Insect Visitors and Potential Pollinators of *Orchis militaris* (Orchidaceae) in Southern Belgium

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Abstract

As part of a research project on the food deception strategy in *Orchis militaris* (L.), the objective of this study was to identify insect visitors and potential pollinators of this orchid species in Belgium. In 2013, insects were collected over 2 d per site in five localities distributed in Southern Belgium (Wallonia). A total of 104 insects belonging to 49 species were caught. Dipterans were the most abundant visitors (50% of total specimens), followed by Hymenopterans (32%). *Rhingia campestris* Meig., *Bombylius venosus* Mikan, *Apis mellifera* (L.), and *Bombus lapidarius* (L.) were the most abundant species. Only five specimens bore one to more than 10 pol-linia: four honeybees (*A. mellifera*) and one bumblebee worker (*B. lapidarius*). These two species should be considered as potential pollinators in the study area, but probably not confirmed ones.

Key words: *Orchis militaris*, pollinator, Apidae, *Apis mellifera*, *Bombus lapidarius*

Orchidaceae are one of the most diversified Angiosperm families, with more than 27,000 accepted names (*The Plant List 2013*). The majority of orchid species rely on biotic pollen vectors to reproduce (*Nilsson 1992*), so do 88% of flowering plant species (*ollerton et al. 2011*). Usually, plants attract pollinators with floral signals that advertise rewards (*Schiestl and Johnson 2013*). However, some plant species also display attractive floral signals but don’t provide any reward (or low or wrong reward) (*Jersákova et al. 2009*); they are called deceptive species (*Renner 2006*). Deceptive pollination occurs in about 30% of orchid species (*Jersákova et al. 2006, Claessens and Kleyen 2011*), and the commonest systems involve the exploitation of the food-seeking behavior of the pollinators (food deception—*Jersákova et al. 2006*).

Under natural conditions, insect visits on rewardless flowers are rare events difficult to record (*Cozzolino et al. 2005, Claessens and Kleyen 2011*), making direct observation time consuming (*Widmer et al. 2000*). Indirect approaches have been developed to study more easily orchid-pollinator relationships: pollinia carried by insects (caught on rewarding plants growing in the vicinity of studied orchid populations) allow the identification, through molecular techniques, of the corresponding orchid species (*Widmer et al. 2000*); pollinia found on insects can also be identified through specific morphological features (*Singer et al. 2008, Micheneau et al. 2009*); scales and hairs found on pollinated stigmas can also allow the identification of the corresponding insect species (*Micheneau et al. 2009,* Videotape observations are another alternative to direct observations (*Micheneau et al. 2009, Lortie et al. 2012, Nakase and Suetsugu 2016*), but observation-based techniques don’t allow accurate identification of numerous insects (especially dipterans and hymenopterans). The latter have to be collected and examined carefully in order to draw up accurately visitor and potential pollinator lists.

In the genus *Orchis* Tourn. ex L. (Orchidaceae), none of the species produce nectar and the majority depends on food deception to attract pollinators (*scopece et al. 2014*). Visitor and pollinator guilds have been studied more or less extensively according to the species (e.g. *van der Cingel 1995, Berger 2003, 2004; Claessens and Kleyen 2011*). In *O. militaris* (L.), most data were collected in Austria by *Voith* (1987) and in the United Kingdom by *Farrell* (1985). Other contributions come from *Berger* (2003, 2004), *Ebert and Rennwald* (1993), *Petit* (1998), *Voith* (1999), etc. Among (altitudinal) regions, a plant species can be pollinated by different insect species. This spatial variation in pollinator identity is well known in various species, including orchids (e.g. *Schatz et al. 2005, Gomez and Zamora 2006, Gonzalez et al. 2014, Sun et al. 2014, Gross et al. 2016*). The aim of this study was thus to determine the guilds of floral visitors and potential pollinators to *O. militaris* in a region that was not widely studied so far: Southern Belgium (Wallonia).

