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BD and SA conceived the general strategy; SA performed the observations and experiments; SA and BD interpreted the results; SA wrote the manuscript

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Competing interests

BD is an editor-in-chief of the *Acta Agrobotanica*; SA: no competing interests have been declared

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ORIGINAL RESEARCH PAPER

Floral phenology and pollen production in the five nocturnal *Oenothera* species (Onagraceae)

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Abstract

Blooming phenology and pollen production in the five *Oenothera* species were investigated during the period of 2013–2015 in the Lublin area, SE Poland. The blooming period was relatively long, and flowering usually started in the middle or late June and lasted until late July or the middle of August. The *Oenothera* species studied exhibited nocturnal anthesis, i.e., the flowers opened in the late evening and lasted overnight until the early morning hours. Plants developed a great number of flowers per individual and per unit area (on average, 158 and 4,136, respectively), and this feature appeared to be species-specific. It was demonstrated that the blooming phase had an impact on the mass of anthers and pollen produced per flower in all *Oenothera* species. In general, the greatest mass of anthers and pollen was observed at the beginning of blooming, and with the progress of flowering, the values decreased. However, statistical differences were found for *O. flaevingina*, *O. paradoxa*, and *O. rubricaulis*. The mass of pollen produced per unit area was also a species-specific characteristic and was related to the abundance of flowering. The greatest amount of pollen was produced by *O. flaevingina* (30.6 g m⁻²), which was almost three times more than that produced by *O. rubricaulis* (10.9 g m⁻²). The protein content of pollen grains was relatively low and on average amounted to 15.4%. The *Oenothera* species examined in this work may be considered valuable pollen yielding plants. Nevertheless, given the invasive potential of species from subsect. *Oenothera*, precautions are suggested during cultivation and/or planting these taxa in bee pastures, in order to prevent uncontrolled spread into new areas.

Keywords

bee pasture; evening primroses; flowering; pollen yield; protein

Introduction

The genus *Oenothera* L. (evening primroses, Onagraceae) is large and heterogeneous, consisting of about 145 species subdivided into 18 sections. Species of *Oenothera* are known nearly worldwide, including Europe, and they are considered to have primarily originated from North, Central, and South America [1–3]. Most of the species easily adapt in a wide range of habitats and climatic zones, ranging from low-elevation hot deserts to montane temperate and subtropical forests, subalpine conifer forests, and eastern deciduous forests [4]. Of these, about 60 taxa have been reported to occur in the flora of Poland, and most of them belong to the subsection *Oenothera* [3,5,6].

Species of *Oenothera* are herbaceous plants, including short-lived perennials, facultative biennials, or uncommon winter annuals, which occur in primary or secondary open habitats [1]. The taxonomy of the genus is very complex, especially with regard to

Oenothera subsection *Oenothera*. Species from this subsection of the genus cross freely producing offspring that differ from both parents [3]. Consequently, numerous, closely similar but morphologically separated hybrids may be formed [1,3,5]. According to Cullen et al. [7], 21 *Oenothera* species have been documented as cultivated in Europe. These mostly includes crop plants used for the production of the essential γ -linolenic fatty acid from their seeds (e.g., [8,9]). Moreover, many other secondary metabolites of *Oenothera*, such as ellagitannins (e.g., oenothlein A or B, which have antioxidant and antitumor properties) are of increasing significance (e.g., [10]). Although research regarding evening primroses has been performed for more than 120 years since de Vries's first publication in 1895, most of the studies on *Oenothera* have focused on genetic and cytological analyses. As a result, the genus became a notable model plant system in the modern synthesis of evolutionary biology [4,11].

From the ecological point of view, there are only few studies available describing in detail the flowering biology of evening primroses in the broader context of general biodiversity maintenance (see, e.g., [12–14]). Given recent evidence of a reduction of pollinator biodiversity and density at a global scale (e.g., [15–17]), knowledge regarding the usefulness of both crop and wild entomophilous plants is essential in order to stabilize pollination networks and to improve food resources for bees and other wild pollinators [18–20]. In order to achieve this, detailed investigations of the flowering biology as well as quantitative and/or qualitative analyses of primary floral rewards are highly desirable [17–19]. Such observations include flowering phenology (commencement and duration of flowering), and evaluations of nectar and pollen production. As emphasized by many authors, the period and duration of flowering and abundance of blooming are crucial in order to select appropriate plant species for the improvement of bee pastures [20,21], whereas total sugar and pollen yield are needed to control food resources for pollinators [22,23].

