Study of the efficiency of creeping clover pollination (*Trifolium repens* L.) by honey bees (*Apis mellifera* L.)

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Abstract. The creeping clover (*Trifolium repens* L.) is a natural allotetraploid allogamous species and is characterized by an entomophilic xenogamous pollination method. One of the reasons for the low yield of clover seeds is the insufficient provision of its flowering grass stands with pollinating insects. The main pollinators are representatives of the order of hymenoptera insects (*Himenoptera*) from the family of true bees (*Apidae*) – the honey bee *Apis mellifera* L. and various species of bumblebees (*Bombus*). Studies have shown that in the conditions of the Central Non-Chernozem region of Russia, the role of bumblebees in the pollination of creeping clover did not exceed 12-13%. In the presence of bumblebees in different growing seasons in the amount of 30-80 to 110-170 individuals/ha during the mass flowering of creeping clover, they - ensured the formation of seed yields of no more than 90 kg/ha. It is established that honey bees perform up to 87-88% of clover pollination. The highest collections of creeping clover seeds are formed at the flight density of bees of 18-21 thousand individuals per hectare. This makes it possible to form a biological clover yield over 720 kg/ha. To ensure such a rich flight of bees, it is necessary to have at least 2-3 strong bee colonies on clover crops.

1 Introduction

The problem of increasing the seed productivity of creeping clover, considering the high ecological adaptation potential of this crop, can be solved only if a set of agrotechnical methods is applied in a targeted manner, primarily contributing to the formation of grass stands with the maximum number of well-developed heads per unit area and creating conditions for their pollination [1, 2].

To increase the seed production efficiency, detailed knowledge of the method of species reproduction, based on the study of its biological characteristics, is necessary. In various varieties of creeping clover, inflorescences usually consist of 40-70 small flowers (in a wider range, the variation interval is from 20-30 to 105 pieces). Theoretically, the

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maximum number of seeds that can develop in one flower, based on the number of eggs present, can be six to seven pieces [3]. Nevertheless, under natural conditions, the seed set ability at the stage of preliminary fertilization is already limited by the sterility of up to 20-30% of eggs. In addition, if any conditions do not correspond to the optimum, for example, the illumination for full-fledged photosynthesis at the post-fertilization stage, it can lead to the abortion of a large proportion of already fertilized eggs and developing seeds [4]. At low temperatures, at least a 30% decrease in the potential seed set was caused by the result of inadequate growth of pollen tubes [6]. Due to the degeneration of a part of already fertilized fertile ovules due to genetic factors, including those associated with homozygotization of lethal and semi-lethal genes (primarily during self-pollination), the development of the embryo or endosperm may also stop [5]. In creeping clover, the successive opening of the flowers occurs as they form from the lower part of the inflorescence, that is, from the base to the top during several days. After the beginning of their flowering, if they are not pollinated, they remain open for 5-8 days, but the number of seeds set in them decreases by 60% in case of pollination delay by the fifth day [6].

Creeping clover is considered a natural allotetraploid allogamous species with a xenogamous method of insect pollination [7]. At the same time, the creeping clover (*Trifolium repens* L.) flowers are hermaphrodite, that is, bisexual, and a small part of them can self-fertilize [8]. It was found that in isolated sites without insect access, a limited process of self-fertilization of creeping clover occurred, resulting in a seed yield of 1.37 kg/ha [9]. Nevertheless, self-fertilization leads to a decrease in heterozygosity and heterogeneity of populations, it is accompanied by inbreeding depression and causes a serious drop in agronomic economic indicators in progeny as a result of obtaining homozygous plants [8, 10, 11]. To prevent this phenomenon, the creeping clover evolutionarily has a gametophytic system of self-incompatibility in self-pollination, based on multiple oppositions of the S locus allele, which maintain high genetic differentiation in all populations [8, 10]. Gametophytic self-incompatibility in creeping clover prevents self-fertilization, but not self-pollination and germination of its own pollen. Thus, on intact flowers, a significant stigma saturation with pollen grains was achieved after the first visit of the bee (up to 280 grains) due to self-pollination. Additional visits did not significantly increase the density of pollen grain deposition, but improved pollen quality in terms of increasing the number of sprouted pollen tubes reaching the eggs. When the bee visited the clover inflorescences, it was noted that the pollen grains left on the stigmas were both from other flowers of the same plant, and foreign (gaitonogamous and autogamous) in various proportions, an average of 115 pieces at a time [8]. For guaranteed pollination, it is necessary for visiting insects to deposit a sufficient amount of compatible pollen on the stigma from other plants to obtain maximum fertilization (optimal pollination efficiency). In the context of light saturation of the stigmas with their own pollen, its presence does not suppress cross-pollen activity, but represents a restriction for pollination, which requires repeated visits by entomophiles to each flower to achieve maximum fertilization [8].

