



## POLLINATION STRATEGY CHOICE FOR ALFALFA

KONSTANTIN S. ARTOKHIN<sup>1\*</sup> AND ALEXANDER N. POLTAVSKY<sup>2</sup>

<sup>1</sup>Agroliga of Russia Inc., Chehova, 71, Rostov-on-Don, Russia.

<sup>2</sup>Southern Federal University, Bolshaja Sadovaja, 105/42, Rostov-on-Don, 344006, Russia.

### AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

### ARTICLE INFORMATION

#### Reviewers:

- (1) Anonymous.
- (2) Anonymous.

*Original Research Article*

*Received 18<sup>th</sup> January 2018*

*Accepted 28<sup>th</sup> March 2018*

*Published 30<sup>th</sup> March 2018*

### ABSTRACT

The pollination strategy choice for alfalfa depends on two main factors: pollinator density in agro-ecosystems; and their faunal formation in the field. The natural occurrence of pollinators was estimated in southern Russia and found to be of sufficient quantity to achieve complete alfalfa pollination. For solitary wild bee pollinator species was shown to establish highly mobile nomadic populations in agro-ecosystems.

**Keywords:** Seed alfalfa; bee pollinators; solitary wild bees; agro-ecosystems; southern Russia; nomadic bee populations; neonicotinoids.

### 1. INTRODUCTION

Alfalfa is a cross-pollinated entomophilous plant. Its sticky and heavy pollen and flower structure obstruct self-pollination. Pollination can occur only after the forcible opening of the flower by an insect and the extrusion of the pistil and stamen from the boat. When the flower opens the pistil's stigma touches the insect's body. The stigma is scraped open and the pollen, collected by the insect from another flowers, adheres to the stigma.

Followers contact with insect's body the pistil's column strikes to the flower's sail. In an opened alfalfa flower the pistil column then tightly presses against the sail and the stamens cover the stigma from all sides. Due to this structure alfalfa flower pollination can occur only once at the moment of the

boat opening. After the strike against the sail the stigma becomes concave form and so creates a humid cell, which assists the pollen germination (Lubenets et al, 1974).

Most researchers consider that alfalfa flower to be opened only by means of a suitable pollinator [1,2]. This was demonstrated by the special experiments with alfalfa isolated from pollinators. In Russia, scientists revealed solitary bees and bumble-bees to be pollinators of alfalfa [3,4,5]. The honey bee in Russia is a noneffective pollinator of alfalfa, as it does not collect the nectar through the pharynx of the flower, but through the fissure between the paddle and the sail, without opening the flower. Although Y.A. Pesenko [6] noted 250 species of 70 genera of Apidae, most of these species are not effective pollinators. The fauna and population density of bee

\*Corresponding author: Email: artokhin@mail.ru;

species differ in different climatic zones of the Palaearctic region, and effective pollination is carried out by not more than 10 species [7,8].

In the Palaearctic the most numerous and effective pollinators of alfalfa are oligotrophy bees on Leguminosae [9,5,6]. Under nature conditions of the steppe zone of the northern Caucasus on the seed alfalfa 60 species of that solitary bees were observed [10]. Y. A. Pesenko [5] noted for the northern Caucasus 62 species of solitary bees, while V.I. Golikov [11] noted 89 species for the north-west foothills alone.

Most bee species pollinators of alfalfa make their nests in the soil, preferring sites with dense soil and thin natural vegetation, although also settle on alfalfa fields [3,5]. Bees from the Megachilidae family generally construct their nests in elevated cavities.

Assuming the established settlement of bee populations, many studies recommend creating favorable conditions for their concentration near alfalfa fields. They recommend sowing the alfalfa seeds fairly close to the natural steppe reservations in long narrow fields of not more than 5-10 hectares [12, 13].

This recommendation derives from the bees ability to collect pollen and nectar from a distance of no greater than 200 m from the nest. It is considered that for highly intensive agriculture the role of wild pollinators is decreasing, indicating the need to establish artificial rearing of a few pollinators in order to solve the pollination problem [5,6]. The ability of many bee species to create densely populated colonies is exploited for their cultivation in the USA and Canada [14,7]. In the 1980s the rearing of leafcutter bee (*Megachile rotundata*) was begun in Russia. One of the problems accompanying the introduction of this technology was that of an increase in harmful activity of nesting parasites near concentrations of bees [15,13].

Practically all studies have specified the priority of pollination but not the agrotechnology of seed alfalfa [16]. This priority based on the choice of one of two main pollination strategies: 1) application of natural populations of wild solitary bees, 2) colonization of artificially reproduced bee species. Employing of the two different species of pollinators concomitantly is impractical as they are in competition for food sources [7].