Materials and Methods

Study Species

*O. militaris* (Fig. 1) is a perennial herb with a Eurasian-Mediterranean distribution area (*Kretzschmar et al. 2007*). The species is confined to calcareous soils and occurs in sunny or partially shaded sites. It ranges from sea level to 2,200 m and it can grow in
According to Alexandersson and Ågren (1996), a population was

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\text{(Claessens and Kleynen 2011). Low levels of fruit set}
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Wilcock 1998, Metsare et al. 2015) and non-autogamous (Delforge

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\text{2012). It blooms from April to June \(-\text{July} (Delforge}
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\text{2012). O. militaris appears as a moderately long stem, generally varying from 20 to 45 cm, with a single inflorescence bearing 10\text{-}40 hermaphrodite flowers (Farrell 1985, Delforge 2012). It blooms from April to June \(-\text{July} (Delforge}
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\text{2012). O. militaris is assumed to be self-compatible (Neiland and}
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\text{Wilcock 1998, Metsare et al. 2015) and non-autogamous (Delforge}
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\text{2012), and it depends on the food deception strategy to attract pollinators (Claessens and Kleynen 2011). Low levels of fruit set}
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\text{(<30\%)} \text{are common (Tremblay et al. 2005, Claessens and Kleynen}
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**Fig. 1. O. militaris visited by A. mellifera (Wijre-akkers, Netherlands, 1 June 2010, photographer: Jean Claessens).**

Study Sites

The study was carried out in 2013 in five Belgian populations (Table 1). According to Alexandersson and Ågren (1996), a population was defined as a group of inflorescences separated from their closest conspecific by at least 100 m. All populations were at least 5 km apart, except those of Visé, which were 800 m apart (but separated by a canal 140 m wide). The biotopes were various: recolonized slag heaps, semi-dry calcareous grassland, mesic grassland, thickets and deciduous forestry plantations.

**Insect Collection**

Field work took place during sunny and warm (at least 18 °C) days with little or no wind, between 8.00 a.m. and 8.00 p.m. between 27 May and 17 June (Table 1). We made around 30 total hours of captures (180 10-min censuses), thus \(\sim 6 \text{ h} (36 \text{ 10-min censuses})\) per population. Each population was studied during 2 consecutive days, and the 6 h were regularly distributed throughout the 2 days. During 10-min censuses, taking a standard route (100 m) across the population, we used a butterfly net to catch all insects that landed on O. militaris inflorescences. We put them in individual pill-boxes containing a piece of cotton wool soaked with ethyl acetate. Pill-boxes were conserved in a cool box (EDA Plastiques, Oyonnax, France) with cold accumulators (Kern Frio S.A., L’Hospitalet de Llobregat, Spain) and then in a freezer at \(- 18 \text{ °C} \). When insects bore pollinia, we used morphological characters in order to distinguish the pollinia of O. militaris from those of other orchids found in the study sites and their surroundings (Anacamptis pyramidalis (L.) Rich., Neotria ooea (L.) Bluff & Fingerh., Ophrys apifera Huds., O. Ophrys insectifera (L.), Orchis anthropophora (L.) All., and Platanthera chlorantha (Custer) Rchh.). Insects were mounted and identified. Most of the specimens are kept at the Biodiversity Research Centre (Université catholique de Louvain); some dipterans are stored at the Royal Belgian Institute of Natural Sciences.

**Results**

We caught a total of 104 insects belonging to 49 species, 21 families and four orders (Table 2). Most of the specimens were dipterans (50\%) and hymenopterans (32\%). Rhingia campestris Meigen (14 individuals) and Bombylus venosus Mikan (10 individuals) were the most abundant dipterans; Apis mellifera (L.) (8 individuals) and Bombus lapidarius (L.) (6 individuals) were the most abundant hymenopterans. Some lepidopterans and coleopterans were also collected. Syrphids were the most diverse family with 12 different species. Pollinia (only from O. militaris) were only found in hymenopterans: four honeybee workers (A. mellifera) bore respectively 13, 12, 3 (attached to the clypeus) and 2 pollinia, and one bumblebee worker (B. lapidarius) bore 1 pollinium. A. mellifera was caught in three sites and B. lapidarius in four of the five sites. Because ethyl acetate fumes rapidly dissolve the viscidium glue (Peter and Johnson 2013), we failed to determine the position of pollinia on four of the five insects. A number of insects were also observed but not caught on O. militaris inflorescences: Clytus arietis (L.) (Coleoptera, Cerambycidae), Harmonia axyridis Pallas (Coleoptera, Coccinellidae), Eristalis sp. (Diptera, Syrphidae), Sarcophaga sp. (Diptera, Sarcophagidae), Pyrausta aurata Scopoli (Lepidoptera, Crambidae), Polyommatus icarus Rottemburg (Lepidoptera, Lycaenidae), Vanessa sp. (Lepidoptera, Nymphalidae), and Anthocharis cardamines (L.) (Lepidoptera, Pieridae).

