Pollination of *Trifolium hybridum* by *Megachile rotundata*[1]

D.T. Fairey[2] and L.P. Lefkovitch[3]

ABSTRACT

Alsike clover (*Trifolium hybridum* L.) is an obligate, outcrossing entomophilous species. This study was conducted to determine whether the leafcutting bee (*Megachile rotundata* Fab.) could be used as an effective pollinator of this crop. This was tested in cage and field studies. There was approximately eight times the seed yield in cages with leafcutting bees in comparison with seed yield in their absence. Under open field conditions, the mean seed yield was 345 kg ha^{-1} in areas where leafcutting bees were provided (i.e., leafcutting bees together with other pollinators), and 228 kg ha^{-1} where leafcutting bees were not provided (i.e., other pollinators). The viable cell production for leafcutting bees foraging on the alsike clover exceeded 92%; about one-third of the progeny was female, and there were approximately 2.5 times as many cells at the end of the season as were introduced. The pollen in the diet of the developing larvae was predominantly that of alsike clover. Based on these studies, leafcutting bees are recommended as pollinators for alsike clover.

Additional index words: **Alsike clover, leafcutting bee, pollinators, seed yield.**

INTRODUCTION

In cross-pollinated crops such as alsike clover, *Trifolium hybridum* L. grown for seed, it is essential that appropriate insect pollinators are active during the flowering season. Currently, the honey bee, *Apis mellifera* L., is recommended for this crop (Crane and Walker, 1984), but if the leafcutting bee, *Megachile rotundata* Fab., presently used largely for pollinating lucerne, *Medicago* spp., is also effective, the result would be an added insurance for consistent seed yield. That this insurance is desirable can be seen from the fact that of the 10-year average (Fairey and Fairey, 1991) of approximately 14,000 tonnes of clover seed exported annually from Canada, approximately 2,300 tonnes are of alsike clover seed, of which 2,186 tonnes (94%) are from the Peace River region.

There is another economic benefit to be obtained from the use of leafcutting bees; their cells from the Peace River region are in demand both in domestic and international markets because they are not infected with chalkbrood, *Ascosphaera aggregata* (see Fairey and Lieverse, 1992). This demand can be met more readily if the leafcutting bee can utilise pollen and nectar from crops in flower at times not always coinciding completely with that of lucerne.

In the Peace River region, the leafcutting bee successfully pollinates lucerne, and also has pollinated clovers (*Trifolium* spp.), birdsfoot trefoil (*Lotus* spp.) and sainfoin (*Onobrychis* spp.) in experimental conditions (plots 10.5 m x 0.6 m). Since total area of these plots was about 0.5 ha (Fairey and Lefkovitch, 1989), the present study was initiated to determine whether successful cross-pollination would also occur in large fields (50 to 65 ha) of these legumes, particularly the clovers. This was investigated by observations on the pollination of alsike clover by leafcutting bees as follows:

(1) by comparing the size of the corolla tube length of florets of alsike clover and the mouth parts of the leafcutting bee;
(2) by determining the extent of cross-pollination, as measured by seed yield, attributable to this insect in caged alsike crops;
(3) by comparing seed yield in two separated areas in alsike clover fields, only half of which was provided with leafcutting bees.

Further support for the effectiveness of the leafcutting bee as a pollinator of alsike clover is the ability to reproduce and increase successfully, coupled with the identity of the pollen that it provides for its larvae.

MATERIALS AND METHODS

Experiments were carried out in small plots of alsike clover that were enclosed within cages, and in a 50 and 65 ha alsike clover seed field. The cage studies were performed in 1988 at the Agriculture Canada Research Station, Beaverlodge, Alberta (55° 13' N, 119° 26' W); the field studies were done near Rycroft, Alberta (55° 45' N, 118° 43' W) in 1989, with further observations in 1990 and 1991. All leafcutting bees came from the population maintained since 1966 at the Beaverlodge Research Station for which the natural male-to-female ratio is 2:1. Samples of the alsike clover seed crop were harvested when more than 80% of the seed heads were dark brown to black. All data were analysed using Genstat 5 (Lawes Agricultural Trust, 1987).

Morphological measurements

Ten racemes, one per plant, of the cultivar Dawn, were taken from a 0.25 ha plot at the Agriculture Canada Research Station, Beaverlodge. These racemes were fixed

[1] Contribution Nos BRS92-07 and R-120a.
[2] Agriculture Canada, Research Branch, Research Station, Box 29, Beaverlodge, Alberta, Canada TOH OCO.
[3] Agriculture Canada, Research Branch, Research Programme Service, Statistical Research Section, Bldg 54, Ottawa, Ontario, Canada KIA OC6. Accepted for publication 9 February 1993.

in FAA (formaldehyde-acetic acid-alcohol) solution. Corolla tube lengths of 10 florets, from each of the 10 racemes, were measured.

