EXPERIENCE WITH USING BUMBLEBEES AS POLLINATORS IN THE REGENERATION OF THE GENETIC RESOURCES OF SOME FORAGE LEGUMES

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Summary

In our study the effectiveness of the bumble bees as pollinators of several species of the genera *Trifolium* L. (*T. rubens*, *T. ambiguum*, *T. hybridum*, *T. repens*, *T. pratense*, *T. medium*), *Medicago* L. (*M. romanica*, *M. polychroa*, *M. falcata*, *M. sativa*) and *Lotus corniculatus* was evaluated over a 5-year period at the Research Institute of Plant Production, Piestany. Those species were propagated in technical isolation separately or in combinations using *Bombus terrestris* for pollination. In the years 2000, 2001 *Bombus pascuorum* and *B. lapidarius* were used for pollination of *T. pratense* and *M. falcata*. The results showed that bumblebees could serve as effective pollinators of some perennial forage legumes with the aim to obtain sufficient amount of seeds for their maintenance in a gene bank. The existing differences in attractiveness between various plant genera may influence the amount of harvested seed in the case of their presence under the same coverage. Better seed yields per plant were obtained in *Medicago* in comparison to *Trifolium* species when grown together in the same enclosure. The effect of *Bombus* species on the seed weight of *T. pratense* was not significant. However, additional research is required to improve our knowledge in the reproductive biology and pollination requirements inclusive specific demands on the insect agent.

Keywords: forage legumes, multiplication, pollination, *Bombus* spp.

INTRODUCTION

Regeneration of germplasm is one of the most critical processes involved in the conservation of plant genetic resources and in the gene bank management. Even under optimum *ex situ* storage conditions, the viability of seeds declines and the genetic diversity is lost. Thus, the monitoring of viability and timely regeneration of seeds must be the prior activity of all gene banks (Richards 2001).

Maintenance of perennial forage legumes, which mostly need insect cross-pollination and are self-incompatible, is more difficult and costly than that of self-pollinated crops. The complexity and expense of regeneration are higher for species, which are insect pollinated.

Regeneration of sufficient number of plants in isolation is a possible solution to maintain the genetic integrity of increased perennial forage legumes. Principally, there are three possible ways of isolation to achieve it: technical, space isolation and manual pollination.

Manual pollination being thorough and precise it is also time consuming with high demand for labour. On the other hand, natural pollinators are more efficient and effective in producing quality germplasm than pollinating by hand.

Isolation in space may be possible, if

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specific areas for the multiplication of genetic resources are reserved. Risk arises in the case of forage crops as cross-pollination with wild-growing species and possibly with cultivated species grown on neighbouring fields may occur. Also the inter- as well as intra-specific competition for pollinating insects may play its negative role if space isolation is used.

The possible solution how to maintain the genetic integrity of increased forage legumes seems to be through the regeneration of sufficient number of plants in technical isolation. The use of screened cages with specially designed hives for honey, leafcutter or bumble bees for several insect-pollinated crop species is now common (Ellis et al. 1981, Ptacek 1987, 1999, Richards 1995). From several insect species bumble bees, *Bombus* spp. are recognized as efficient and effective pollinators of many crop species. Bumblebees have some obvious advantages over honeybees for pollinating plant germplasm in cages. Under limited space condition various *Bombus* species can pollinate successfully even those plant species which do not belong among their favourite food sources in the open. Ptacek (1983), Ptacek et al. (1984) obtained satisfactory results in cage pollination of alfalfa secured not only by *B. lucorum* and *B. terrestris*, but also by *B. lapidarius*, *B. pratorum* and *B. sylvarum*. To save on resources, different crop species may be planted under one enclosure (Engels and Rao 1995).

The aim of this study was (1) to obtain the basic information on agronomics and pollination requirements of some perennial forage legumes, (2) to compare the possibilities to pollinate various species of forage legumes in one enclosure, (3) to find out those *Bombus* species which are the most suitable for pollination of particular forage legumes, (4) to improve the regeneration procedures for the concerned crops.

