

CHARACTERISTICS OF BLOOMING AND POLLEN IN FLOWERS OF TWO *Syringa* SPECIES (f. Oleaceae)

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Abstract

The observations were conducted in long-term sequence studies in the years 2006, 2009, 2013, in the Lublin area, Poland (51° 16' N, 22° 30' E). The flowering phenology, diurnal pattern of blooming, pollen production and insect visitation to the shrubs of *Syringa oblata* Lindl. var. *dilatata* (Nakai) Rehd. and *S. meyeri* 'Palibin' Schn. were examined.

Syringa oblata var. *dilatata* and *S. meyeri* 'Palibin' bloomed from the mid May till mid June. The species are characteristic of early morning diurnal pattern of blooming, with approx. of 60% of daily instalment of flowers opened before 9.00 (GMT + 2h). Both species studied had the corolla tube 2-fold deeper during the pollen shedding phase compared to bud stage (mean = 14.9 mm ± 3.2 SD vs. 7.8 mm ± 2.8). No species effect was found for the size of anthers, for the mass of pollen produced in anthers and for the pollen viability. A constant number of 2 stamens in the flowers of *Syringa* species entailed the pollen yield was derivative mainly to the number of developed flowers. Therefore significant differences were noted for the pollen yield between individual shrubs (mean 0.9 kg for *S. meyeri* 'Palibin', and 8.1 kg/ha for *S. oblata* var. *dilatata*).

The *Syringa oblata* var. *dilatata* and *S. meyeri* 'Palibin' due to their attractive flowering period, and the abundance of blooming are suitable for different ornamental designs in urban areas. Unfortunately, despite the entomophilous flower traits, the insect visitors appeared sporadically.

Key words: *Syringa oblata* var. *dilatata*, *S. meyeri* 'Palibin', phenology, abundance of blooming, mass of pollen

INTRODUCTION

The genus *Syringa* L., lilacs, includes about 30 species, of woody, multi-stemmed shrub plants in the family Oleaceae [1]. The species are native to the mountain areas of south-eastern Europe and eastern

Asia and within the range of their origin are considered as insect pollinated [2]. Lilacs, due to delicious fragrant and abundant blooming, are one of the most widely cultivated ornamental trees and shrubs in temperate regions of the world [3]. Several hundred cultivars have been developed through extensive hybridization and artificial selection [4]. The plants (e.g. *Syringa vulgaris*) have been used ethnobotanically to reduce fever and treat malaria, as a perfume and a tonic, and in homeopathy [5].

The flowers are tiny, from the basic single, and semi-double to double. The genus *Syringa* is divided into two subgenera, and one of these *Ligustrina* L. has flowers with corolla tube shorter than corolla lobes. The second subgenus *Syringa* L. has generally corolla tube longer than calyx [6]. The single flowers of *Syringa* species are reported to be visited by hummingbirds, bees and butterflies [7]. In the former Soviet Union, the *S. vulgaris* was mentioned as nectariferous [8]. In Poland *S. reticulata* is regarded as melliferous plant [9,10] and is attractive for bees [7]. The pollen grains of the *Syringa* type are noted in bee products [11,12].

In Poland different ornamental species are recognised as a source of nectar for insect visitors [13–15]. The species from the genus *Syringa* are very popular in different garden designs mainly in urban areas [6,16], it means reasonable, when a native knowledge is not yet available, to fulfil the gap in the aspects of lilac's blooming biology.

The main objective of the study was to 1) examine floral phenology and diurnal dynamics of flowering for two ornamental shrubs species *Syringa oblata* var. *dilatata* and *S. meyeri* 'Palibin' 2) estimate the quantity of pollen, and 3) to monitor the activity and spectrum of insect visitors.

MATERIALS AND METHODS

Study sites and study species. The observations were conducted in long-term sequence studies in 2006, 2009, 2013, in the Lublin area, Poland (51° 16' N, 22° 30' E). The shrubs of *Syringa oblata* Lindl. var. *dilatata* (Nakai) Rehd. and *S. meyeri* 'Palibin' Schn. were grown at the arboretum section in the Botanical Garden Maria Curie-Skłodowska University. The species are known as frost resistant, they are decorative both in blooming and autumn leaf colour change phases, and are recommended as ornamentals. The experimental individuals shrubs were grown on a loess originated soil. Standard plant protection treatments were applied, when necessary.

