

## Cockroaches as Pollinators of *Clusia* aff. *sellowiana* (Clusiaceae) on Inselbergs in French Guiana

BLANKA VLASÁKOVÁ<sup>1,2,\*</sup>, BLANKA KALINOVÁ<sup>3</sup>, MATS H. G. GUSTAFSSON<sup>4</sup>  
and HOLGER TEICHERT<sup>5</sup>

<sup>1</sup>Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-12801 Prague 2, Czech Republic, <sup>2</sup>The Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Květnové náměstí 391, CZ-25243 Průhonice, Czech Republic, <sup>3</sup>Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-16610 Prague 6, Czech Republic, <sup>4</sup>Herbarium, Systematic Botany, Department of Biological Sciences, University of Aarhus, Ole Worms Allé, Building 1137, 8000 Århus C, Denmark and <sup>5</sup>Institute of Systematic Botany and Ecology, University of Ulm, Albert-Einstein-Allee 11, D-89081 Ulm, Germany

Received: 14 February 2008 Returned for revision: 2 April 2008 Accepted: 16 May 2008 Published electronically: 20 June 2008

- **Background and Aims** A report is made on a new species of *Clusia* related to *C. sellowiana* that dominates the vegetation of the Nouragues inselberg in French Guiana. The focus is on the pollination biology and on the remarkable relationship of this plant species to *Amazonina platystylata*, its cockroach pollinator. This appears to be only the second record of pollination by cockroaches.
- **Methods** Pollination ecology was investigated by combining morphological studies, field observations and additional experiments. Floral scent was analysed by gas chromatography–mass spectrometry. The role of acetoin, the major component of the scent of this species of *Clusia*, in attracting pollinators was examined in field attraction experiments. The ability of cockroaches to perceive acetoin was investigated by electroantennography (EAG).
- **Key Results** The *Clusia* species studied produces seeds only sexually. Its nocturnal flowers are visited by crickets, ants, moths and cockroaches. A species of cockroach, *Amazonina platystylata*, is the principal pollinator. The reward for the visit is a liquid secretion produced by tissues at the floral apex and at the base of the ovary. Although the cockroaches have no structures specialized for pollen collection, their body surface is rough enough to retain pollen grains. The cockroaches show significant EAG reactions to floral volatiles and acetoin, suggesting that the floral scent is a factor involved in attracting the cockroaches to the flowers.
- **Conclusions** The results suggest that the plant–cockroach interaction may be quite specialized and the plant has probably evolved a specific strategy to attract and reward its cockroach pollinators. Acetoin is a substance involved in the chemical communication of several other cockroach species and it seems plausible that the plant exploits the sensitivity of cockroaches to this compound to attract them to the flowers as part of the pollination syndrome of this species.

**Key words:** *Clusia*, cockroaches, acetoin, pollination, floral scent, floral reward, plant–animal interaction, inselberg, French Guiana, *Amazonina platystylata*.

### INTRODUCTION

Diversification of angiosperms is associated with pollination by animals, in particular insects (Eriksson and Bremer, 1992). Co-evolution can promote specialized foraging behaviour, consequently leading to changes in both floral and insect traits. There are four orders of insect pollinators whose association with plants is often evident in the floral traits: Coleoptera, Diptera, Hymenoptera and Lepidoptera (Faegri and van der Pijl, 1979). Pollination by species of other insect groups is poorly known, mostly because the flowers and insects involved are small and inconspicuous but very likely also because of preconceived notions about the mechanisms of ‘proper pollination’. Members of these other insect orders probably serve as pollinators far more often than has been inferred. This study presents a unique pollination system from tropical South America where a possibly new species of shrub, *Clusia* aff. *sellowiana* (Clusiaceae), is pollinated by cockroaches (Blattodea). Cockroaches are an ancient group of insects comprising several thousand

described species (Mackerras, 1970; Grandcolas, 1996; Beccaloni and Eades, 2007). Plant material, including floral resources, form part of the diet of many cockroach species (Perry, 1978; Schal *et al.*, 1984). Therefore it would seem that cockroaches have the potential to act as pollinators, but so far cockroach pollination has been described only once: in *Uvaria elmeri* (Annonaceae) in Malaysia (Nagamitsu and Inoue, 1997).

*Clusia* (Clusiaceae) is a Neotropical genus of trees, shrubs and hemi-epiphytes comprising close to 300 described species, the vast majority of which are dioecious. The genus is remarkable for its diverse floral morphology, occurrence of crassulacean acid metabolism, and unusual adaptations in terms of plant–pollinator relationships (Maguire, 1979; Bittrich and Amaral, 1997; Gustafsson, 2000; de Mattos and Lüttge, 2001; Gustafsson and Bittrich, 2002; Scarano, 2002; Lüttge, 2007). Most previous studies of the pollination biology of *Clusia* have concerned species that produce floral resin as a pollinator reward. This is an otherwise very rare adaptation to attract pollinators. The resin serves as a floral reward for certain groups of bees that use

\* For correspondence. E-mail vlasakb@gmail.com

it for nest construction. In addition, pollen and nectar have been described as floral resources in other species of *Clusia* (Martins *et al.*, 2007).

The species of *Clusia* under study is known only from inselbergs of French Guiana. These monolithic rock outcrops represent a unique type of dry habitat with a harsh microclimate and plant species' composition quite distinct from that of the surrounding vegetation. The plant communities of the inselbergs are rich in specialized life forms and include many endemic species (Porembski and Barthlott, 2000). The isolation of the plant communities provides an opportunity to study species reproduction and survival in naturally fragmented ecosystems (Franceschinelli *et al.*, 2006).

We explore the breeding system and flowering phenology of *Clusia* aff. *sellowiana* and examine the relationship with its pollinator. This relationship bears several notable characteristics: (1) the plant species under study is a member of a family that is known for specialized relationships with its pollinators; (2) the pollinators belong to ancient insect group for which a role in pollination has been noted only once before; and (3) the plant–insect relationship is described from an isolated environment of a tropical inselberg. This study presents data suggesting that *Clusia* aff. *sellowiana* uses a unique strategy to attract cockroach pollinators, and the degree of specialization in this plant–cockroach relationship is discussed.

## MATERIAL AND METHODS

### Study site

The study was undertaken on the Nouragues inselberg, a massive granitic outcrop in the Nouragues Natural Reserve, French Guiana (4°5' N, 52°41' W). The area lies in the tropical wet climatic region (2990 mm mean annual rainfall, 26.3 °C mean annual temperature). Rainfall reaches its peak in May (407 mm) and a dry period occurs between August and November (minimum monthly rainfall 88 mm; Grimaldi and Riéra, 2001). The inselberg rises 410 m above sea level and its microclimate is characterized by wide daily variation in temperature (18–55 °C) and humidity (20–100 %; Sarthou, 1992). The surface mostly consists of exposed rock, with shallow soil confined to rock depressions (Barthlott and Porembski, 2000; Porembski, 2005). Major floristic research has been carried out in the area during the past 30 years (Granville and Sastre, 1974; Sarthou and Villiers, 1998; Sarthou, 2001; Sarthou *et al.*, 2003).

