II JORNADAS DE POLINIZACIÓN EN PLANTAS HORTÍCOLAS

Jornadas organizadas por el CIFA de La Mojonera y La Cañada IFAPA en colaboración con Agrobío

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Septiembre 2006

7. Cría y uso de los abejorros para la polinización de los recursos genéticos de legumbres del forraje.

7. Rearing and using bumblebees for pollination of forage legume genetic resources.

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Elektronic publication: http://www.agrobio.es/noticias/noticias.htm Newly on:

The pollination effectiveness of bumblebees makes them important pollinators of specific plant species. On this way, bumblebees ensure a great deal of seeds and fruits of cultivated as well as wild flora. They contribute to production of food resources for the human population and by the transfer of pollen to the gene diversity in nature.

Recently, there are several species of *Bombus* sensu stricto managed in laboratories with the aim to use them in greenhouses. In Europe this is the case of *Bombus terrestris* L. that was able to breed in the given laboratory conditions and became the first completely man–managed species. Currently, *B. terrestris* has been used to pollinate tomatoes or peppers. We found the species to be efficient pollinator of alfalfa either in the field or in enclosure, when we did research in seed production in legumes in the Forage Crops Research Institute in Troubsko, near Brno, CR. In the open it also visited fields of red clover. Later on, when red clover was grown together with alfalfa in enclosure, the seed yield of alfalfa was more satisfactory. We did the trial in the Plant Production Institute in Piestany, Slovakia, in order to multiply the genetic resources of legumes. Two species were then grown together with the aim to reduce the costs of bumblebee pollination. From that time we knew, that the availability of other bumblebee species would be convenient. Round 10 recent years I have been trying to apply the laboratory methods known for *B. terrestris* to other species. Prior to discuss some experiences gained on this way let us summarize the main points of the "terrestris" method we used to apply:

1. The laboratory conditions for *B. terrestris* queens to start colonies are 29 – 30 ^{o}C, about 70 % of air humidity, darkness, suitable containers with a layer of material absorbing faeces, feeders providing constantly approximately 60% sugar solution with the 10 % of fructose and 90 % of sucrose in the dry matter. Solution should be stabilized by 0,1 % of Na–benzoate. Queens should be fed fresh or deep frozen honeybee pollen pellets pressed slightly into small plastic caps. After two–day periods pollen should be changed for the fresh one. Continuous access to quality food and cleanness is a must for successful laboratory management.

2. Queens awaken from diapauses should be given refreshment under laboratory temperature, in darkness. First, they may be left in groups, supplied with sugar solution and pollen ad lib. After round a week some of them can became aggressive, so the queens should be separated. The temperature for brood rearing is 29-30 ^{o}C.

3. Starting the first brood cell, the most important point**,** can bee achieved by several procedures:

Putting several (2–3) freshly emerged honeybee workers to the queen. Within round 10 next days most of the healthy queens will construct the cell and lay eggs on the bottom of the rearing box (Fig. 1). Then the bees should be taken away.

Keeping two queens together in the common rearing box. If they remain free, most of them behave friendly for the first time, but later on one of them becomes dominant, lays eggs and then kills the other. To prevent such losses, the firm double screen should separate the queens, so that they can easily perceive but not directly touch one another. During the following week or 10 days at least one of them will start egg lying. This one ought to be left alone and the other should be transferred into the society of another similar queen. The method of separated pairs can be used successfully also for the artificially over–wintered queens to get them over the refreshment period. At the start of the breeding time only the temperature and humidity should increase. The two methods just described are applicable if there is no simultaneous colony available, which can serve as a source of the living material.

Queens "ripening" either individually or in divided couples can be given one (or several) preferably young male cocoon (which must be fastened to the underlay). Those queens, ready to lay eggs, begin to incubate the added cocoons and soon construct egg cell(s) on them. The males, which emerge from the cocoons later, have to be put away immediately. Similarly, the queen– as well as worker cocoons can be used. Also here, the emerged adults should be taken away.

Workers from alien colony can be added to help the starting queen, too. Only one, preferably freshly emerged worker should be used. This should not be done before the queen has her own brood in the stage of larvae; otherwise the added worker becomes an egg–layer and produces her own haploid (male) brood. Then, the queen doesn't develop her physiological stage normally and the starting period is lost. When the queen has her brood round a week or 10 days old, she behaves dominantly to the added worker. The presence of the worker has then beneficial influence on the development of the first portions of brood. Workers e.g. seem to be able to cope with the (a bit) worse quality of pollen and can use it to feed larvae. So when there are worker bees, the care for the colonies becomes simpler.