The species composition of wild pollinating insects found on flowering crops of creeping clover is very diverse in the zonal section and is largely determined by the development of territories and the anthropogenic load on agricultural landscapes. In different regions of Russia, representatives of more than 53 generic taxonomic groups from various families have been registered on flowering grass stands of this crop. In the forest-steppe zone, 103 bee species from 27 genera of 7 families were identified on crops of perennial legumes, including *Colletidae* – 5 species, *Andrenidae* - 28 species, *Halictidae* - 24 species, *Melittidae* - 2 species, *Megachilidae* - 18 species, *Anthophoridae* - 12 species, *Apidae* - 14 species [12]. Of the wild pollinators, the most effective and numerous are representatives of hymenopteran (*Hymenoptera*, *Apoidea*) – various species of solitary and social bees (*Anthophila*), bumblebees (*Bombus*). At the same time, to obtain an economic

result from the pollination work of wild bees, their density is not less than 5.38 thousand units/ha of individuals. The actual availability averaged 0.83 thousand bees/ha, or 15 % of the required saturation [12]. As a result, the increase in yield due to pollination by honey bees on entomophilic crops is closely correlated with the flight density of these insects (r = 97,8–94,4%) [13]. At the same time, it should be noted that according to the records, the density of bees increases markedly in the border land of sowing, decreasing towards its center. In large fields, the concentration of pollinators might be at the boundaries of the plots, that is, the so-called edge effect is manifested [13, 14, 15].

When studying the entomofauna of industrial crops of creeping clover in Nepal, only 20 species of insect pollinators from three orders were identified: hymenopteran (*Hymenoptera*) accounted for 69%, lepidopteran (*Lepidoptera*) – 27% and dipteran (*Diptera*) – 4% [16]. In Europe, in conditions of high land development and widespread use of chemicals, which led to a decrease in the number of natural fauna, the main pollination work is carried out by honey bees. Thus, in Denmark, the pollinators mainly consisted of honey bees, several species of bumblebees (*Bombus terrestris*, *Bombus lapidarus*, *Bombus sylvarum*, *Bombus pascorum* and *Bombus muscorum*), two taxa of butterflies (*Autographa gamma* and *Pieris rapae*) and one species of solitary wild bees (*Halictus tamulorum*). Several species of hover flies were also observed, but it was unclear whether they functioned as pollinators [14].

In a comparative assessment of the efficiency of the representatives of wild entomofauna and domestic bees in the export of apiaries to the fields in Europe, it was found that honey bees (*Apis mellifera*) in quantitative terms accounted for 88% of the total number of all insect pollinators of this crop [17]. In New Zealand, without the use of bees, pollination of clover flowers was at an extremely low level – 5%. When apiaries were exported to sowings, an increase in pollination was observed from 5 to 95% [18]. The high efficiency of bees in pollination is also due to the fact that during one flight they collect pollen from only one plant species [19]. It is established that wild insects cannot provide effective pollination of clover in the Non-Chernozem zone, and honeybees should be considered as its main pollinators [20].

Purpose of the work. To study the efficiency of pollination work of honeybees (*Apis mellifer*a L.) on seed grass stands of creeping clover (*Trifolium repens* L.), in the conditions of the Central Non-Chernozem region of Russia.