### Species description

*Clusia* aff. *sellowiana* (Clusiaceae, sect. *Oedematopus*) is a dioecious shrub, usually 2–10 m tall. Leaves are obovate to elliptic, 8.5–14.0 × 4.0–7.5 cm, with attenuate base and petioles 1.0–1.2 cm long. Latex is white, oxidizing yellow. On the Nouragues inselberg, this species was originally misidentified as *Clusia minor* L. (sect. *Retinostemon*), a distantly related, apomictic species. Recent phylogenetic analysis based on internal transcribed spacer (ITS) sequences (M. H. G. Gustafsson, unpubl. res.) demonstrates

that it is most closely related to *C. sellowiana* Schtdl. (sect. *Oedematopus*), but apparently represents a species new to science. Many other herbarium collections of *C. minor* from French Guiana may in fact belong to this new species. *Clusia* aff. *sellowiana* dominates the shrub vegetation of the inselberg, which mainly occurs in soil-filled depressions. Locally, the plants expand vegetatively by producing adventive roots from branches.

### General description, floral morphology and scent analysis

To provide the material needed for the morphological descriptions, flowers were collected early in the morning and fixed in FAA (70 % ethanol, glacial acetic acid and 37 % formaldehyde in the proportions 7 : 1 : 2). For light microscopy examination, buds and open flowers were dehydrated in an ethanol–tertiary butyl alcohol (TBA) series to pure TBA, and via a 1:1 TBA–paraffin oil mixture to pure paraffin oil. The material was transferred to a 1 : 1 paraffin oil–paraffin mix at 60 °C, and finally to pure melted paraffin. Microtome sections were stained with safranin (in 50 % ethanol) and counter-stained with Astra Blue (in aqueous solution).

To localize scent secretory tissues, a 0.01 % solution of Neutral Red was used (Vogel, 1963; Effmert *et al.*, 2005). Fresh pistillate and staminate flowers were immersed in the solution and left for 20 min. The flowers were then inspected under a stereomicroscope and photographed.

To determine floral scent composition, floral volatiles were trapped by the dynamic headspace method (Raguso and Pellmyr, 1998). Flowers were first enclosed in a headspace chamber made from baking foil lightly wrapped around them. One hour later, a battery-operated pump was turned on, drawing the air enriched by flower volatiles from the headspace chamber through a filter made from a 0.5-mm glass tube containing an adsorbent consisting of a mixture of Tenax TA 60/80 (25 mg) and Carbopack B 60/80a (40 mg). The air was drawn through the tube for 2 h. The volatiles adsorbed on the filter were recovered in a two-step elution using 0.3 and 0.2 mL of high-grade acetone, respectively. The samples were analysed by gas chromatography–mass spectrometry (GC-MS) by Dr Roman Kaiser (Givaudan Schweiz AG, Switzerland) on a Thermo Finnigen Voyager Mass Spectrometer combined with a Trace GC 2000 Series and the Xcalibur software (Thermo Electron Corporation). A DB-WAX column 30 m × 0.32 mm i.d., film thickness 0.25 µm (J&W Scientific) and splitless injection mode was used. The temperature regime was 50 °C – 2' iso – 2.5 °C min<sup>-1</sup> – 230 °C – 40' iso. The compounds were identified by comparison of their mass spectra and retention times with those of authentic reference samples generated from reference compounds.

Specimens were deposited in the herbarium of the Natural History Museum, Czech Republic (PR 720199).

### Flowering phenology of the population and events in a single flower

The flowering phenology was studied from late-September to November in 2005 and late-July to mid-September in

2006. In total, 81 plants were included in the study (41 male and 40 female). Plants were checked every 2 weeks for the presence of remnants of flowers and inflorescences (peduncles and scars from fallen inflorescences). The phenological phase was expressed as the cumulative percentage of wilted flowers. Because inflorescences of a single individual are formed at approximately the same time, this method gives an accurate estimate of a plant's phenological phase.

In 19 inflorescences, individual flowers were examined daily for a period of 50 d in order to follow the development of the inflorescence and individual buds. The course of anthesis was observed during numerous day and night observations.

### Reproduction

The possibility of asexual reproduction by apomixis (agamosperry) was examined by covering 16 inflorescences on four pistillate plants by nylon bags (four inflorescences on each plant). Prior to bagging, the buds were counted. At the end of the flowering season the inflorescences were examined for the presence of fruits.

Another 22 female inflorescences (120 buds) were inspected weekly in order to determine the fruit set, i.e. how many buds actually produced fruits. To estimate the number of seeds, 24 fruits were dissected. Mean seed set was calculated by relating the average number of seeds per fruit to the average number of ovules per flower.

Pollination by wind was investigated using pollen traps (Dafni, 1992). These consisted of a microscope slide covered by a thin layer ( $\pm 1$  mm) of gelatin–fuchsin. Each slide was placed in a bag made of fine mesh ( $1 \times 1$  mm), which prevented insect contact. The traps were then attached in a horizontal position among branches of flowering individuals in the vicinity of an inflorescence or infructescence. In total, 30 traps were exposed in five plants for 43 h between 1700 h of the first day to 1200 h of the day after the next day, corresponding to the length of anthesis of an individual flower. The slides were examined for pollen grains under a light microscope. The number of pollen grains was related to the area based on the size of the gelatine–fuchsin layer.

### Flower visitors

A preliminary screening of insect visitors and their behaviour was carried out during numerous days and nights from September to November 2005. Flower visitors were collected for later identification. Because no day visitors were observed in this screening, subsequent quantified observations were made only at night, specifically during eight nights in August and September 2006. The total observation time was 42 h; 10.5 h for staminate and 31.5 h for pistillate flowers. The flowers were inspected every 15 min using a red light to avoid disturbing the insects. Visitors were noted when they were in contact or within 5 cm of the reproductive parts of a flower. The number of inspections where a visitor was encountered was estimated. Visitation rate differences between sexes were compared using a Chi-square test on the contingency

table. The analyses were run in S-PLUS ver. 6.2 (Insightful Corp., Seattle, WA, USA).

In September 2006, 11 cockroaches were captured after they had contacted staminate flowers and these were used to determine pollen load (Dafni, 1992). The animals were immobilized with ethyl acetate and washed with few drops of 96 % ethanol. The drops were spread on a small area of a Petri dish covered with a thin layer of gelatin–fuchsin and the ethanol was allowed to evaporate. In the laboratory, the layer of gelatin–fuchsin was excised, melted on a microscope slide and examined under a light microscope for the presence of pollen grains.