Combinations of the methods described are commonly used according to the actual situation.

Queens caught in nature just after having left hibernacula can be given the refreshment period in divided couples. Those having already searched for nesting site are usually ready to lay eggs and can be put together with a male cocoons.

4. **Colonies having the first generation of adult workers** should be transferred into the laboratory hives. The space needed for further development of the good *terrestris* colonies amounts round 10 – 12 litter. In detail hives are designed to meet the specific demands of the manager. Currently they should allow the manipulation with the colony, its aeration and feeding. Pollen can be fed pressed in the plastic tubs, put close to the brood. For colonies with workers even dry pollen pellets can be fed which are available commercially. They only have to be moistened again to the original stage (+20% of water).Naturally, such a pollen has to be good quality. Sugar solution feeders can be either external or inside the hive. For the beginning colonies the temperature should be kept at 29 ^{o}C, later on the populated colonies can be kept at the room temperature.

5. Obtaining new young queens in laboratory is possible either from colonies, which produce them naturally, or as a deliberate process from any viable colony. The first case is simpler and the amount of queens usually larger. But not all colonies in the laboratory produce queens. The latter way consists in removing the queen when the colony has round 20 workers. Then the orphan bees produce young queens from the part of the diploid brood (Fig.9). The supply of food for colonies feeding queen larvae should be plentiful. If the queens producing colony is populous, its hive can be coupled to another empty one to get more space for the large amount of bees of all castes, as well as for easier manipulation. Freshly emerged queens consume large amount of pollen, so it has to be available in several feeders. This activity continues during the 4 first days of their life. On the day 4 and 5 day they do cleaning and orientation flights.
If there is a need for more precise control of developing queens, they can be more easily removed from colonies in the stage of cocoons and let in groups under 30 ^{o}C, 70 % humidity and darkness, to emerge. The presence of 2 worker bees is favourable to keep all clean. Well supplied with pollen the queens are left under this conditions till the 6th day, when they are transferred into the mating cage.

6. Fertilized queens can be obtained in laboratory, too. From the 6th day onward they fly out for mating. So those, which are just trying to leave the hive, are usually 5 or more days old. They should be caught and transferred into the mating room, to the presence of unrelated males. As mating chamber an aquarium (or even a cucumber glass) can be used covered by mesh allowing ventilation. The bottom of a mating room should be supplied with a sheet of paper to absorb faeces. Feeders must not be forgotten. Fertilization occurs under light conditions, but for *terrestris* artificial light seems to be enough. It is advisable to follow the behaviour of bees in mating space. The connected couples should be removed and transferred into suitable boxes, several together. After mating has finished (10 min. – 1 hr.), males should be removed. Queens equipped with sugar feeder should be left in darkness under the room temperature for the following 3 days. Then those having filled their honey stomach with sugar (looking heavy and cumbrous) can be stored in cold for further use.

7. The storing conditions are 4 ^{o}C, maximum possible air humidity, and quietness (Fig. 11). About 3 month of diapauses seem to be enough for queens to follow the scheme of the normal nesting behaviour. The longest survival we could observe was 8 months.

8. Queens awake by an increase of temperature and the new circle of rearing colonies can be started. If some of queens do not wake for a long period, they can be treated with CO_2. The queen should be put in a small glass or proof and as a source of carbon dioxide an empty soda bottle can serve. Puffing the gas onto the queen should be quite light, just to let her to fall asleep. Than the queen should be let to wake again. On this way queens can be forced to start nesting even without any diapauses. On the other hand the narcoses seem to shorten the life span of queens.

We tried to apply the above–described general scheme of rearing B. terrestris to other species. From of the group of so called “pollen storers” we could experiment with *B. lucorum, B. lapidarius, B. hypnorum, B. pratorum, B. soroeensis,* and from the group of “pocket makers” with *B. pascuorum, B. hortorum, B. humilis, B. ruderarius, B.sylvarum.* In the recent 2 years we obtained also 2 queens of *B. ruderatus* and 3 speciemens of *B. subterraneus*, which are considered to be very scarce in our country. As the new approach we tried also the collaboration between different species in our efforts to encourage queens to lay eggs in laboratory. Summarizing the recent experiences, together with our earlier knowledge, we came to following recommendations taking into account also the pollinating abilities of the individual species:

B. terrestris' pollinating abilities are generally known. It pollinated well *Medicago sativa*, but not *Medicago falcata* in our trials in cages. Outside, on red clover large workers visited flowers properly while the smaller ones robbed nectar through the openings on the corollas.
The species became the model for the laboratory rearing technique.