Species of *Oenothera* are generally considered valuable pollen-giving plants (e.g., [12,24]) as well as important components in the ecological restoration of post-exploitation areas [25,26]. However, a dynamic spread of *Oenothera* in Europe has been observed in the last 200 years [27,28], and numerous representatives are currently considered active invasive species, especially in Central and Eastern Europe [29]. All these observations indicate that much of the available data on *Oenothera* are contradictory and/or incomplete, especially concerning the flowering biology and pollen production. The present work was therefore undertaken to: (i) examine flowering phenology and (ii) pollen production and quality of the five *Oenothera* species occurring in Europe. Additionally, we were especially interested to determine whether pollen production differs between phenological phases and growing seasons for the same plant species.

Material and methods

Study site and plant species

The investigations of flowering and pollen production were carried out during the period 2013–2015 at the Botanical Garden of Maria Curie-Skłodowska University in Lublin, SE Poland (51°15'44" N, 22°30'48" E). Five species of *Oenothera* (subsect. *Oenothera*) differing in their status in Poland and geographical distribution were studied [3,30]. These included: *O. casimiri* Rostański (apophyte), *O. flaemingtonia* Hudziok (antropophyte), *O. nuda* Renner ex Rostański (antropophyte), *O. paradoxa* Hudziok (antropophyte), and *O. rubricaulis* Kleb (apophyte). The species used in the current study are biennials, therefore every year preceding investigations experimental plots were established using seeds obtained in 2012 from natural populations in SE Poland (Lublin and Mielec). Seeds were sown onto a light substrate at the end of each April. After germination, the seedlings were planted out in September. Each experimental plot contained approximately 20–30 individuals per species, per year. All plants were grown in experimental plots (approx. 2 m² in two replicates for each species) on loess soil (pH of 6–7) at the site which is fully exposed to the sun.

Phenology and abundance of flowering

Detailed observations on the flowering phenology of the five *Oenothera* species were performed. During the seasons of 2013–2015 flowering onset and termination were recorded in order to determine duration of flowering. In addition, the onset of each stage of flowering was established according to the protocol described by Denisow and Bożek [31]. Therefore, three phenological phases were determined for each study species; these were: (i) the *beginning* of the flowering phase (i.e., when 10% of individuals started blooming), (ii) the *full* blooming phase (i.e., when 70% of individuals bloomed), and the *end* of blooming phase (i.e., when almost 90% of individuals finished blooming).

In order to establish the abundance of flowering in the 2013 and 2014 seasons, the number of buds and developed flowers on individual ($n = 15\text{--}25$ per species, per year), as well as the number of individuals on the experimental plots was counted. These results were then converted to the number of flowers per 1 m². Additionally, in 2013 and 2014, in order to determine the length of anthesis of a single flower, 15 buds (per year/per species) was randomly marked approximately 3 days before anthesis. Then, twice a day, i.e., in the morning (ca. 9:00 a.m.) and evening (ca. 7:00 p.m.), the progress of flowering was noted.

Pollen production and pollen protein content

Total pollen mass available to floral insect visitors was examined in the 2013 and 2014 seasons, using a modified ether method described in detail by Denisow [23]. Given the relatively long duration of blooming of the *Oenothera* species studied, pollen production was estimated for three pheno-phases, i.e., the beginning, full, and end of blooming. Additionally, the size of anthers (expressed as the fresh and dry mass of anthers per flower) was also obtained. The mature but unopened anthers from 10 flowers ($n = 80$) were removed from buds and were placed in glass containers of known mass. Four replicates were used for each blooming phase and study species. Subsequently, samples were transferred to a dryer (Elcon CL 65) at 33°C. Following drying for 7–14 days, the pollen was washed out from anthers using 70% ethanol, and then several times with pure ether. The accuracy of this pollen extraction was checked using an Olympus (Japan) SZX12 stereomicroscope. Next the pollen samples were weighed using a WPS 36 electronic balance (Radwag, Poland), and pollen production was calculated for one flower and for 1 m².

A test for protein content in pollen grains was also performed in the five *Oenothera* species in 2014. For this purpose, dry pollen samples from the full blooming phase ($n = 3$ per species) were analyzed according to the Kjeldahl method (PN75/A-04018, ISO, AOAC-920-123).

Data analysis

Data are presented as mean values \pm SD. Parametric statistical ANOVA procedures were used to assess differences in the mean values of the analyzed criteria (number of flowers, mass of anthers and pollen, and protein content in pollen) between species, and within species between study years and/or blooming phases. When statistically significant differences were noted, post hoc comparison was made using Tukey's HSD test. The level of statistical significance for all the analyses was set at $p = 0.05$. Data analyses were performed using Statistica 13.1 software (Statsoft, Poland).