### Role of acetoin in attraction of insect visitors to flowers: field experiments

The GC-MS analysis of the scent sample trapped in 2005 showed that 50 % of the scent is composed of acetoin (see Table 1). In 2006, two field experiments were run to examine the potential role of this compound in insect attraction. In the first experiment, a paper model of a flower soaked with pure acetoin (Fluka Analytical/Sigma-Aldrich) was hung in a *Clusia* plant 2 m away from the nearest flower. In this way, we excluded the possibility that cockroaches were attracted to the real flowers and

TABLE 1. Chemical composition (in relative amounts) of the floral scent of *Clusia aff. sellowiana*

| Constituent                                   | Relative amount (%) |
|---|---------------------|
| Benzenoids                                    |                     |
| Benzaldehyde                                  | 0.10                |
| Benzoic acid                                  | 0.09                |
| Phenol  | 0.07                |
| Fatty acids derivatives                       |                     |
| 2-Methylbutyric acid                          | 0.06                |
| 2,3-Butandiol                                 | 0.10                |
| 3-Octanone                                    | 0.10                |
| 5(Z)-Dodecenal                                | 19.00               |
| Acetic acid                                   | 1.00                |
| Acetoin                                       | 50.00               |
| Butyric acid                                  | 0.80                |
| Caproic acid                                  | 0.20                |
| Caprylic acid                                 | 0.40                |
| Decanal                                       | 1.00                |
| Dodecanal                                     | 0.10                |
| Heptan-3-one                                  | 0.20                |
| Heptanal                                      | 0.20                |
| Heptanoic acid                                | 0.20                |
| Hexanol                                       | 0.05                |
| Nonanal                                       | 0.60                |
| Nonanoic acid                                 | 0.50                |
| Octanal                                       | 0.40                |
| Octanol                                       | 0.10                |
| Propionic acid                                | 0.40                |
| Undecanal                                     | 0.10                |
| Isoprenoids                                   |                     |
| 6-Methyl-5-hepten-2-one                       | 1.00                |
| $\alpha$ -Pinene                              | 0.07                |
| (E)-9(10)-Epoxy-6,10-dimethyl-5-undecen-2-one | 0.05                |
| (E)-Geranylacetone                            | 2.00                |
| Limonene                                      | 0.10                |
| Menthol                                       | 0.20                |

contacted the model by chance. The model was inspected every 15 min for the presence of any animal on it or in its vicinity. The experiment was performed over three evenings for 10.5 h in total. In the second experiment, a vial containing 1 mL of acetoin was exposed in the vicinity of a male flowering plant. The vial was illuminated by red light. A digital camera was set to take pictures every 15 s without flash. This experiment lasted for 2 h from 1930 h to 2130 h. The differences between visitation rates of the paper model, vial and real flowers of both sexes were evaluated using a Chi-square test on the contingency table using the S-PLUS software.

#### *Electroantennographic (EAG) recordings*

In order to test whether headspace volatiles and acetoin are perceived by cockroaches, electroantennographic recordings (EAG) were performed on adults. A series of solutions containing 1 ng to 10 µg of acetoin was prepared in hexane. From each respective solution, 1 µL was loaded on a filter paper disc placed in a Pasteur pipette. After solvent evaporation, the Pasteur pipette was sealed using Parafilm and stored in a freezer until use. In addition, 10 µL of headspace samples obtained from flowers were applied into Pasteur odour cartridges and treated similarly as described for acetoin odour cartridges. Prior to each experiment, the Pasteur pipettes were taken from the freezer and allowed to equilibrate with the laboratory temperature. Isolated cockroach antennae, the tips of which were cut off, were used for EAG recordings. The cut tip was connected to a glass Ag/AgCl microelectrode filled with Ringer solution. The reference electrode was slipped over the base of the antenna. The electrical signal from the antenna was amplified using a high-impedance amplifier ( $10^{14}$  ohms, Syntech). Signals were then fed into a computer. Antennae connected to the electrodes were placed in a stream of clean air delivered to the antenna through a glass tube (8 mm inner diameter). Stimuli (1 mL of air injected within 1 s into the stream through the Pasteur pipettes loaded with the odour sample) were delivered via a small aperture (3 mm in diameter) located 3 cm from the glass tube outlet. Hexane was used as a control stimulus. An active stimulus changes the electrical potential between the base and tip of the antenna. The amplitude of the EAG potential reflects the activation of olfactory sensory neurons within the antenna; therefore, the EAG amplitudes were evaluated with respect to different stimuli. The EAG responses were recorded and analysed using the Syntech EAG software. After log-normal transformation the data were tested by analysis of variance using the S-PLUS software.

## RESULTS

#### *Floral morphology*

Inflorescences are dichasial cymes, with 2–16 actinomorphic flowers. Like other species of the section *Oedematopus* the flowers do not produce resin. Pistillate and staminate flowers do not differ in the character of

non-reproductive floral parts. Each flower is subtended by two pairs of bracteoles, which are thick, yellow-green, convex and up to 4 mm in diameter. The calyx is formed by two pairs of sepals resembling the bracteoles in colour and shape. They are free, circular-to-elliptic, convex, 3–4 mm wide and 4–5 mm long. The inner sepals have a scarious margin. Petals are four in number, oblong-to-ovate, 4 mm wide, 7 mm long, decussate, coriaceous and pale yellow.

Staminate flowers are approx. 10 mm in diameter and contain 6–16 stamens dehiscing by two lateral slits. Pollen is tricolpate, approx. 13 µm in diameter. A pistillode is absent. Pistillate flowers are only 8 mm in diameter as the petals are erect, tightly surrounding the gynoecium. The gynoecium consists of 4–6 flap-like stigmas (1 mm in diameter), on short styles (1 mm), on top of the superior ovary. The ovary is square in cross-section, 4- or 6-locular and 4 mm in diameter. A single locule contains  $4.79 \pm 1.50$  (mean  $\pm$  s.d.) ovules. Pistillate flowers always have four, stamen-like staminodes opposite the petals (Figs 1, 2). Fruits are fleshy, green-yellow, globose, septifragal capsules, 1.1–1.7 cm in diameter. Seeds are surrounded by a bright orange aril.

Flowers of both sexes produce a liquid secretion. In staminate flowers, there is a distinct secretory organ at the floral apex (Fig. 1A), while in pistillate flowers the secretion is apparently released from tissue at the base of the ovary. The secretory tissues act as a sponge that releases the secretion when pressed upon. According to a preliminary HPLC analysis (B. Vlasáková, unpubl. res.) the secretion contains no sugars.

The Neutral Red test indicated that volatiles are possibly produced by several flower parts; the dye stained areas on petals, stigmas, stamens and staminodes. The petals exhibited colouring in a diffuse pattern where the majority of staining occurred on their adaxial side in the central-upper part. Abaxial parts were stained only along the edge of the petal.

The scent consists of 30 compounds (Table 1), most of which are fatty-acid derivatives. Acetoin was the main constituent (50%) and gives the scent a buttery note. Isoprenoids are represented by only six compounds. Three of them (limonene,  $\alpha$ -pinene and 6-methyl-5-hepten-2-one) are among the most common floral volatiles, occurring in more than 50% of the families of seed plants (Knudsen *et al.*, 2006).

