FLOWERS ECOLOGY AND POLLEN OUTPUT OF *Symphytum officinale* L.

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Received 17 October 2008; accepted 03 November 2008

S u m m a r y

Observations and measurements of *Symphytum officinale* L. were taken in meadows of the Lublin area, in the district of Sławin (51°18' N and 21°32' E) in the years 2006-2007. The surface of the corolla is densely covered with non-glandular and a few glandular trichomes with lipids. The scales situated between stamen heads are densely covered by one-cell trichomes. The apical part of the hair cells is rich in lipids. The inflorescence of *Symphytum officinale* consists of 14.65 flowers on average, with 2-7 open at any one time. The mean mass of pollen delivered was 9.87 mg per 100 anthers. The mean mass of pollen produced in anthers averaged 27.15% of the total dry anther weight. The amount of pollen delivered per inflorescence was 9.26 mg. The estimated magnitude of pollen flow in successive flowering stages differs and is 0.17 kg per 1 ha in the initial phase of blooming. At full bloom *Symphytum officinale* yield from 6.50 to 17.64 kg per 1 ha of the community. Towards the end of the flowering period the estimated pollen flow is 2.95 kg per 1 ha, on average. The dominant pollinators of *Symphytum officinale* were different *Bombus* spp. The taxon is the spring source of nectar and pollen. Besides sugars and proteins, it is highly probable that lipids are important attractants. *Symphytum officinale* should be also considered as a supplementary source of food for *Apis mellifera.* Honeybees mainly collect nectar using the holes made by bumblebees in the base of the corolla. A direct access to the flow, however, is often used by *Apis mellifera* or *Bombus* spp.

Keywords: blooming, flower morphology, pollen production, *Symphytum officinale*.

INTRODUCTION

Comfrey (*Symphytum officinale* L.) is a herbaceous perennial plant, native to Europe and Asia. In Poland the taxon commonly occurs in various regions. It is associated with the *Molinio-Arrhenetheretea* class. It predominantly grows in meadows mainly on moist, fertile soil which has a high water level. It can also be found in damp habitats beside rivers (Zarzycki et al. 2002). Although comfrey has been used as a food crop, in the past 20-30 years, scientific studies reported that the plant may be carcinogenic. It appears to cause liver damage and tumors in rats (Sadowska 2004). Pyrrolizidine alkaloids are present mainly in the roots. The herb may still be used but for external purposes only (Sanderski 2004).

Symphytum officinale L. is a self-incompatible species and is known as a forage plant (Rawski 1947, Ostrowska 1981, Lipiński 1982, Koter 1984, Goulson et al. 1998, Stpiczyńska and Masierowska 2004). Nectar secretion and nectar chemistry were described by Stpiczyńska (2003). The nectar is sucrose predominated with smaller

amounts of fructose and glucose. The nectar contains low amounts of amino acids. In the Ukraine the general sugar efficiency was estimated to be 300 kg per 1 ha (Bodnarczuk et al. 1993), while in Poland it was 100 kg per 1 ha (Kołtowski 2006). Flowers attract pollinators usually by nectar and pollen but some offer specialized lipids (Buchmann 1987). Pollen has two main functions in ecosystem: 1) It is essential for pollination thus the reproduction of plants and 2) it serves as food for pollinators. Palinological analysis identified comfrey as a food plant for *Bombus terrestris* (Teper 2004). Pollen grains were also found in different bee products e.g. honey, beebread (Demianowicz and Warakomska 1973, Warakomska 1985). This confirms that the species is a source of pollen for *Apis mellifera*.

The aim of the study was to determine the pollen production as well as to describe some morphological traits of the flower. Additionally I determined the pattern of insect visits to *Symphytum officinale*.