Samples of 100 leafcutting bees of each sex pollinating alsike clover in the field were trapped with a hand-net, killed in an atmosphere of ethyl acetate to facilitate relaxation before dissecting, fixing and mounting the mouth parts on a microscope slide (Gauld and Bolton, 1988). Length measurements of both plant and insect specimens were made under a dissecting microscope.

Cage studies

The role of cross-pollination in seed set was assessed by enclosing the crop in cages, 1.2 m x 1.2 m x 1.2 m in size, and comparing the within cage seed yield from those where leafcutting bees were introduced, with those from which insect pollinators were excluded. Two cultivars of alsike clover, 'Dawn' and 'Aurora', were used. There were two treatments: in Trt1, 200 viable cells were incubated and released into each cage at weekly intervals commencing at the first bloom and continuing until the crop was ready for harvest; in Trt2, leafcutting bees and all other pollinating insects were excluded from the cage. There were 7 repeats for each treatment in each cultivar. The entire crop in each cage was harvested, threshed, and the air-dry weight of the clean seed recorded. These data were analysed using a generalised linear model (McCullagh and Nelder, 1989) assuming a constant coefficient of variation, and with cultivar, treatment and their interaction as the factors of interest, and the cages representing replication.

Field studies

Two alsike clover fields (50 and 65 ha, respectively) were used during the 1988 growing season. Each field was divided into two rectangular areas of equal size, one for each of two treatments, with an isolation strip of about 300 m between them.

1. Trt1 consisted of 20 shelters for leafcutting bees placed in one half of the field. Prior to being used, bee cells were incubated at 30°C. Incubation trays were placed in the shelters after the onset of male emergence at day 18 of incubation (ca. July 1). Commencement of incubation was timed to ensure the introduction of bees into the field when about 10 percent of the alsike clover crop was in bloom. Incubation trays were removed from the shelters about 10 days later, after the bees had emerged from their cells. A stocking rate of 20,000 viable cells per shelter (equivalent to 50,000 viable cells per hectare of crop) was used. Wood framed shelters, each 1.2 m x 1.2 m x 1.2 m, with polyethylene panels were used. All shelters were placed at least 100 m away from the headlands; the distance between shelters was increased from the recommended 70 m for the region to 100 m so as to isolate shelters and minimise drift of bees from shelter to shelter (Fairey and Lieverse, 1986). The culture and management of leafcutting bees, as well as the methods of isolation of shelters from each other, were identical to those described for lucerne (Fairey and Lieverse, 1986). All other management procedures for leafcutting bees were in accordance with those delineated for the region for pollination of lucerne and are described in detail elsewhere (Fairey, Lieverse and Siemens, 1984).
2. Trt 2 consisted of 20 stakes arranged similarly to the shelters placed in the other half of the field, but bees were not provided.

Other pollinating insects were not excluded in both treatments; in fact, owing to circumstances beyond our control, honey bee hives were present in adjacent areas. This split plot design was chosen because of the need to isolate the stakes (i.e. the native/other pollinators) from the shelters (i.e. the native/other pollinators together with the leafcutting bees). Thus the treatments are confounded with the half-fields.

Four 1 m^2 sub-samples of the crop 15 m away from the north, south, east and west of each shelter/stake, were harvested. The samples were threshed and the air-dry weight of the clean seed was recorded. These data were analysed using a generalised linear model assuming a constant coefficient of variation, with location (fields), treatment and their interaction as the factors of interest.

These field studies also included an investigation of the reproduction of the leafcutting bee, and the viability and the sex ratio of the progeny. In addition, this assessment was extended to the two subsequent growing seasons of 1989 and 1990 at one of these locations (the 50 ha field). The viability and sex ratio data were analysed with generalised linear models assuming a binomial error distribution.

Observations on the pollinating activity of leafcutting bees were made at frequent intervals during the growing season. After the pollination season was completed (ca. August 20 in all three years), nesting boxes were brought indoors for storage at 17 to 20°C to allow the larvae within the cells to complete their development. Cells were then removed from the nesting material, tumbled to remove excess leaf debris, and the total weight of cells from each shelter recorded. The weights of six 100 cell+dockage subsamples were determined, and the number of viable male and female cells in these determined after incubation (Fairey, Lefkovitch and Lieverse, 1989). The total viable cell number was calculated from these data. The number of viable cells out of the 100 is assumed to have a binomial distribution, and data were analysed using a generalised linear model with the weight of the subsample as a covariate. The number of females in the viable cells was also analysed assuming a binomial distribution to determine the sex ratio. The ratio of the number of viable cells to the initial 20,000 was analysed assuming a gamma error distribution.