#### *Flowering phenology of the population and events in a single flower*

The development of a single flower from bud emergence to anthesis takes 2 months. Inflorescences of a single individual are formed simultaneously. Although all the plants of the population do not initiate buds at the same time, most appear during a 1.5-month period from July to mid-August. This variation among individuals is evident in Fig. 3 as a high standard deviation from the mean phenological phase from late-July to early September (time 1 through to time 5 in Fig. 3). On average, only  $3.43 \pm 5.76$  (mean  $\pm$  s.d.) flowers opened per day per plant.

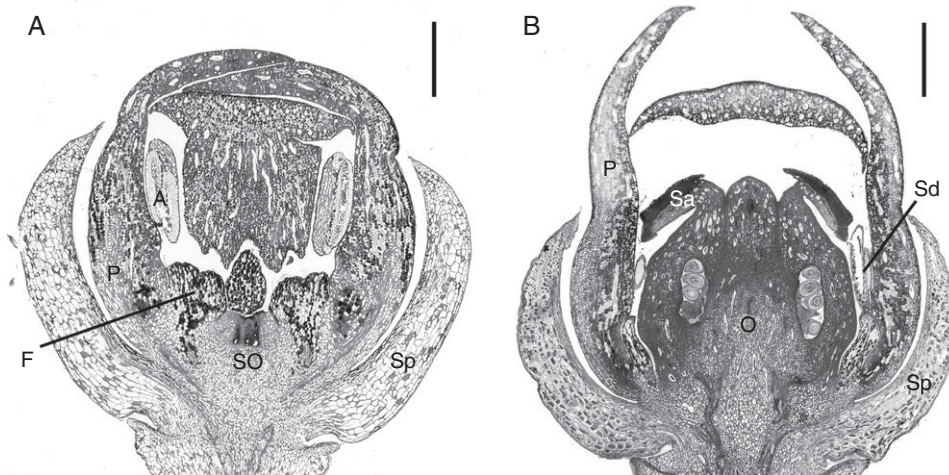


FIG. 1. *Clusia* aff. *sellowiana*. Sections of floral buds. (A) A staminate floral bud, near-median longitudinal section; and (B) a pistillate floral bud, median longitudinal section. Abbreviations: A, anthers; F, staminal filaments; O, ovary; P, petals; Sa, stigma; Sd, staminodes; SO, secretory organ; Sp, sepals. Scale bars = 1 mm.

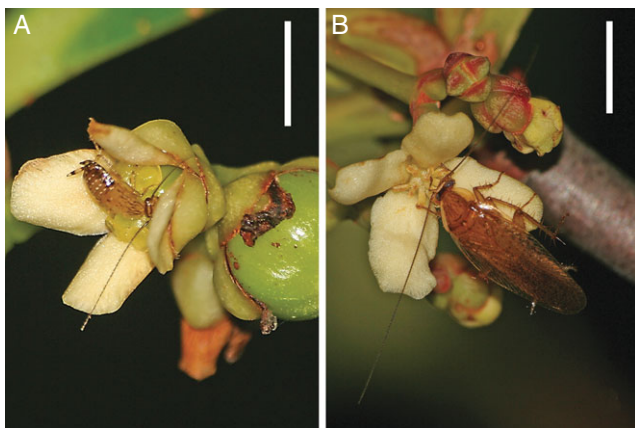


FIG. 2. The cockroach *Amazonina platystylata* visiting flowers of *Clusia* aff. *sellowiana*. (A) A nymph on a pistillate flower; and (B) an adult on a staminate flower. Scale bars = 5 mm.

The proportion of flowering male to female plants during the main flowering season seems to be close to 1. Apart from the main flowering season, flowers also appear sporadically throughout the rest of the year. However, most individuals on the Nouragues inselberg do not flower at all (B. Vlasáková pers. obs.).

Flowers of *C. aff. sellowiana* are nocturnal. The character of anthesis differs between the sexes. Staminate flowers open earlier than pistillate flowers, beginning late in the morning but not reaching full anthesis until 1800 h. Anthers are not visible before 1700 h and do not open until the flower is fully expanded. Pollen is released via lateral slits. The production of scent starts between 1700–1800 h. The flowers remain open throughout the night and wither during the morning of the following day. In contrast, pistillate flowers do not reach full anthesis until 1900 h. They remain open for two consecutive nights and the day in between.

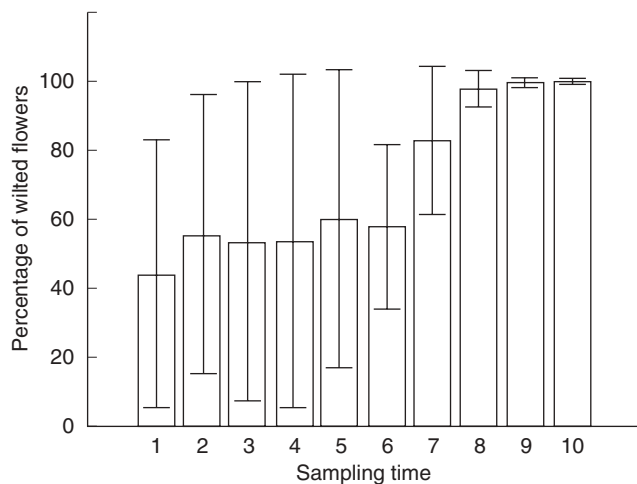


FIG. 3. Flowering phenology of *Clusia* aff. *sellowiana* on an inselberg in French Guiana. Readings were taken every 2 weeks from late-July to early September in 2006 (time 1–5) and from mid-September to early November in 2005 (time 6–10). Columns indicate the mean percentage of wilted flowers,  $\pm$  s.d. Casual observations made at other times of the year indicated that flowers occur sporadically throughout the whole year, but the main flowering season lasts from July to November.

### Reproduction

Seeds are produced sexually and apomixis does not occur in this species. Only three fruits were produced from the 89 buds in the 16 inflorescences covered by nylon bags; all three occurred on one plant. Most probably, these fruits were the result of violation of the bag's integrity. Pollination by wind is highly improbable. Only 12 grains of *C. aff. sellowiana* were found on the 30 pollen traps. This corresponds to a density of 11.24 pollen grains  $m^{-2}$  and suggests that the plant is pollinated by animals. Both fruit set and seed set of open-pollinated flowers were rather low: 51 out of 120 buds (42.5%) produced fruit. Taking into account that a single ovary contains

22.60 ± 5.68 ovules and one capsule contains 19.00 ± 3.66 (means ± s.d.) seeds, we calculate that 35.73 % of the ovules produce seeds.