MATERIAL AND METHODS

Observations and measurements of *Symphytum officinale* L. were taken in the meadows of the Lublin area (51°18'N and 21°32'E) which is in the district of Sławin. The study was done in the years 2006-2007. All observations on flowering biology and insect forages were done according to the methods of Jabłoński and Szklanowska (1997). The date and duration of blooming was recorded. The pattern of insect visits was observed in the initial phase and full phase of blooming. Some features of flowers morphology were observed using the stereo-microscope and light microscope (LM Nikon Eclipse E-200). Analyses referred to different flower structures. Some scale sections were tested for lipids and starch using the Sudan III solution and Lugol solution, respectively.

The average number of flowers which develop in the lifetime of a plant, was determined from counts on 30 individuals, the number of flowers per cymes, and the number of inflorescences per plant. The population densities were determined on the basis of 30 random circular areas 0.1 m^2 (36.7 cm in diameter). The total number of flowers was calculated by multiplying the number of plant individuals with the average number of flowers per plant. This was then converted to number of flowers per 1 m^2.

In order to rate the abundance of pollen production, the method of Warakomska (1972) as modified by Szklanowska (1995) was used. Well developed flower buds were collected. The stamen heads were dissected and placed in tarred vessels (n = 100) prepared for this purpose. Measurements were done in four replications. Anthers were assayed for fresh and dry matter and for the mass of pollen delivered. Pollen was taken out of anthers with ether and ethyl alcohol. Pollen viability was tested using standard acetocarmine-stained slides.

Observations of insect intensity were carried out on 1 m^2 plots with five replications in the full blooming period. They were carried out for three days between 8 and 10 June in 2006 and between 26 and 28 May in 2007. The number of working pollinators were counted every other hour from 7.00 h to 20.00 h (EET).

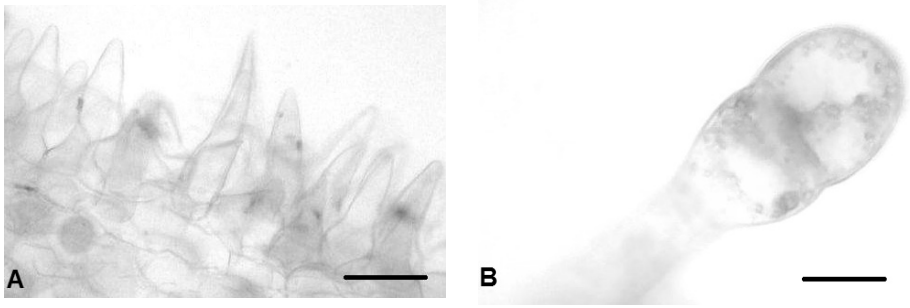
The results were analyzed statistically using one-way ANOVA. Duncan's test at a significance level α=0.05 was used to measure the significance of differences between means.

RESULTS

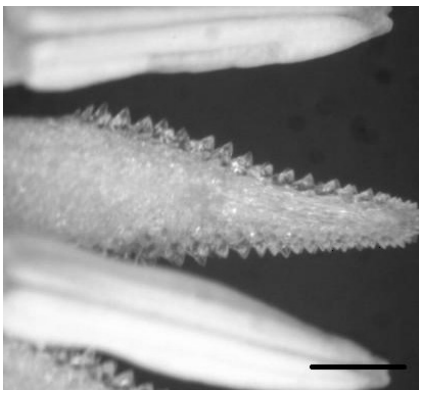
In south-eastern Poland the flowering of *Symphytum officinale* started in late May in 2006 and in the beginning of May in 2007. The full blooming phase lasted approx. 3-4 weeks. The fall of flowering took place in late July (2007) and in the middle of July (2006).

The bell-shaped flowers with pedicels are arranged in cymes. Bisexual flowers are purple at the bud stage and become light purple while the corolla is opened. The flower development from bud stage till corolla opening lasts approx. 6-7 hours. The corolla has five sepals and is connate at the base. It has five stamens inserted midway down the corolla tube and five scales. The surface of the corolla is densely covered by non-glandular, one-cellular trichomes sharp at the tip (Fot. 1A). These trichomes are 68-180 μm in length. A few glandular trichomes are present as well (Fot. 1B). These are multicellular with a roundish head at the top and stains with Sudan III.

