	0.4	—	—	—	—	—
Nonanoic acid	tr	—	—	0.6	—	—	0.1	0.3	—
Capric acid	—	—	—	0.6	—	—	—	0.1	—
Aliphatic alcohols									
2-Oxopentan-3-ol	—	—	—	—	2.9	—	—	—	—
1-Hexanol	—	1.5	—	0.9	—	—	—	0.6	0.6
1-Octen-3-ol	—	—	—	—		—	—	0.3	—
Heptan-1-ol	—	—	—	—	—	—	—	0.1	—
Butan-2,3-diol	—	—	—	1.5	3.3	—	—	—	—
Nonan-1-ol	—	—	—	0.4	—	—	—	0.1	—
Aliphatic aldehydes									
Octanal	—	—	—	0.2	—	—	—	—	—
Nonanal	3.7	1.8	0.2	—	1.5	—	9.6	0.9	1.0
Decanal	0.5	0.3		0.6	1.3	0.2	1.8	—	1.0
Aliphatic esters									
Ethyl acetate	7.7	14.2	—	—	—	94.9	52.7	—	—
Ethyl isobutyrate	—	—	—	—	3.3	—	—	—	—
Propyl acetate	—	0.7	—	—	8.2	—	4.3	—	—
Ethyl butyrate	—	—	—	—	1.8	—	—	—	—
Ethyl 2-methylbutyrate	—	—	—	—	6.2	—	—	0.3	—
Methyl-2-ethylcaproate	—	—	—	0.6	—	—	—	0.1	0.3
Ethyl isovalerate	—	—	—	—	1.5	—	—	—	—
2-Methylbutyl acetate	—	—	—	—	1.5	—	—	—	—
Ethyl caproate	—	—	—	—	0.7	—	—	—	—
Ethyl tiglate	—	—	—	—	0.7	—	—	—	—
(*E*)-2-Hexen-1-yl acetate	—	0.6	—	—	—	—	—	—	—
(*Z*)-3-Hexen-1-yl acetate	3.8	—	0.6	—	—	—	—	—	—
Aliphatic ketones									
Butane-2,3-dione	8.3	9.6	—	—	—	—	—	—	—
Pentane-2,3-dione	2.2	1.4	5.1	—	..	—	—	—	—
Octan-3-one	—	—	—	3.9	—	—	—	0.1	—
Heptan-2,3-dione	—	1.1	2.5	—	—	—	—	—	—
Acetoin	14.6	15.3	5.9	—	35.9	—	—	10.1	1.5
2-Hydroxypentan-3-one	1.8	1.7	0.3	1.8	9.8	—	—	0.9	—
2-Hydroxy-5-methylhexan-3-one	—	—	—	—	0.3	—	—	—	—
Aromatic compounds									
p-Cymene	—	—	—	—	—	—	—	—	0.2
Benzaldehyde	3.6	2.7	4.8	0.4	0.8	2.3	10.2	—	—
Methyl benzoate	0.3	0.3	—	—	—	—	—	—	—
Phenylacetaldehyde	0.1	0.1	—	—	—	—	0.4	—	—
Ethyl benzoate	22.1	26.3	0.5	—	0.5	—	0.1	1.3	—
1,2-Dimethoxybenzene	7.3	5.9	0.2	4.0	0.7	0.4	12.5	2.5	1.9
Propyl benzoate	0.4	0.7	—	0.2	—	—	—	0.1	—
Methyl salicylate	0.1	0.1	—	0.4	—	—	tr	0.1	—
Isobutyl benzoate	tr	0.1	—	—	—	—	—	—	—
Benzyl alcohol	0.7	0.4	0.1	—	—	—	—	—	0.6
Phenylethyl alcohol	0.5	0.3	tr	0.6	—	—	1.9	0.1	1.8
Methyleugenol	2.2	0.3	tr	0.5	—	0.3	0.9	0.3	0.5
Methylisoeugenol	1.0	3.2	tr	—	—	—	0.1	—	—
Elemicin	0.3	0.8	—	1.8	—	0.1	0.2	0.6	—
Estragole	—	—	—	0.2	—	—	—	—	—
Irregular terpenoids									
(*E*)-4,8-Dimethyl-1,3,7-nonatriene	—	—	—	—	—	—	—	2.0	—
6-Methyl-5-hepten-2-one	0.5	0.7	1.0	2.6	2.9	0.7	1.9	0.5	1.5
Monoterpenes									
Myrcene	tr	—	—		—	—	—		1.0
Limonene	tr	—	—	0.2	0.2		tr	0.1	0.3
(*E*)-Ocimene	1.0	0.6	0.1	—	—	—	—	7.4	1.5
(*E*)-Ocimene epoxide	—	—	—	—	0.2	—	—	—	—

TABLE 3. Continued.