#### Flower visitors and their behaviour

Both staminate and pistillate flowers were visited by the same array of insect species: cockroaches (Blattodea), crickets (Gryllidae), ants (Formicidae) and small moths (Lepidoptera). All visits occurred during the night. Although pistillate flowers remained open during the day, they were never visited by any animal in this period. Both adults and nymphs of the cockroach *Amazonina platystylata* Hebard (Blattellidae, Pseudophyllodromiinae) were the most frequent visitors to the flowers of both sexes (Fig. 2, Table 2). Their contact with reproductive parts was noted in 20.33 % of all observations. Staminate flowers were contacted more frequently than pistillate flowers at 46.34 % and 14.18 %, respectively ( $\chi^2 = 27.67$ , d.f. = 1,  $P < 0.001$ ).

The first individuals of *A. platystylata* appeared shortly after dusk at around 1930 h and visits occurred throughout the entire night. The cockroaches were apparently rewarded for their visits by the liquid secretion produced by the flowers that accumulated at the base of stamens or staminodes; it appeared that they were feeding on it. During this behaviour the animals came in contact with opened anthers and their head and lower side were dusted with pollen. In pistillate flowers, the secretion was more difficult to access because it was released at the base of the staminodes, which is enclosed by the petals. Cockroaches that tried to reach these parts remained in contact with the stigmas for a considerable time. A single visit lasted 2.09 ± 3.00 min (mean ± s.d.). Maximum observed time at one flower was 12 min. The pollen load estimated per cockroach was 23.40 ± 15.80 grains (mean ± s.d.). However, we noted that our extraction method considerably under-estimated the pollen load. Two of the examined cockroaches were later investigated under a scanning electron microscope. In spite of the prolonged handling (transport to the laboratory and preparation for the SEM), there were still numerous pollen grains attached to the cockroaches' bodies (B. Vlasáková pers. obs.).

Visits of insects belonging to other taxa were so rare that they were not encountered during the established observation periods. Crickets (Gryllidae) were the most

common during preliminary screening; in staminate flowers, these fed on anthers and pollen. After reaching the flower they destroyed a large portion of the androecium. By feeding on anthers their head and their lower side were dusted with pollen. In spite of this, their role as pollinators seems to be very limited because their visits to receptive pistillate flowers were very rare. In female plants as well as in male plants they also fed on petals of freshly wilted flowers. Cockroaches belonging to the species *Epilampra sodalis* (Blaberidae, Epilamprinae) visited only staminate flowers, where they fed on anthers in a similar fashion to that of the crickets; we never observed them in contact with pistillate flowers. Ants (*Pheidole* sp., *Crematogaster* sp. and *Campanotus* sp.) visited both pistillate and staminate flowers, where they seemed to feed on the floral secretion. Thus, they were in contact with both stigma and pollen. However, it is not likely that individual ants cover an area large enough to get in contact with both male and female flowers with a frequency sufficient to affect the reproductive success because the flower density is low in both space and time. Small nocturnal Lepidoptera visited flowers on occasion, but the contact appeared to be solely accidental as they never showed any behaviour that indicated they were acting as pollinators.

#### Role of acetoin in attraction of insect visitors to flowers: field experiments

In the 42 inspections during the experiment when a model flower soaked in acetoin was used, a cockroach was observed only once to be in contact with the model and once in its vicinity. In the second experiment, where a vial with acetoin was used, cockroaches were recorded in 87 (19.25 %) of the 452 inspections. In 50 inspections (11.06 %) they were directly in contact with the vial, where they were mostly observed examining its mouth but they never tried to enter the vial (Table 2). The visitation rate (the number of contacts) differed statistically among staminate flowers, pistillate flowers, the vial and the model ( $\chi^2 = 42.21$ , d.f. = 2,  $P < 0.001$ ).

#### EAG experiments

Headspace volatiles from both male and female *Clusia* flowers elicited higher responses in cockroach antennae than the hexane control. EAG reactions to volatiles that

TABLE 2. Observed number of *Amazonina platystylata* visits to flowers, to paper models soaked with acetoin, and to vials containing acetoin. Percentages are derived from the total number of inspections made during observation of the particular object

| Observed object   | Total no. of records | Contacts with object |      | Appearance in the vicinity |      | Total |      |
|-------------------|----------------------|----------------------|------|----------------------------|------|-------|------|
|                   |                      | No.                  | %    | No.                        | %    | No.   | %    |
| Staminate flower  | 41                   | 19                   | 46.3 | 0                          | 0    | 19    | 46.3 |
| Pistillate flower | 141                  | 20                   | 14.2 | 25                         | 17.7 | 45    | 31.9 |
| Flowers in total  | 182                  | 37                   | 20.3 | 21                         | 11.5 | 64    | 35.2 |
| Paper model       | 42                   | 1                    | 2.4  | 1                          | 2.4  | 2     | 4.8  |
| Vial with acetoin | 452                  | 50                   | 11.1 | 37                         | 8.2  | 87    | 19.3 |

emanated from both flower sexes were almost equipotent (Fig. 4). Both male and female cockroaches responded to flower stimuli (data not shown), suggesting that both sexes of *A. platystylata* might play a role in pollination. EAG experiments with acetoin were performed only on males. Acetoin, the major compound in the headspace volatiles of both staminate and pistillate flowers, was highly effective in eliciting EAG responses in cockroach antennae ( $F = 23.42$ , d.f. = 4,  $P < 0.001$ ). Increased doses of acetoin elicited increased EAG responses (Fig. 5). The threshold for EAG responses, i.e. responses significantly higher than the hexane control, was observed to be at 100 ng. Saturation of the EAG response with increased dose of acetoin (the concentration at which there was no further increase in amplitude) was not observed up to 10  $\mu\text{g}$ . Higher doses of acetoin were not tested in order

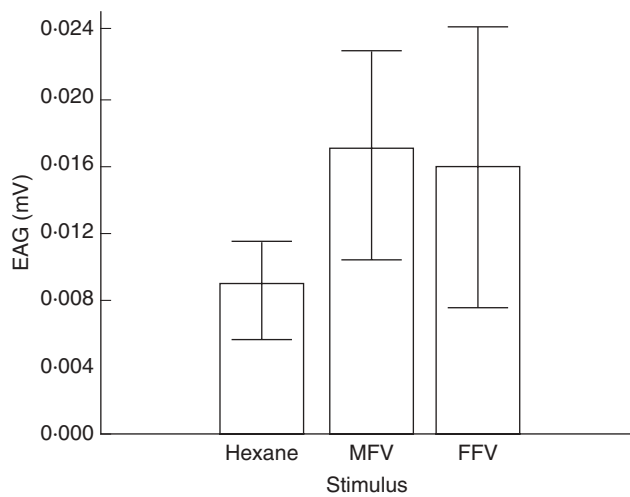


FIG. 4. Electroantennographic responses of male antennae of *Amazonina platystylata* to a hexane control, and to male and female flower volatiles (MFV, FFV, respectively). Means ( $\pm$  s.d.) are from seven recording experiments.

