Bombus terrestris L (Hymenoptera: Apidae)

Pollination services, a business challenge

Rearing Methods and Techniques

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The potential value of bumblebees as pollinating insects in agriculture has been recognized for a long time. The vegetable, fruit and seed growers using bumblebees for pollination benefit from lower production costs, increased yields, and an improved quality of their products. Bumblebees have many characteristics that make them more efficient in pollinating crops than honeybees. They visit twice as many flowers per minute, can carry relatively bigger loads, find it easier to operate in greenhouses and tunnels, remain active at extreme weather conditions, require a friendlier pest control and can pollinate flowers with deep corollas. In addition, the application of bumblebees for pollination has stimulated growers to change towards bio control methods for crop protection. The consumers and the environment also benefit from this development: consumers get a better, tastier, and healthier product, whilst fewer residues of harmful pesticides are released into the environment.

Bumblebees can pollinate crops like tomato, pepper, eggplant and many more (see table).

The bumblebee *Bombus terrestris* has distinguished itself as pollinator of glasshouse plants, especially tomato. In 2004, 40000 ha of tomato crops were pollinated, with a crop value of €12000 million (Velthuis et all. 2006).

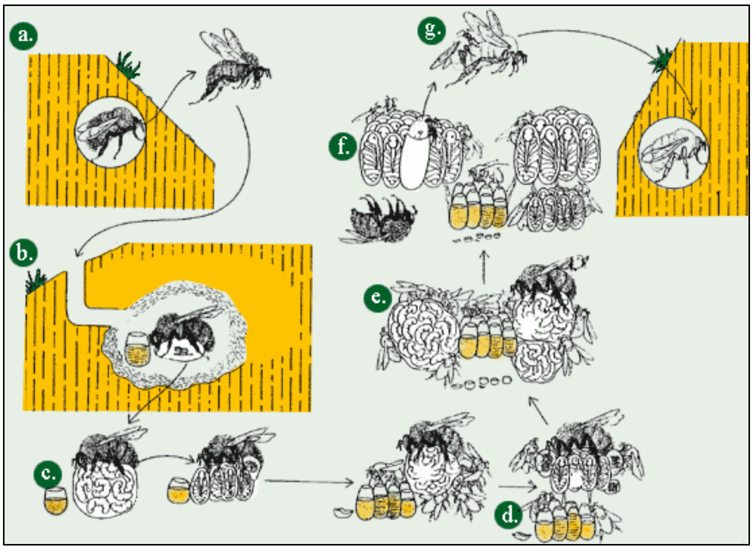
B. terrestris is aparted by approximately ten subspecies and many of them have been used in the early years of commercial rearing. From the commercial point of view, however, *B. t. dalmatinus* proved to have superior characteristics. It produces the largest colonies, and the success rate in the rearing is the highest. It has, therefore, become the dominant subspecies in the rearing industry. Today, *B. terrestris* colonies (mainly *B. t. dalmatinus*) are used for commercial pollination not only in its Eurasian area of distribution, but also in East Asia (Japan, South Korea, China), and South America (Chile) (Velthuis and all 2006). It is remarkable that the distribution area of this subspecies is Greece and Turkey. This fact makes the choice to establish a company in our country very important. Especially in Lesvos the wild species B. terrestris is abundant probably due to the good weather conditions or to the islands geographical position.

Crops commercially pollinated by bumblebees.

Crop	Latin name
tomato	*Lycopersicon esculentum*
pepper (sweet, hot)	*Capsicum annuum*
eggplant	*Solanum melongena*
melon	*Cucumis melo*
watermelon	*Citrullus lanatus*
cucumber	*Cucumis sativa*
courgette (zucchini)	*Cucurbita pepo*
strawberry	*Fragaria x ananassa*
raspberry	*Rubus idaeus*
blackberry	*Rubus fruticosus*
currant (red, black)	*Ribes sativum, R. nigrum*
cranberry	*Vaccinium macrocarpon*
blueberry (highbush, lowbush, rabbiteye)	*Vaccinium corymbosum, V. angustifolium, V. ashei*
apple	*Malus domestica*
pear	*Pyrus communis*
cherry	*Prunus cerasus, P. avium*
kiwifruit	*Actinidia deliciosa*
peach	*Prunus persica*
apricot	*Prunus armeniaca*
plum	*Prunus domestica*

All bumblebee producers today have developed their own rearing systems, which are kept primarily secret. Nevertheless, the scientific literature gives some information on bumble bee-keeping and so we have information about early queen producing, mating, managing diapause and the founding of new colonies, which are major problems during the year round rearing.