Cell subsamples were taken from shelters used in

1990 in the field study to monitor reproduction of this pollinator on an alsike clover crop. The pollen rations from 100 cells were removed and faecal plates were cut out from another 100 cells. The Erdtmann acetolysis technique (Kapp, 1969) was used to clean, separate and fix pollen grains from this material in silicone oil. Five pollen slides per sample, i.e., 500 slides each from the pollen rations and faecal plates were prepared. From each of these two categories, 100 slides were selected at random for microscopic examination. The identity of the pollen was made by comparison with pollen of known identity. For each slide, the first 100 grains were identified as either belonging to the candidate crop alsike clover, or to crops of other kinds.

RESULTS

Morphological measurements

The range of the sample of 100 corolla tube lengths was 2.56 - 4.96 mm; the mean was 3.87, standard error 0.11. Length measurements for the mouthparts of leafcutting bees are given in Table 1. These measurements revealed that this insect can easily reach the nectar at the base of the corolla tube.

Table 1. Measurements of mouth parts of leafcutting bees[1]. Means (mm) and standard errors in parenthesis.

	Glossa	Prementum	Mentum	Total
Males	2.335	1.612	0.527	4.481
	(0.015)	(0.014)	(0.006)	(0.019)
Females	2.368	1.639	0.546	4.560
	(0.015)	(0.014)	(0.006)	(0.019)

[1] 100 of each were measured.

Cage studies

In Table 2, the analysis of deviance and the estimated means and standard errors for the seed yield are summarised for the two treatments and cultivars. The probabilities given provide evidence supporting treatment and cultivar differences, but none for their interaction. The significant differences in seed yield observed between cultivars can be attributed, at least in part, to the fact that the stand of cv. Aurora appeared to be more uniform and vigorous than the stand of cv. Dawn before commencement of treatment. There was approximately eight times the seed yield in cages with bees as compared to those without, emphasising the importance of insects in this predominantly cross-pollinated species.

Table 2. Seed yield of two cultivars of alsike clover in cages with and without leafcutting bees.

a. Analysis of deviance

	d.f.	Deviance	Probability
Cultivar (C)	1	2.3444	0.019
Treatment (T)	1	24.2442	<0.001
C*T	1	0.1655	0.513
Residual	24	0.3731	

b. Mean yields (kg ha^{-1})

Cultivar	With bees	Without bees
Aurora	270.15	24.86
	(62.16)*	(5.75)
Dawn	136.57	28.15
	(31.52)	(6.41)
Mean	203.36	26.50
	(34.85)	(4.30)

* Standard errors are approximate, since model is not linear.

Field studies

Good seed yields were produced under field conditions where leafcutting bees were provided, and there is evidence supporting the pollinating activity of the leafcutting bees (Table 3), although to a lesser extent than in the cage studies. Average seed yields with and without leafcutting bees were 345 kg ha^{-1} and 228 kg ha^{-1}, respectively.

The viable cell production for leafcutting bees foraging on alsike clover exceeded 92% (Table 4); while it was not influenced by location, there was evidence to support a difference between years. About one third of each population consisted of females; this proportion was not influenced either by year or location. There were approximately 2.5 times as many cells at the end of the season as were introduced; this increase factor also apparently was not influenced by year or location.

The pollen examination from the faecal plates and the pollen balls consisted respectively of 93.8% (se = 0.2%) and 88.6% (se = 0.3%) alsike clover.

Table 3. Seed yield in alsike clover fields with and without leafcutting bees.

a. Analysis of deviance

	d.f.	Mean deviance
Location (L)	1	1.6183
Treatment (T)	1	3.2988
L*T	1	2.4573
Residual	74	0.3771

b. Mean yields (kg ha^{-1})

Location	With bees	Without bees	Mean
1	352.8	295.0	323.1
	(48.4)*	(40.2)	(31.3)
2	337.2	157.5	245.0
	(48.8)	(21.2)	(26.1)
Mean	345.2	228.0	
	(34.4)	(23.0)	

* Standard errors are approximate, since model is not linear.

DISCUSSION

The mouth parts of leafcutting bees are of a size capable of extracting nectar from alsike clover florets, and an increased seed production was associated with the provision of leafcutting bees for pollination. In the cage studies, up to eight times the seed yield was obtained in cages containing the bees. Under field conditions, the yield in the vicinity of the leafcutting bees was higher than elsewhere, although a good set was also obtained in those areas. This may have been a consequence of feral pollinators and also the presence of honey bee colonies in adjacent fields. The good seed set in the fields used in the present study is attributable to the activity of the leafcutting bee in Trt1, and to other pollinators including the leafcutting bee in Trt2. It is interesting to note that in Trt1, other pollinators were seldom observed near the leafcutting bee shelters, and that the activity of the leafcutting bees was revealed by concentric 'seed rings' of dark brown seed heads which appeared around the shelters in mid-July; this effect gradually disappeared by mid-August. This delineation of pollination territory is not uncommon, and has been observed in other studies (e.g. Fairey *et al.*, 1989). Further evidence for leafcutting bee activity in the alsike clover crop is the predominance of alsike pollen in the bee bread prepared for their offspring, and in the faecal pellets ejected by the larvae. Thus the cell increase of 2.5 times occurred with a diet where pollen was predominantly that of alsike clover.