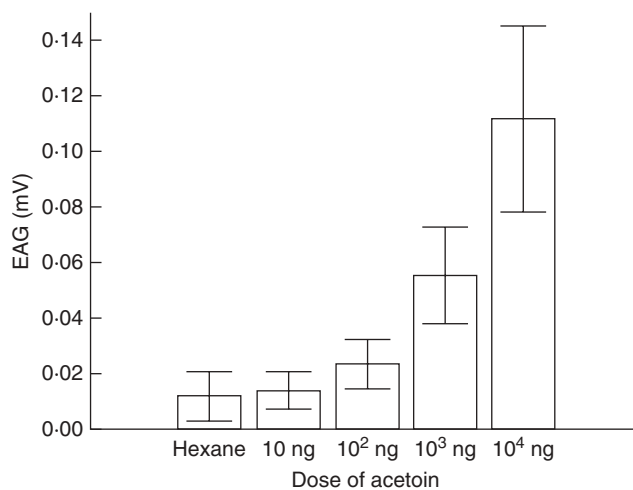


FIG. 5. Electroantennographic responses of male antennae of *Amazonina platystylata* to a hexane control and to different doses of acetoin. Means ( $\pm$  s.d.) are from five experiments.

to avoid contamination of the apparatus. The data are consistent with the hypothesis that *A. platystylata* cockroaches have receptors for *Clusia* flower volatiles and for acetoin on their antennae.

## DISCUSSION

Although apomixis occurs in the genus *Clusia*, it has been recorded in only three species (Maguire, 1976; M. H. G. Gustafsson, unpubl. res.) and we found no indication of such asexual seed production in *C. aff. sellowiana*. Our data demonstrate that pollination is carried out by insects, not by wind, and that the principal pollinator on the Nouragues Inselberg is the cockroach *Amazonina platystylata*. Cockroach pollination has previously been documented only once: Nagamitsu and Inoue (1997) described the case of *Uvaria elmeri* (Annonaceae), a hermaphroditic and self-incompatible plant species with protogynous flowers that is pollinated by cockroaches and drosophilid flies and provides pollen as the floral reward.

Adults and nymphs of *A. platystylata* visit both staminate and pistillate flowers, where they exploit a floral exudate and have prolonged contact with the reproductive floral parts. Although this cockroach species has no specialized structures for transferring the pollen, it was demonstrated that the body surface of this insect retains pollen grains. In general, cockroaches can be very mobile (Schal *et al.*, 1984) and this is also true for *A. platystylata*. Adults fly, while wingless nymphs move by long jumps. We assume that they can easily cover the distances between flowering individuals, which are usually between 2–15 m apart.

Fruit set has been studied for very few species of *Clusia*. For beetle-pollinated *C. criuva*, fruit set was found to be 90 % (Correia *et al.*, 1993) but only 22 % was documented for *C. hilariana* (de Faria *et al.*, 2006); *Clusia aff. sellowiana* falls near the lower end of this range. We infer that the other insect visitors observed visiting the flowers of this species of *Clusia* cannot mediate pollen transfer to the extent that would be needed to explain the fruit production observed. More likely some of them (*Epilampra sodalis* and crickets) have a negative impact on reproduction success because they consume anthers and pollen.

### Floral reward

The nature of the floral secretion is unknown but according to preliminary HPLC analysis it contains no sugars. Indirect evidence based on staining with Neutral Red suggests that the secretion may contain non-volatile oils. Central parts of the flowers, where the secretion is released, were intensively stained. Apart from osmophores, Neutral Red also reveals nectaries and oil bodies (Stern *et al.*, 1986). The occurrence of non-volatile oils as reward is known from several angiosperm families (Simpson and Neff, 1981) including *Clusiaceae* (Nogueira *et al.*, 1998). In the genus *Tovomita*, which is closely related to *Clusia*, droplets of fragrant oil are produced by filaments or staminodes. In general, oil production in flowers has always been associated with bee pollination (Simpson

et al., 1977; Steiner and Whitehead, 1991; Nogueira et al., 1998; Sersic and Cocucci, 1999).

Floral resources are of particular importance in a dioecious animal-pollinated species where plants must ensure pollen transport between males and females. Perhaps this is one of the reasons why dioecy is rare among angiosperms (Renner and Ricklefs, 1995; Heilbut, 2000), and the fact that this breeding system prevails in *Clusia* is another exceptional characteristic of the genus. Knowledge of floral rewards in *Clusia* is strongly biased towards the role of resins (Martins et al., 2007). However, other rare adaptations associated with floral rewards have also been documented, including floral automimicry in species where pollen is offered to pollinators (e.g. *C. criuva*; Correia et al., 1993). As floral morphological diversity is exceptionally high in *Clusia* (Gustafsson and Bittrich, 2002), many unusual plant–pollinator interactions are probably still to be discovered among the species of this genus.

The closest relatives of *C. aff. sellowiana* belong to sections *Oedematopus*, *Criuvopsis* and *Criuva*, which form a monophyletic group in the phylogenetic trees presented by Gustafsson and Bittrich (2002) and Gustafsson et al. (2007). Among the pollinator rewards commonly seen in *Clusia*, resin appears to be completely absent in this group, and nectar, if it occurs at all, is certainly very sparse. Pollen has been found or assumed to be the reward in several species (e.g. *C. criuva* and *C. amazonica*; Correia et al., 1993; M. H. G. Gustafsson, pers. obs). The small, bud-like flowers of most species of sect. *Oedematopus* have no obvious floral reward, and although several species are widespread and common in Neotropical rainforests, the pollination biology is completely unknown. The limited information on pollination biology of this group of plants makes it very difficult to reconstruct the ancestral condition in this respect, which is necessary in order to elucidate how cockroach pollination evolved in *C. aff. sellowiana* or its ancestors.

#### *The role of scent in attracting pollinators*

EAG reactions of *A. platystylata* to floral volatiles demonstrated that flowers of both sexes emit olfactorily active compounds. Both male and female cockroaches responded to flower stimuli, suggesting that both sexes of *A. platystylata* might be attracted by the scent and subsequently carry out pollination.

Information on scent composition in *Clusia* is limited. The chemical profiles of floral scents in different species can be the result of taxonomic affinities, adaptation to pollinators or both. Sixteen species of *Clusia* from different sections were analysed for floral scent composition by Nogueira et al. (2001). Of these, *Clusia criuva* (sect. *Criuvopsis*), which is beetle-pollinated (Correia et al., 1993), is the one most closely related to the species in this study, but the two have no scent constituents in common.

The scent of *C. aff. sellowiana* contains a large proportion of acetoin. This compound does not occur in the scent of any species of *Clusia* analysed to date (Nogueira et al., 2001). Acetoin is involved in sexual chemical signalling of several cockroach species (Sreng, 1993; Nojima et al., 1999). It seems to be a common male pheromonal

substance of many species in subfamily Oxyhaloinae (Blaberidae). In this cockroach group, acetoin is secreted by male abdominal glands and attracts receptive females for mating. However, the gland secretion may have additional functions. In *Nauphoeta cinerea* it also attracts males and larvae, suggesting a dual role as stimulant for receptive females and as feeding stimulant for larvae and males (L. Sreng, CNRS-Université de Provence, France, pers. comm.).