Different areas of the world employ one or the other approach, but in general the massive artificial

colonization of either the leafcutter bee or the honey bee has led to the virtual extinction of local bee fauna.

The second strategy (colonization) is preferable only in cases in which the density of natural populations of bee-pollinators and the effectiveness of their work is estimated, as insufficient. Although some studies in Russia have connected a poor harvest of alfalfa seeds with the limited role of pollinators [4], there are currently no accurate field estimates of pollinator density and no such data are available in Russian references.

In the USA this second approach has led to the total failure of exploitation of natural populations of pollinators, in favor of commercial cultivation of several species of wild bees. The leader of this movement is Dr. Karen Strickler, an adjunct instructor at the College of Western Idaho and the owner of Pollinator Paradise - a company that is dedicated to achieving effective pollination through the commercial management of native pollen bees [17,18, 19]. This tendency continues in the USA still. Wild solitary bees and honey bees are considered to be a perspective operating agents for pollination, used commercially in crop production [20]. But in other regions of the world investigators recognize a high importance of protection the wild pollinators in natural ecosystems: "The crop pollination by insects estimated approximate \$361 billion of crop production worldwide. To ensure higher crop production, it is mandatory to conserve the native pollinators, and manage wild bees and other non-hymenopteran pollinators for wider ecosystem stability and food security" [21].

The studies of bee population density in Russia can be divided into two periods: pre-quantitative and quantitative. During the pre-quantitative period relative measurements were used for the estimation of insect density. The accumulated data from different studies could be easily compared. We consider, however, that such estimations are flawed, despite appearing in every publication of this period. The flawed hypothesis regarding alfalfa pollinator deficit, which was not proved, but slowly became accepted nonetheless, arose during that period.

In this article we focus attention on the need for a correct assess of the density of bees pollinator populations in agro-ecosystems.

## 2. MATERIALS AND METHODS

There are two methods for insect density assesment in the field: 1) marking with secondary catching; 2)

population exhausting [22]. The population exhausting method is more acceptable to pollinators density estimation. It performs sequential sampling on one experimental field at constant time intervals. The appropriate sampling site for catching coefficient determination and for regular monitoring of insect density has been determined as an area of 100 m long and 2 m wide, which the collector tracks twice per each sampling effort. A sample unit comprises the number of bees caught on the site during a 10 minutes period. The absolute density of the bees is assessed on the basis of individual catch coefficients (ICC), computed from 20-30 sequential samples by asymptotic function:  $Y = A + D * 10^{-cx}$  [6], where: "Y" is number of specimens caught during one sample; "A" is the sample size, equal to the number of bees that invaded to sampling site from the surrounding sites during a sampling interval; "D" is the reduced of sample size during sampling period:  $D = X_1 - X_i$ ; "c" – constant; "x" – sample number. The individual catch coefficient is then computed according to the formula:  $ICC = 1 - 10^{-c}$ . Bees density on the sampling site calculated as:  $H = M / ICC$ ; "M" – mean sample size.

The asymptotic function is used because it is impossible to carry out an ideal exhaustion survey due to the migration of bees onto the explored site during assessment.

It is impossible to compute the ICC for bees on the alfalfa field due to the lack of the basic condition necessary for this method: the stable position of insects (no moving) during the sampling period. Consequently the data samples for ICC computing need to be collected from a small and comparatively isolated bee population, with the ICC later applied for estimation of the bee population density in the surrounding alfalfa fields.

In Russia the ICC has been computed by only two investigators to date and both obtained similar results: Y.A. Pesenko (1972) computed ICC for natural ecosystems and K.S. Artokhin [10] for agro-ecosystems.

There is also great importance to the specific phase of alfalfa blossoming. The most appropriate phase is that of mass blossoming, when the bee complex has already been completely developed (they have made their nests).

The degree of alfalfa flower pollination was defined for 10 model stems in each part of the experiment in the lower and upper layers of vegetation throughout the whole period of blossoming of alfalfa plants, every 2-3 days in the evening, when bee activity decreases. We counted the number of flowers opened

by bees and total blossoming, and determined the pollination percentage according to ratio.

Bee productiveness was estimated according to the number of flowers, opened during one minute, timed by stop-watch. The bees were then caught for species determination. Bee activity was estimated by observing the nests and noting time of absence of bees. We also noted the number of bee working days during the blossoming period.