Results

The flowering seasons of *Oenothera* species investigated in the present paper began in the middle or late June and lasted until late July or mid-August. In general, the time and duration of blooming differed between study species and between years (Fig. 1). Individuals of *O. casimiri*, *O. flaevingina*, and *O. nuda* were the first to bloom, followed

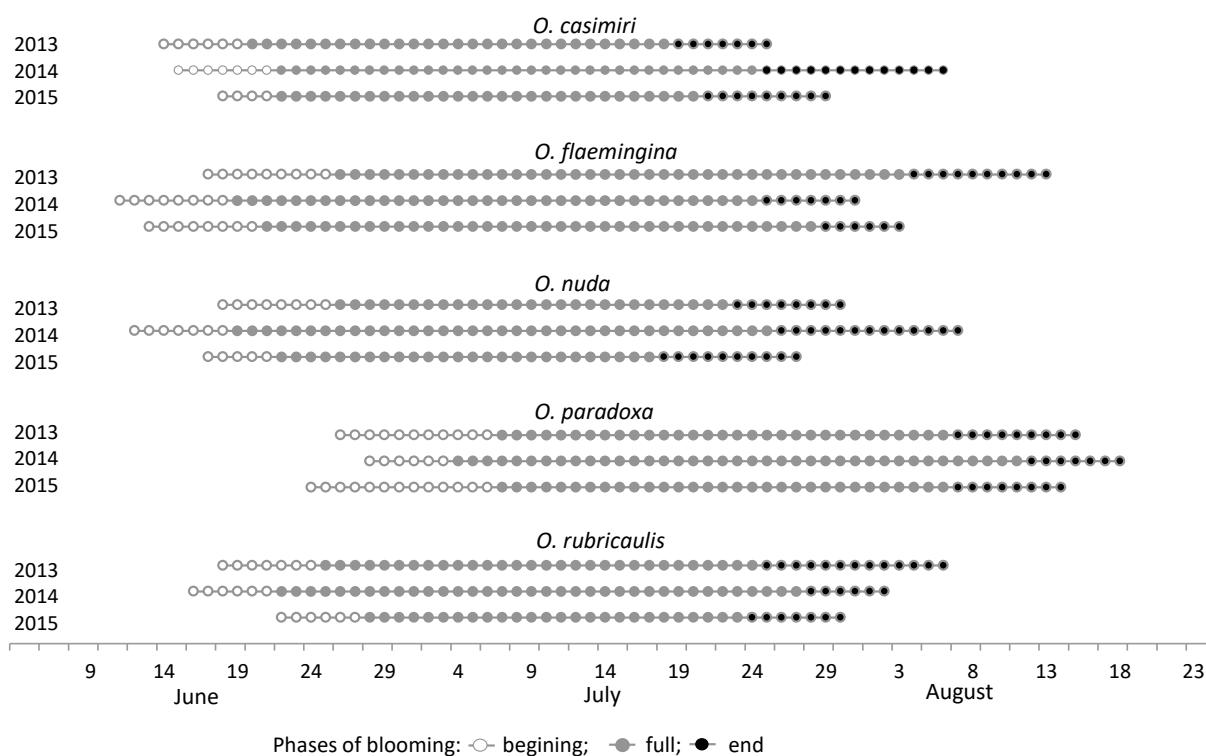


Fig. 1 Blooming phenology of the five *Oenothera* species in 2013–2015 seasons in Lublin, SE Poland.

by *O. rubricaulis* and *O. paradoxa*. The study species exhibited a relatively long duration of blooming (47.6 ± 5.9 days; means calculated across study species and years). The longest flowering period was noted for *O. flaevingina* in 2013 (57 days), whereas the shortest one was for *O. rubricaulis* in 2015 (38 days). In addition, the duration of blooming of *O. paradoxa* was relatively constant between growing seasons and lasted for 50–51 days.

The flowers of all five species exhibited nocturnal anthesis; i.e., the flowers opened in the late evening hours (8:00 p.m. – 9:00 p.m.) and lasted only until the early morning hours (7:00 a.m. – 9:00 a.m.). Therefore, the anthesis of a single flower was relatively short and lasted 10.3 ± 3 h (mean \pm SD, calculated across plants species and 2 study years). The flowers of *Oenothera* are arranged in a raceme inflorescence, and the flowering progresses acropetally, i.e., from the bottom to the top of the inflorescence. However, for all species examined, it was specific that 3–5 flowers were at bloom at the same time within a particular inflorescence. The number of flowers developed per individual and per unit area was species-specific [$F(4, 159) = 11.25, p < 0.001$ and $F(4, 159) = 11.93, p < 0.001$, respectively; Tab. 1]. The lowest number of flowers per individual was recorded for *O. rubricaulis* (88.0 ± 42.1), whereas the highest was observed for *O. paradoxa* (191.7 ± 81.7). The year effect on the number of flowers per individual was found for *O. casimiri* [$F(1, 31) = 36.39, p < 0.001$], *O. flaevingina* [$F(1, 31) = 10.57, p < 0.003$], *O. nuda* [$F(1, 30) = 15.84, p < 0.001$], and *O. rubricaulis* [$F(1, 36) = 11.40, p < 0.002$], whereas in *O. paradoxa*, the number of flowers per inflorescence was not affected by the year of observation [$F(1, 26) = 2.93, p = 0.099$].