Alsike clover is an obligate, outcrossing entomophilous species (Clarke and Malte, 1913). Florets are largely self incompatible and so must receive pollen from another plant (McGregor, 1976). Since cross-pollination should exceed 90% in seed fields (Frankel and Galun, 1977), the present paper has demonstrated that the leafcutting bee is capable of bringing this about; further, it can also reproduce successfully, similar to levels on lucerne (Fairey *et al.*, 1989). Its adoption for use in alsike clover seed fields will provide a pollination insurance, especially in the absence of other pollinators.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of J.A.C. Lieverse and O. Lee. Messrs Tom Steigmeier and Mel Melkovitch made their alsike clover seed fields available for these studies.

Table 4. Reproduction of leafcutting bees foraging on alsike clover.

a. Binomial analysis	d.f.	Viability deviance	Viability probability	Proportion of females deviance	Proportion of females probability	Cell increase deviance	Cell increase probability
Year	2	15.488	< 0.001	13.283	0.103	0.02031	0.477
Location	1	3.316	0.069	4.671	0.206	0.01116	0.522
Residual	28	47.543		72.589		0.02676	

b. Means Year	Viability	Proportion of females	Cell increase
88	0.958	0.27	2.511
	(0.007)*	(0.02)	(0.107)
89	0.950	0.31	2.330
	(0.010)	(0.03)	(0.135)
90	0.923	0.36	2.528
	(0.012)	(0.03)	(0.146)

* Standard errors are approximate, since model is not linear.

REFERENCES

1. Clark, G.H. and Malte, M.O. 1913. Alsike clover (*Trifolium hybridum* L.). In: Fodder and pasture plants, 108-112. Department of Agriculture, Government of Canada, Ottawa, Ontario, Canada.

2. Crane, E. and Walker, P. 1984. Pollination directory for world crops, 45-46. International Bee Research Association, Bucks, UK.

3. Fairey, D.T. and Fairey, N.A. 1991. Forage seed production in Canada. (Abstract) Supplement to *Journal of Applied Seed Production* 9: 66.

4. Fairey, D.T. and Lefkovitch, L.P. 1989. Alternative floral sources for leafcutting bees. NRG 89-01 and ESRC C-068. Invited paper, Canadian Alfalfa Seed Council, 8th Annual Alfalfa Seed School, Winnipeg, Manitoba, Jan 12-14, pp 9-12.

5. Fairey, D.T., Lefkovitch, L.P. and Lieverse, J.A.C. 1989. The leafcutting bee, *Megachile rotundata* (Fab.): a potential pollinator of red clover. *Journal of Applied Entomology* 107: 52-57.

6. Fairey, D.T. and Lieverse, J.A.C. 1986. Cell production by the alfalfa leafcutting bee (*Megachile rotundata* F.). *Journal of Applied Entomology* 102: 148-153.

7. Fairey, D.T. and Lieverse, J.A.C. 1992. Quality of leafcutting bee cells produced in the Peace River region during the 1991 growing season. Publ. 92-01, Alberta Alfalfa Seed Producers' Association, Peace Branch, Peace River, Alberta, Canada.

8. Fairey, D.T., Lieverse, J.A.C. and Siemens, B. 1984. Management of the alfalfa leafcutting bee in north west Canada. NRG 84-21, Agriculture Canada, Beaverlodge, Alberta, Canada.

9. Frankel, R. and Galun, E. 1977. Pollination mechanisms, reproduction and plant breeding, 281 pp. Springer-Verlag.

10. Gauld, I.D. and Bolton, B. 1988. The Hymenoptera, 48-57. Oxford University Press, Oxford, UK.

11. Kapp, R.O. 1969. How to know pollen and spores, 249 pp. Wm. C. Brown Company, Dubuque, Iowa, USA.

12. Lawes Agricultural Trust. 1987. Genstat 5 reference manual. Genstat 5 Committee, Statistics Department, Rothamsted Experimental Station, Harpenden, Hertfordshire AL5 2JQ, UK. Clarenden Press, Oxford, UK.

13. McCullagh, P. and Nelder, J.A. 1989. Generalised linear models (2nd edition). Chapman and Hall, London.

14. McGregor, S.E. 1976. Insect pollination of cultivated crop plants. Agriculture Handbook; USDA Publication 496, Washington, DC, USA.