The pollination studies in Russia were carried out in 27 administrative districts in the Rostov-on-Don region, 12 districts in the Stavropol region and 16 districts in the Krasnodar region during 1978–2017. The observations were statistically processed using regression correlation and dispersion methods (Snedecor, 1961) in "Excel".

The systematics of Apoidea were according to the: Annotated catalogue of the Hymenoptera of Russia [23].

### 3. RESULTS AND DISCUSSION

#### 3.1 Nomadic Populations of Bee Pollinators

Meteorological conditions throughout the period of investigation were variable. In the hot and dry years the number of bee pollinators increased, in one sample reaching 59 specimens. Moreover, the proportion of the mass species: *R. canus* and *M. leporina* increased too. The total fauna of solitary bees on alfalfa fields at the northern Caucasus of Russia is represented by 60 species (Table 1). Despite of the relatively high diversity of pollinator fauna on alfalfa, there were eight dominate species, while all others represent only 4 % (Table 2). All the dominant species are soil-nesting.

Throughout the alfalfa blossoming different bee species participated differently in pollination. Thus, at the end of the first blossoming of alfalfa there were numerous *R. canus*; while at the beginning of the first blossoming and the end of second one *A. flavipes* and *A. ovatula* were prominent. The bee species *A. labialis* and *E. clypeata* were active only during the first alfalfa blossoming, while the proportion of *M. clavicornis* and *M. leporina* species remained fairly constant throughout the entire blossoming period.

The duration of blossoming periods of the first and second alfalfa harvests is much shorter, than the flying period of most pollinators (Table 2). Independent of the isolation level between the fields

of the first and second blossoming, pollinator density increases during the latter.

The flying period of pollinators in southern Russia is between April and September (Table 2), but the bees appearing the alfalfa fields only during the blossoming period, which depends on the harvests in June or July (Fig. 1). The maximum density of pollinators is at the end of June and beginning of July, which coincide with the blossoming of second and intermediate harvests. The maximum density of bees in the agricultural landscape of the northern Caucasus observed in May, then gradually decreases.

Estimation of the natural bee-pollinator's density in agro-ecosystems has reveal that the highest density of bees is in alfalfa fields. In the other crops and even in the natural steppes, where the Apidae species diversity is very high, the percentage of alfalfa pollinators does not exceed 12%, with density of 2100 specimens per hectare. Moreover, the alfalfa pollinators density adjacent to forests line and virgin lands is very low – 1%; and for the entire agricultural landscape it does not exceed 20%. This disparity between the pollinator reserves at natural sites and in alfalfa fields appears to be explained by their aggregation from a wide area of the surrounding agricultural landscape.