**Discussion**

In this study, O. militaris attracted a variety of visitors, mainly hoverflies (32 individuals), bumblebees (16 individuals) and bee flies (13 individuals), probably through the display of general floral signals (typical for rewarding plants) such as showy flower colors, presence of a spur, etc. Rewarding plants attract insects and non-rewarding O. militaris individuals flowering nearby benefit from chance visits (Jersáková et al. 2009). All caught dipterans and lepidopterans have to be considered as visitors (sensu Schatz et al. 2005) since they bore no pollinia. To our knowledge, dipterans and butterflies are never cited as pollinators in the literature. The former visit various orchid species for nectar (absent in deceptive species like
Table 1. Characteristics of *O. militaris* populations

| Site no. | Municipality     | Site name                         | Coordinates       | Altitude (m) | Population size | Date of insect collections |
|---------|------------------|-----------------------------------|-------------------|--------------|-----------------|----------------------------|
| A       | Visé             | Friche du canal Albert            | 50° 46'16" N 5° 41'04" E | 55           | ~10,000         | 4–5 June 2013              |
| B       | Visé             | Oseraie de Lanaye                 | 50° 46'45" N 5° 40'59" E | 65           | ~1,500          | 6–7 June 2013              |
| C       | Virton           | Contournement de Virton           | 49° 34'38" N 5° 30'58" E | 250          | ~150            | 16–17 June 2013            |
| D       | Musson           | Crassier de Musson               | 49° 33'09" N 5° 43'04" E | 290          | ~230            | 27–28 May 2013             |
| E       | Rouvroy          | Nature reserve ‘Raymond Mayné’    | 49° 30'34" N 5° 28'53" E | 295          | ~80             | 11–12 June 2013            |

