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Some Additional Notes on Pollination of Red Clover

L. H. Pammel
Iowa State College

L. A. Kenoyer
Iowa State College

SOME ADDITIONAL NOTES ON POLLINATION OF RED CLOVER.

L. H. PAMMEL AND L. A. KENOYER.

Many years ago Charles Darwin conducted some experiments to determine whether red clover is self fertile. This investigator found that when insects were excluded, red clover did not produce a single seed, while flowers exposed to insects produced an average of twenty-seven seeds per head. The experiments of Darwin and many of the older investigators who conducted experiments with red clover were faulty since they did not release the anthers from the keel. The experiments of Frandsen, according to Lindhard,[18] Waldron,[7] Kirchner,[4] Witte,[6] Fruwirth,[3] Pammel and King,[11] Sirrine,[5] Beal,[9] Cook,[1] Shamel[12] and Bolley[13] confirmed the results of the work of Darwin.[19] These authors find that bumble bees and in some cases honey bees are the important pollinators. Several years ago H. S. Coe, H. D. Hughes, L. H. Pammel, J. N. Martin and J. N. Westgate published a lengthy account of the pollination of the red clover.* The more important conclusions arrived at are given in the following summary from the bulletin, "Red Clover Seed Production."

A study of the cytology of red clover flowers shows that many of them contain infertile ovules. The percentage of infertile ovules is greater in the first crop than in the second crop. In the first crop many plants produce 100 per cent of infertile ovules, while in the second crop the percentage of infertility ranges from none to a high per cent. The percentage of infertile ovules in red clover is probably correlated with moisture conditions.

The pollen grains of red clover are very sensitive to moisture. On account of this there can be little effective pollination when the flowers are wet. Germination of the pollen grains takes place only within a limited range of variation in the water supply. It is probably true that the only function of the stigma is that of supplying the requisite amount of water to the pollen for germination.

*Red Clover Seed Production, Bull. U. S. Dept. Agr., Contr. Bur. Pl. Ind., 289:1-31.

The time between pollination and fertilization varies with the temperature of the atmosphere. The time between pollination and fertilization in July is approximately 18 hours, while in October it varies from 35 to 50 hours. An examination of 30 flowers which had been self-pollinated for 55 hours showed good germination on the stigmas but no fertilization. The pollen tubes made a slow growth and none exceeded 4 mm. in length. In flowers which had been self-pollinated for 90 hours one pollen tube attained a length of 7.5 mm., while the rest were 5 mm. or less in length. The pistils of red clover average about 12 mm. in length. Eggs were found to be disintegrating four days after the flower opened.

The self-pollination and cross-pollination experiments which were conducted in the field checked up very closely with the results obtained from the cytological studies. The average yield of seed obtained on heads which were not pollinated, and on heads which were self-pollinated in different ways, was less than one-half of 1 per cent. This small amount of seed may be accounted for by the occasional access of bees to these heads for a very short time on account of rains or grasshoppers mutilating the tarlatan which was used to cover the heads.

The bumblebee is an efficient cross-pollinator of red clover. Bumble bees are able to pollinate from 30 to 35 flowers a minute.

The honey bee proved to be as efficient a cross-pollinator of red clover as the bumble bee in 1911. In 1911 when the precipitation was considerably below normal in June, July and August and but few nectar producing plants were to be found at the time, honey bees collected a large amount of pollen from red clover. In order to collect pollen they must spring the keels of the flowers. In doing this they cross pollinate the flowers.

A clover cross-pollenizing machine which was offered for sale on the market did not prove to be an efficient cross-pollinator of red clover.

The various types of hand operated brushes which were used did not prove efficient as cross-pollinators of red clover. In nearly all cases where these brushes were used the seed yield was decreased instead of increased. This was undoubtedly due to the bristles of the brushes injuring the flowers since the average seed yield of the plots given in Table VIII is lower than that given in Table IX. The plots given in Table VIII received

three treatments with the brushes while the plots in Table IX received but one treatment.