**Table 1. Apoidea species in alfalfa agro-ecosystems of the northern Caucasus, 1978-2017**

<b>Fam. Colletidae</b>	<b>Fam. Megachilidae</b>
1. <i>Colletes marginatus</i> Smith, 1846	29. <i>Anthidium florentinum</i> (Fabricius, 1775)
Fam. Andrenidae	30. <i>Anthidiellum strigatum</i> Panzer, 1805
2. <i>Andrena wilkella</i> (Kirby, 1802)	31. <i>Osmia coerulescens</i> Linnaeus, 1758
3. <i>A. dorsata</i> (Kirby, 1802)	32. <i>O. parvula</i> (Dufour & Perris, 1840)
4. <i>A. flavipes</i> Panzer, 1799	33. <i>Archimegachile flavipes</i> Spinola, 1838
5. <i>A. labialis</i> (Kirby, 1802)	34. <i>Megachile argentata</i> (Fabricius, 1793)
6. <i>A. ovata</i> (Kirby, 1802)	35. <i>M. centuncularis</i> (Linnaeus, 1758)
7. <i>Melitturga clavicornis</i> (Latreille, 1806)	36. <i>M. maritima</i> Kirby, 1802
Fam. Halictidae	37. <i>M. versicolor</i> Smith, 1844
8. <i>Halictus asperulus</i> Pérez, 1895	38. <i>M. rotundata</i> Fabricius, 1787
9. <i>H. kessleri kessleri</i> Bramson, 1879	39. <i>M. circumcincta</i> Kirby, 1802
10. <i>H. maculatus</i> Smith, 1848	40. <i>M. ericetorum</i> Lepeletier, 1841
11. <i>H. subauratus</i> Rossi, 1792	41. <i>M. lagopoda</i> Linnaeus, 1761
12. <i>H. eurygnathus</i> Bluethgen, 1931	42. <i>Stelis breviscula</i> Nylander, 1848
13. <i>H. rubicundus</i> Christ, 1791	43. <i>Coelioxys afra</i> Lepeletiere, 1841
14. <i>Lasioglossum discum</i> (Smith, 1853)	44. <i>C. obtusa</i> Pérez, 1884
15. <i>L. malachurum</i> (Kirby, 1802)	Fam. Anthophoridae
16. <i>L. calceatum</i> (Scopoli, 1763)	45. <i>Eucera clypeata</i> Erichson, 1835
17. <i>L. albipes</i> (Fabricius, 1781)	46. <i>E. curvitaris</i> Mocsáry, 1879
18. <i>L. pauxillum</i> (Schenck, 1853).	47. <i>E. nitidiventris</i> Mocsáry, 1879
19. <i>L. brevicorne</i> (Schenck, 1869)	48. <i>E. pollinosa</i> Smith, 1854
20. <i>Sphecodes monilicornis</i> Kirby, 1802	49. <i>E. interrupta</i> Baer, 1850
21. <i>Nomiapis diversipes</i> (Latreille, 1806)	50. <i>Anthophora erschowi</i> Fedtschenko, 1875
22. <i>N. bispinosa</i> (Brulle, 1832)	51. <i>A. radoszkowskyi</i> Fedtschenko, 1875
23. <i>Rophites canus</i> (Eversmann, 1852)	52. <i>A. monacha</i> (Erichson, 1849).
24. <i>Sphecodes schenckii</i> Hagens, 1882	53. <i>Nomada fucata</i> Panzer, 1798
Fam. Melittidae	54. <i>N. stigma</i> Fabricius, 1804
25. <i>Melitta leporina</i> Panzer, 1799	55. <i>N. flavopicta</i> (Kirby, 1802)
Fam. Apidae	56. <i>N. zonata</i> Panzer, 1798
26. <i>Bombus sylvarum</i> Linnaeus, 1761	57. <i>Ammobatoides abdominalis</i> (Eversmann, 1852)
27. <i>B. terrestris</i> Linnaeus, 1758	58. <i>Tetraloniella dentata</i> (Germar, 1839)
28. <i>Apis mellifera</i> Linnaeus, 1758	59. <i>Amegilla magnilabris</i> (Fedtschenko, 1875)
	60. <i>A. quadrifasciata</i> (de Villers, 1789)

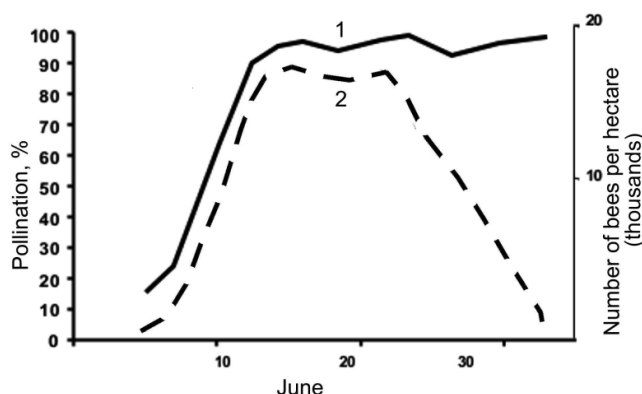


Fig. 1. Dynamics of pollination (1) and bees density (2) on the first year field and yield of alfalfa seeds in the first harvest of alfalfa (Rostov-on-Don region, 1983) [24]

Table 2. Dominate species of alfalfa pollinators at the Rostov-on-Don region in XX-XXI

Family	Species	% of total bee number on alfalfa fields		Flying period (months)
		1978-2000	2001-2017	
Halictidae	<i>Halictus eurygnathus</i>	8	15	V – IX
	<i>Halictus malachurum</i>	3	6	IV – IX
	<i>Rophites canus</i>	37	2	VI – VII
Andrenidae	<i>Andrena flavipes</i>	18	27	IV – IX
	<i>Andrena ovatula</i>	7	13	IV – VIII
	<i>Melitturga clavicornis</i>	7	14	VI – VIII
Melittidae	<i>Melitta leporina</i>	10	12	VI – VIII
Anthophoridae	<i>Eucera clypeata</i>	2	7	V – VIII

The high density of pollinators on the first year alfalfa, far remote from natural sites, is typical for the northern Caucasus and has been explained as due to the migration ability of alfalfa pollinators. Bee migration activity is clearly discernible in rice fields, where there is no blossoming vegetation closer than a few kilometers. The richest harvests of alfalfa seeds (700–1200 kg per hectare) in the south of Russia is obtained from the rice fields, where the alfalfa was sowed in the course of a crop rotation. We conclude therefore, that the distribution of seed alfalfa in agroecosystems should be determined by crop rotation, rather than locating alfalfa fields adjacent to natural nesting sites of bees.