METHODS

The effectiveness of bumble bees as pollinators of several species of genus *Trifolium* L. (*T. repens* L., *T. pratense* L., *T. medium* L., *T. rubens* L., *T. ambiguum* L., *T. hybridum* L.,), *Medicago* L. (*M. romanica* Prodan, *M. polychroa* Grossh., *M. falcata* L., *M. sativa* L.) and *Lotus corniculatus* L. was evaluated over a 5-year period at the Research Institute of Plant Production, Piestany. These species were multiplied in technical isolation separately or in combinations. In the years 1997, 1998, and 1999 *Bombus terrestris* colonies were used for pollination, in the following years, also *B. pascuorum* and *B. lapidarius* were used for pollination of *T. pratense* and *M. falcata*.

Plants were planted out individually on 2 m x 3 m beds and the first or the second cropping season year was used for seeds production. Isolators were assembled and installed in the time of flower bud formation, and hives with bumblebees were put into cages at the beginning of flowering. The flowering period lasted 6 to 8 weeks, depending on the particular plant species.

Colonies of bumblebees were prepared in laboratory conditions. Given the restricted space and consequently limited food sources from plants on beds, smaller units were prepared. In each of them a laying queen or workers and brood in larval stage were present. In order to minimise initial stress after release, the colonies was supplied with a portion of pollen dough. Inside the isolators sugar solution was supplied ad libitum.

RESULTS AND DISCUSSION

In 1997 seed production per plant of 10 white clover (*Trifolium repens* L.) varieties grown outside and in enclosures were compared. The varieties grown in isolators yielded more seed in 7 of all cases (Fig. 1),

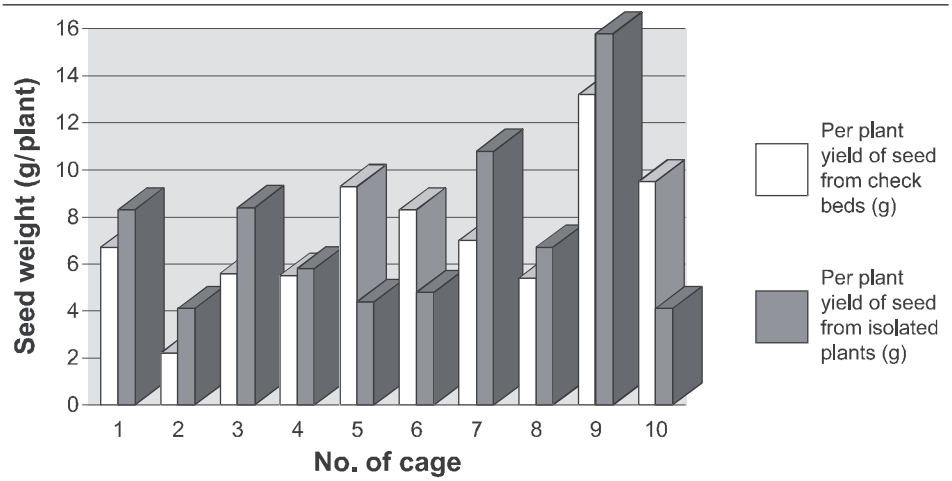

Fig. 1 Weight of white clover seeds (1997)

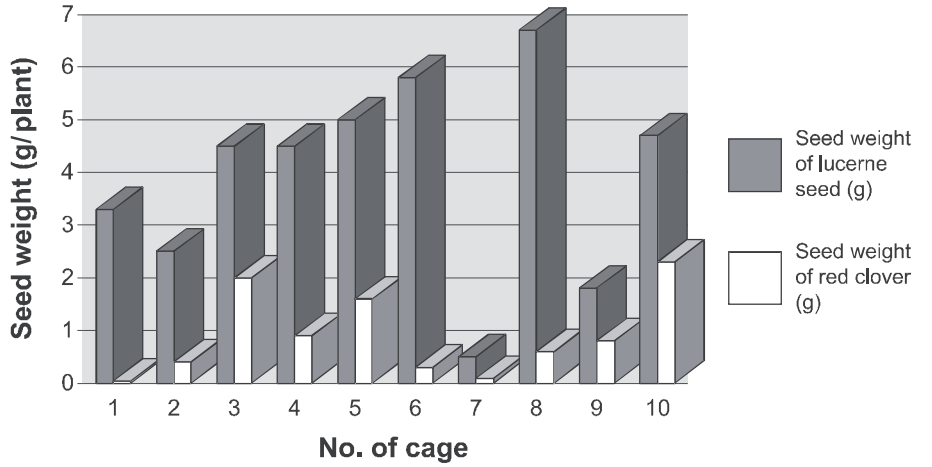

Fig. 2 Weight of lucerne and red clover seeds (1998)