Physiology- Life cycle



During the blooming stage, bumblebees live in colonies. This stage is about three or four months long. All through this period, the whole colony evolution takes place; it starts with a single individual, the queen, and it ends with hundreds of dead bumblebees that have finally make up the hive population.

As soon as blooming starts in winter or spring, queens wake up after their hibernation and they start looking for a proper place to begin their colony.

Bombus terrestris usually look for shelter in burrows in the ground, sometimes they go into old mouse holes. Afterwards, the queen comes out to collect food. The queen moulds a wax cup for honey, big enough to store the quantity she will need. She builds a small ball of pollen, and then she covers it with a wax layer. On this wax, the queen builds the first small cells, and she lays up to eight eggs inside each one of the cells. The queen rests on the cells, breeding the brood thanks to her high body temperature. Larvae find food inside the pollen ball on which they are placed.

Three weeks after, the first eggs hatch and the first adult workers emerge. Soon these workers will take over from the queen the duties of collecting pollen and caring for the hive. Later on, the queen will not fly out any more, and she will be devoted to egg laying and breeding them. Workers will be in charge of enlarging and isolating the wax structure and also feeding the larvae with honey and pollen.

After a while, no more workers emerge, we find instead new queens and male bees. The old queen usually dies about that time. Males stay a few days in the hive and then they leave forever.

After emerging, during the first week queens feed themselves with the pollen and honey supply that they find in the nest. Therefore, they can build a thick greasy layer on their bodies that will be essential to survive during the long solitary hibernation stage. Soon, young queens will come out of the hive for their nuptial flight. They are able to store sufficient sperm in her sperm pouch, or spermatheca to fertilize all the eggs she will lay for the rest of her life. After that, young queens leave the maternal nest forever to look for a sheltered place to hibernate.

Young queens' departure indicates the end of the bumblebees' colony. Nearly day by day, workers stop collecting food, and they do not take care of the larvae and pupae and they die.

The beginning. Obtaining colonies.

Finding natural colonies.

It is difficult to locate bumblebee colonies in their natural habitat, but using natural colonies is one of the best methods to begin. It can be achieved by tracking workers visually when they move in or out of places that are not evident foraging sites. The location of nests is highly variable and may include in or on the ground, holes of trees, or even the roof and wall insulation of human dwellings. Larger colonies become more conspicuous with the increased forager traffic they generate especially during the summer (Thrasivoulou et all 1998). Disturbing the vegetation around the location where the nest entrance is suspected will force foragers to search for its position and, thus, may help pinpoint the entrance faster. Once a natural colony is found, it is usually necessary to transfer it to a nest box. Instructions how to transfer a wild colony are available in http://www.bombus.de/Umsiedelung.aspx.

However, the chances of finding natural colonies are low. Furthermore only well developed colonies, with high foraging activity, will normally be found.