The quantification data on bees in alfalfa fields bears a close relation to the alfalfa blossoming periods, but not to bee phenology. Although, bee activity period lasts 2–6 months (from April to September), the solitary bees are found in the alfalfa field only during the blossoming period. At the beginning of this period there are not enough bees, with their number reaching a maximum during mass blossoming, and then decreasing toward the end of blossoming (Fig. 2).

From our findings we hypothesize the existence in an agrolandscape of nomadic populations of solitary bees (Fig. 3). The behavior of this faunistic complex of wild bees, we suggest, would appear to radically change the present conception of pollinator behavior. We recommend to analyse this hypothesis for practical use at different regions of the world.

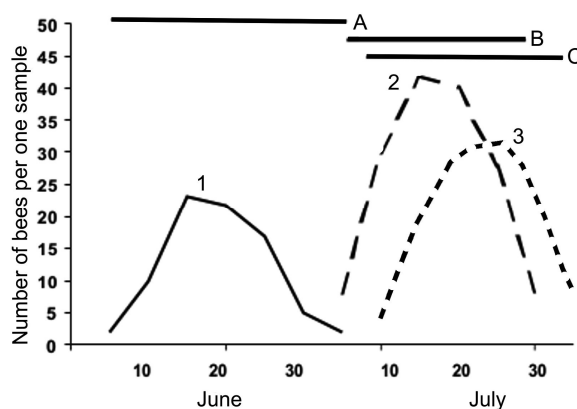
### 3.2 Effectiveness of Apidae Species as Pollinators of Alfalfa

Pollinator effectiveness is determined as the number of opened flowers depending on the following factors: 1) the speed of flower visits by bees; 2) the proportion of successfully opened flowers; 3) the length of flying activity of bees during the day; 4) the length of flying activity of bees during the blossoming period; 5) pollinator density in alfalfa fields.

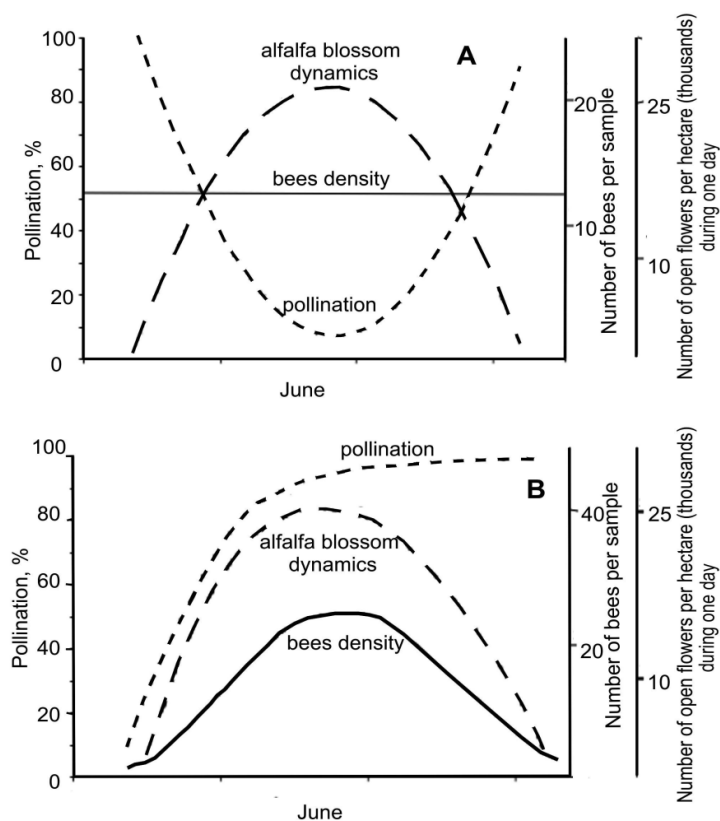
Factors 1-4 and their dependence on local geography and ecology have been well investigated. According to V.I. Zharinov (1978) – in the forest-steppe zone of Ukraine one female of *M. leporina* opens 14-18 flowers during one minute, 4-4.5 thousand flowers

during one day and 52-82 thousand of flowers throughout the period of blossoming. Female of *R. canus* pollinates 28-45 thousand of flowers throughout the season. In Poland and Hungary *R. canus* opens 7-9 flowers per minute, *A. ovatula* – 6-7,

*A. flavipes* – 6-7, *M. leporina* – 9-16, *M. clavicornis* – 15-17 (Dylewska et al., 1970; Mocsar, 1959). It has been calculated that 100% pollination of one hectare of alfalfa requires 14-15 thousand female bees [25]; in another studies – 4-5 thousands (Voloshina, 1981).