The androecium in *Oenothera* consists of eight stamens; the filaments are distinct and the anthers are large and elongated. The pollen grains are connected to each other by viscin threads in clusters. The release of pollen from anthers in the species studied began in the bud stage 24–36 hours before anthesis.

The detailed data concerning the influence of blooming phase on the size of anthers (expressed as the fresh and dry mass of anthers per flower) and pollen production are shown in Tab. 2. In general, the blooming phase had an impact on the size of anthers and/or mass of pollen produced per flower in all the *Oenothera* species. The largest anthers and greatest pollen production were observed at the beginning and/or full blooming

Tab. 1 Number of flowers per individual and per 1 m² in the five *Oenothera* species in Lublin, SE Poland.

Species	Year	Number of flowers per individual	Number of flowers per 1 m ²
<i>O. casimiri</i>	2013	272.0 ^a ±97.4	7,888.0 ^a ±2,823.7
	2014	102.7 ^b ±57.5	2,567.2 ^b ±1,437.3
	Mean	189.9 ^A ±116.9	5,308.2 ^A ±3,499.6
<i>O. flaemingina</i>	2013	229.0 ^a ±107.8	5,725.0 ^a ±2,695.3
	2014	130.2 ^b ±51.1	3,385.2 ^b ±1,327.9
	Mean	184.1 ^A ±99.1	4,661.4 ^{AB} ±2,455.8
<i>O. nuda</i>	2013	152.5 ^a ±52.2	4,116.7 ^a ±1,410.9
	2014	90.3 ^b ±32.4	2,617.7 ^b ±939.8
	Mean	123.3 ^B ±53.7	3,414.1 ^{BC} ±1,415.2
<i>O. paradoxa</i>	2013	225.9 ^a ±76.9	5,914.0 ^a ±1,992.6
	2014	172.7 ^a ±79.9	4,834.7 ^a ±2,237.2
	Mean	191.7 ^A ±81.7	5,220.1 ^A ±2,179.9
<i>O. rubricaulis</i>	2013	107.3 ^a ±44.8	2,789.0 ^a ±1,164.8
	2014	66.5 ^b ±26.0	1,530.8 ^b ±598.6
	Mean	88.0 ^B ±42.1	2,193.4 ^C ±1,125.7

Data represent mean values ±SD. Means within the columns with the same lowercase letter do not differ significantly between study seasons within species, whereas means within the columns with the same capital letter do not differ significantly between study species at $p < 0.05$, based on Tukey's HSD test.

Tab. 2 The fresh and dry mass of anthers and mass of pollen per flower of five *Oenothera* species in three blooming phases in Lublin, SE Poland.

Species	Blooming phase	Fresh mass of anthers per flower (mg)	Dry mass of anthers per flower (mg)	Mass of pollen per flower (mg)
<i>O. casimiri</i>	Beginning	22.9 ^a ±5.4	5.5 ^a ±0.8	3.9 ^a ±0.5
	Full	17.4 ^b ±1.7	4.4 ^b ±0.3	3.5 ^a ±0.4
	End	16.2 ^b ±4.1	4.2 ^b ±1.0	3.6 ^a ±1.1
<i>O. flaemingina</i>	Beginning	40.6 ^a ±6.6	9.8 ^a ±0.5	7.4 ^a ±0.5
	Full	34.0 ^b ±2.5	8.1 ^b ±0.8	6.6 ^a ±0.7
	End	26.2 ^c ±1.7	6.2 ^c ±0.5	5.2 ^b ±0.7
<i>O. nuda</i>	Beginning	32.6 ^a ±2.0	7.9 ^a ±0.2	6.5 ^a ±0.4
	Full	32.3 ^a ±2.8	7.8 ^a ±0.9	5.6 ^a ±1.2
	End	28.6 ^b ±1.9	6.6 ^b ±0.5	5.3 ^a ±0.7
<i>O. paradoxa</i>	Beginning	29.6 ^a ±6.9	6.8 ^a ±0.8	5.1 ^a ±0.8
	Full	29.3 ^a ±5.7	6.8 ^a ±0.6	5.6 ^a ±0.5
	End	18.1 ^b ±4.8	4.2 ^b ±1.1	3.7 ^b ±0.7
<i>O. rubricaulis</i>	Beginning	26.1 ^a ±3.6	6.2 ^a ±0.6	5.0 ^a ±0.5
	Full	25.1 ^a ±0.9	5.9 ^{ab} ±0.2	5.0 ^a ±0.2
	End	25.4 ^a ±3.8	5.5 ^b ±0.3	4.5 ^b ±0.3