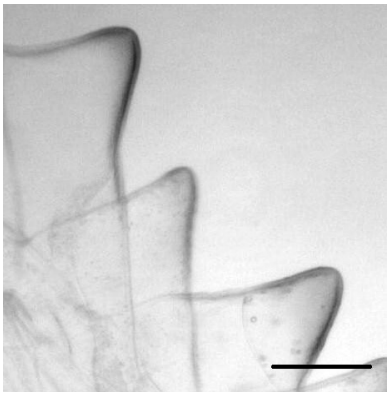
The scales are white and are situated between stamen heads (Fot. 2). The adaxial surface of scales is covered by densely arranged one-cell trichomes (Fot. 3). The apical part of the hair cells is rich in lipids which are stained with Sudan III. The reaction for Sudan III depends on the stage of blooming. In bud stage the concentration of lipids is only in the apical part of the cells. In the first to the second day of anthesis, the concentration of lipids is



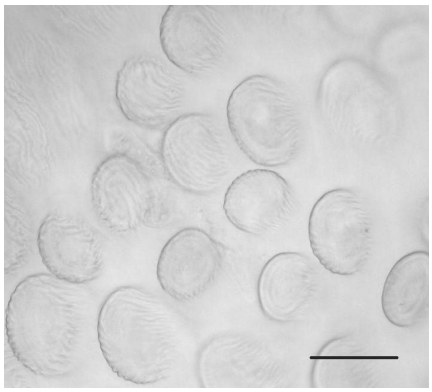
Fot. 1. The A- non-glandular trichomes and B -glandular trichomes on the adaxial surface of *Symphytum officinale* corolla Bar = 30 μm - A; Bar = 50 μm- B.



Fot. 2. The inside of a corolla tube with anthers before pollen exposure, also the crystal shining scales with densely arranged. trichomes



Fot. 3. Lipids stained with Sudan III accumulated in the apical part of scale cells. Bar = 30 μm.



Fot. 4. The papillate stigma surface covered with bubble cells. Bar = 60 μm

higher. Treatment with IKI does not indicate the presence of starch. The single style is two lobed with the papillate surface covered by bubble cells (Fot. 4). Under weather conditions ca. 20°C and 50-60% rh the flowers remain open for 2 days. In colder weather (< 18°C and 65-70% rh) the life span was 3-4 days.

The abundance of blooming depended on the season (Table 1). The inflorescence of *Symphytum officinale* consists of a mean of 14.65 flowers, with 2-7 open at any one time. The estimated number of flowers per plant was 160.45 and the number of flowers arranged per plant was 35% lower in 2006 then in 2007. The number of flowers per 1 m^2 varied from 1.02 thousands to 4.06 thousands and averaged 2.08 thousands in 2006. In 2007 the mean number of flowers was 4.76 thousands per 1 m^2 and varied from 0.58 thousand to 13.7 thousands.

The fresh and dry mass of anthers were statistically higher in 2007 then in 2006 (Table 2). The anthers release pollen simultaneously and very rapidly. During sunny, dry weather pollen is released within a few hours. Usually with pollen exposure the anther tissue changes colour from white to brown. The mean fresh mass of 100 anthers was 110.4 mg, and the hydration of anther tissues was from 66.5 to 67.5%. The mean dry mass was 36.3 mg per 100 anthers. The mean mass of pollen delivered was 9.97 mg per 100 anthers and was significantly higher in 2007. The weather conditions modified the efficiency of the anther tissue, and differences of mass of pollen delivered between years were found. The mass of pollen produced in anthers averaged 27.15% of the total dry anther weight. The pollen viability was very high and averaged 98.9%. Pollen output varied substantially with flowering abundance (Table 3). The amount of pollen delivered was 6.21 mg per inflorescence in 2006 and was twice as high in 2007. The estimated magnitude of pollen flow in successive flowering stages differed and was 0.17 kg per 1 ha in the initial phase of

Table 1.