**Fig. 2.** The season dynamics of alfalfa pollinators density. First harvest (1), second harvest (2) and intermediate harvest (3). Blossoming periods: A – first harvest, B – intermediate harvest, C – Second harvest [24]



**Fig. 3.** Two models of alfalfa pollination by the natural populations of solitary bee species: A – hypothesis of settled populations (Bees density constant); B – hypothesis of nomadic populations (Experimental data) [24]

The larger bees are more effective pollinators, such as *M. clavicornis*, *M. leporina*, *E. clypeata* (Table 3). They spend less time opening the flowers. Their effectiveness depends on their actual working time, when they open the flowers and of the number flying days during the blossoming period. The latter depends on weather conditions and how the bees endure them.

In the northern Caucasus of Russia *M. leporina* displays the highest number of flying days and on windy and cloudy days it is practically the only pollinator of alfalfa, as it known for the Ukraine too [26].

### 3.3 Estimation of Pollinator Quantity

On the basis of ICC=0.1, obtained both analytically and graphically, we computed the resources of bee-pollinators on alfalfa in the seed-production farms of southern Russia (Table 4). This resulted in a reliable estimation of the bees population density.

Our analysis indicates that the bees need to pollinate about 25 million alfalfa flowers in order to obtain a yield of about 100 kg/hectare. From our data on the different bee species pollination effectiveness we

calculated the necessary number of females of each species in order to obtain such yield (Table 4). We found that the current wild pollinators of alfalfa under the conditions of a steppe zone in south of Russia are enough to provide a potential harvest of seeds to 3000 kg /hectare, which greatly exceeds that of the actual harvest in farms.

Efforts concentrated on other problems related to seed alfalfa pollination (pest control and harvesting) in Russia have increased the seed yield from 90 to 400-500 kg/hectare, and in some cases up to 1000 kg/hectare, without the introduction of *M. rotundata*.

### 3.4 Completeness of Pollination

A systematic approach requires a general consideration of the "alfalfa flower – pollinator" system. The pollination of flowers with a particular dynamics is result of their interaction. The alfalfa flower is organized in such a unique way that its pollination can be precisely and unambiguously determined by visual observation: it is pollinated or not (see Introduction). To date, however, there has been almost no use in Russia of this indication to test the hypothesis regarding a possible lack of sufficient bees in agro-ecosystems.

**Table 3. Effectiveness of alfalfa pollination by the main solitary bee species in Russia**

Species	Days	Working time during one day (hours)	Number of the tripped flowers by one female during:			Number of the tripped flowers by one female during the blossoming period (thousands)
			Minute	Hour	Day	
<i>Halictus sp.</i>	22	4,5	7	420	1890	41,58
<i>Rophites canus</i>	19	4,0	6	360	1440	27,36
<i>Andrena flavipes</i>	20	5,5	8	480	2640	52,80
<i>Andrena ovatula</i>	18	4,5	7	420	1890	34,02
<i>Melitturga clavicornis</i>	21	5,0	17	1020	5100	107,10
<i>Melitta leporina</i>	24	5,0	15	900	4500	108,00
<i>Eucera clypeata</i>	16	4,0	12	720	2880	46,08

**Table 4. Potential alfalfa yield, provided by the main pollinators in Russia, 1978-2017**

Species	Female's number to obtain 100 kg of seeds per hectare	Real number of females – result of sampling (ex./hectare)		Mean yield of seeds, provided by the real number of bees (kg/hectare)	
		1978-2000	2001-2017	1978-2000	2001-2017
<i>Halictus sp.</i>	600	1960	1200	327	200
<i>Rophites canus</i>	900	6530	230	726	25
<i>Andrena flavipes</i>	470	3220	2700	685	570
<i>Andrena ovatula</i>	730	1295	1300	177	180
<i>Melitturga clavicornis</i>	230	1270	970	552	420
<i>Melitta leporina</i>	230	1875	1200	815	520
<i>Eucera clypeata</i>	550	350	250	64	45
Total:				3346	1960

At the beginning of the alfalfa blossoming season bee density in the field is not high, and despite the small number of flowers their pollination is not higher, then 50%. Furthermore, following a sufficient arrival of bees, the dynamics of their increased number is accompanied by the dynamics of alfalfa blossoming. With the decrease in flowers in the field, the number of bees gradually decreases too.