*The population size is given in terms of number of inflorescences.*

Table 2. Identity and number of insects caught on *O. militaris* inflorescences

| Order       | Family       | Taxon                                | A | B | C | D | E |
|-------------|--------------|--------------------------------------|---|---|---|---|---|
| Coleoptera  | Elateridae   | Indet.                               | 1 |   |   |   |   |
|             | Scarabaeida  | *Phyllopertha horticola* (L. 1758)   | 2 | 2 |   |   |   |
|             |              | *Valgus hemipterus* (L. 1758)        | 2 |   |   |   |   |
| Diptera     | Agromyzidae  | Indet.                               | 1 |   |   |   |   |
|             | Asilidae     | *Neotamus cf. socius* (Loew 1871)    | 1 |   |   |   |   |
|             | Bombylidae   | *Bombylius major* (L. 1758)          | 3 |   |   |   |   |
|             |              | *B. venosus* (Mikan 1796)            | 1 | 1 | 6 |   |   |
| Empididae   | *Empis discolor* (Loew 1856)         | 1 |   |   |   |   |
|             | *Empis femorata* (F. 1798)            | 1 |   |   |   |   |
|             | *Empis livida* (F. 1798)              | 1 |   |   |   |   |
|             | *Hybos* sp. |                                      | 1 |   |   |   |   |
| Scathophagidae | *Scathophaga stercoraria* (L. 1758) | 1 |   |   |   |   |
| Syrphidae   | *Chrysotoxum cautum* (Harris 1776)    | 3 | 1 |   |   |   |
|             | *Episyrphus balteatus* (De Geer 1776) | 1 |   |   |   |   |
|             | *E. cf. socius* (Loew 1871)           | 1 |   |   |   |   |
|             | *Episyrphus cf. socius* (Loew 1871)   | 1 |   |   |   |   |
|             | *Helophilus pendulus* (L. 1758)       | 1 |   |   |   |   |
|             | *Merodon equestris* (F. 1794)         | 1 |   |   |   |   |
|             | *Scathophaga stercoraria* (L. 1758)   | 1 |   |   |   |   |
| Hymenoptera | *Andrena carantonica* (Perez 1902)    | 1(♀) |   |   |   |   |
|             | *Andrena flavipes* (Panzer 1799)      | 1(♀) |   |   |   |   |
|             | *Andrena nigronea* (Kirby 1802)       | 1(♀) |   |   |   |   |
| Apidae      | *A. mellifera* (L. 1758)              | 4(♀) | 3(♀) |   |   |   |
|             | *Bombus hypnorum* (L. 1758)           | 1(♀) |   |   |   |   |
|             | *B. lapidarius* (L. 1758)             | 2(♀) | 1(♀) | 2(♀, ♂) |   |   |
|             | *Bombus lucorum* (L. 1761)            | 1(♂) |   |   |   |   |
|             | *B. pascuorum* (Scopoli 1763)         | 1(♀) |   |   |   |   |
|             | *Bombus terrestris* (L. 1758)         | 1(♀) |   |   |   |   |
|             | *Bombus terrestris* (L. 1758)         | 4(♂, ♀) |   |   |   |   |
|             | *Colletes confusa* (Nylander 1852)    | 1(♀) |   |   |   |   |
| Colletidae  | *Lasiothrix fulgicorne* (Kirby 1802)   | 1(♀) |   |   |   |   |
| Halictidae  | *Lasiothrix fulgicorne* (Kirby 1802)   | 1(♀) |   |   |   |   |
|             | *Lasiothrix malachurum* (Kirby 1802)   | 1(♀) |   |   |   |   |
|             | *Lasiothrix pareulum* (Schönk 1853)   | 1(♀) |   |   |   |   |
|             | *Sphecodes sp.*                       | 1(♀) |   |   |   |   |
| Megachilidae| *O. bicolor* (Schrank 1781)           | 1(♀) |   |   |   |   |
| Lepidoptera | *Crambidae* | *Pyrausta sp.*                       | 1 |   |   |   |   |
|             | *Geometridae* | *Siona lineata* (Scopoli 1763)     | 1 |   |   |   |   |
|             | *Hesperiidae* | *Erynnis tages* (L. 1758)         | 1 |   |   |   |   |
|             | *Nymphalidae* | *Colias io* (L. 1758)              | 1 |   |   |   |   |
|             | *Noctuidae* | *Lasiommata megera* (L. 1767)       | 1 |   |   |   |   |
|             | *Pieridae* | *Conepithrix erynnis* (L. 1758)     | 1 |   |   |   |   |

*Formicidae were present in all the populations but weren’t caught. ♀ = female; ♂ = male; ♂ = worker.*
Table 3. Non-exhaustive list of insects observed on *O. militaris* inflorescences in different countries