The EAG data are also consistent with the hypothesis that *A. platystylata* cockroaches probably possess specific receptors for acetoin on their antennae. The field experiments with synthetic acetoin do not give unequivocal evidence, but the lower visitation rates might be the result of the experimental conditions. Cockroaches might be attracted by the acetoin but quickly leave the model as they find no reward, and are therefore difficult to observe at the model flower. The relatively low numbers of cockroaches that were observed visiting the model might also be the result of non-optimal concentrations. In many Lepidopteran species, a high concentration of sex pheromones repels rather than attracts the insects (Carde and Elkinton, 1984). Furthermore, acetoin may not be the only compound that attracts cockroaches to the flowers.

There is no published information about chemical signalling in *A. platystylata* and therefore it is not possible to say without further evidence whether the plant does, in fact, release and possibly mimic a compound that is involved in the intraspecific chemical signalling of its pollinator. The floral scent composition could have simply evolved as a reflection of the sensitivity of the pollinator to this chemical signal and not as a pheromone mimic. It is well known that preferences of pollinators are a strong force in the evolution of the floral scents and often result in rapid and independent evolution of similar scents in very distantly related families (Whitten et al., 1986; Teichert et al., 2008).

#### *Specialization in the plant–cockroach relationship*

Plants often specialize in a narrow array of pollinators (Bawa, 1990), but because there are no previous reports on pollinators of *C. aff. sellowiana* from other localities we cannot evaluate the degree of dependence at the species scale. However, on the local scale of the Nouragues inselberg, *C. aff. sellowiana* seems to be dependent on cockroaches for pollination.

The geographic distribution of *C. aff. sellowiana* is certainly much smaller than that of *A. platystylata*, which covers a large part of South America (including Venezuela, Guyana, Suriname, French Guiana, Brazil, Peru and Argentina; Hebard, 1929; Grandcolas, 1994; Beccaloni and Eades, 2007). Therefore it is not probable that the survival of this cockroach species is completely dependent on the *Clusia* under study. However, *A. platystylata* does not feed on pollen, a rich source of energy that is often exploited by insect pollinators (Roulston et al., 2000; Lopes and Machado, 1998). This suggests that the floral secretion may be of particular importance to the cockroaches and the



plant–pollinator relationship may be specialized at least to some degree.

#### ACKNOWLEDGEMENTS

We thank Philippe Grandcolas for identification of the cockroach specimens, Roman Kaiser for scent analysis and Anni Sloth for laboratory assistance. We thank Petr Sklenář, Scott Mori and Bob Lupo for helpful comments on the research and valuable remarks on the manuscript. Thanks also to Pierre Charles-Dominique and our French colleagues at Les Nouragues Station for their hospitality and the permission to work there. This study was supported by the Fund for Neotropical Plant Research of The New York Botanical Garden, the Grant Agency of the Charles University (152/2005), the Czech Science Foundation (206/03/H137), the Grant Agency of the Czech Academy of Sciences (Z40550506) and the Ministry of Education, Youth and Sports of the Czech Republic (MŠMT 6293359101).