The pollination dynamics is asymptotic. During the early alfalfa blossoming period pollination is not complete, but following mass blossoming it reaches 90% and remains at this level till the end of blossoming. It should be noted that the bees exploit all available food resources and during the second half of the blossoming season they complete their pollination of all open flowers before midday. In all cases they do not fly in the field after 18.00-19.00.

Thus, a major factor in the dynamics of pollinator numbers is correlated with food. Consequently, bees occupy seed alfalfa crops only during the blossoming phase. The key feature of pollinator fauna formation that its a mobile element in structure element of agro-ecosystem. Bees begin to occupy alfalfa fields within several days after blossoming begins. It has been shown that a distance of several kilometers is not an obstacle to the bees.

Potential yields of seeds, which could provide the natural pollinators at the northern Caucasus, much greater than produced by farmers. In the agro-ecosystem of the region the greatest density of bee pollinators was observed only in alfalfa fields.

In recent years, due to the decreasing of number of specialized farms that cultivate seed alfalfa and other herbs, the mean density of regional populations of the most important bee pollinator - *R. canus*, has also considerably decreased. At the same time, due to frequent applications of neonicotinoid insecticides against pests on grain crops, a decrease has been observed in all other species of Apoidea - pollinators of alfalfa. Concomitantly, the significance of the nomadic populations of bee-pollinators in agro-ecosystems increases due to the instability of seed production.

We consider that both natural and anthropogenic changes in pollinator fauna could lead to radical changes in the nature of the region in the structure of its agro-ecosystems and in a reduced production of entomophilous herbs.

### 3.5 Protection for Pollinators

The effect of insecticides on the fauna of pollinators presents a problem worldwide. Due to of this

problem, in 2013 insecticides of the neonicotinoid class (imidacloprid, thiacloprid, thiamethoxam, clothianidin, acetomiprid), being especially dangerous to bees, were banned in the European Union. An important aspect of neonicotinoids is that following their application to the blossoming plants they retain their toxicity to pollinators much longer than insecticides of other chemical classes – pyrethroids and organophosphates. It should also be noted that the regulation of neonicotinoid application relates only to protection for honey bees. The protection of wild solitary bees has been little discussed in depth to date.

In many regions of the world there are hundreds of bee species representing a natural biological resource of huge ecological significance. We have shown the negative effect of the neonicotinoids applied to crops, on pollinators and on the harvest of entomophilous plants. Such toxic influence affects only the fields, sprayed with neonicotinoids, but also the adjacent fields. Spraying neonicotinoids on wheat against of corn bug larvae on a farm leads the death of solitary bees, to the disappearance of pollinators in the next farms, and to a decrease in the harvest of sunflower and alfalfa (Artokhin, et. al., 2013). Only during the treatment of wheat and sunflower seeds the pollinators are not poisoned by the neonicotinoid toxin.

## 4. CONCLUSIONS

An assessment of pollinator density in southern Russia and their effectiveness indicates that there are enough bees in agro-ecosystems to enable the complete pollination of alfalfa. The pollination strategy in Russia can thus exploit the natural resources of pollinators.

The pollination strategy for alfalfa in the USA is based on a different principle: intensification of seed alfalfa production on comparatively small sites employing a technology of colonization of artificially reproduced bees and alfalfa sorts with short blossoming period. This led to a deadly competition between the expansive leafcutter bees and the wild solitary bees, up to the complete extinction of the latter in some agro-ecosystems (Strickler, Cane, 2003).

In Russia the technology of bee colonization is not well developed. Alfalfa seed production takes place extensively in large alfalfa fields (60-120 hectares), with a tendency to specialization by seed producers.

Neither in Russia nor in the USA have accurate assessments been performed of the solitary bees density in agro-ecosystems. The hypothesis of “an



initial lack of wild bees” has not been proven anywhere. On the contrary, our study has shown existence of a high density of solitary bees in agro-ecosystems in southern Russia. As “nomadic populations”, they migrate widely in the steppe regions and quickly aggregate in the fields with blossoming alfalfa. These nomadic populations guarantee the production of alfalfa seeds up to 3000 kg/hectare. The limiting factors to obtaining a high yield of alfalfa seeds in southern Russia are those of the pest complex and harvesting techniques.

Russia is one of the few countries in which the production of seeds of entomophilous herbs on the basis of natural pollinator resources and without expensive technologies of the artificial cultivation of leafcutter bees and other bee species is possible.