B. lucorum is the species able to fly even under very limited light intensity (working illumination in growth chamber). It can be used to pollinate *Coronilla varia*, *Medicago sativa*, *Lotus sp.*, *Mellilotus sp.* On *Trifolium pratense* workers usually rob nectar by biting openings on flower corollas.
The technique of rearing colonies in lab may be the same as it was described for *terrestris,* with the exception of using the honeybee workers. If we don't have parallel *lucorum* nest to stimulate brood rearing in queens, most of them will accept also *terrestris* male cocoon to do this. But currently, they do not collaborate with *terrestris* workers. Earlier, under laboratory conditions young colonies switched very soon to production of sexuals and failed to develop. The situation changed under lowered rearing temperature. The species prefers the room temperature round 22 ^{o}C. In 2 last yeas, keeping this condition, we obtained more numerous colonies, which produced several tenths of females. We did not deliberate experiments but the orphan colonies do not seem to produce young queens in this species. A serious problem remains in wintering young queens – we had severe mortality of hibernating queens in comparison to *terrestris*.

B. lapidarius is able to pollinate a wide range of plant species. From those multiplied in cages it prefers *Lotus*, *Coronilla*, *Trifolium pratense,* but can be used also for *Trifolium repens*.
Following the laboratory method of rearing colonies, we obtained very satisfactory results. By starting of brood rearing *lapidarius* queen responsed similarly as *terrestris* did, (again with the exception of honeybee workers, which the queens did not like, and this seemed to be the case in any other bumblebee species, too). On the other hand *lapidarius* queens regularly accepted *terrestris* cocoons for incubation (Fig.2). Most of queens having their first brood in larval stage did accept even a young *terrestris* worker, which normally helped the queen with the care of *lapidarius* brood (Fig.3). Orphan colonies reared only workers from the diploid brood, so we were able to obtain young queens from untouched colonies, only. Queens and males did copulate in limited space under natural light condition from window. We noticed severe losses on queens during wintering according to the *terrestris* conditions, without knowing the reason so far.
We were able to rear several colonies of second generation in this species.

The other three species mentioned bellow do not play an important role in pollination of cultivated plants in our country, but they have their significance in keeping the genetic diversity of other enthomophilous plant species. In enclosure they probably would be able to adapt themselves to various situations. For instance *B. pratorum* currently does not visit *Medicago sativa* in the open, but in enclosure one colony was able to pollinate it successfully and even to raise proper brood on its pollen.

What the laboratory rearing concerns**,** we don't have satisfactory results in ***B. hypnorum***. In starting period some queen accepted *terrestris* cocoon, the others did not. Mostly they failed to feed larvae properly, and colonies did not develop. Also queens of ***B. pratorum*** reacted similarly. They started nesting quite successfully in pairs (round 50%), though queens did not fed their brood well. We had only one exception in the year 2005, when one queen did breed the strong colony that produced round 40 young queens. They mated in an aquarium on the table at the window and looked heavy before the cool storage. Nevertheless, none of them survived. We gained some positive results with ***B. soroeensis*****.** In recent 3 years from 12 queens in trials 5 started brood rearing and from those 4 reared workers and small colonies. 2 of them produced young queens (8 and 1 resp.), 1 colony reared males only, and the last one, showing promising development, was lost accidentally. Probably, the cause of only partial rearing success in the three species just mentioned may be either the improper composition of pollen fed, or/and the rearing temperature, similarly as in *B. lucorum*.

From the species of so–called pocket–makers ***B. pascuorum*** gave the best results. Queens obtained in nature could be left either in free– or in separated couples (Fig. 4). The method gave round 70 % of positive results if the submissive queens were put together to make couples of second level.
On the other hand queens cannot feed larvae from the pollen reserves. After a queen constructs pocket on the brood cell (larvae had hatched from eggs), pollen pellets have to be inserted carefully into the pocket once or twice a day (Fig. 5) This has to be done by someone of the staff, what causes this phase of colony development time demanding. For workers in the developing phase of colony cannot feed larvae from pollen reserves either, it is advisable to transfer the young colony (Fig. 6) in the open. (The hive must have some suitable insulating material protecting the nest from loss of temperature.) It is surprising, that outside colonies know how to use the pollen pressed in plastic caps, which are put close to the brood. Such colonies use even the commercially available dry bee pollen after they it was properly moistened. Workers collect a portion of the needed pollen on the right plant species and the colony uses the surplus to develop much more brood. Continuous supplemental feeding of sugar is necessity, too.