It will not be necessary to describe the method of pollination since this has been done in several papers by Hermann Mueller, L. H. Pammel and Charlotte M. King, Roberts[15] and others. We might note in this connection that any insect with sufficient weight to press down the keel of red clover can pollinate it. For this reason various butterflies observed on red clover like the large monarch butterfly (*Danais archippus*), the cabbage butterfly (*Pieris rapae* and *P. Brassicae*), the yellow butterfly (*Colcus philodice* and *C. eurytheme*) as well as certain Noctuidae which are especially common in September, are not normal pollinators. Certain Coleoptera also visit red clover but they are not normal pollinators though they sometimes no doubt transfer pollen from other plants. The normal pollinators are bumble bees, of which we have observed the following at Ames: The *Bombus pennsylvanicus*, *B. fervidus*, *B. americanus;* and the honey bee *Apis mellifica*. The bumble bees are generally not common on the first crop. In 1915 we observed two bumble bees over an area sixteen by sixteen feet in ten minutes on a partly cloudy afternoon.

In order to determine the length of the corolla tubes under the most favorable weather conditions we had H. L. Rothschild measure the corolla tubes of 1,000 corollas of first crop clover and J. H. Frazier measured the corolla tubes of second crop clover. The conditions for the development of red clover flowers were most favorable this year (1915). Rothschild found the average length of first crop to be 9.52 mm. and Frazier found the corolla tubes of 500 second crop to have an average length of 9.42 mm. The Rothschild measurements were made between June 27 and July 6 and the Frazier measurements between July 10 and 15. For the purpose of obtaining an average the length of ten flowers of each head was measured.

LENGTH OF COROLLA TUBES AND SIZES OF HEAD IN MILLIMETERS.

FIRST CROP CLOVER.

No. Flowers	1	2	3	4	5	6	7	8	9	10	Average	Height of Head
1	10.	9.5	9.	9.2	9.	9.4	8.	9.	9.5	9.2	9.2	27.5
2	8.	8.	7.5	8.	8.	8.	8.	8.	9.	8.1	8.1	30.
3	10.	10.5	10.5	10.5	10.5	10.	10.5	9.5	9.5	11.	10.25	30.
10	10.5	10.	10.	10.5	11.	11.	10.5	10.5	10.5	10.	10.4	31.
13	8.5	8.5	9.	8.5	8.	9.	9.	8.	9.	8.5	8.6	23.

SECOND CROP CLOVER.

1	8.	7.5	8.	8.5	7.5	7.5	8.	8.	9.	8.	8.	22.
3	10.	9.5	11.	10.	10.5	10.	10.	9.5	9.5	10.	10.	28.
6	12.	12.	12.5	12.	12.5	12.5	12.	12.5	12.	12.2	12.2	
12	11.	11.	10.5	10.5	11.	11.	10.5	11.	10.5	10.5	10.2	33.
42	9.	9.5	8.	9.	9.	9.	8.5	9.	9.	9.	8.	31.
45	8.5	8.5	8.5	8.5	8.5	9.	8.5	9.	9.	9.	8.7	31.

The measurements reported by Pammel, and by us in 1911 and those made by Rothschild and Frazier show a pretty close agreement. The longest corolla tube reported was of second crop clover. The general average is, however, somewhat shorter than first crop. This statement seems to bear out the one usually made by beekeepers that the corolla tubes of second crop clover are shorter than for the first crop. The difference is, however, slight and the amount of nectar collected by honey bees from the second crop because of the shortness of the corolla tube is practically nil generally.

It might, however, be noted that a number of investigators referred to in the paper, "Pollination Studies of Red Clover," state that red clover is self fertile. This is not borne out by the studies of H. S. Coe referred to in the above paper; moreover, J. N. Martin in the same paper points out that sterile ovules in red clover are of common occurrence. "During the first crop many plants produce 100 per cent of infertile ovules, with such plants the presence of bees is not a matter of importance for the ovules have no reproductive cells, hence there can be no fertilization and no production of seeds. During the second crop when the season is generally dry and favorable for seed setting, there is some infertility ranging from a low percentage or none in some plants to a high percentage in others. It is very probable that this infertility of ovules is to a greater or less degree a hereditary character and that the selection of a high yielding strain will consist, among other features, in se-

lecting those plants with the least tendency toward infertility."

Quoting from the paper, "Red Clover Seed Production," the results published by previous investigators on cross pollination and self-pollination of red clover do not agree. Frandsen and Fruwirth also show that pollen must come from an entirely separate plant in order to fertilize the ovules of red clover flowers.