	Male					Female			
	TD		SOL			TD		SOL	
Compounds	Intact	Intact	Intact	Cut	Intact	Intact	Intact	Cut	Intact
trans-Linalool oxide (furanoid)	—	—	—	1.5	0.3	—	—	0.3	0.5
cis-Linalooloxide (furanoid)		—	—		—	—	—	0.1	0.3
Linalool	4.2	1.7	tr	0.5	6.2		0.1	19.1	0.5
trans-Linalool oxide (pyranoid)	0.2	0.2	—	—	—	—	—	—	—
cis-Linalooloxide (pyranoid)	0.5	—	—	0.4	—	—	—	0.1	—
Cryptone		—	—	1.4	—	—	—	—	—
Sesquiterpenes									
α-Copaene	—	—	—		—	—	—	—	1.6
β-Cubebene	—	—	—	—	—	—	—	—	2.3
β-Caryophyllene	0.2	0.5	tr	1.1	—	—	—	—	16.2
Alloaromadendrene	—	—	—	0.6	—	—	—	—	—
Humelene	—	—	—	—	—	—	—	—	1.0
Germacrene D	—	—	—	—	—	—	—	—	3.9
α-Farnesene	0.1	0.1	—	0.2	—	—	—	1.5	6.3
Caryophyllene epoxide	—	—	—	0.4	—	—	—	0.1	—
Nitrogen compounds									
(*E*)-2-Methylbutyraldoxime-*o*-methyl ether		—	25.8	—	—	—	—	—	1.3
(*Z*)-2-Methylbutyraldoxime-*o*-methyl ether		—	44.2	—	—	—	—	—	6.9
2-Methylpropyl aldoxime (*E* + *Z*)	—	0.3	0.9		—	—	—	—	—
Tetramethylpyrazine	1.1	0.9	—	—	—	—	—	—	—
Phenylacetalaldoxime *o*-methyl ether	—	—		0.6	—	—	—	—	0.6
Benzyl nitrile	tr	—		—	—	—	0.1	—	—
2-Methylbutylaldoxime (*E* + *Z*)	—	0.3	0.7	—	—	—	—	—	—
Indole	0.9	0.5	—	—	—	tr	0.2	—	—
Sulfur-containing compounds									
Isopropylisothiocyanate	0.3	1.7	5.6	6.6	4.7	—	—	29.3	24.2
Isobutylisothiocyanate		0.2		1.8	0.7	—	—	1.8	1.5
2-Methylbutylisothiocyanate	3.0	1.1	1.4	61.4	4.1	—	0.1	17.8	19.4
Unknown compounds	—	—	—	—	—	—	1.0	—	—
Emission rate (ng·flower^{-1}·h^{-1})	2120	3765	2345			249	131		

est trees (Bawa and Opler, 1975), but lower than the 74% of flowers recorded for dioecious plants in general (Sutherland and Delph, 1984). The lack of seed set in flowers that had stigmas removed in the bud stage indicates that fruit set is not due to classical agamospermy (although agamospermy induced by pollination cannot be excluded by this experiment). However, since agamospermy would essentially clone females, the male-biased sex ratio in this population indicates that seeds are produced through sexual reproduction. We suspect that the actual sex ratio in the population is close to 50 : 50, but apparently male-biased because some female trees were nonreproductive in dry years. An alternative possibility, that female trees mature more slowly (Armstrong and Irvine, 1989), was not supported because mean stem diameters did not differ between flowering individuals of the sexes (Table 1)

