Pollen quality and pollen productivity of blue honeysuckle species and varieties

M S Yamburov, A S Prokopyev, S A Suchkova, S B Romanova, O D Chernova, T N Kataeva and T Z Abzaltdenov

National Research Tomsk State University, 36 Lenin ave., Tomsk, 634050, Russia

E-mail: yamburov@mail.ru

Abstract. Studies to assess the pollen quality and pollen productivity of blue honeysuckle were conducted on the ecosystem dendrological territory of the Siberian Botanical garden of TSU (Tomsk). Objects of research: 8 varieties and 4 species of blue honeysuckle – ‘Velvet’, ‘Berel’, ‘Vasyuganskaya’, ‘Zolushka’, ‘Lazurnaya’, ‘Ogneny Opal’, ‘Selena’, ‘Tomichka’, Lonicera altaica, L. edulis, L. kantschatica, L. turczaninovii. It was found that the ‘Ogneny Opal’ and ‘Velvet’ varieties have low fertility, and the ‘Berel’ and L. edulis have an average fertility. Other varieties and species of honeysuckle have high pollen fertility. The viability of pollen with high values is more than 60 % – ‘Berel’, ‘Vasyuganskaya’, ‘Lazurnaya’, L. turczaninovii, L. kantschatica, with average values from 40% to 60 % – ‘Selena’, ‘Tomichka’ and L. altaica, with low values – less than 40 % – ‘Velvet’, ‘Zolushka’, ‘Ogneny Opal’ and L. edulis. High pollen productivity – more than 20,000 pollen grains per flower – ‘Tomichka’, L. kantschatica, L. turczaninovii and L. edulis, average productivity - from 10,000 to 20,000 pollen grains – ‘Berel’, ‘Vasyuganskaya’, ‘Zolushka’, ‘Lazurnaya’, ‘Selena’ and L. altaica, low productivity – less than 10,000 pollen grains per flower – ‘Velvet’, ‘Ogneny Opal’. It is recommended to use at least 10% of varieties with high pollen viability and pollen productivity as pollinators when creating industrial honeysuckle plantations: ‘Lazurnaya’, ‘Vasyuganskaya’, and ‘Berel’.

1. Introduction

It was found that the features of the structure and physiology of the flower are characteristic of cross-pollinated plants in the terms of reproductive biology studies of the honeysuckle species and varieties in the Tomsk region. The fruit setability in natural and artificial self-pollination is low, while cross-pollination is high in intraspecific, interspecific, and free pollination (Gidzyuk, 1978; 1981).

Experiments at the Moscow state University Botanical garden and the main Tsitsin Botanical garden of the Russian Academy of Sciences have shown that honeysuckle is strictly cross-pollinated: the stigma in protogynous flowers is shown much earlier than the Bud and anthers of the same flower open, which indicates that it is adapted to pollination with another pollen (Skvortsov and Kuklina, 2002).

The study of blue honeysuckle varieties (Lonicera caerulea L.) biological features of productivity formation was carried out in 1994-2004 in the Central Siberian Botanical garden SB RAS (Boyarskikh, 2017). It was found that pollen fertility in blue honeysuckle varieties of different ecological and geographical origin varies significantly. Low fertility and very weak pollen production are typical for some representatives of L. caerulea subsp. altaica from the Ore Altai. It was found that the use of varieties with a high content of sterile pollen as pollinators leads to a decrease in fruit setability and a decrease in their mass. All the studied varieties of L. caerulea were self-fertile. Free pollination in the
forest-steppe zone conditions of the South of Western Siberia provided an average of 38-64% of fruit set during the years of research. A decrease in fruit setting by more than 50% and reduction in fruit weight and seed productivity compared to free pollination were revealed when crossing closely related varieties of blue honeysuckle. Similar anomalies in the growth of pistil tissues pollen tubes were observed in the variants of crossing closely related blue honeysuckle varieties and autogamy, which were apparently related to gametophytic control of self-incompatibility. Varieties were selected to ensure more productive pollination when grown together (Boyarskikh, 2017).

The Central Siberian Botanical garden SB RAS also conducted detailed studies of the reproductive biology of L. caerulea in various ecological and geographical conditions (Kulikova, 2017). L. caerulea introducers of Kamchatka and Primorye origin in the conditions of right-bank forest-steppe of the Ob region form high-fertile pollen; low-fertile and sterile forms were found among plants from the Rudny Altai, as well as in their offspring. Larger fruits are formed under the influence of complex anomalies associated with active tectonic processes in micro populations of L. caerulea subsp. altaica, more seeds are set in the fruits, which have greater germination and germination energy compared to plants growing in the background (Kulikova, 2017).