Data represent mean values (calculated across two study seasons) ±SD. Means within the columns with the same lowercase letter do not differ significantly between blooming phases at $p < 0.05$, based on Tukey's HSD test.

phase, and together with progress of flowering, the values decreased significantly at the ends of the blooming phase. Exceptionally, this pattern was not observed for the fresh mass of anthers per flower in *O. rubricaulis* [$F(2, 21) = 0.19, p = 0.83$], as well as the mass of pollen per flower in *O. casimiri* [$F(2, 18) = 0.36, p = 0.70$] and *O. nuda* [$F(2, 19) = 3.36, p = 0.06$; Tab. 2].

The species effects were established for the size of anthers, expressed as the fresh and dry mass of anthers per flower [$F(4, 113) = 25.79, p < 0.001$ and $F(4, 113) = 32.56, p < 0.001$, respectively]. However, significant differences between study years for the fresh mass of anthers per flower were observed only for *O. casimiri* [$F(1, 22) = 17.91, p < 0.001$] and *O. rubricaulis* [$F(1, 22) = 6.90, p < 0.02$], whereas the year effect for the dry mass of anthers per flower was only noted for *O. casimiri* [$F(1, 22) = 11.23, p < 0.003$].

It was also found that the mass of pollen produced per one flower of *Oenothera* examined in the present study was species-specific [$F(4, 103) = 29.38, p < 0.001$; Tab. 3]. The greatest amount of pollen was produced by a single flower of *O. flaemingina* (i.e., 6.4 ± 1.1 mg), whereas the lowest value was noted in *O. casimiri* (i.e., 3.7 ± 0.6 mg). Remarkably, the year effect of the mass of pollen produced per flower was established only for *O. casimiri* [$F(1, 19) = 8.06, p < 0.02$], whereas these values were relatively constant between growing seasons for *O. flaemingina* [$F(1, 20) = 1.53, p = 0.23$], *O. nuda* [$F(1, 20) = 0.18, p = 0.678$], *O. paradoxa* [$F(1, 20) = 0.29, p = 0.594$], and *O. rubricaulis* [$F(1, 19) = 1.16, p = 0.295$; Tab. 3]. On the other hand, however, the mass of pollen produced per 1 m² varied significantly between study years for all *Oenothera* species (Fig. 2). Furthermore, the mass of pollen produced per 1 m² was also species-specific [$F(4, 100) = 21.36, p < 0.001$], and the greatest amount of pollen was produced by *O. flaemingina* (i.e., 30.6 ± 10.3 g/m²), which was almost 3 times more than that produced by *O. rubricaulis* (10.9 ± 3.1 g/m²). The average protein content of pollen grains differed between *Oenothera* species [$F(4, 10) = 8.48, p < 0.003$], and the highest protein content was observed in the pollen of *O. paradoxa* ($17.72 \pm 2.06\%$), and the lowest in pollen of *O. casimiri* ($13.61 \pm 0.55\%$; Tab. 4).

Tab. 3 The fresh and dry mass of anthers and mass of pollen per flower of five *Oenothera* species during 2013–2014 seasons in Lublin, SE Poland.

Species	Year	Fresh mass of anthers per flower (mg)	Dry mass of anthers per flower (mg)	Mass of pollen per flower (mg)
<i>O. casimiri</i>	2013	15.7 ^a ± 2.9	4.2 ^a ± 0.7	3.4 ^a ± 0.6
	2014	22.1 ^b ± 4.4	5.2 ^b ± 0.8	4.1 ^b ± 0.6
	Mean for years	18.9 ^A ± 4.9	4.7 ^A ± 0.9	3.7 ^A ± 0.7
<i>O. flaemingina</i>	2013	32.8 ^a ± 6.1	8.3 ^a ± 1.4	6.7 ^a ± 1.1
	2014	34.5 ^a ± 8.4	7.8 ^a ± 1.8	6.1 ^a ± 1.1
	Mean for years	33.6 ^B ± 7.3	8.1 ^B ± 1.6	6.4 ^B ± 1.1
<i>O. nuda</i>	2013	30.5 ^a ± 3.1	7.6 ^a ± 1.0	5.9 ^a ± 1.0
	2014	31.9 ^a ± 2.5	7.2 ^a ± 0.6	5.7 ^a ± 0.9
	Mean for years	31.2 ^B ± 2.9	7.4 ^B ± 0.9	5.8 ^B ± 1.0
<i>O. paradoxa</i>	2013	23.2 ^a ± 1.1	5.8 ^a ± 0.5	4.6 ^a ± 0.6
	2014	27.5 ^a ± 1.1	5.9 ^a ± 2.1	4.8 ^a ± 1.4
	Mean for years	25.4 ^C ± 7.9	5.8 ^C ± 1.5	4.7 ^C ± 1.1
<i>O. rubricaulis</i>	2013	24.1 ^a ± 1.2	5.8 ^a ± 0.4	4.8 ^a ± 0.4
	2014	26.9 ^b ± 3.5	5.9 ^a ± 0.6	4.9 ^a ± 0.5
	Mean for years	25.5 ^C ± 2.9	5.9 ^C ± 0.5	4.9 ^C ± 0.4