Flowering observations. During the growing seasons detailed observations of flowering biology were conducted. The onset and length of blooming period was recorded. The observations of the diurnal model of blooming were made in 2006 and 2009. Protocols described by Jabłoński and Szklanowska [17] and Denisow [18] were applied.

The diurnal pattern of flowering was expressed as the percentage of newly opened flowers in relation to the total number of flowers opened during the day. The development of flowers was noted in one-hour intervals, between 8.00 and 20.00 h (GMT + 2h). To determine flower life-span, the buds ($n = 10$ per each cultivar) were randomly chosen on different individual shrubs. The buds were marked just before opening and their development was observed. The flower life-span was defined as the period from bud-opening till the corolla withering. As the weather conditions were changeable during flowering periods the impact of air temperature on the flower longevity was assessed.

The average number of flowers produced per each individual shrub was established. The abundance of blooming was determined on the basis of the number of flowers per inflorescence ($n=30$), the number of inflorescences per shoot ($n=15$), and the number of shoots per shrub.

Pollen production was determined for the full blooming phase assayed using the standard method described in details by Denisow [19]. The fresh and dry mass of anthers with pollen was determined (ELCON CL 65 dryer, at ca. 33°C), and then the pollen was rinsed from anthers once with pure ether (1–2 ml) and then 4–6 times with 70% ethanol (2–8 ml). Four replications $n = 100$ anthers were collected every year. The mass of produced pollen was calculated for 100 anthers, and per shrub. Pollen viability was tested in standard acetocarmine slides. The secretion of nectar was checked, according to Jabłoński [20] but not quantified in this study.

Insect activity. Simultaneously to the blooming observations the pattern and intensity of insects visits were noted for three consecutive days at one-hour intervals, at the time of the most intensive forage activ-

ity of pollinators, between 8.00 and 20.00 h (GMT + 2.00 h). The daily pattern of insect visitation for the full flowering period was recorded in 2006 and 2009 for the individual shrubs.

Data analysis. Data are presented as means with SD. The standard analysis of variance was applied [21]. *Post hoc* comparison of means for the number of flowers, the pollen production and viability was tested by the Duncan t-test. The flower life-span and the corolla length were compared using the independent samples t-test. The level of statistical significance for all analyses was at $\alpha = 0.05$. All analyses were performed using Statistica 6.0 (StatSoft Poland, Krakow).

RESULTS

The detailed dates of seasonal phenology are shown in Table 1. During the study period, *Syringa oblata* var. *dilatata* started to bloom insensibly earlier (2006), simultaneously (2009) or insensibly latter (2013) compared to *S. meyeri* 'Palibin'. Generally, the flowering began in mid May and lasted till mid June. The duration of flowering varied from 21–24 days (*Syringa oblata* var. *dilatata*) and from 22–29 days (*S. meyeri* 'Palibin'). The flowers opening noted was slightly more intense for the *S. meyeri* 'Palibin' with approx. of 60% of daily instalment of flowers have been peaking at 8.00 (Fig. 1). Most flowers of *Syringa oblata* var. *dilatata* were opened between 8.00–9.00 (GMT + 2h). In the following hours (10.00–18.00), ca. 1–10% of flowers were opened in one hour intervals.

The species effect for the flower longevity was found ($t = 0.356$, $df = 37$, $p < 0.025$). Irrespective of the weather conditions, a longer life-span was characteristic for a single flower of *S. oblata* var. *dilatata* (mean = 5.9 ± 2.2 SD) than a single flower of *S. meyeri* 'Palibin' (mean = 4.5 ± 1.7 SD). The duration of floral longevity was also dependant on the air temperature ($t = 0.156$, $df = 37$, $p < 0.041$).