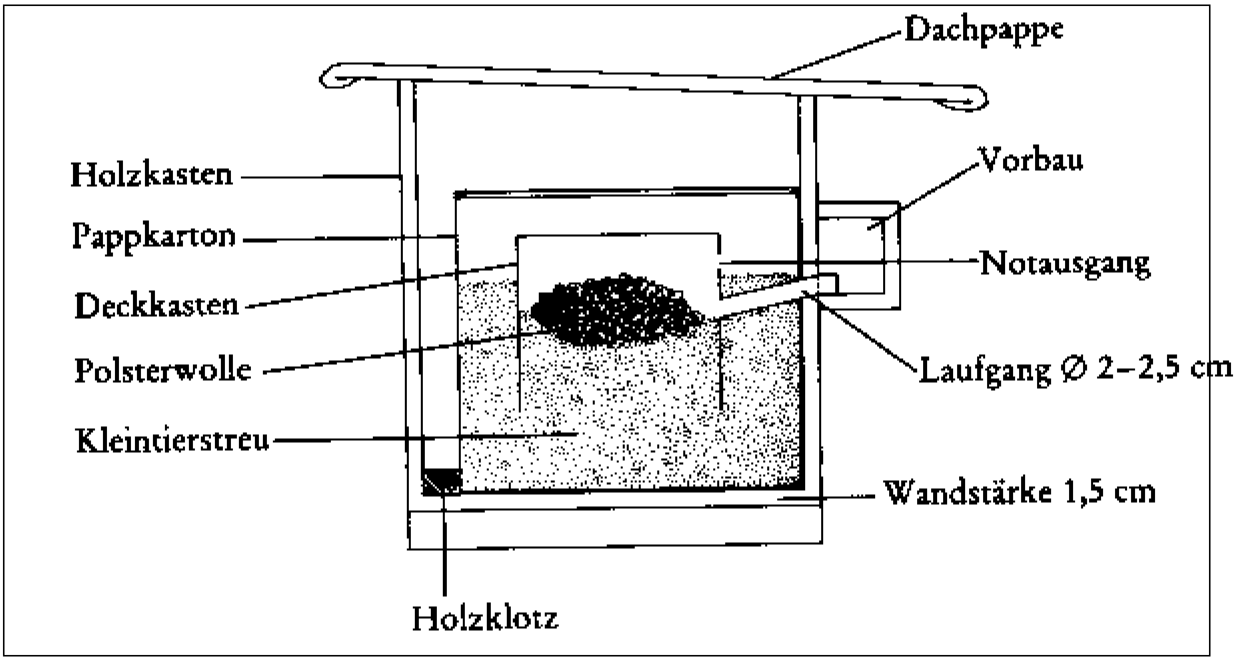
Nest boxes.

In areas where bumblebees are abundant, setting out nest boxes (domiciles) in the field to promote the establishment of bumblebee queens is the method that requires the least effort.

There are many descriptions of how to build nest boxes but overall a nest box of internal dimensions 15 cm x 15 cm x 15 cm is usually sufficient as it accommodates a colony of up to 150 workers. The box can be made out of any type of material (plywood, concrete, polystyrene, plastic) (Macfarlane et al. 1984), but should be constructed in such a way to allow the water vapour to escape, as excessive humidity makes colonies susceptible to mould. The inside surface needs a 2-cm thick layer of insulation, such as water-repellent upholsterer's cotton (Hobbs et al. 1960) or non-surgical white bleached cotton. The bleached cotton has the advantage of allowing easy detection of nest parasites. Cotton wool is not recommended, as bumblebees tangle their feet in it (Pelletier 2003).

In the Mediterranean and Aegean coastal regions *Bombus terrestris* queens emerge from diapause in autumn (September-November), whereas in inland regions and in Europe they emerge from diapause in spring (February-March) (Goesterit et all 2005). Velthuis et all. (2006) claim that in southern European populations of B. terrestris aestivate rather than hibernate. This is very important, because this fact appoints the period that we should place the nest-boxes in the field

Nest boxes should be placed close to abundant sources of spring food, as queens appear to search for cavities near rewarding habitats. Prairies, forests, bogs, and margins of intensively managed fields are generally poor sites. Meadows and especially botanical gardens (where food is plentiful but natural nesting sites are limited) are good sites. It is also advisable to set boxes in sites where pesticide use and possible vandalism are minimal (Pelletier 2003).



Nest boxes can be placed underground, above-ground, and on trees.

Instructions how to build an underground nest box are available in http://www.arminkrenz.de/erdnest.htm.

