

Pollinators of *Lavandula angustifolia* Mill., an important factor for optimal production of lavender essential oil

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Abstract

Lavender essential oil is widely used in pharmacy, perfumery and the food industry. It is one of the key essential oils in aromatherapy due to its valuable pharmacological properties. The producers of lavender essential oil are well aware that the greatest quantity of oil is obtained near the end of the inflorescence anthesis and that oil quantity is correlated with the pollination as unpollinated flowers drop down. In addition, it has been demonstrated that oil quality is also highest at the end of the flowering period, related to the gradual increase of monoterpenes (particularly the valuable linalool) and the decrease of sesquiterpenes during flower ontogenesis. The aim of this preliminary study was to measure the occurrence of spontaneous self-pollination in *Lavandula angustifolia* Mill. and to identify external pollinators. The field experiments were performed in a lavender plantation near Gorna Lipnitsa Village, north Bulgaria and in the ex-situ lavender collection in the experimental plot of the Botanical Garden of Sofia University. It was revealed that spontaneous self-pollination did not occur in flowers from which external pollinators had been excluded. Exposed flowers were pollinated by polylectic insects, such as honeybees, several species of bumblebees and butterflies. Wild pollinators (particularly bumblebees) dominated over honeybees at both study sites. Our observations showed that all pollinators actively collected nectar. The pollen baskets of most bees were full, indicating the active consolidation of pollen adhering to the pollinators' bodies. Although lavender growers tend to place beehives in the fields for optimal essential oil production, it is also crucial to conserve wild pollinators.

Keywords

Apis mellifera, *Bombus*, Bulgaria, Coleoptera, Diptera, Hymenoptera, Lepidoptera, pollination

Introduction

Lavender essential oil, extracted from *Lavandula angustifolia* Mill. (synonyms *L. vera* DC., *L. officinalis* Chaix), is of economic importance as it is widely used in pharmacy, perfumery and the food industry. It is one of the key essential oils in aromatherapy due to its valuable pharmacological effects (Clarke 2002, 2009; Lawless 2013; Buckle 2014; Salehi et al. 2018; Gallotte et al. 2020).

According to lavender essential oil producers, the greatest quantity of oil is obtained near the end of the inflorescence anthesis and the oil quantity correlates with the pollination as unpollinated flowers drop down (Pavlovi Food Industries Ltd. and S. Stanev, personal communication). In addition, it has been demonstrated that the increase in oil quality at the end of the flowering period is related to the gradual increase of monoterpenes (particularly the valuable linalool) and the decrease of sesquiterpenes during flower ontogenesis (Détár et al. 2021). Although more data are available about the pollination of *Lavandula latifolia* Medik. (Herrera 1987, 1988, 1989, 1990a, 1995, 2000, 2001, 2005, 2021), insect pollination of *L. angustifolia*, as a factor for the fruit-set, is surprisingly poorly studied (Benachour 2017; Gilpin et al. 2017). Bulgaria is one of the main lavender oil producers in the world, along with France, the UK, China and Spain (Zagorcheva et al. 2013; Stanev et al. 2016; Salehi et al. 2018). Despite this, there is no information about *L. angustifolia* pollination in Bulgaria. In addition, little is known about the occurrence of spontaneous self-pollination in the various cultivars of *L. angustifolia* (Romanenko and Buyukli 1980).

The aim of this preliminary study was to determine the occurrence of spontaneous self-pollination in *L. angustifolia* and to identify its insect pollinators.

Material and methods

There are six cultivars of the perennial shrub *L. angustifolia* grown in Bulgaria. They have differing flower yields and also differ in the yield, content and composition of their essential oils. For the unique quality and economic value of their oil to be retained, the different cultivars must be propagated vegetatively, not from seed. Cultivars are difficult to identify by morphological characters and molecular markers are needed (Stanev et al. 2016; Zagorcheva et al. 2020). Therefore, in this study, we specify the identity of this plant to species level only.

Study sites

The field experiments were performed in July (setting up pollinator exclusion experiments on selected flowers and conducting observations of pollinator activity) and September (collecting the pollinator-excluded flowers) of the year 2020. The location of the experiments was the experimental plot, ex situ collection of the Botanical Garden



Figure 1. *Lavandula angustifolia* inflorescence excluded from pollinators.

of Sofia University (GPS: 42°41'48.7"N, 23°20'02.3"E, 600 m above sea level) and a lavender plantation near Gorna Lipnitza Village, north Bulgaria (GPS: 43°19'45.1"N, 25°24'18.2"E, 180 m above sea level).