## ACKNOWLEDGEMENTS

The research was carried out within the framework of a State project of the Russian Federal Ministry of Science and Education #6.6222.2017/8.9: “Development of strategy, methods and technologies of preservation and rational application of biological diversity in environment of natural and urbanized territories of a steppe zone of the European part of Russia”.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Tysdal HM. Is tripping necessary for seed setting in alfalfa? Amer. Soc. Agron. J. 1940;32:570-585.
2. Zalesky A. Alfalfa investigation. 11. Population and seed settings in alfalfa strains. J. Agric. Sci. 1956;48:236-244.
3. Blagoveshenskaja NN. The main Apidae-species – pollinators of alfalfa in Uljanovsk region. Abstract of candidate dissertation. Leningrad. 1954;19. (In Russian).
4. Zharinov AI. Influence of bee’s pollination on the seeds production of alfalfa. Agricultural Biology. 1975;10(6):931-933. (In Russian).
5. Pesenko YA. About the formation of the solitary bees population on alfalfa fields. Zoological Magazine. 1976;55(6):856-859. (In Russian).
6. Pesenko YA. Alfalfa leaf cutter bee (*Megachile rotundata*) and its breeding for alfalfa pollination. Leningrad: Nauka. 1982;136. (In Russian).
7. Bohart GE. Management of wild bees for the pollination of crops. Annual Review of Entomology. 1972;17:287-312.
8. Ponomarev AN. Ecology of entomophyllous pollinations of alfalfa (*Medicago sativa*). Pollinations ecology. Perm. 1975;1:5-36. (In Russian).
9. Popov VV. Bees and their interactions with blossoming plants and the problem of alfalfa pollination. Entomological Review. 1956; 35(3):582-598. (In Russian).
10. Artokhin KS. Entomocoenose of alfalfa: Monitoring and managing. Rostov-on-Don. 2000;200. (In Russian).
11. Golikov VI. Ecological base for pollination of some field and garden cultures by Apidae at the North-West Caucasus. Krasnodar. 2000;192. (In Russian).
12. Zinchenko BS. The ways for rational application of pollinators on seed alfalfa. Selection and seeds production. Republican Scientific Collection. 1977;37:72-76. (In Russian).
13. Zinchenko BS. Enemies of alfalfa pollinators. Apiculture. 1981;10:29. (In Russian).
14. Stephen WP, Bohart GE, Torchio PF. The biology and external morphology of bees. With a synopsis of the genera of Northwestern America. Corvallis: Oregon State Univ. 1969; 140.
15. Zinchenko BS, Korbetskaja LA. Parasites and predators of alfalfa pollinators. Apiculture. 1978;6:10-11. (In Russian).
16. Pesenko YA, Radchenko VG. Application of bees (Hymenoptera, Apoidea) for alfalfa pollination: Main directions, managing, methods for estimation of the wild bees resources and effectiveness of pollinators. Entomological Review. 1992;71(2):249-266. (In Russian).
17. Strickler K. The impact of flower standing crop and pollinator movement on alfalfa seed yield. Environ. Entomol. 1999;28(6):1067-1076.
18. Strickler K, Freitas S. Interactions between floral resources and bees in commercial alfalfa seed fields. Environ. Entomol. 1999;28(2): 178-187.
19. Strickler KV, Cane JH, (Eds.). For Non-native crops: Whence pollinators for the future? Thomas Say Publications, Entomological Society of America. 2003;204.
20. Brunet J, Syed Z. Enhancing pollination by attracting & retaining leaf cutting bees (*Megachile rotundata*) in alfalfa seed production fields. Proceedings for the 2017 Winter Seed School Conference, January 29-31, Las Vegas, Nevada. 2017;67-73.

21. Khan MS, Yogi MK. Insect crop pollinators. Industrial Entomology. Springer, Singapore. 2017;397-412.
22. Southwood TRE. Ecological methods. London. 1968;391.
23. Annotated catalogue of the Hymenoptera of Russia. V. 1. Symphyta and Apocrita: Aculeata. Proceedings of the Zoological institute of the Russian Academy of sciences. Supplement 6. Saint Petersburg: Russian collection SPb. 2017;475.
24. Artokhin KS, Poltavsky AN. Formation of the fauna of bees (Hymenoptera, Apoidea) in agrocoenoses of alfalfa and the choice of pollination strategy. Bulletin of the Dnepropetrovsk University. Biology. Ecology. 2003;11(1):66-75.
25. Zharinov AI, Kluy VS. Alfalfa. Kiev. 1983;240. (In Russian).
26. Zharinov AI. Melitta – is a valuable alfalfa pollinator. Apiculture. 1978;6:13. (In Russian).