The considerable elongation of the corolla tube was observed between the swollen bud phase and the pollen presentation phase of the flower development. Both species studied had the corolla tube 2-fold deeper during the pollen shedding phase compared to the swollen bud phase (mean = $14.9 \text{ mm} \pm 3.2$ SD vs. $7.8 \text{ mm} \pm 2.8$) ($t = 0.456$, $df = 66$, $p < 0.032$).

The lilac flowers are arranged in compound panicle. The single inflorescence of *S. meyeri* 'Palibin' grouped more flowers (mean = 138.7) than *S. oblata* var. *dilatata* (mean = 115.8) and the difference was statistically significant ($F_{1,68} = 29.22$, $p = 0.023$).

New flowers were constantly developed on each individual inflorescences and shrubs. The number of flowers per shrub was species related (Table 1). Also year-to-year variations were found for both species. The shrubs of *S. oblata* var. *dilatata*, developing 49,400

flowers per shrub, flowered 8-fold more abundant than *S. meyeri* 'Palibin'. Both species developed the lowest number of flowers in the year 2006.

The flowers of both species are bisexual. The calyx is four-lobed, and the four-lobed corolla is gamopetalous with a cylindrical basal tube. The centrally positioned gynoecium consists of one pistil with two-lobed stigmas. Two stamens are fused with the corolla at the base. The release of pollen began approx. 3–4 hours after anthesis. Most anthers dehisced between 12.00 and 15.00 (GMT + 2h). Usually pollen was released simultaneously in 4 anthers per single flower. At a mean air temperature 25–30°C the pollen release was very intensive and entire quantity of pollen per anther was released in 2–3 hours. During temperature drop at a mean 15–20°C the process was slower and lasted 5–8 hours.

The statistical analysis of the mass of pollen produced in anthers revealed significant year effect, for *S. oblata* var. *dilatata* ($F_{2,11} = 6.59$, $p = 0.0321$) and for *S. meyeri* 'Palibin' ($F_{2,11} = 12.14$, $p = 0.016$). Both species

reacted similarly for the external environmental factors and produced the lowest amounts of pollen in the year 2013, which was approx. twice lower compared to values obtained in the other years. Also the lowest pollen viability was noted in 2013. No species effect was found for the size of anthers, measured as the dry mass of anthers ($F_{1,17} = 3.29$, $p = 0.321$), for the mass of pollen produced in anthers ($F_{1,17} = 1.98$, $p = 0.421$) and for the pollen viability ($F_{1,16} = 2.23$, $p = 0.081$).

The mass of pollen produced per inflorescence depended on both the number of flowers developed and the efficiency of archesporial tissues. The average pollen output was from 9.6 – 29.1 mg per inflorescence of *S. oblata* var. *dilatata*, and from 9.6–28.5 mg per inflorescence of *S. meyeri* 'Palibin'. The mass of pollen produced per individual shrub substantially related to the abundance of blooming. The average estimated pollen yield, in the full flowering phase, amounted 0.9 kg for *S. meyeri* 'Palibin', and 8.1 kg for *S. oblata* var. *dilatata* per individual shrub.