The relative efficiency of the bumble bee and honey bee as cross pollinators of red clover has also been discussed by scientific investigators as well as by agricultural papers and bee keepers. Bee men generally agree that the Italian races of honey bees can extract nectar from red clover flowers. Little, however, has been said about the ability of the honey bee to collect pollen from red clover.

In view of the above diverse opinion in regard to self-pollination and cross pollination of red clover, a number of experiments were outlined in order to determine (1) whether red clover flowers were self fertile, (2) if self fertile, whether any effective method of self pollination could be found which would be applicable for use on a field scale; and (3) the relative efficiency of the bumble bee and honey bee as cross pollinators of red clover.

In the matter of the bumble bee as a cross pollinator of red clover, in addition to the foregoing data the following additional data will be of interest.

TABLE GIVING THE RESULT OF EXPERIMENTS CONDUCTED IN 1911 AND 1913.

Year	Location	Bumble Bee Cage		Field Conditions	
		No. of Heads	Average	No. of Heads	Average
1911	Ames	311	30.4	1302	52.5
1913	Ames (First crop)	970	13.3	79	12.9*
1913	Le Mars (First crop)	242	49.7	244	5.3*
1913	Harpers Ferry (First crop)	250	46.6	237	9.1*
	(Second crop)	220	20.3	244	45.0
1913	Essex (First crop)	200	34.5	224	36.1
1913	Knoxville (First crop)	245	50.5	250	54.9
	General average		28.5		41.0

*It is not surprising that the average is smaller in bumble bee cage for reason that the bumble bee does not work as well under restraint.

On the whole, bumble bees are shown to be quite efficient pollinators of red clover.

H. S. Coe summarized the previous investigations in regard to the honey bee as follows:

HONEY BEES AS CROSS POLLINATORS OF RED CLOVER

The ability of the honey bee to cross pollinate red clover has been discussed by scientific investigators and bee keepers for some time. Those who do not believe that the honey bee is able to pollinate red clover base their statements for the most part on the fact that the proboscis of the honey bee is not long enough to reach the nectar located at the base of the staminal tube. Some investigators and bee men state that some strains of the Italian race of honey bees are able to obtain some nectar from red clover flowers while other investigators say that honey bees collect pollen from red clover flowers and thereby cross pollinate them.

According to Knuth[20] the proboscis of the honey bee is 6 mm. in length, which is 3.6 mm. shorter than the average length of the corolla tubes of first crop red clover flowers. Pammel states that nectar lies from 7 to 9 mm. deep. Honey bees may be able at times to obtain some nectar from the sides of the staminal tubes of red clover flowers when a large amount is secreted, or when the flowers are not in an upright position. On pulling the red clover flower out of the calyx the nectar is visible to the naked eye. J. H. Frazier found that in rainy weather the nectar may be reached at a depth of 6 mm. from the upper part of the tube of the corolla, but in dry weather the nectar was found at a depth of 8 mm. Chemical analyses of the nectar washed from clover corollas, however, show that in rainy weather it is not very rich in sugar. Indeed, much of the accumulated liquid may be rain water drawn by capillarity into the corolla. It was also found that the amount of nectar secreted varied greatly. After a heavy dew on July 20, 1915, the amount varied from a small drop at the base of the tube to 6 mm. The tightly fitting calyx evidently crowded the nectar up. The small corolla tube causes the nectar to rise by capillarity.

Opinions differ as to whether honey bees actually collect nectar. Dadant[17] makes the statement that honey bees do sometimes collect nectar because of the shorter corolla tubes. The same statement is also made by A. I. Root.[21]

C. E. Bartholomew, who has charge of the college apiary, informs us that he saw bees gather nectar from red clover in the college apiary. He states that bees usually gather pollen and because the bee is highly specialized that some bees gather pollen, others only nectar. The statement is made by bee authorities that both honey and pollen may be gathered at the same time. Our observations made in the college apiary indicate that 80 per cent of the bees had pollen on their legs and on 20 per cent of the bees pollen could not be seen. Several bees without pollen were tested for the presence of honey in the honey bag, but we could not detect it. It is interesting to note that honey bees were abundant on the first crop of red clover in the college apiary, but on August 11, bees were as numerous as one to the quadrat (4 feet square) on a field a quarter mile from the apiary. Of nine that were captured seven were found to have pollen on their legs. Microscopic examination showed this to be red clover pollen. On subsequent days, bees were rather frequently seen on red clover. Some of them were evidently gathering nectar, others pollen. In 1914, a very dry season, honey bees were more numerous on red clover than in 1915. At one time during August, 1914, the month of their greatest abundance, as many as nineteen were counted on one quadrat. This may account for the difference in the pollination experiments noted later.