Spontaneous self-pollination test

At Gorna Lipnitza Village, the occurrence of spontaneous self-pollination in *L. angustifolia* was tested by excluding pollinators (Fig. 1) from 10 inflorescences (a total of 638 flowers). Additionally, a control was established: in order to test the effect of the pollinator exclusion on the fruit-set, a free-pollinated inflorescence was covered and its fruit-set was tested.

Pollinator composition and activity

A pollinator activity index, modified from Kozuharova and Firmage (2007), was calculated as the number of recorded pollinator visits to one inflorescence divided by the recording time in minutes, the result being multiplied by 60 minutes. The formula used is as follows: $PA = (N/T) \cdot 60$, where PA = pollinator activity index, N = number of recorded pollinator visits to one inflorescence, T = time of observation (minutes).

Observations were made for a total of 465 minutes over four days in the Botanical Garden of Sofia University and 293 minutes over four days in the lavender plantation near Gorna Lipnitza Village. The weather conditions during the observations were

similar and comparable. The pollinators were identified in the field or later in the laboratory from photos. Since it is not possible to distinguish with certainty between some *Anthidium* spp. and *Bombus* spp. in the field and from photos, we present them as undistinguished pairs. Their individual foraging behaviour was recorded and documented by pictures and videos taken with a Nikon D5100 camera.

Results

Spontaneous self-pollination test

At the experimental plot, the fruit-set of all flowers from which pollinators were excluded (N = 683) was 0% while that of the control (N = 64) was 85.9%. This strongly suggests an absence of spontaneous self-pollination.

Pollinator composition and activity

Botanical Garden ex-situ collection

During our study, the only visitors to lavender that we observed in the Botanical Garden in June – July 2020 were bees (Hymenoptera): *Anthidium manicatum* (Linnaeus, 1758), *Lasioglossum* spp. *Bombus pascuorum* (Scopoli, 1763), *Bombus terrestris* (Linnaeus, 1758) and/or *B. lucorum* (Linnaeus, 1761) and some unidentified bumblebees, *Apis mellifera* (Linnaeus, 1758) (Table 1). Interestingly, *Anthidium manicatum* (Linnaeus, 1758) and/or *A. florentinum* (Fabricius, 1775) and *Lasioglossum* spp. bees appeared only in June. Since these were not recorded subsequently, their average contribution was calculated to be low (Table 1). In July, pollinators were bumblebees and honeybees (Table 1).

Table 1. Pollinators of *Lavandula angustifolia* in the Botanical Garden of Sofia University.

		Observations in 2020					
Date		19.06.2020	14.07.2020	15.07.2020	16.07.2020	17.07.2020	
Recording time		74 min	119 min	118 min	116 min	112 min	
Temperature		26 °C	24 °C	26 °C	24 °C	25 °C	
Clouds (scale 0–10)		1	0	0	2	1–6	
Wind		1 m/s	1 m/s	1 m/s	2 m/s	3 m/s	
Family	Species	Activity index (AI)					Average AI
		Order Hymenoptera					7.68
Megachilidae	<i>Anthidium manicatum/florentinum</i>	5.7	0.0	0.0	0.0	0.0	1.14
Halictidae	<i>Lasioglossum</i> spp.	1.6	0.0	0.0	0.0	0.0	0.32
Apidae	<i>Bombus pascuorum</i>	0.0	1.0	1.5	1.0	2.7	1.24
Apidae	<i>Bombus terrestris/lucorum</i> complex	0.0	3.0	2.0	2.6	3.2	2.16
Apidae	<i>Bombus</i> sp.	0.0	2.5	3.1	1.6	1.6	1.76
Apidae	<i>Bombus</i> sp. div.: total	0.0	6.6	6.6	5.2	7.0	5.08
Apidae	<i>Apis mellifera</i>	0.0	1.0	1.0	2.6	1.1	1.14

Table 2. Pollinators of *Lavandula angustifolia* in the lavender plantation near Gorna Lipnitza Village.