The abundance of *Symphytum officinale* blooming in 2006-2007 in Lublin, Poland.

Year of study	Number of stems/m^2			Number of flowers per					
				inflorescence		plant		1 m^2 (thousands)	
	min – max	mean	±Sd	mean	±Sd	mean	±Sd	min – max	mean
2006	8 – 32	16.3a	±4.2	13.6a	±3.2	127.4 a	±21.4	1.02 – 4.06	2.08
2007	3 – 71	24.6b	±8.3	15.7a	±4.1	193.5 b	±42.5	0.58 – 13.74	4.76
mean		20.45		14.65		160.45			3.42

Means within a column followed by the same letters do not differ significantly at α= 0.05.

blooming (Fig. 1). At full bloom *Symphytum officinale* yield from 6.50 to 17.64 kg per 1 ha of growth. Towards the end of the flowering period the estimated pollen flow was 2.95 kg per 1 ha on average.

The intensity of pollinators differed between years and was higher in 2007 than in 2006. The mean number of working pollinators in the full blooming period was 4.3 per 1 m^2 in 2006 and 7.2 per 1 m^2 in 2007. The dominant pollinators of *Symphytum officinale* were different *Bombus* spp. (especially *B. terrestris*). During the day the peak activity of *Bombus* spp. was in the morning hours

Table 2.

The fresh and dry mass of anthers and the mass of pollen in anthers of *Symphytum officinale* in 2006-2007 in Lublin, Poland.

Year of study	Mass of 100 anthers (mg)				% H_2O	Mass of pollen per 100 anthers			Viability %
	Fresh		Dry			mg		%	
	mean	±Sd	mean	±Sd		mean	±Sd		
2006	108.5_a	±12.6	35.2_a	±3.1	67.6	9.1_a	±1.8	25.96	98.3
2007	112.3_b	±11.9	37.4_a	±3.7	66.7	10.6_b	±2.4	28.34	99.6
mean	110.4		36.3		67.15	9.87		27.15	98.9

Means within a column followed by the same letters do not differ significantly at α= 0.05.

Table 3.

The pollen production of *Symphytum officinale* in 2006-2007 in Lublin, Poland.

Year of study	Mass of pollen delivered per		
	10 flowers (mg)	Inflorescence (mg)	m^2 (g)
2006	4.57	6.21	0.95
2007	5.30	12.32	2.52
mean	4.93	9.26	1.74

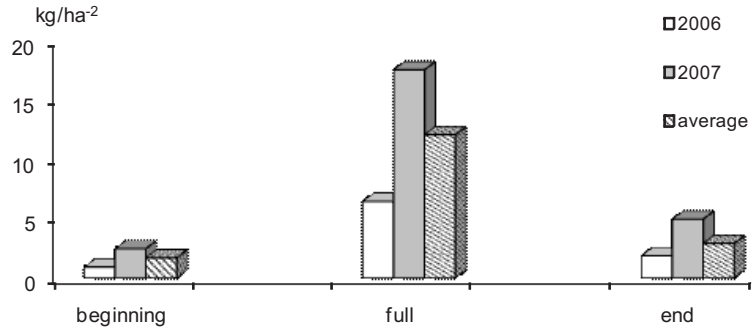


Fig. 1. The mass of pollen delivered in kg per 1 ha in succesive stages of blooming of patches with *Symphytum officinale* in years 2006-2007 in Lublin.