Pollen productivity and pollen morphology in the varieties ‘Atut’ and ‘Duet’ (L. kamtschatica (Sevast.) Pojark) were studied in 2004-2006 at the experimental farm of the Felin agricultural University in Lublin (Bożek, 2007). It was found that 10 flowers of both studied varieties produced 11.42 mg of pollen, and the average pollen yield per 1 ha of a perennial plantation was 30.04 kg. Pollen from the observed plants was readily collected by honeybees. It was found that pollen grains of both varieties are almost flattened. They can be described as large considering their size ($P = 47.55$ microns, $E = 60.37$ microns). Pollen viability for both varieties is high, about 95% (Bożek, 2007).

The study shows that, despite the very high pollen viability (on average 90%), the percentage of pollen germination was very low and ranged from 3.3 to 34.5%. It was found that the varieties differed significantly in the number of pollen grains in flowers when evaluating pollen productivity. The ‘Morena’ variety had the highest number of pollen grains (45375), while the ‘Fialka’ variety had the lowest number (2750). Observations of the growth of pollen tubes after open (cross) pollination and self-pollination allowed us to calculate the pollen germination index (PGI), used to assess the fertility of varieties. It was found that only the ‘Doch Velikana’ variety showed a PGI > 2 out of 40 varieties studied, which confirms the trend towards self-fertilization of this variety (Bieniasz, 2019).

The purpose of these studies was to determine the pollen quality and pollen productivity of blue honeysuckle and select varieties for effective pollination of industrial plants.

2. Research objects and methods

Samples were collected to study the quality and quantity of pollen productivity of blue honeysuckle species and varieties on the ecosystem dendrological territory of the Siberian Botanical garden of Tomsk State University (Tomsk, Russia).

Objects of research were 8 varieties and 4 species of blue honeysuckle – ‘Velvet’, ‘Berel’, ‘Vasyuganskaya’, ‘Zolushka’, ‘Lazurnaya’, ‘Ogreny Opal’, ‘Selena’, ‘Tomichka’, Lonicera altaica, L. edulis, L. kamtschatica, L. turczaninovii.

Pollen fertility was determined by histochemical reaction to the aceto-orcein dye. Fully expanded anthers were used for analysis. At least 300 pollen grains of each species were analyzed to determine fertility. Freshly collected pollen grains were placed on a slide and stained with aceto-orcein, covered with a slide after 3-5 minutes and examined under a light microscope at 900x magnification. Fertile pollen was colored carmine red, while sterile pollen remained unpainted (Singh, 2002).

The study of pollen seed germination was carried out according to the method of D. A. Trankovskiy (Barykina et al., 2004). The basis of the nutrient medium is a 1% agar-agar solution with the addition of sucrose of various concentrations (1, 5, 10, 15, 20, 30%). Mineral salts were used as additional components of the medium according to the Brewbaker and Kwack method in the following concentrations: $H_3BO_3$ – 0.01 %; $Ca(NO_3)_2$ • 4$H_2$O – 0.03 %; $MgSO_4$ • 7$H_2$O – 0.02 %; $KNO_3$ – 0.01 %.
(Brewbaker, Kwack, 1963). Pollen was considered to be sprouted if the size of the pollen tube exceeded the diameter of the pollen grain.

The number of pollen grains was calculated using a Goryaev hemocytometer-chamber (MiniMed, Russia) and the method of determining the amount of pollen in a known volume of A. Godini liquid (Godini, 1981) with our modification (Yamburov et al., 2014).

3. Results

In terms of pollen fertility, the studied varieties and species of honeysuckle can be divided into 3 groups: 1) with high values – fertility is above 90%, 2) medium values – fertility is from 70% to 90%, and 3) low – fertility is less than 70%. 2 varieties have low fertility: ‘Ogneny Opal’ and ‘Velvet’, while ‘Berel’ and edible honeysuckle (L. edulis) have medium fertility. All other studied varieties and species of honeysuckle have high pollen fertility (table 1).

Table 1. Indicators of the male varieties and species of honeysuckle reproductive sphere.

| Varieties and species | Pollen fertility, % | Pollen viability, % | Number of pollen grains in a flower (in 5 anthers), pcs. |
|-----------------------|---------------------|---------------------|----------------------------------------------------------|
| ‘Velvet’              | 95.5                | 29.2                | 4570 ± 2647                                              |
| ‘Berel’               | 70.0                | 61.7                | 17014 ± 7160                                             |
| ‘Vasyuganskaya’       | 97.5                | 64.1                | 12032 ± 4524                                             |
| ‘Zolushka’            | 96.7                | 37.5                | 18187 ± 3426                                             |
| ‘Lazurnaya’           | 97.4                | 76.0                | 11555 ± 2343                                             |
| ‘Ogneny Opal’         | 61.8                | 18.4                | 4183 ± 1315                                              |
| ‘Selena’              | 19.5                | 53.1                | 10444 ± 1520                                             |
| ‘Tomichka’            | 91.3                | 51.0                | 23449 ± 4586                                             |
| Lonicera altaica      | 92.9                | 44.3                | 15069 ± 1878                                             |
| L. edulis             | 87.9                | 30.2                | 21028 ± 4633                                             |
| L. kamtschatica       | 98.2                | 70.0                | 21563 ± 1954                                             |
| L. turczaninovii      | 93.1                | 77.9                | 21148 ± 5600                                             |