Data represent mean values ±SD. Means within the columns with the same lowercase letter do not differ significantly between years within a plant species, whereas means within the columns with the same capital letter do not differ significantly between study species at $p < 0.05$, based on Tukey's HSD test.

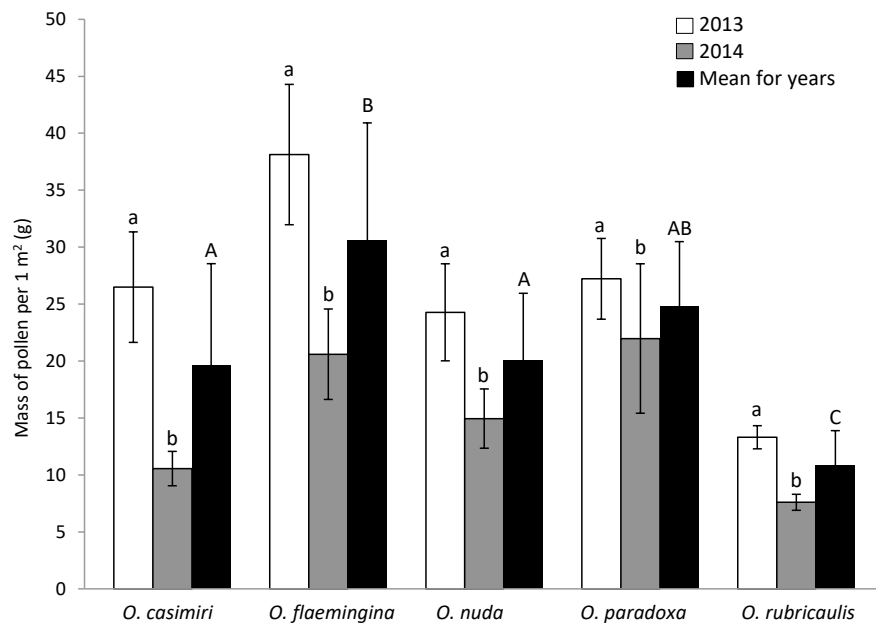


Fig. 2 The mass of pollen produced by five *Oenothera* species per 1 m² during the 2013–2014 seasons in Lublin, SE Poland. Data represent mean values \pm SD (vertical bars). Means with the same lowercase letter do not differ significantly between study seasons within species, whereas means with the same capital letter do not differ significantly between study species at $p < 0.05$, based on Tukey's HSD test.

Tab. 4 Average protein content in pollen grains of five *Oenothera* species.

Species	Protein content (%)
<i>O. casimiri</i>	13.61 ^a \pm 0.55
<i>O. flaemingina</i>	16.55 ^{bc} \pm 0.64
<i>O. nuda</i>	14.89 ^{ab} \pm 0.19
<i>O. paradoxa</i>	17.72 ^c \pm 2.06
<i>O. rubricaulis</i>	14.24 ^{ab} \pm 0.33

Data represent mean values \pm SD. Means within the columns with the same lowercase letter do not differ significantly between study species at $p < 0.05$, based on Tukey's HSD test.

Discussion

Our results indicate that in SE Poland, the flowering period of *Oenothera* species studied occurs in the middle of June and lasts until late July and/or the middle of August. This flowering period is consistent with that reported for other species from subsect. *Oenothera*, occurring both in Poland [24] and Eastern and Central North America [32: p. 106–107]. According to Szklanowska and Czubacki [12], however, the duration of flowering of several species of subsect. *Oenothera* (including *O. paradoxa*) is much longer (up to 20–28 days longer), and begins in early July and usually terminates in the middle of September. In general, the variability in the output and length of flowering is not an unusual plant feature, and has in fact been observed by many authors even for the same plant species and/or cultivars (e.g., [19,33]). These differences in the duration of blooming could be related to phenotypic plasticity, differences among genotypes, and/or various abiotic factors, such as soil type, water availability, or the ever-changing weather conditions [34–36].