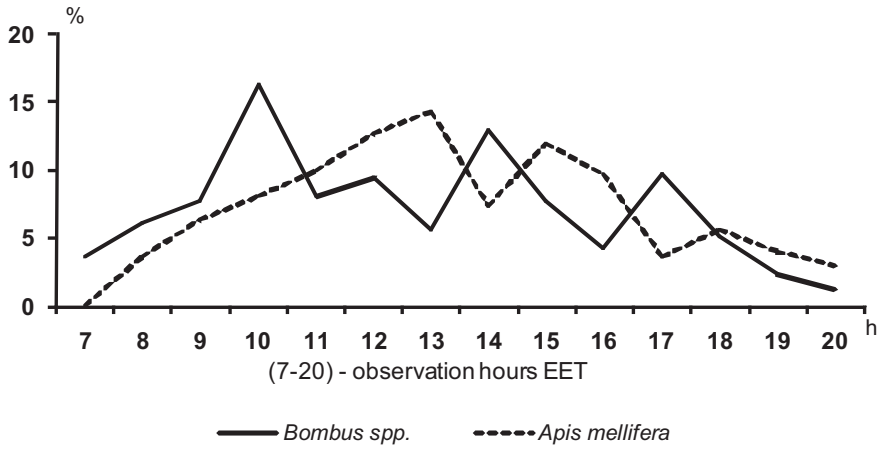


Fig. 2. Diurnal dynamics of forage intensity of *Bombus* spp. and *Apis mellifera* on *Symphytum officinale* in Lublin (means from 2006-2007). The mean number of insects shown in % of the total number of insects foraged during the whole day. Means are calculated for m^2 in 1 h intervals.

(7:00-10:00). Between 10:00 and 12:00 a considerable decrease in activity was found (Fig. 2). The next two peaks were created in early and late afternoon (14.00-15.00 and 17.00-18.00). *Apis mellifera* was active from about 8:00 till the afternoon, with a peak around midday. Honeybee activity increased substantially when bumblebee activity decreased. Short-tongued species (e.g. *Apis mellifera*) used holes nibbled by bumblebees in the base of the corolla to obtain nectar, but direct access to the corolla was used as well by bumblebees or honeybees.

DISCUSSION

The effective flowering of *Symphytum officinale* occurs from third decade of May till the middle of June and lasts approx. 4 weeks. That is a week shorter then established by Ostrowska (1981) for northern Poland. The abundance of flowering in a natural environment community, is very changeable between seasons. The influence of weather conditions for different aspects of flowering was described by Jabłoński and Szklanowska (1997) for many other species.

The glands containing lipids were found in flowers of *Symphytum officinale*. These highly specialized oil-secreting organs are present in a few families e.g Orchidaceae and Scrophulariaceae (Buchmann 1987). The structure of the gland and its density on the surface of the corolla as well as its oil secreting function, may be an adaptive trait to pollination by specific groups of insects. In *Symphytum officinale* lipids were present mainly in glandular trichomes on the surface of the corolla and in the cells of scales. In botany literature scales are described as morphological elements protecting nectar. The lipids found in the scales probably react as a visible attractant. It may be recognized easily by the omatidia because it is shiny. The oils content may also act as an additional flower reward helping the gene flow. This factor helps to increase the probability of effective pollination, mainly in flowers with an untypical corolla.

The corolla of *Symphytum officinale* is deep and the energy cost of lipid

production is compensated for by the higher chance of pollinator visits.

The main pollinators of *Symphytum officinale* were bumblebees. The number of bumblebees visiting *Symphytum officinalle* varied significantly between seasons within the study area. This may be the result of the different density of nests and the colony abundance of each year. The other possible reason is abundant flowering of neighboring plants (e.g *Polygonum bistorta*). The differences in number of visits between initial and full phase, tend to be connected with the floral display and with reduction of travel cost between flowers. Furthermore, the average size of bumblebee colonies change throughout the season and also could affect the forage abundance. The access to flower reward in *Symphytum officinale* is in the conventional manner and by holes nibbled in the base of the corolla. Every pollinator species is known to take advantage of these holes as a pathway, despite the proboscis longevity. The phenomena was described widely in many species with deep corolla tubes. It may have negative implications for the reproductive success of the species as the "robber" omits the effective pollination path (Faegri, van der Pijl 1979, Kołtowski 2004). In the *Symphytum officinale* long-tongued as well as short-tongued species (e.g. *Apis mellifera*) use holes but also use direct access to the corolla, thus pollination is not excluded.