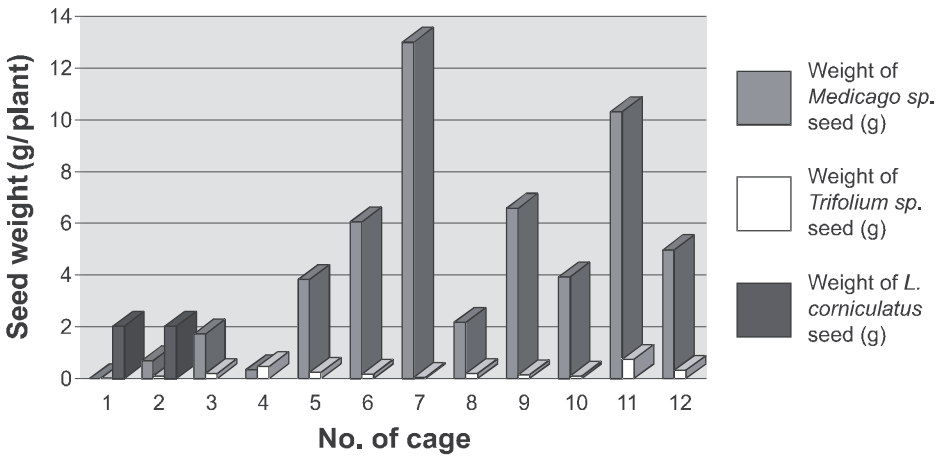

Fig. 3 Weight of *Medicago*, *Trifolium* species and *Lotus corniculatus* seeds (1999)

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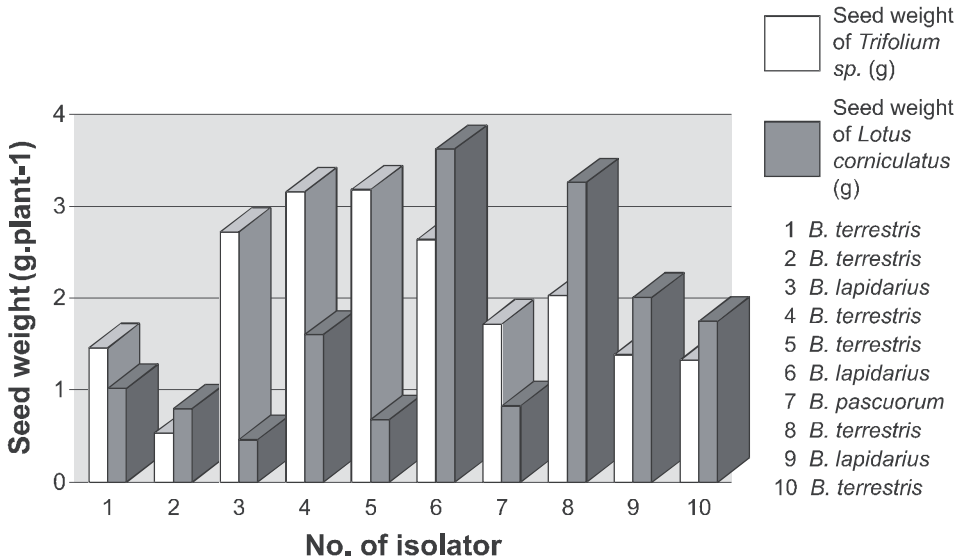

Fig. 4 Weight of *Trifolium* species and *Lotus corniculatus* seeds (2000)

Fig. 5 The arrangement of the trials

but generally, the yields under coverage were similar to those in the open air.

The next year alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) varieties were placed in ten enclosures. Better average results per plant were obtained in alfalfa in comparison with red clover (Fig. 2).

In 1999 combinations of several *Medicago* (*M. romanica* Prodan, *M. polychroa* Grossh., *M. falcata* L., *M. sativa* L.) and *Trifolium* (*T. rubens* L., *T. ambiguum* L., *T. hybridum* L., *T. repens* L., *T. pratense* L.) species (Fig. 3) grown in one enclosure have shown, that higher average seed yields per plant were obtained mostly in *Medicago* in comparison to *Trifolium* species. When alfalfa and red clover were grown in isolators together with *Lotus corniculatus*, bird's-foot trefoil gave the most satisfactory results. According to the results of both years *Bombus terrestris* preferred to forage on *Medicago* species rather than on clovers.