Pollen viability (the ability to germinate, forming a pollen tube), as well as fertility, can be divided into 3 groups: 1) with high values – above 60 % (‘Berel’, ‘Vasyuganskaya’, ‘Lazurnaya’, L. turczaninovii, L. kamtschatica), 2) with average values from 40 % to 60 % (‘Selena’, ‘Tomichka’ and L. altaica), 3) low values – less than 40 % (‘Velvet’, ‘Zolushka’, ‘Ogneny Opal’ and L. edulis) (figure 1 and 2, table 1). Low pollen viability indicates that these varieties and species have problems in the development of pollen grains at the later stages of microsporogenesis and this may be both a specific characteristic of the variety and the response of the species or variety to adverse factors (higher sensitivity), for example, to a lack of soil moisture.
Pollen productivity differs significantly in varieties and species - from 4.5 thousand to 23.4 thousand pollen grains in 1 flower (in 5 anthers). The studied varieties and species of honeysuckle are divided into 3 groups: 1) high pollen productivity – more than 20,000 pollen grains per flower (‘Tomichka’, L. kamschatka, L. turczaninovii, L. edulis), 2) average productivity – from 10,000 to 20,000 pollen grains (‘Berel’, ‘Vasyuganskaya’, ‘Zolushka’, ‘Lazurnaya’, ‘Selena’ and L. altaica), 3) low productivity – less than 10,000 pollen grains per flower (‘Velvet’, ‘Ognyeny Opal’).

The study showed that promising pollinating varieties for the creation of industrial honeysuckle plantations can be varieties with high and medium pollen productivity, and high fertility and pollen viability according to 3 indicators (fertility, viability and pollen productivity), – ‘Berel’, ‘Vasyuganskaya’, ‘Lazurnaya’. Unpromising as pollinating varieties are varieties with low viability and productivity of pollen – ‘Velvet’, ‘Ognyeny Opal’.

Thus, it is recommended to use at least 10% of varieties with high pollen viability and pollen productivity as pollinating varieties when creating industrial plantations: ‘Lazurnaya’, ‘Vasyuganskaya’, ‘Berel’. In protective strips, one can use species of honeysuckle: L. kamschatka and L. turczaninovii which have high viability and pollen productivity.

Acknowledgments
The study was performed within the framework of a state assignment of the Ministry of Science and Higher Education of the Russian Federation (project No. 0721-2020-0019).

References
[1] Gidzyuk I K 1978 Blue-fruited garden honeysuckle (Tomsk, USSR: Publishing house of the Tomsk University)
[2] Gidzyuk I K 1981 Honeysuckle with edible fruits (Tomsk, USSR: Publishing house of the Tomsk University)
[3] Skvortsov A K and Kuklina A G 2002 Blue honeysuckle. Botanical study and prospects of culture in the middle zone of Russia (Moscow, Russia: Nauka)
[4] Boyarskikh I G 2017 Features of the blue honeysuckle Lonicera caerulea L reproductive biology Agricultural biology 52(1) 200-10
[5] Kulikova A I 2017 Features of the Lonicera caerulea S. L. reproductive biology in various ecological and geographical conditions (Novosibirsk, Russia)
[6] Božek M 2007 Pollen productivity and morphology of pollen grains in two cultivars of honeyberry (Lonicera kamschatcatica (Sevast.) Pojark.) Acta Agrobotanica 60(1) 73-7
[7] Bieniasz M, Dziedzic E and Słowiń G 2019 Biological features of flowers influence the fertility
of Lonicera spp. cultivars *Horticulture, Environment, and Biotechnology* **60**(2) 155-66

[8] Singh R J 2002 *Plant Cytogenetics. Second Edition* (Boca Raton, Florida, US: CRC Press)

[9] Barykina R P, Veselova T D, Devyatov A G, Jalilova Kh Kh, Ilina G M and Chubatova N V 2004 *Handbook of Botanical microtechnics Fundamentals and methods* (Moscow, Russia: MSU publishing house)

[10] Brewbaker J L and Kwack B H 1963 The essential role of calcium ion in pollen germination and pollen tube growth *American Journal of Botany* **50**(9) 859-65

[11] Godini A 1981 *Counting pollen grains of some almond cultivars by means of a haemocytometer. GREMPA, colloque* (Paris: CIHEAM-Options Mediterraneennes: Serie Etudes) p 83-6

[12] Yamburov M S, Astafurova T P, Zhuk K V, Romanova S B and Smolin V M 2014 The effects of drought and flood stress on pollen quality and quantity in *Clivia miniata* (Lindl.) Bosse (Amaryllidaceae) *Biomedical & Pharmacology Journal* **7**(2) 575-80