		Observations in 2020				
Date		8.07.2020	9.07. 2020	10.07. 2020	11.07. 2020	
Recording time		68 min	75 min	75 min	75 min	
Temperature		26 °C	24 °C	25 °C	27 °C	
Clouds (scale 0–10)		1	6	1–5	0	
Wind		1 m/s	4 m/s	2 m/s	1 m/s	
Family	Species	Activity index (AI)				Average AI
		Order Hymenoptera				12.3
Apidae	<i>Bombus pascuorum</i>	1.8	0.8	2.4	2.4	1.85
Apidae	<i>Bombus terrestris/lucorum</i> complex	2.6	0.8	0.8	1.6	1.45
Apidae	<i>Bombus niveatus</i>	1.8	3.2	3.2	2.4	2.65
Apidae	<i>Bombus</i> sp.	2.6	1.6	0.8	0.8	1.45
Apidae	<i>Bombus</i> sp. div.: total	8.8	6.4	7.2	7.2	7.4
Apidae	<i>Apis mellifera</i>	4.4	5.6	4.8	4.8	4.9
		Order Lepidoptera				0.43
Sphingidae	<i>Macroglossum stellatarum</i>	0	0	0	0.1	0.03
Lycaenidae	<i>Plebejus argus</i>	0.1	0.1	0.1	0	0.08
Pieridae	<i>Pieris rapae</i>	0.1	0.0	0.1	0	0.05
Pieridae	<i>Pontia edusa</i>	0	0.1	0.1	0.1	0.08
Papilionidae	<i>Iphiclides podalirius</i>	0	0.1	0	0.1	0.05
Nymphalidae	<i>Melanargia galathea</i>	0.2	0.1	0.3	0.1	0.15
Order Diptera						0.08
Bombyliidae	<i>Bombylius</i> sp.	0.1	0.1	0	0.1	0.08
		Order Coleoptera				0.05
Cantharidae	<i>Rhagozycha fulva</i>	0.1	0.1	0	0	0.05

Gorna Lipnitza Lavender Field

Bees (Hymenoptera) were the main lavender pollinators at the plantation near Gorna Lipnitza. Flower visits were dominated by bumblebees, followed by honeybees. However, some butterflies, moths, bee-flies and beetles were also recorded visiting the flowers as follow: Hymenoptera: *B. pascuorum*, *B. terrestris* and/or *B. lucorum*, *B. niveatus* Kriechbaumer, 1870 and some unidentified bumblebees, *A. mellifera*; Lepidoptera: *Macroglossum stellatarum* (Linnaeus, 1758), *Plebejus argus* (Linnaeus, 1758), *Pieris rapae* (Linnaeus, 1758), *Pontia edusa* (Fabricius, 1777), *Iphiclides odalirius* (Linnaeus, 1758), *Melanargia galathea* (Linnaeus, 1758); Diptera – *Bombylius* sp.; Coleoptera – *Rhagozycha fulva* (Scopoli, 1763) (Table 2).

Discussion

Spontaneous self-pollination

Our experiments revealed a complete absence of spontaneous self-pollination in the studied *L. angustifolia* plants. This plant is known to have adaptations to avoid self-pollination, such as heterostyly and various systems of genetic self-incompatibility, as well as male sterility (Bujukli 1970). Other research states that, while *L. angustifolia* is

normally considered an allogamous species, autogamy is possible and the mode of pollination depends on the degree of heterozygosity of the plants involved (Romanenko and Buyukli 1980). Cytoplasmic male sterility (CMS) is reported for *L. angustifolia* and even used for selection purposes (Gostev et al. 1976). CMS plants do not produce and release functional pollen following a dysfunction in the respiratory cell metabolism of the anther tapetum during sporogenesis, promoting cross-pollination (Richards 1997). The number of fruits in the freely-pollinated control was less than the total number of flowers. Due to complex factors, not all ovules of *L. latifolia* mature to seeds (Herrera 1990b) and it is still unclear whether this is resource- or pollen-limited.

Pollinator composition and activity

Bumblebees dominated the pollinators at both experimental plots, followed by honeybees (Tables 1 and 2). The average activity index of bumblebees in the Botanical Garden was 5.08 and at the lavender plantation near Gorna Lipnitsa, it was 7.4 (Tables 1 and 2). The average activity index of honeybees was 1.14 and 4.9, respectively (Tables 1 and 2). This is not surprising because beehives are common in the agricultural fields and the distance that honeybees can fly to their food resource is greater than that of wild pollinators, reaching 2–3 km (Steffan-Dewenter and Tscharntke 2000). Our results correspond to previous findings as it is shown that lavender (*Lavandula* spp.) flowers attract more bumblebees (*Bombus* spp.) than honeybees (*A. mellifera*) (Herrera 1990a, Balfour et al. 2013, Benachour 2017). Our research also confirms the conclusions of other studies that nectar is the main reward taken by bees (Herrera 1989; Balfour et al. 2013; Benachour 2017). Only a few bumblebees were observed to have full pollen baskets and they filled them mainly by consolidating the pollen that adhered to their bodies while flying from flower to flower.