Significant differences in the mass of pollen delivered within the studied years confirm that the feature is dependent on external factors (Szklanowska 1995). The averaged mass of pollen delivered by 10 flowers of *Symphytum officinale* is higher then established by Koter (1984). The possible reason for the differences is the method applied in the study. The efficiency of archesporial tissue averaged 27.15% and was lower than established by Szklanowska (1995) for the Ranunculaceae family. At full bloom *Symphytum officinale* yield from 6.0 to 17.7 kg per 1 ha of the growth community. That is lower than meadow *Caltha palustris* yield (Szklanowska 1995). *Symphytum officinale* should be considered as a supplementary source of food for *Apis mellifera.* The taxon is also a spring source of sugars, proteins, and probably lipids, for different *Bombus* spp. As assumed by different authors (e.g. Corbet 2000, Goulson 2003, Denisow 2006) every taxon in the natural environment should be considered as indirectly benefiting pollination.

CONCLUSIONS

— *Symphytum officinale* attract pollinators by nectar, pollen and the lipids present in scales.
— The mean mass of pollen delivered by 100 anthers was 9.87 mg and significantly varied according to abiotic factors. The efficiency of archesporial tissues averaged 27.15%.
— The mean pollen efficiency fluctuated in the years of the study. According to the blooming phase it was 0.17 kg per 1 ha in the initial phase of blooming, 6.50 to 17.64 kg per 1 ha in the full bloom phase and 2.95 kg per 1 ha towards the end of the flowering period.
— Pollen grains of *Symphytum officinale* are characterized by high viability.
— The dominant pollinators of *Symphytum officinale* are *Bombus* spp. taxon is also a supplementary source of flow for *Apis mellifera*.

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CECHY EKOLOGICZNE KWIATÓW I POŻYTEK PYŁKOWY *Symphytum officinale* L.

D e n i s o w B .

S t r e s z c z e n i e

Obserwacje i pomiary prowadzono w latach 2006-2007 w Lublinie, w dzielnicy Sławin (51°18'N and 21°32'E). Korona *Symphytum officinale* L. jest pokryta włoskami mechanicznymi oraz nielicznymi włoskami o charakterze wydzielniczym. Osklepki gęsto pokryte są jednokomórkowymi włoskami zawierającymi substancje o charakterze lipidów, które zgromadzone są w szczytowej części komórek. Kwiatostany *Symphytum officinale* zawierają przeciętnie 14,65 kwiatów, z których jednocześnie kwitnie 2 - 7. Przeciętna masa pyłku wytwarzanego w 100 pylnikach wynosi 9,87 mg, co stanowi 27,15% suchej masy pylników. Jeden kwiatostan dostarcza średnio 9,26 mg pyłku. Oszacowana wydajność pyłkowa *Symphytum officinale* w środowisku naturalnym, w poszczególnych fazach kwitnienia, wynosi 0,17 kg z 1 ha w początkowej fazie od 6,50 do 17,64 kg z 1 ha w okresie pełni kwitnienia oraz 2,95 kg w końcowej fazie. Głównymi zapylaczami kwiatów są różne gatunki z rodzaju *Bombus,* które wykorzystują zarówno nektar jak i pyłek. Atraktantem w kwiatach poza węglowodanami i białkami są również lipidy. *Symphytum officinale* można uznać za uzupełniające źródło pożytku dla pszczoły miodnej.

Słowa kluczowe: kwitnienie, morfologia kwiatów, wydajność pyłkowa, *Symphytum officinale*.