The ***pascuorum*** colonies fed outside produce several tenths of queens. They can be taken from hives just after their emergence (are grey), fed quality pollen in laboratory, and mated with the chosen mails and treated in the way as in was recommended for *B. terrestris* (Fig. 7).
B. pascuorum seems to be very adaptive species. It is able to pollinate a vide range of entomophilous plants. In cages it is the case of all clovers, *Medicago sativa*, *Lotus spp., and Anthylis vulneraria*. As it is common, easily available, friendly and giving good results, it became the model for its group of bumblebees – the pocket makers.

B. hortorum is the typical red clover species it has the longest tongue among the prevailing species. It is the best one to pollinate tetraploid cultivars of *Trifolium pratense*. For starting colonies in laboratory the way of free– or divided couples can be recommended (Fig. 8) Ca. 30 – 50 % of queens can be expected to lay eggs and the first clump of larvae must be fed into the pockets. Like in *pascuorum* the outside development of colonies seems to be adviseable. We do not have any own experiences with the treatment of young queens, as all of our colonies were used for pollination in cages, where they failed to develop.

B. sylvarum becomes scarce in our country and we try to rear it laboratory, just to save it. We have no strict way to rear the species, till now. Round 40 % of queens constructed egg cell in divided pairs, the larvae have to be fed artificially into pockets. Some queens accepted *pascuorum* cocoon to start breeding. Last year, two colonies placed outside developed successfully under supplementary feeding and produced queens and males (Fig. 8)

We started cage pollination with bumblebees on alfalfa and red clover in early 80ties, last century, at the Research Institute for Fodder Crops in Troubsko, near Brno, in former Czechoslovakia. The seed yields obtained were the same of higher in comparison to those under open pollination. By means of trials with white flowering alfalfa we came to conclusion, that there was no danger of unwanted pollen contamination from outside into cages made of plastic mesh.

In Slovakia, at the Institut for Plant Production, Piestany, 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 (2 x 3 m, pollinated by *B. terrestris*) yielded more seed in 7 of all cases, but generally, the yields under coverage were similar to those in the open air. In following years bumblebee pollination in enclosure was used as a common method for safe propagation of seeds for the gene bank.

The summarizing informative list of plant species grown under enclosure during the last 8 year brings table 1. It includes also species of bumblebees for each plant species, which got right in the trials, or were seen at least by us as pollinators of the given species in nature.

Practical notes for using bumblebees to pollinate in enclosure.

- The colony used has to have brood in larval stage what encourages workers to collect pollen.
- With some exceptions the colony should have supplemental feeding of sugar and if possible also a reserve of pollen near to brood–nest (e.g. pressed into plastic tube) to avoid stress and loss of brood because of starvation. Bumblebees currently do prefer natural sources of pollen and visit flowers to collect it, so the supplement normally has no influence on the pollination. Feeders and some times also the hive–boxes must be protected from the access of ants!
- If possible, hives should be sheltered from direct sunshine (Fig 11). The warmer the weather the more important this recommendation.
- The strength of a colony should be in accordance to the possible food supply of the covered field. For e.g. 10 m^2 of well flowering red clover is enough a colony of *B. pascuorum* at the beginning of the development, having round 12 worker bees, brood and the queen, whereas for a larger glasshouse, let us say 200 m^2 of some *Brassica sp*. seed field, a well progressed colony of *B. terrestris* with round 50 workers, queen and worker brood should be used. The colony used need not to be a complete one in all cases, often a divide – several workers and a part of brood – will do. In special situations even males, flying in the cage will pollinate the flowers.
- Before inserting bumblebee colony, plants in enclosure should be partially in flower, so that bees visit them and do not search for escape.
- In one cage several plant genera can be pollinated together by one bumblebee colony, what lowers the costs of both, bees and equipment (Fig. 12).

- The plants must be treated against aphids before bees come in, as aphid population, well protected against enemies, does multiply explosively in the cage, what results in very low seed setting and almost no yield.
- Hives must be protected from rain (even an artificial one - from watering) and must not stay in water.
- Plants in cages should be grown freely, with enough air and sunshine coming among them; eventually even with an artificial underpinning, what results in the highest possible seed yield.
- Sometimes the separated gathering of seeds according to their maturity can result in increase of total yield due to prolonged flowering and prevention of falling down of overripe.
- It is advisable that plant breeder (or producer) does collaborate very closely with the manager of bumblebees, as the pollination “made–to–measure” is the right way to success.

Acknowledgement

This article came into existence thanks to the financial support from the project NAZV IR440114.

The photos of bumblebees were enabled by the sources covered by the project GA AVCR IAA4055403.