As observed for other dioecious species (Tollsten and Knudsen, 1992; Dufaÿ et al., 2004; Fussel et al., 2007), the scents of male and female flowers of *D. natalensis* are generally similar in terms of chemical composition. The number of compounds recorded for female flowers (55) was lower than in male flowers (77), but this could be due to the lower rates of emission from female flowers, which makes it less likely for trace compounds to be detected by GCMS methods. There was some variation among the samples from different trees of the same sex (Table 3). This probably reflects differences in flower ages as well as differences between the two methods of analysis (thermally desorbed samples on tenax : carbotrap vs. solvent eluted samples on Poropak). Nectar microbial fermentation may account for the very high percentages of ethyl acetate, acetoin, and acetic acid recorded in some samples. Nevertheless, many compounds were consistently retrieved in relatively large proportions from virtually all samples. These include two ketones (2-hydroxy-3-pentanone and acetoin), a benzenoid compound (1,2-dimethoxybenzene), an aromatic alcohol (methyleugenol) and two isothiocyanates (2-methylbutyl isothiocyanate and isopropyl isothiocyanate) (Table 3).

The chemical composition of the floral scent of both sexes of *D. natalensis* is highly unusual on account of the presence of isothiocyanates. These compounds arise from the glucosinolate pathway that is known to occur in the Putranjivaceae, but were nevertheless an unexpected component of floral scent. A previous report of this class of compound in a bat-pollinated species (Knudsen and Tollsten, 1995) led us to consider whether *D. natalensis* might additionally be visited by bats. This possibility cannot be firmly excluded, but we consider it unlikely for two reasons: first, we never observed visits by bats, despite camping directly beneath the canopy of several flowering *D. natalensis* trees over several years, and, second, because the flowers of this species are too small to accommodate the snouts of fruit bats, the only local bats known to visit flowers in Africa.

In Brassicaceae, emission of isothiocyanates is known to increase when plants are damaged (Tollsten and Bergstrom, 1988), which may also have been the case during our initial sampling of *Drypetes* from cut branches, even though the flowers were not damaged directly. The two scent samples taken from flowers on cut stems had the highest proportion of isothiocyanates. However, subsequent samples taken from *D. natalensis* flowers on intact stems also contained significant

proportions of isothiocyanates, suggesting that floral emission of isothiocyanates is constitutive and not simply an induced response to stem damage. We did consider the possibility that pollinators, particularly beetles, damage the flowers, but even freshly opened flowers that had not yet been visited by insects produced the characteristic odor of isothiocyanates. This suggests that *Drypetes* flowers, unlike leaves of Brassicaceae, have the capacity to predetonate the "mustard bomb" without the need for physical rupturing of cells by animals.

It has been established that some insects are capable of detecting isothiocynates by antennal olfaction. Studies have demonstrated electrophysiological activity in the antennae of some insects that are specialist herbivores on Brassicaceae when exposed to isothiocyanates (Barker et al., 2006; Renwick et al., 2006). Allyl isothiocyanate emitted from leaves is attractive to a pollen-feeding sap beetle and seed-feeding weevil, both of which feed mainly on Brassicaceae (Free and Williams, 1978). However, the functional role, if any, played by isothiocyanates emitted from *D. natalensis* flowers is still unclear. The results of our olfactometer tests indicate that cetoniid beetles use scent as a cue for locating *D. natalensis* flowers. However, this does not mean that the beetles were responding to isothiocyanates per se. Many of the aliphatic and aromatic esters, alcohols, and aldehydes in the floral scent of *D. natalensis* are potentially attractive to cetoniid beetles (Donaldson et al., 1990; Leal et al., 1994; Johnson et al., 2007). An alternative possibility is that isothiocyanates function to defend the flowers against insect herbivory. Cetoniid beetles, in particular, have strong biting mouthparts (this may account for the damage to the nectar-discs visible in Fig. 1E, F), and it is not implausible that isothiocyanates play a role in defending flowers against excessive damage by these or other insects. Several recent studies have shown that secondary compounds in flowers can function to repel or modify the behavior of florivores, such as pollen thieves and nectar robbers (Johnson et al., 2006; Kessler et al., 2008).

This study is the first step toward understanding the reproductive and chemical ecology of *D. natalensis*. We have identified the major pollinators of this species, shown that beetle pollinators are attracted to the overall scent and characterized its chemical composition. The apparent constitutive emission of isothiocyanates from *D. natalensis* flowers is particularly intriguing and its possible function will be investigated further using electrophysiological and behavioral tests with both pollinators and florivores.

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