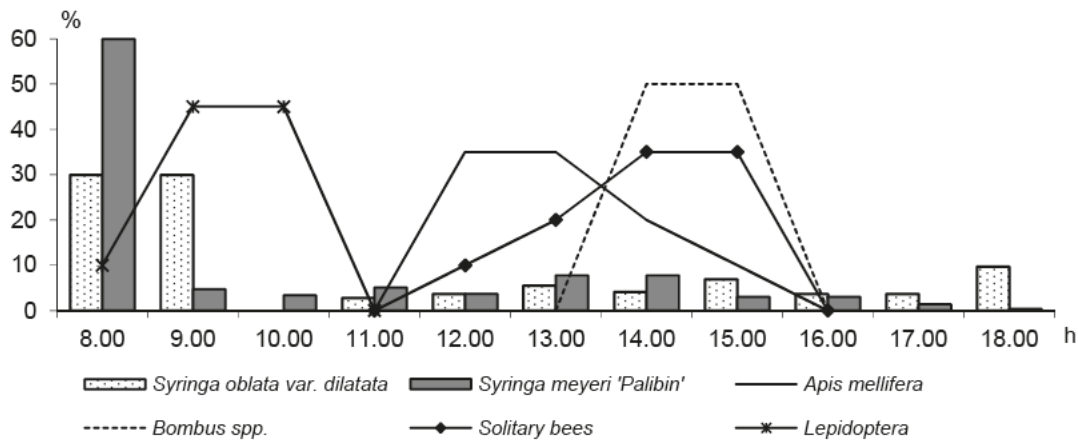


Fig. 1. Diurnal pattern of flowering of two *Syringa* species expressed as the number of flowers opened in one hour intervals in relation of the total opened during the day and the diurnal activity of insect visitors observed in SE Poland (means calculated from 2006 and 2009).

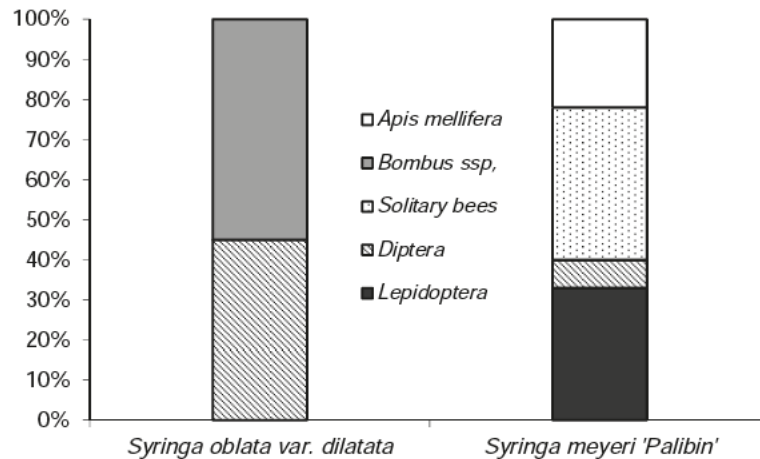


Fig. 2. The participation of insect visitors on the flowers of two *Syringa* species observed in SE Poland (means calculated from 2006 and 2009).

Table 1
Flowering period, features of blooming and abundance of flowering in two *Syringa* species
in the years 2006, 2009 and 2013, observed in SE Poland

Species	Year	Flowering period	Flower life-span (days)		Length of corolla (mm)		Number of flowers per inflorescence		Number of flowers per shrub (thous.)	
			air temperature		phase		min–max	mean	min–max	mean
			10–15°C	20–25°C	bud	pollen sheath				
<i>Syringa oblata</i> var. <i>dilatata</i>	2006	13.05–2.06	5.1	4.2	7.9	15.3	61 – 167	110.6 _a	0.5 – 169.0	30.8
	2009	9.05–1.06	7.2	5.4	8.3	16.1	54 – 214	142.6 _b	0.8 – 182.5	62.5
	2013	18.05–10.06	8.1	6.1	6.4	13.1	47 – 121	94.3 _a	0.7 – 153.8	54.9
	mean		6.8	5.2	7.5	14.8		115.8 _A		49.4
<i>Syringa meyeri</i> 'Palibin'	2006	22.05–12.06	4.2	2.4	8.0	15.1	126 – 180	150.6 _b	1.1 – 10.8	4.2
	2009	12.05–9.06	6.4	4.1	9.1	15.8	134 – 199	163.6 _b	1.8 – 13.5	7.4
	2013	15.05–12.06	7.6	4.1	7.1	14.2	102 – 136	101.8 _a	1.0 – 12.5	6.4
	mean		6.1	3.5	8.1	15.0		138.7 _B		6.0
Mean			6.5	4.4	7.8	14.9		127.3		27.7

Means in columns with the same letters are not significantly different at $\alpha = 0.05$ (Duncan t-test)

Table 2
The fresh and dry mass of anthers, the mass of produced pollen, total pollen yield and pollen viability
in two *Syringa* species in the years 2006, 2009 and 2013, observed in SE Poland