| Order          | Family       | Taxon                          | Country (ISO code) | References |
|----------------|--------------|--------------------------------|--------------------|------------|
| Coleoptera     | Cantharidae  | *Cantharis lateralis* (L. 1758) | RU                 | 12         |
|                |              | *Cantharis rustica* (Fallen 1807) | GB                 | 9          |
| Cerambycidae   |              | *Pachyta quadrimaculata* (L. 1758) | RU                 | 10         |
|                |              | *Rutpela maculata* (Poda 1761)   | GB                 | 9          |
| Cetoniidae     |              | *T. hirta* (Poda 1761)           | RU, RU, AT         | 10*, 12*, 15* |
| Chrysomelidae  |              | *Cassida viridis* (L. 1758)      | GB                 | 9          |
|                |              | *Smaragdina salicina* (Scopoli 1763) | RU             | 12         |
| Malachiidae    |              | *Malachius bispustulatus* (L. 1758) | RU                 | 12         |
|                |              | *Malachius coccineus* (Waltl 1838) | RU                 | 12         |
| Oedemeridae    |              | *Oedema nobilis* (Scopoli 1763)  | RU                 | 10, 12     |
| Diptera        | Bibionidae   | *Bibio marci* (L. 1758)         | GB                 | 9          |
|                | Bombyliidae  | *B. major* (L. 1758)            | AT                 | 13         |
|                |              | *B. venosus* (Mikan 1796)       | AT                 | 13         |
|                | Calliphoridae| *Onesia* sp.                    | GB                 | 7          |
|                | Empididae    | *Empis tessellata* (F. 1794)    | FR, GB             | 3, 9       |
|                | Muscidae     | *Musca* sp.                     | AT                 | 13         |
|                |              | *Thricops seminicornes* (Wiedemann 1817) | GB         | 7          |
|                | Opomyzidae   | *Opomyza germinationis*         | GB                 | 7          |
|                | Scathophagidae| *S. stercoraria* (L. 1758)   | GB                 | 7          |
| Syrphidae      |              | *Baccha elongata* (F. 1775)     | GB                 | 7          |
|                |              | *C. cautum* (Harris 1776)       | GB                 | 9          |
|                |              | *Chrysotoxum octomaculatum* (Curtis 1837) | AT         | 13         |
|                |              | *E. balteatus* (De Geer 1776)   | GB, AT             | 9, 13      |
|                |              | *Erirstalis pertinax* (Scopoli 1763) | GB             | 9          |
|                |              | *Leucozona lucorum* (L. 1758)   | GB                 | 7          |
|                |              | *Melanostoma mellinum* (L. 1758) | GB                 | 7          |
|                |              | *Melanostoma scalare* (F. 1794) | GB                 | 9          |
|                |              | *Platycerus ambiguus* (Fallen 1817) | AT           | 13         |
|                |              | *P. scutatus* (Meigen 1822)     | GB                 | 7          |
|                |              | *R. campestris* (Meigen 1822)   | GB                 | 7          |
| Hemiptera      | Cercopidae   | *Cercopis vulnerata* (Rossi 1807) | GB                 | 9          |
|                | Scutelleridae| *Eurygaster* spp.               | GB                 | 9          |
| Hymenoptera    | Andrenidae   | *A. cananitica* (Perez 1902)    | AT                 | 13         |
|                |              | *Andrena cineraria* (L. 1758)   | RU                 | 12*        |
|                |              | *Andrena curvungula* (Thomson 1870) | FR          | 8*         |
|                |              | *A. ensinella* (Stoeckhert 1924) | AT                 | 13*, 14*   |
| Apidae         |              | *Anthophora aestivalis* (Panzer 1801) | AT           | 13*        |
|                |              | *Anthophora borealis* (Morawitz 1864) | RU         | 10*, 12*   |
|                |              | *A. mellifera* (L. 1758)         | FR, FR, NL, GB, AT | 2*, 3*, 5*, 9*, 13*, 14* |
|                |              | *B. lapidarius* (L. 1758)       | FR, GB, FR, GB, AT | 3, 7, 8, 9, 13 |
|                |              | *B. lucorum* (L. 1761)          | GB                 | 7, 9       |
|                |              | *B. pascuorum* (Scopoli 1763)   | FR, GB             | 3*, 9      |
|                |              | *B. pratorum* (L. 1761)         | GB, AT             | 1*, 9      |
|                |              | *B. terrestris* (L. 1758)       | GB, AT             | 9*, 13     |
|                |              | *Bombus vestalis* (Geoffroy 1785) | GB          | 1*         |
|                |              | *Ceratina callosa* (F. 1794)    | AT                 | 13         |
|                |              | *Ceratina cyanae* (Kirby 1802)  | AT                 | 13         |
|                |              | *Eucera* sp.                    | FR                 | 3, 4       |
|                |              | *N. fabriciana* (L. 1767)       | BE                 | 11*        |
|                |              | *N. ruficorns* (L. 1758)        | BE                 | 11*        |
|                |              | *N. succincta* (Panzer 1798)    | BE                 | 11*        |
|                |              | *Tetralonia* sp.                | FR                 | 3          |
| Formicidae     |              | *Myrmica ruginoda* (Nylander 1846) | GB          | 7          |
| Halictidae     |              | *Halictus simplex* (Bluethgen 1923) | AT             | 13*, 14*   |

(continued)
O. militaris, which exploit the food-seeking behavior of insects and lure visitors—Jersákovi et al. 2006), substances secreted by the stigma, pollen or occasionally to find a shelter; lepidopterans seek nectar essentially in species with a long spur (Berger 2003), like Gymnadenia spp. or Platanthera spp. Caught and observed coleopterans were “simple visitors” (sensu Berger 2003); they apparently landed randomly on inflorescences and never actively visited flowers—Schatz et al. 2005). 