2 Materials and Methods

The research was carried out on the experimental field of the Yermolino EF in the Dmitrovsky district of the Moscow region on seed crops of creeping clover of the VIK 70 variety. The cultivation technology is generally accepted for the crop in the Non-Chernozem zone. The intensity of pollinator flight was considered on two sites of 50 m^2 (band width of 1 m, length – 50 m in the direction from the edge to the depth of the field) three times a day: at 9 ^{00} – 9 ^{30} am, 01$^{.00}$ -01 ^{30} pm and 05 ^{00} – 05 ^{30} pm. Bees were counted during the route passage for 10 minutes. Bumblebees and wild solitary bees were counted for 30 minutes in the intervals of 9-11 am and 04-06 pm. On the sites of 100x4 m from the edge to the center of the field, the area of clover seed crops in different years ranged from 2 to 5 hectares. Hives with bees were displayed on the edge of the field in the amount of 4 to 8 pieces, and one year - 500 m from the crops - an apiary from a greenhouse farm of 120 hives with Carpathian bees. Also, at a distance of about 1 km from the crops, there were up to 22 more beehives on household plots. According to surveys of beekeepers-owners of apiaries, in different years, bee breeds were identified: mainly Carpathian and cross-breed colonies, as well as Central Russian.

3 Results and Discussion

The main clover pollinators are bumblebees, honey bees and solitary bees. It should be noted that the work of one bumblebee can be equated with the work of 2.5 bees [20]. Nevertheless, in conditions of clover seed production concentration, wild insects do not play a significant role in pollination due to their decreasing number and significant fluctuations in the number over the years. Several species of bumblebee have been recorded on creeping clover sowings, of which four have been identified: ground (*B. terrestris*), garden (*B. hortorum*), meadow or common (*B. pratorum*) and carder (*B. muscorum abricus*). As studies have shown, bumblebees in their presence in different growing seasons in the amount of 30-80 to 110-170 - individuals/ha during the mass flowering of creeping clover at different times of the day provided the formation of seed yields of no more than 90 kg/ha. At the same time, the use of honeybees for pollination under the same conditions allowed to form a yield of more than 720 kg/ha. The analysis showed that the role of bumblebees in creeping clover pollination did not exceed 12-13%. Wild solitary bees were recorded as single specimens. It should also be noted that the presence of representatives from the order *Lepidoptera* in the fields, mainly from the family of blue (*Lycaenidae*), is mostly associated only with nectar collection. Butterflies extract nectar with long, thin proboscis and have minimal contact with anthers, and their visits to pollinate flowers do not have a significant effect. In this regard, the provision of pollination of clover seed sowings by honeybees should be considered as a mandatory agricultural approach.

The development of bee colonies and the efficiency of their work are associated with the phenology of honeybees, determined by weather factors. Studies have shown that the maximum development of bee colonies, mass flight and high flight activity of bees were observed from mid-June, when the sum of effective temperatures (>5^0 C) reached 563^0 C. Moreover, this indicator changed slightly over the years, in the corridor of extreme values from 535.3 to 581.7^0C, which fell within the 13-14-day interval. At the same time, the usual timing of the beginning of creeping clover flowering is noted in the first half of June, that is, in conditions of a shortage of pollinators. That is why, there is a need to regulate the - period of clover flowering. One of the ways to combine the mass flowering of creeping clover with intense flight and high pollinator efficiency is spring mowing of the grass stand. This technique manages to shift the flowering period and make this phase more friendly, which contributes to the simultaneous maturation of the heads. The optimal period of creeping clover mowing in the conditions of the Central region of the Non-Chernozem zone of Russia was established by us in the period from May 25 to June 5. The grass stand mowing at this time (the budding phase – the beginning of flowering) restrained crop development for up to 10 days, which allowed combining the peak activity of the mass departure of bees and the clover flowering. As a result, the process of pollination and seed setting took place in more favorable conditions, when the daytime air temperature reached 20-25 °C and above, which is the thermal optimum for the release of nectar by clover. This contributed to an increase in seed setting ability by 4-9% and head contamination by 10-15%. In addition, after mowing, there was an increase in the number of clover heads by 35-49% compared to the un-mown grass stand. In different years, from 967 to 1280 pieces/m^2 or more heads were formed in clover sowings. In the end, when mowing the grass stand from May 25 to June 5, the highest yield of clover seeds was obtained - 285-315 kg/ha, or 28-41% higher than on the un-mown grass stand. It should be noted that the effectiveness of mowing increased in the years with cold and rainy spring, when the mass departure of pollinators was delayed for up to two weeks. When mowing the grass stand under such conditions, there was an increase in the yield of clover seeds by 58%.