Species	Year	Dry mass of 100 anthers (mg)	Mass of pollen per				Viability (%)
			100 anthers (mg)		inflorescence (mg)	shrub (kg)	
			min–max	mean			
<i>Syringa oblata</i> var. <i>dilatata</i>	2006	29.2 _b	7.1–11.5	9.4 _b	20.8	5.8	89.4 _b
	2009	32.1 _b	9.2–12.3	10.2 _b	29.1	12.8	82.3 _b
	2013	19.8 _a	4.8–7.7	5.1 _a	9.6	5.6	62.5 _a
	mean	27.0 _A		8.2 _A	19.8	8.1	78.1 _A
<i>Syringa meyeri</i> 'Palibin'	2006	31.4 _b	7.8–11.2	9.0 _b	27.1	0.8	87.3 _b
	2009	26.9 _{ab}	8.2–12.8	8.7 _b	28.5	1.3	85.4 _b
	2013	23.4 _a	4.1–6.9	4.7 _a	9.6	0.6	52.4 _a
	mean	27.2 _A		7.5 _A	21.7	0.9	75.0 _A
Mean		27.1		7.9	20.8	4.5	76.6

Means in columns with the same letters are not significantly different at $\alpha = 0.05$ (Duncan t-test)

DISCUSSION

In the environmental conditions of south-eastern Poland *Syringa oblata* var. *dilatata* and *S. meyeri* 'Palibin' bloomed from mid May till mid June. This is about one-three weeks later than an average blooming period registered for western Poland [6]. The timing and duration of a species' flowering may be influenced by biotic and/or abiotic factors [22], and they indicate the discrepancies in flowering between species, seasons and sites.

The diurnal pattern of blooming was similar for both species. Most flowers were opened in the morning hours between (8.00–9.00 h), that indicates their

rise in the early morning or even at night. There are no adequate data about diurnal rhythm of *Syringa* spp. blooming found in the literature. Some authors insist on genetic determination or even phylogenetic constraints of the trait [17,22]. However, the impact of environmental factors on the diurnal pattern of blooming was found for different species [18,23,24]. In our study no substantial difference for the pattern of diurnal sequence of flower opening in *Syringa* spp. was revealed between subsequent days or growing seasons, suggesting a very high degree of genetic influence.

The flowers of *Syringa* species studied conforms to the general pattern of the family Oleaceae

[1]. The corolla forms a deep tube (10–17 mm). The characteristic was described by Lipiński [7], who pointed out that the feature may limit the access of insect visitors to floral reward. Flowers of the studied *Syringa* species offered both nectar and pollen, although we quantified only pollen reward in this study. Different groups of insect visitors were observed on flowers, including Apoidea bees, although their density was extremely low (only 1–2 visitors per hour). We observed lepidopterans foraging on flowers of both *Syringa* species early in the morning only. Szafer and Wojtusiakowa [25] consider the lilacs among typical lepidopterans pollinated species. The adult butterflies were able, using their long proboscis, to reach the nectar hidden at the base of deep corolla tube of lilacs observed. The short-tongue visitors occasionally foraged for nectar, and only in mid-day hours. The flowering of studied lilacs overlap with many other shrubs and trees present in nearby surrounding. Some of them (e.g. *Cotoneastr* spp., *Deutzia* species) are regarded as very good flow species [10], and in the selection between rewarding and nectar-less plants visiting insects could avoid less attractive lilacs.

The studied species produced numerous flowers per inflorescence (94.3–163.6) and the number differed between species and among growing seasons. *Syringa* species and cultivars are known of producing terminal panicles which differ in the number of flowers [6]. The number of flowers developed per inflorescence is considered a genetic feature [22] and also is highly dependant on weather factors [17]. Studies of trees grown in temperate climate showed that differentiation of flower organs continues through two growing seasons [26]. In *Syringa vulgaris* the flower primordia differentiation and flower organ development starts in midsummer and lasts until autumn and is completed in six to eight weeks during the spring following the year of initiation [27]. In our study both *Syringa* species developed the smallest number of flowers in 2013, when flowering followed the summer and autumn of 2012, with the lack of precipitation (August/September *c.a.* 180–240 % lower than in long-term). This indicates the drought as limiting factor for the bud formation in examined *Syringa* species and consequently for the abundance of blooming.