Some other studies with pollination of forage legumes showed similar results. Hofbauer and Pridal (1997) found that bumble bees, such as *B. terrestris* and

B. lucorum operate less effectively in isolators on red clover than *B. lapidarius*, *B. pascuorum* or *B. sylvarum* do. Brodie (1996) compared several species of bumblebees in the open and observed that *B. terrestris* never foraged on *T. pratense*. *T. pratense* was foraged in her study only by *B. pascuorum*. Also Ptacek (1987) found that *B. pascuorum* and *B. sylvarum* were more effective on *Trifolium pratense* and *B. terrestris* on alfalfa.

Basically on these statements, together with *B. terrestris* also *B. pascuorum* and *B. lapidarius* were used as pollinating agents in the cages with red clover next two years.

In 2000, combinations of *L. corniculatus* with *T. pratense* or *T. repens* were evaluated. There were no considerable differences between particular forage legumes in seed weight, but the higher average seed yield per plant was obtained in *Trifolium* species. In contrast to the previous findings, no negative effect of *Bombus* species on seed weight of *T. pratense* (Fig. 4) was observed. Only in some cases red clover pollinated by *B. lapidarius* and *B. pascuorum* gave better seed yield than by *B. terrestris*. According to the results both plant species seem to be similarly attractive for any of the bumblebee species used for pollination. In 2001 mainly *B. lapidarius* was used for pollination of *T. pratense* and *M. falcata* grown separately that time. In that year higher seed yield was obtained in red clover.

The simple evaluation of the results that we were able to do so far does not allow much definite conclusions. Obviously, the technical isolation can be used for regeneration of genetic sources because it brings at least as high seed yields as the pollination outside. The comparison of plants on the level of the genera, especially in the case of *Trifolium* and *Medicago*, hides the possible interspecific differences. Nevertheless, it can be concluded, that *B. terrestris* might not be a suitable pollinator in cases where *Medicago* together with *Trifolium* with long corolla tubes should be grown in one enclosure. *Trifolium* species can be grown with *Lotus* species regardless of the bumblebee species. Using of some "non terrestris" species may be convenient for enabling the pollination "made-to-measure".

CONCLUSIONS

According to the results from this study bumblebees can serve as pollinating agents of some perennial forage legumes in enclosures and thus ensure the sufficient amount of seeds for the maintenance of a given plant population in gene banks. Growing more than one plant species under the same coverage in order to lower the expenses is possible. However, remarkable differences in seed yields between *Trifolium* spp. and *Medicago spp*. grown together show that the process itself has to be studied more accurately and from the point of view both of the plants and the bees. Additional knowledge of the reproductive biology and pollination requirements of plants in the enclosures would be helpful, as well as the improvement of bumblebee management in order to allow the choice among several species.

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DOŚWIADCZENIE Z UŻYCIEM TRZMIELI JAKO ZAPYLACZY W ODNAWIANIU ZASOBÓW GENETYCZNYCH KILKU STRĄCZKOWYCH ROŚLIN PASTEWNYCH

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Streszczenie

W 5-letnich badaniach (1997-2001) prowadzonych w Naukowym Instytucie Produkcji Roślinnej w Piestanach (Czechy) oceniano efektywność trzmieli jako zapylaczy kilku gatunków koniczyny (*Trifolium rubens*, *T. ambiguum*, *T. hybridum*, *T. repens*, *T. pratense*, *T. medium*), lucerny (*Medicago romanica*, *M. polychroa*, *M. falcata*, *M. sativa*) i komonicy rożkowej - *Lotus corniculatus*. Rośliny uprawiano pod izolatorami oddzielnie lub w różnych kombinacjach używając do ich zapylania trzmiela ziemnego (*Bombus terrestris*). W latach 2000-2001 do zapylania *T. pratense* i *M. falcata* użyto dodatkowo trzmiela rudego (*Bombus pascuorum*) i kamiennika (*B. lapidarius*). Badania wykazały, że trzmiele mogą być wykorzystane jako efektywne zapylacze trwałych pastewnych roślin motylkowych dla uzyskania dostatecznej ilości nasion na potrzeby banku genów.

Słowa kluczowe: pastewne rośliny motylkowe, zapylanie, *Bombus* spp.