#### LITERATURE CITED

- Barthlott W, Porembski S. 2000. Why study inselbergs? In: Porembski S, Barthlott W, eds. *Inselbergs. Biotic diversity of isolated rock outcrops in tropical and temperate regions*. Heidelberg: Springer-Verlag, 1–6.
- Bawa KS. 1990. Plant–pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics* 21: 399–422.
- Beccaloni G, Eades DC. 2007. Blattodea species file online, version 1.2/3-1. <http://Blattodea.SpeciesFile.org> (accessed 13 Feb. 2008).
- Bittrich V, Amaral MCE. 1997. Floral biology and pollination biology of *Clusia* species from the Grand Sabana (Venezuela). *Kew Bulletin* 51: 681–694.
- Carde RT, Elkinton JS. 1984. Field trapping with attractants: methods and interpretation. In: Hummel H, Miller T, eds. *Techniques in pheromone research*. New York: Springer-Verlag, 111–131.
- Correia MCL, Ormond WT, Pinheiro MCB, de Lima HA. 1993. Estudo da biologia floral de *Clusia criuva* Camb. Um caso de mimetismo. *Bradea* 24: 209–219.
- Dafni A. 1992. *Pollination ecology: a practical approach*. New York: IRL Press at Oxford University Press.
- Effmert U, Grosse J, Röse UR, Ehrig F, Kägi R, Piechulla B. 2005. Volatile composition, emission pattern, and localization of floral scent emission in *Mirabilis jalapa* (Nyctaginaceae). *American Journal of Botany* 92: 2–12.
- Eriksson O, Bremer B. 1992. Pollination systems, dispersal modes, life forms, and diversification rates in angiosperm families. *Evolution* 46: 258–266.
- Faegri K, van der Pijl L. 1979. *The principles of pollination ecology*. Oxford: Pergamon Press.
- de Faria APG, Matallana G, Wendt T, Scarano FR. 2006. Low fruit set in the abundant dioecious tree *Clusia hilariana* (Clusiaceae) in a Brazilian restinga. *Flora* 201: 606–611.
- Franceschinelli EV, Jacobi CM, Drummond MG, Resende MFS. 2006. The genetic diversity of two Brazilian *Vellozia* (Velloziaceae) with different patterns of spatial distribution and pollination biology. *Annals of Botany* 97: 585.
- Grandcolas P. 1994. Les blattes de la forêt tropicale de Guyane Française: structure du peuplement (Insecta, Dictyoptera, Blattaria). *Bulletin de la société Zoologique de France* 119: 59–67.
- Grandcolas P. 1996. The phylogeny of cockroach families: a cladistic appraisal of morpho-anatomical data. *Canadian Journal of Zoology* 74: 508–527.
- de Granville JJ, Sastre C. 1974. Aperçu sur la végétation des inselbergs du sud-ouest de la Guyane Française. *C. R. Société de biogéographie* 439: 53–58.
- Grimaldi M, Riéra B. 2001. Geography and climate. In: Bongers F, Charles-Dominique P, Forget PM, Théry M, eds. *Nouragues. Dynamics and plant–animal interactions in a neotropical rainforest*. Dordrecht: Kluwer Academic Publishers, 9–18.
- Gustafsson MHG. 2000. Floral morphology and relationships of *Clusia gundlachii* with a discussion of floral organ identity and diversity in the genus *Clusia*. *International Journal of Plant Sciences* 161: 43–53.
- Gustafsson MHG, Bittrich V. 2002. Evolution of morphological diversity and resin secretion in flowers of *Clusia* (Clusiaceae): insights from its sequence variation. *Nordic Journal of Botany* 22: 312–312.
- Gustafsson MHG, Winter K, Bittrich V. 2007. Diversity, phylogeny and classification of *Clusia*. In: Lüttge U, ed. *Clusia: A woody neotropical genus of remarkable plasticity and diversity*. Berlin: Springer-Verlag, 95–116.
- Hebard M. 1929. Previously unreported tropical American Blattidae (Orthoptera) in the British museum. *Transactions of the American Entomological Society* 55: 345–388.
- Heilbuth JC. 2000. Lower species richness in dioecious clades. *American Naturalist* 156: 221–241.
- Knudsen JT, Eriksson R, Gershenzon J, Ståhl B. 2006. Diversity and distribution of floral scent. *The Botanical Review* 72: 1–120.
- Lopes AV, Machado IC. 1998. Floral biology and reproductive ecology of *Clusia nemorosa* (Clusiaceae) in northeastern Brazil. *Plant Systematics and Evolution* 213: 71–90.
- Lüttge U. (ed.). 2007. *Clusia: a woody neotropical genus of remarkable plasticity and diversity*. Heidelberg: Springer-Verlag.
- Mackerras MJ. 1970. Blattodea. In: Lawrence J, Britton E, eds. *The insects of Australia: a textbook for students and research workers*. Carlton, Victoria: Melbourne University Press, 262–274.
- Maguire B. 1976. Apomixis in genus *Clusia* (Clusiaceae) – preliminary report. *Taxon* 25: 241–244.
- Maguire B. 1979. On the genus *Clusia* (Clusiaceae) in Mexico. *Taxon* 28: 13–18.
- Martins RL, Wendt T, Margis R, Scarano FR. 2007. Reproductive biology. In: Lüttge U., ed. *Clusia: a woody Neotropical genus of remarkable plasticity and diversity*. Berlin: Springer-Verlag, 73–94.
- de Mattos EA, Lüttge U. 2001. Chlorophyll fluorescence and organic acid oscillations during transition from CAM to C<sub>3</sub>-photosynthesis in *Clusia minor* L. (Clusiaceae). *Annals of Botany* 88: 457–463.
- Nagamitsu T, Inoue T. 1997. Cockroach pollination and breeding system of *Uvaria elmeri* (Annonaceae) in a lowland mixed-dipterocarp forest in Sarawak. *American Journal of Botany* 84: 208–213.
- Nogueira PC de L, Marsaioli AJ, Amaral MCE, Bittrich V. 1998. The fragrant floral oils of *Tovomita* species. *Phytochemistry* 49: 1009–1012.
- Nogueira PC de L, Bittrich V, Shepherd GJ, Lopes AV, Marsaioli AJ. 2001. The ecological and taxonomic importance of flower volatiles of *Clusia* species (Guttiferae). *Phytochemistry* 56: 443–452.
- Nojima S, Sakuma M, Nishida R, Kuwahara Y. 1999. A glandular gift in the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae): the courtship feeding of a female on secretions from male tergal glands. *Journal of Insect Behavior* 12: 627–640.
- Perry DR. 1978. *Paratropes bilunata* (Orthoptera: Blattidae): an outcrossing pollinator in a neotropical wet forest canopy. *Proceedings of the Entomological Society of Washington* 80: 657–658.
- Porembski S. 2005. Floristic diversity of African and south American inselbergs: a comparative analysis. *Acta Botanica Gallica* 152: 573–580.
- Porembski S, Barthlott W. 2000. *Inselbergs. Biotic diversity of isolated rock outcrops in tropical and temperate regions*. Ecological Studies 146. Heidelberg: Springer-Verlag.
- Raguso RA, Pellmyr O. 1998. Dynamic headspace analysis of floral volatiles: a comparison of methods. *Oikos* 81: 238–254.
- Renner SS, Ricklefs RE. 1995. Dioecy and its correlates in the flowering plants. *American Journal of Botany* 82: 596–606.
- Roulston TH, Cane JH, Buchmann SL. 2000. What governs protein content of pollen: pollinator preferences, pollen–pistil interactions, or phylogeny? *Ecological Monographs* 70: 617–643.
- Sarthou C. 1992. *Dynamique de la végétation pionnière sur un inselberg en Guyane Française*. PhD Thesis, Université Paris 6, France.
- Sarthou C. 2001. Plant communities on a granitic outcrop. In: Bongers F, Charles-Dominique P, Forget PM, Théry M, eds. *Nouragues. Dynamics and plant–animal interactions in a neotropical rainforest*. Dordrecht: Kluwer Academic Publishers, 65–78.
- Sarthou C, Villiers JF. 1998. Epilithic plant communities on inselbergs in French Guiana. *Journal of Vegetation Science* 9: 847–860.

- Sarthou C, Villiers JF, Ponge JF. 2003.** Shrub vegetation on tropical granitic inselbergs in French Guiana. *Journal of Vegetation Science* **14**: 645–652.
- Scarano FR. 2002.** Structure, function and floristic relationships of plant communities in stressful habitats marginal to the Brazilian Atlantic rainforest. *Annals of Botany* **90**: 517–524.
- Sersic AN, Cocucci AA. 1999.** An unusual kind of nectary in the oil flowers of *Monttea*: its structure and function. *Flora* **194**: 393–404.
- Schal C, Gautier JY, Bell WJ. 1984.** Behavioural ecology of cockroaches. *Biological Review* **59**: 209–254.
- Simpson BB, Neff JL. 1981.** Floral rewards: alternatives to pollen and nectar. *Annals of the Missouri Botanical Garden* **68**: 301–322.
- Simpson BB, Neff JL, Seigler DL. 1977.** *Krameria*, free fatty acids and oil-collecting bees. *Nature* **267**: 150–151.
- Sreng L. 1993.** Cockroach mating behaviors, sex-pheromones, and abdominal glands (Dictyoptera, Blaberidae). *Journal of Insect Behavior* **6**: 715–735.
- Steiner KE, Whitehead VB. 1991.** Oil flowers and oil bees – further evidence for pollinator adaptation. *Evolution* **45**: 1493–1501.
- Stern WL, Curry KJ, Whitten WM. 1986.** Staining fragrance glands in orchid flowers. *Bulletin of the Torrey Botanical Club* **113**: 288–297.
- Teichert H, Dötterl S, Zimma B, Ayasse M, Gottsberger G. 2008.** Perfume-collecting male euglossine bees as pollinators of a basal angiosperm: the case of *Unonopsis stipitata* (Annonaceae). *Plant Biology*. In press.
- Vogel S. 1963.** Duftdrüsen im Dienste der Bestäubung. über Bau und Funktion der Osmophoren. *Abhandlungen der Mathematik-Naturwissenschaftlichen Klasse, Akademie der Wissenschaften, Mainz* **10**: 1–165.
- Whitten WM, Williams NH, Armbruster WS, Battiste MA, Strekowski L, Lindquist N. 1986.** Carvone oxide: an example of convergent evolution in euglossine pollinated plants. *Systematic Botany* **11**: 222–228.