In the flowers of studied *Syringa* species most anthers dehisced in mid-day hours. This is a trait common in many plant species [19], and is derivative to the mechanisms of anther dehiscence that is related to daily cycles of declining the relative humidity and rising the air temperature that enhance evaporative water loss from the locular fluid and accelerate the structural changes in plant tissue that lead to dehiscence [28,29].

According to our knowledge, the study are the first on the pollen production of *Syringa* species. The

mass of pollen offered by the anthers of *Syringa oblata* var. *dilatata* and *S. meyeri* ‘Palibin’ was similar but the value differed among years of study. Jędrzejuk and Szlachetka [27] revealed the differentiation of pollen mother cells in anthers during bud breaking after winter dormancy in *Syringa vulgaris*. The air temperature drops during bud cracking in the studied *Syringa* species were noted in the year 2013, and resulted in the decrease in both the mass of pollen produced in anthers and the pollen viability. The noted dependence indicates that the microsporogenesis and the pollen production in the studied *Syringa* species is sensitive for external factors, i.e low temperatures.

A constant number of 2 stamens in the flowers of *Syringa* species entail the pollen yield is derivative mainly to the number of developed flowers. Therefore significant differences were noted between pollen yield per shrub (0.9–8.1 kg/ha). The impact of the abundance of blooming for the total pollen yield was underlined by different authors [23,30].

The average mass of pollen produced in the anthers of studied lilacs was 7.5–8.2 mg per 100 anthers. The value is similar to obtained from anthers in *Rosa multiflora* (9.5 mg per 100 anthers), in *Chaenomeles* sp. (3.6–11.5 mg) or in *Aesculus hypocastanum* (6.5–10.6 mg per 100 anthers), but not in flowers [30–32]. The dependence is clear comparing the androecium (2-stamens in *Syringa* vs. multi-stamens in *Rosa* or vs. 5-9-stamens in *Aesculus*).

In conclusion, the *Syringa oblata* var. *dilatata* and *S. meyeri* ‘Palibin’ due to their attractive flowering period, and the abundance of blooming are suitable for different ornamental designs in urban areas. Unfortunately, despite the entomophilous flower features, due to very deep corolla tube, the insect visitors used the floral reward offered sporadically.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: designed the experiments: BD; performed the experiments: MSA; analyzed the experimental data: BD; wrote the paper: BD, MSA

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Charakterystyka kwitnienia i pyłek w kwiatach dwóch gatunków z rodzaju *Syringa* (f. Oleaceae)

Streszczenie

W latach 2006, 2009, 2013, na terenie Lublina (51° 16' N, 22° 30' E) badano fenologię, dzienny rytm kwitnienia, obfitość kwitnienia, produkcję pyłku krzewów *Syringa oblata* var. *dilatata* oraz *S. meyeri* 'Pali-bin'. Monitorowano również zainteresowanie owadów pożytkiem. Badane krzewy kwitną na przełomie maja i czerwca. Kwiaty charakteryzują się wczesnym dobowym rytmem rozkwitania (ok. 60% dobowej porcji kwiatów rozkwita do 9.00). Rurka korony w fazie prezentacji pyłku jest przeciętnie 2-krotnie dłuższa niż w fazie nabrzmiałego pąka. Wielkość pylników, masa

produkowanego pyłku oraz żywotność pyłku nie różniły się istotnie pomiędzy gatunkami. Stała liczba pręcików (2) w kwiecie powoduje, że wydajność pyłkowa krzewów zależy głównie od liczby wytworzonych kwiatów. Stąd notowano znaczne różnice w produktywności pyłku, średnio 0.9 kg/ha z krzewów *S. meyeri* 'Palibin', oraz 8.1 kg/ha z krzewów *S. oblata* var. *dilatata*.

Krzewy *Syringa oblata* var. *dilatata* i *S. meyeri* 'Palibin', ze względu na atrakcyjny termin kwitnienia oraz obfite kwitnienie nadają się do wykorzystania w założeniach miejskich. Niestety, kwiaty pomimo typowych cech entomofilnych są sporadycznie odwiedzane przez owady.

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