Plants differ greatly in terms of the number of flowers displayed in a particular growing season. In the case of biennial taxa, the degree of flowering of an individual is strongly influenced by nutrient availability in soil, and other environmental conditions, in particular, the duration and age of the plant during exposure to low temperatures, required for bud and inflorescence formation [37,38]. We noted that the five species of *Oenothera* produced numerous flowers per individual, and the density of flowers per 1 m² exceeded 2,100 flowers for *O. rubricaulis* and even 5,300 flowers for *O. casimiri* (see Tab. 1). Furthermore, the abundance of flowering differed significantly between species, and pronounced intraspecific variations between growing seasons were also observed; the latter difference, however, was not statistically significant for *O. paradoxa*. The intraspecific differences in the abundance of flowering between study years were probably caused by variable atmospheric conditions during vegetation periods. Indeed, it has been shown experimentally that environmental and cultivation requirements for flower-induction differ greatly in *Oenothera* species [4,39]. The latter authors also documented that the response of an individual plant to the vernalization process is highly variable within *Oenothera*, and this response is not uniform even within a

particular species. Accordingly, our results are consistent with other studies on species from subsect. *Oenothera*, which demonstrated similar number of flowers produced per individual and noted great variations in the abundance of flowering between consecutive years of study (e.g., [12]).

The species of *Oenothera* examined represent a highly specialized pattern of flower opening. We noted that flowers open exclusively in the late evening hours (8 p.m. – 9 p.m.), and last only for one night until early morning, resulting in a relatively short anthesis of a single flower (ca. 10 h). Moreover, we recorded that 3–5 flowers were in bloom at the same time within a particular inflorescence. This characteristic pattern of nocturnal synchronization in flower opening and duration of anthesis may have ecological and physiological significance. On the one hand, it may directly impact on the number of flowers per individual (i.e., the size of floral display) and the degree of generalization in the group of floral visitors attracted (nocturnal vs. diurnal). On the other hand, however, it is suggested that on a given day, a certain amount of nectar and pollen is available to floral visitors in a particular inflorescence. Indeed, this complex pattern of floral reward distribution has been proposed to determine the movement of potential pollinators between individual plants, thereby promoting cross-pollination [14]. Moreover, according to the nomenclature proposed by Gentry [40], these *Oenothera* represent a “cornucopia” pattern of flowering, namely, an extended flowering period with only a few flowers open at the same time on a particular individual. According to the latter author, this strategy is implemented mostly by generalist plants in temperate regions, and probably ensures repeatable service of floral visitors, even when the rate of flower production decreases together with the progress of the blooming period.

The knowledge of the species’ pollen and nectar production is essential in order to determine the amount of these rewards that could be offered to floral visitors, including potential pollinators. In our complementary project [14], we have demonstrated that the *Oenothera* species studied produce copious amount of floral nectar (up to 26.8 mg per flower), which is rich in proteins and a great range of amino acids. Here, we present the details regarding pollen production at the intra- and interspecific level in these five *Oenothera* species. In general, the flowers of these species presented substantial amounts of pollen to floral visitors, however, statistically significant interspecific differences were noted. In fact, the mass of pollen produced per flower is a genetically-determined feature. This characteristic has frequently been documented to vary significantly between species, cultivars of even varieties [19]. However, the mass of pollen produced per flower observed in the present work generally concurs with that recorded by Szklanowska and Czubacki [12] in several other species from subsect. *Oenothera*. According to Szklanowska [41], the mass of pollen produced per flower is highly affected by the size of pollen sacs. However, atmospheric conditions during flower bud formation as well as soil and habitat type have also been reported to directly impact on the mass of pollen produced [42,43]. Interestingly, in our study the dry mass of anthers and pollen produced per flower were both relatively constant between growing seasons within *Oenothera*, and statistically significant year-to-year differences were recorded only in *O. casimiri*. This result may suggest a high resistance in anther size and output of the archesporial tissue of *Oenothera* as a result of ever-changing environmental conditions.

Our results show that blooming phase probably has an impact on the size of anthers (expressed as the fresh and dry mass of anthers) and pollen produced per flower in *Oenothera*. In general, the highest values were noted at the beginning of blooming, and together with progress in the blooming period the values decreased. These relationships were true for the size of anthers in all *Oenothera* species, whereas a significant decrease in the mass of pollen produced per flower was noted only in *O. flaevingina*, *O. paradoxa*, and *O. rubricaulis*. It is notable, however, that statistical differences in the mass of produced pollen were found at the end of the blooming phase, and no variations between the beginning and full blooming phases were observed for any species. These differences in the size of anthers and pollen produced per flower could be related to different intraplant resource availability across the blooming period. Given that in the *Oenothera* species we studied the flowers are arranged within racemes, and the blooming period is relatively long (up to 57 days), a particular inflorescence bears both maturing fruits as well as developing flowers and buds. It is thus reasonable to suggest that distinct flowering pattern, resulting in a proximal-to-distal competition

for resources among developing fruits, flowers, and/or buds, may be responsible for decreasing availability of floral resources at the end the blooming phase. In fact, a similar relationship between flowering phenology and several functional floral traits, including floral resources (e.g., quantity of nectar or pollen), has been documented by several authors in other plant species (e.g., [44,45]).