Table 1.: The list of plant species, which were pollinated in cages and bumblebees, which can pollinate them.

Plant species	**Suitable species of bumblebees**
Anthyllis vutneraria	*B. pascuorum, B. hortorum*
Coronilla varia	*B, terrestris, B. lucorum, B. lapidarius, B. pascuorum*
Lotus corniculatus,	*B. lapidarius, B. lucorum, B. terrestris, B. pascuorum*
Medicago falcata	No possitive result with bumble bees
Medicago sativa	*B. terrestris, B, lucorum, B. pascuorum, B. lapidarius*
Medicago polychlora	
Medicago romanica	
Medicago varia	*B. terrestris, B. lucorum, B. pascuorum, B. lapidarius*
Mellilotus alba	*B. lucorum, B. terrestris, B. lapidarius*
Trifolium ambiguum	*B. pascuorum, B. lapidarius, B. hortorum*
Trifolium aureum	*B. pascuorum, B. lapidarius*
Trifolium medium	*B. hortorum, B. pascuorum*
Trifolium pratense	*B. hortorum, B. pascuorum, B. lapidarius, B. sylvarum, B. terrestris*
Trifolium repens	*B. terrestris B. pratorum, B. pascuorum,*
Trifolium rubens	*B. terrestris, B. lapidarius, B. pascuorum*

Notice: The order of bumblebee species in individual lines is in accordance of their suitability to pollinate the given plant species.

Fig. 1: *Bombus terrestris* queen collaborating with *Apis mellifera* workers

Fig. 2: *B. lapidarius* queen incubation *B. terrestris* cocoon

Fig. 3: *B. lapidarius* queen with *B. terrestris* helper

Fig. 7: Two queens of *B. hortorum* starting breeding as separated couple.

Fig. 4: Two *B. pascuorum* queens as free couple with the brood cell (right at the abdomen of the lower queen).

Fig. 5: The first brood cell with larvae of *B. pascuorum*, with pollen pellets inserted into pockets, and honey pot.

Fig. 6: The young *B. pascuorum* colony with larger pockets filled by pollen. After emergence of the first generation of workers the colony is ready to be situated outside.

Fig. 8: The outside supplementary fed colony of *B. sylvarum* full of young queen cocoons.

Fig. 9: *B. terrestris* new queen larvae and cocoons produced by a colony from which the queen was removed deliberately.

Fig. 7: *B. pascuorum* copulating couple, just being transferred to the special container.

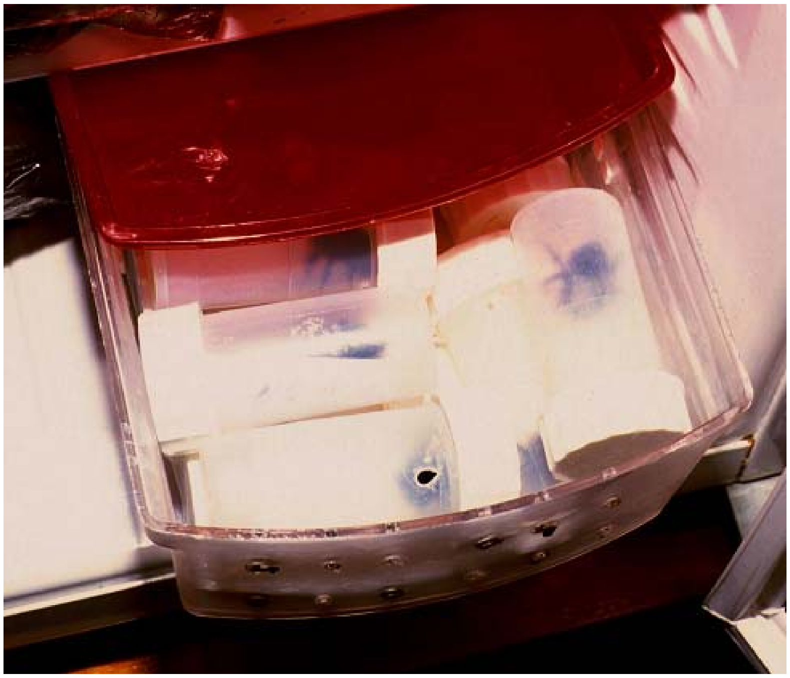
Fig. 10: Diapausing *B. terrestris* queens in refrigerator


Fig 11: The seedbed of *Trifolium pratense* with a bumblebee colony in the shaded hive.


Fig. 12: Inside the cage with *Coronilla varia* and *Lotus corniculatus* grown together and pollinated by the same colony. The hive is protected from the sunshine.