The bumblebees, recorded in the study plots, comprise more than three species. Bumblebees are polylectic foragers, but differ in their requirements: some gather nectar, some pollen, while others may gather both (Goulson 2010). *Bombus terrestris* and *B. lucorum* visit a very wide variety of flowers, but if the corolla tube is deep, they often become nectar robbers. Although *B. pascuorum* visit a wide range of flowers, it is very fond of the flowers of legumes and Dead-nettles (Edwards and Jenner 2005). *B. niveatus* prefer Lamiaceae food plants (Aliyev et al. 2021). Bumblebees forage more efficiently because they have longer tongues and can process lavender flowers three times faster than honeybees (Balfour et al. 2013). In addition, bumblebees, in general and, in particular, the typically dominant *B. terrestris*, emerge as the most efficient pollinator of *L. angustifolia* as revealed by counting pollen grains transported on their bodies in comparison to five other bee visitors of this plant, including honeybees (Benachour 2017).

The butterflies and moths (order Lepidoptera) recorded in the study plots comprise six species belonging to five families.

Macroglossum stellatarum is a fast-flying nectarivorous hawkmoth (Sphingidae) which is abundant throughout Bulgaria. It is popularly known as the “Hummingbird Hawk-moth” owing to its spectacular feeding habits which involve hovering in mid-air

and using its long proboscis to probe flowers for nectar, without any other body parts making contact with the corolla (Goyret and Kelber 2012). Due to its nectar-gathering habits, *M. stellatarum* is noted as a major pollinator of plants with long corolla tubes (Lázaro and Santamaría 2016).

Plebejus argus, one of the most widespread and abundant species of Blue (Lycaenidae) in Bulgaria, is an obligate myrmecophilous species. The spatial distribution of the adult butterflies within a given habitat is primarily correlated with the occurrence of its mutualist host ant, *Lasius niger* (Jordano et al. 1992) and, to a far lesser extent, with the density of nectar sources (Seymour et al. 2003). While *Lavandula* is one of the favoured nectar sources for *P. argus* where available (Seymour et al. 2003), it is visited by the butterfly only if growing in proximity to the patches occupied by the host ant *L. niger*, the latter constraint thus determining the local and probably negligible importance of the butterfly as a pollinator of *Lavandula*.

The remaining four butterfly species, recorded in the study plots, are *P. rapae*, *P. edusa*, *I. podalirius* and *M. galathea*. Despite having rather different pre-imaginal development preferences, as adults, these four butterfly species share two ecological traits. Firstly, they all have a wide geographical and altitudinal distribution across Bulgaria (Buresch and Tuleschkow 1929; Abadjiev 2001), occurring in a broad range of open and semi-open habitats which notably include species-poor agricultural and ruderal landscapes (Z. Kolev, personal observations). Secondly and in keeping with their eurybiotic character, these four species are opportunistic feeders with regards to their use of flowering plants as a source of nectar. Thus, *P. rapae* has been shown to be able to utilise a broad range of native, as well as exotic, flowers with significantly different corolla depths in a complex, anthropogenic urban setting (Lazri and Barrows 1984). However, the value of that species as a pollinator has been questioned, as the study determined that, according to the small number of thereto attached pollen grains, the species may function to a limited degree as a pollinator, but to a much greater extent as a nectar thief (Lazri and Barrows 1984). As a general point, butterflies and moths are seen as far less important in terms of pollinating potential than are Hymenoptera: individuals of *P. rapae* have been shown (Lazri and Barrows 1984) to carry several orders of magnitude fewer pollen grains compared to, for example, honeybees, in which up to 15,000 pollen grains have been counted from a single individual (Kendall and Solomon 1973).

Conclusion

According to the results of the self-pollination test, the flowers of *L. angustifolia* appear to not spontaneously self-pollinate and require insect pollen vectors for their fruit/seed set to occur. Wild bees (particularly bumblebees), as well as other wild pollinators, are predominantly responsible for the pollination of this shrub, with bumblebees shown to be the most efficient pollinators (Balfour et al. 2013). Although lavender growers tend to place beehives in the fields for optimal essential oil production, it is also crucial to conserve wild pollinators.

Negative non-target effects of pesticides are apparent on pollinators and subsequent declines in these insects have been detected particularly in areas of more intensive agriculture (Söderman et al. 1997; Sepp et al. 2004; Biesmeijer et al. 2006; Rundlöf et al. 2008; Brown and Paxton 2009; Grixti et al. 2009; Potts et al. 2010; 2015, Goulson 2013; Goulson et al. 2013, 2015, 2018; Böhning-Gaese et al. 2019). This pollinator crisis thus demands action at many different geographic and political levels and actions for a variety of societal sectors are proposed by Forister et al. (2019). Such actions, however, must necessarily be informed by ongoing and expanding monitoring of insect pollinators. The latter is especially urgent in countries, such as Bulgaria, where such monitoring is yet to be adopted as an essential tool for science-based conservation and where baseline data on the status and population trends of wild pollinators are presently sorely lacking.

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