Our data on mass of pollen produced per plant and per unit area are particularly important for beekeepers and for bee pasture conservation as it provides essential information regarding pollen resources. In this study, we demonstrated that pollen yield per unit area is strongly species-specific, and that the greatest amounts of pollen were recorded for *O. flaevingina* (30.6 g m⁻²) and *O. paradoxa* (24.8 g m⁻²), whereas the lowest was for *O. rubricaulis* (10.8 g m⁻²). Our results clearly show that *Oenothera* species may be considered to be good pollen-yielding plants [22]. Moreover, significant variations within a particular *Oenothera* species in the mass of pollen produced per unit area were observed between study years. These differences are probably related to the variable abundance of blooming noted in this study for plant species across growing seasons, and especially to the number of flowers produced per individual.

The protein content in pollen grains of the five *Oenothera* species was relatively low, compared to data from other plant species (e.g., [23]). It is generally accepted that protein content in pollen is a highly conservative characteristic within families, or even within particular genera (e.g., [46]). Several studies have shown that the protein content in pollen is a selective factor during insect foraging, and that this relationship applies at least to bees and bumblebees [47]. However, these correlations are not always statistically significant [23,48], and some other pollen properties (e.g., scent from the surface of pollen grains) are also considered to participate in floral visitor attraction [47,49]. Furthermore, it is still unclear as to what extent proteins from pollen grains may actually provide a nutritional value in the insect diet, since protein metabolic pathways are quite diverse among insects attracted to the flowers [50]. However, in the present study, a direct relationship between protein content in pollen grains and insect preferences could not be established, and therefore this phenomenon requires more experimental testing.

To conclude, the blooming period of *Oenothera* species studied is relatively long and the amount of pollen produced per flower and unit area is high, suggesting that these species could be considered valuable pollen yielding plants. The specific evening pattern of flower opening suggest that these plants may attract both nocturnal and diurnal insect visitors and hence support the maintenance of general insect biodiversity. Nevertheless, given the invasive potential of *Oenothera*, sustainable management and precautions are suggested in order to prevent uncontrolled spread into new areas in the case of planned cultivation and by introducing these plants into bee pastures.

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Fenologia kwitnienia i produkcja pyłku pięciu gatunków z rodzaju *Oenothera* (Onagraceae), charakteryzujących się nocną antezą

Streszczenie

Badania dotyczące fenologii kwitnienia oraz produkcji pyłku pięciu gatunków z rodzaju *Oenothera* (*O. casimiri*, *O. flaevingina*, *O. nuda*, *O. paradoxa*, *O. rubricaulis*) prowadzono w latach 2013–2015 na terenie Lublina, Polska południowo-wschodnia. Początek kwitnienia obserwowano w połowie lub pod koniec czerwca, kwitnienie było stosunkowo długie i trwało do końca lipca lub połowy sierpnia. Badane taksony cechowała nocna anteza, tzn. otwieranie kwiatów obserwowano późnym wieczorem (ok. godz. 20:00–21:00) i kwiaty żyły do godzin wczesno porannych (ok. godz. 7:00–9:00). Gatunki wytwarzały dużą liczbę kwiatów przypadającą na jednego osobnika oraz na jednostkę powierzchni (odpowiednio średnio 158,2 oraz 4163,5/m²). Wykazano, że faza kwitnienia wpływa na masę pylników oraz na masę pyłku produkowanego w kwiecie. Największą masę pylników i pyłku obserwowano na początku kwitnienia, zaś wraz z postępem kwitnienia masa pylników i produkowanego pyłku spadała; jednakże statystycznie istotne różnice zanotowano wyłącznie w przypadku *O. flaevingina*, *O. paradoxa* i *O. rubricaulis*. Wydajność pyłkowa była cechą gatunkową, związaną z obfitością kwitnienia. Największą masę pyłku dostarczał *O. flaevingina* (30,6 g/m²), niemal trzykrotnie więcej niż wyniosła wydajność pyłkowa *O. rubricaulis* (10,9 g/m²). Zawartość białka w pyłku była stosunkowo niska i przeciętnie wyniosła 15,4%. Badane gatunki mogą być uznane za wartościowe źródło pyłku. Niemniej jednak, z uwagi na wysoki potencjał inwazyjny gatunków z podsekcji *Oenothera* oraz aby przeciwdziałać niekontrolowanemu rozprzestrzenianiu tych taksonów do nowych siedlisk, należy zachować środki ostrożności podczas ich uprawy i ewentualnego podsiewania na pastwiskach pszczelich.