Knuth observes that *Bombus terrestris*, a species of bumble bee found in Europe, pierces the tubes of clover flowers and honey bees later gather nectar through these slits. *Bombus terrestris* has a proboscis from 7 to 9 mm. in length. While working on the experiments given in this bulletin several corolla tubes were observed which had been slit at the base but it can not be stated that these were probably not slit by bees. The structure of the mandibles of the honey bee is not adapted for cutting. Schneck[22] states that Virginia carpenter bee (*Xylocopa virginica*) slits the lower end of the corolla tubes of red clover flowers and that he has observed honey bees obtaining nectar through the slits. Pammel[23] has made similar observations.

In order to determine the efficiency of the honey bee as a cross pollinator of red clover, a cage, 12 feet square and 6 feet high, made of galvanized wire screen having 4 meshes to the linear inch, was erected in the same field as the bumble bee

cage. It was previously determined that a mesh of this size would permit a honey bee, or any insect smaller than a honey bee, to pass through, but would not permit bumble bees to do so. Two weeks before the clover came into bloom a small colony of honey bees was placed in one corner of this cage. The bees soon learned to pass through the screen. By the time the clover began to bloom the bees had become accustomed to the cage and while most of them worked on flowers outside, yet some could always be seen at work on the clover within the cage. Bees working on the clover within the cage were observed to collect pollen from the flowers and carry it to the hive.

As soon as all the flowers in the cage were mature, an area 4 feet square was measured off and all heads within this area were collected, kept separate and threshed by hand. Of the 623 heads collected from this area an average of 37.2 seeds per head was obtained.

The higher yields of seed obtained in the honey bee cage than in the bumble bee cage may be attributed at least in part to the larger number of bees which had access to this clover. However, the ratio of honey bee to bumble bee was no greater in the cages than in the clover fields in the vicinity of Ames, in 1911.

In 1911 the precipitation at Ames, Iowa, was 2.48, 3.83, and 0.39 inches below normal for June, July and August respectively. When the clover was in bloom very few nectar producing plants were to be found. Whether the honey bee would work on red clover to this extent in a year of normal rainfall when the number of other nectar-producing plants is larger, is problematical, but our observations and results show that the honey bee is able to spring the keels of red clover flowers and thereby cross pollinate them.

A repetition of the experiment was made at Ames in 1913 on first crop clover. It was found that 940 heads from the cage containing honey bees and excluding bumble bees yielded an average of 0.8 seeds per head, while 79 heads of the uncovered clover yielded an average of 12.9 seeds and 970 heads from a cage containing bumble bees yielded an average of 13.3 seeds. The summer was rather dry, but rains were more uniformly distributed than in 1911. Perhaps first crop clover is less visited by bees than second crop. In 1914 practically the only time honey bees were seen on red clover was during August.

In 1915 the experiment was repeated. This summer proved to be exceptionally cool and moist, quite the opposite of that of 1911. The cage was erected June 26 and the crop harvested three months later. These three months had an aggregate pre-

cipitation of 17.55 inches, which is much above the normal for Ames. The bees seemed rather bewildered by being enclosed in the cage, and many of them died in the efforts to find their way out. They did not, however, work very freely on the clover heads in the cage, although one or two bees could be seen on these heads at almost any time that the weather was favorable for their activities. It was found that 500 heads in the cage produced an average of 18.7 seeds, while 500 heads in the field twelve feet away from the cage produced on an average of 35.0 seeds.

The results are not such as to give much confidence in the ability of the honey bee as an effective pollinator, under all circumstances, although they do seem to be able to accomplish pollination during some seasons.

DEPARTMENT OF BOTANY,
IOWA STATE COLLEGE.

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