Several factors can explain why an insect is only a visitor and not a pollinator of a plant species (Pellmyr 2002; Shivanna and Tandon 2011). As long as individuals from a given insect species are not documented as having collected at least one pollinium, they should only be considered as ineffective visitors. Caught insects bearing pollinia are potential pollinators. Among them, some species were caught in this study but bore no pollinia (Apidae: Bombus pascuorum Scopoli, B. pratorum (L.), and B. terrestris (L.); Megachilidae: Osmia bicolor Schrank). The low sample size per species (in average, two specimens) could explain why we did not observe pollinia on these species. Moreover, a few potential pollinators from Table 3 are not present or extremely rare in Belgium: Andrena enslinella Stoeckhert (Andrenidae: Rasmont et al. 2013), Anthophora aestivalis Panzer, A. borealis Morawitz (Apidae: Rasmont 2014), and Halictus eurygnathus Bluethgen (Halictidae: Pauly et al. 2016); their role in O. militaris pollination is probably very low in this country. In addition, in the study site “Oseraie de Lanaye” (Table 1), Petit (1998) found specimens from three species of Nomada Scopoli (Apidae: Nomada fabriciana (L.), Nomada ruficornis (L.), and Nomada succincta Panzer) and Hoplitis adunca Panzer (Megachilidae) with O. militaris pollinia. However, none of these species was observed in our study. Finally, as mentioned earlier, A. mellifera was the only species bearing pollinia in common in this study (Table 2) and in previous ones (Table 3).

According to Voß (1987), confirmed pollinators (at least in Lower Austria) are short-tongued hymenopterans from the genera...
Andrenidae: A. enslinella and A. battorifana F.) and Halictus Latreille (Halictidae: H. eurygnathus and H. simplex Blüthgen). A. battorifana (Rasmont et al. 2013), H. eurygnathus, and H. simplex (Pauly et al. 2016) are found in Belgium, but these species were not observed on O. militaris during the censuses. As mentioned in the previous paragraph, A. enslinella is absent from the Belgian territory (Rasmont et al. 2013). Vóth’s conclusion is based upon the presence of pollinia on insects and the similarity between spur length (and lip length) and proboscis length (and body length). According to this interpretation, pollinia adhesion and subsequent transport would be unadapted to the morphology of long-tongued bees (in Vóth’s study, Apidae: A. mellifera, B. lapidarius, and B. terrestris) because they reach the end of the spur before the viscidium adheres to the insect (Vóth 1987, van der Ginkel 1995). However, real pollination events are not described by Vóth (1987) and his conclusion is thus mostly putative. Bateman and Rudall (2014) hypothesized that bumblebees (B. pratorum and B. vestalis Geoffroy) could be confirmed pollinators in a British population. In Vóth’s study, the two mentioned bumblebee species (B. lapidarius and B. terrestris) weren’t pollinators because no specimen bore pollinia (but see Harding 1996) and their proboscis was longer than the spur. B. lapidarius and B. terrestris are long-tongued bees but this is not the case of the short-tongued B. pratorum and B. vestalis (Goulson 2010, Bateman and Rudall 2014). The results of Vóth (1987) and those of Bateman and Rudall (2014) suggest the importance of short-tongued (non-)Apide bees as confirmed pollinators of O. militaris. In general, long-tongued bees, like A. mellifera and B. lapidarius in this study, probably remove pollinia accidentally during their visits. In certain cases, the number of removing events can be high but specimens bearing numerous pollinia should be rare, although we mostly observed honeybees bearing many pollinia. The function of short-tongued bees should be examined in more details through direct observation of pollination events and study of pollination efficiency. The status of incidental pollinators attributed to long-tongued bees could also be confirmed through experiments involving A. mellifera or B. terrestris (e.g. hives provided by Biobest, Westerlo, Belgium). Direct field observations and captures also remain of the greatest importance to determine the role of insect visitors and study the visitor spectrum of a plant species.

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