The biological significance of using a large number of pollinators on creeping clover is due to the fact that bees mainly deposited donor pollen from other plants on the stigmas of

the recipient flower mainly in the first 15-20 inflorescences that they visited during the forage, and then only sporadically until the 50th inflorescence and for maximum access of the germinating pollen tube to the eggs (more than 90%), several visits with new portions of pollen grains are necessary [8, 13]. It is estimated that one bee needs to visit from 468 to 494 clover flowers in one flight to collect the pollen load [21]. For maximum effect, based on the number of seeds obtained as a result of one bee visit (average value = 1.24), it is estimated that 19,420 bees per hectare will be required for an 8-hour foraging day to set the maximum number of seeds per flower in the inflorescence, assuming that no flower received more visits than required [3].

When studying the role of honeybees at different levels of their provision, we found a direct dependence of the seed yield on the intensity of their flight during the clover mass flowering. The relationship of these indicators is described by the regression equation y=88.75+0.00397 x [22]. It expresses the following: starting from the biological yield level of 88.75 kg/ha, a further increase in the flight saturation of bees for every 1000 pcs./ha is accompanied by an additional formation of 3.97 kg of seeds per 1 ha. The correlation analysis confirmed a high dependence of the seed yield on the bees flight density (r=0.965). The substantive test based on criterion F (F=11.24) indicates that the probability of accidentally obtaining a coefficient of this value is less than 5%. The coefficient of determination (r^2) shows that the increase in the yield of clover seeds on a high agricultural background when forming a grass stand with a large number of developed heads is 93% dependent on pollination and only 7% on other factors.

Using the obtained regression equation, considering the potential of creeping clover for seed productivity and the dynamics of crop flowering, it was found that the growth of seed yield stopped after reaching the value of the bees flight density of 177-208 individuals per 100 m^2 of flowering grass stand. At the same time, in years with favorable weather conditions and the number of heads in the grass stand up to 1319 pcs./m^2, 208 individuals of bees per 100 m^2 were sufficient, and in less favorable years, when only 400-700 pcs./m^2 of inflorescences were formed, the bees flight density of 177 individuals per 100 m^2 was sufficient. In other words, the potential of the VIK 70 variety for seed productivity at this border of the agricultural background is exhausted when the flight is saturated with 18-21 thousand bees per hectare of flowering grass stands of creeping clover. To ensure this amount, 2-3 bee colonies are sufficient, having at least twelve bee ways between the frames occupied by the pollination period. Along with pollination, bees collect nectar on clover. It is established that to collect 100 g of nectar, bees must visit about 1 million flowers. To obtain 1 kg of honey, bees bring 50 million portions of nectar to the hive [23].

It should be noted that despite the fact that the creeping clover is a good nectariferous plant bees are willing to work on its sowings. Nevertheless, if there is a competing nectariferous flora within the flight radius of such crops as winter rapeseed (*Brassica napus* var. *napus*), white mustard (*Sinapis alba* L.), Sarepta mustard (*Brassica juncea* (L.) Czem.), eastern galega (*Galega orientales* Lam) and others, blooming at the same time as clover, it is necessary to carry out additional measures to attract pollinators. Along with smell training, the use of Pollinus and Biopolin mks attractants with proven effectiveness is highly effective for attracting pollinating insects, including representatives of wild fauna [24, 25].

4 Conclusion

The organization of pollination of seed production crops of creeping clover by honey bees in conditions of low numbers of wild pollinating insects is a mandatory agricultural approach in the technology of seed production of this crop. In the conditions of the Central Non-

Chernozem region of Russia, the highest collections of creeping clover seeds are formed when the flight is saturated with 18-21 thousand bees per hectare of flowering grass stand, having from 700 to 1300 pcs./m^2 of heads. To ensure such bees flight density, it is necessary to transfer at least 2-3 strong bee colonies per hectare. At the same time, at least twelve bee ways between the frames should be occupied by bees in the hives by the pollination period.

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