Information about nest boxes available in

http://www.bombus.de/Nistkaesten.aspx

http://www.harry-abraham.homepage.t-online.de/nistkastenbestellung.htm.

http://www.hymenoptera.de/html/search/node/nistkasten.

Catching wild queens.

We can collect queens from the field. Those which had emerged from diapause, become active when the pollen sources are available. They fly low to the ground in a zig-zag pattern, looking for a place to nest. It is best to catch them while they have not yet started a nest. Queens with pollen on their legs have already started a nest in the field and are unlikely to resume nesting in captivity (Delaplane). Queens can be caught with an insect net. They should be transported individually in small boxes with lids loosened to provide ventilation and prevent overheating. These queens should be transferred into starter boxes as quickly as possible.

In order to set up a bumblebee-rearing process, companies collected tens of thousands of queens in the early years, preferably those which had just emerged from their hibernation sites. After some time, however, the massive collection of queens from nature evoked protests from the citizens of the countries involved (Özbek, 1993). We believe that for the establishment of a small local company and because we have now plenty information about rearing techniques, the collection of many wild queens it is not necessary.

There is another technique using queens, which have started a nest. Catching queens which collect pollen for their brood and using the CO2 method, we can stimulate them to restart laying eggs by inducing their ovary development. In 1985 Röseler published that a CO2-treatment could also be used to circumvent or break diapause. This proved to be an easier method and, later, became a key instrument in the commercial production of bumblebee colonies (Velthuis et all. 2006). CO2-narcosis of mated queens makes it possible to rear bumblebees continuously by eliminating or truncating queen's hibernation period, according to the demand for colonies.

A necessary tool for this method is a bottle of carbon dioxide (CO2) gas with a regulator connected to a few meter long rubber tubing. The mated queen is placed individually in a glass jar in which she is treated with CO2 for 10-20sec, until she is immobilized. Puffing the gas onto queen should be quite light, just to let her fall asleep.

Then the queen should be let to wake again (Ptacek 2006). Delaphane suggests that the CO2 treatment should be repeated after 24h. Two to four days after the last CO2 treatment, each queen is placed in the starter box to lay eggs. It is very important that activating queens fly and eat pollen and syrup. Ptacek claims that the only disadvantage of the method is that the narcoses seem to shorten the life span of queens.

Professor Guerel from the Akdeniz University in Antalya Turkey (personal interview) argues that in the Mediterranean coastal region queens from the *Arbutus unedo* species can be collected in autumn. This plan is very important for bumblebees because it is the only one that blooms in autumn providing very good quality of pollen.

Buying colonies

As a last resort, colonies can be bought from an industry (e.g., Koppert, Biobest). Those companies have built up to provide year-round bumblebee colonies for greenhouse crops, and scientists in this industry have perfected means of continuously breeding *Bombus terrestris*.

Producing new queens.

By using the commercial colonies described above or from any wild colony which is grown in the nest-boxes, we can produce new queens. When a colony has reached maturity begins producing queens and males. But not all colonies in the laboratory produce queens. There is a split-sex ratio in *B. terrestris*: some colonies produce males in large numbers, while others specialize in queen production (Duchateau et al., 2004).

Queens are reared from the portion of diploid brood available in a colony when it reaches a physiological stage called as the “switch point”. No other young queens emerge then in such a colony as its old queen turns to laying haploid eggs only and so produces males for the rest of her life (Ptacek 2002). This possibility of producing new queens either from commercial or even from any laying queen makes the method of laboratory rearing bumblebees complied. Moreover using “good” colonies we can produce queens on the basis of their favourable properties. It is presumed that the commercial companies have make selection over the last 15 years in order to rear bumblebees with desirable characteristics such as strong, long lasting colonies, which will produce queens and males in the later stages for pollination in the greenhouses (Goesterit 2005).

Another method known from the honeybee-keeping is the production of young queens in a colony by removing its old queen at the stage of 15-30 workers. Under this situation remaining old larvae and those in cocoons develop into workers. The younger ones well fed by workers quickly enlarge their bodies and finely spine typically large cocoons. They also behave according to the queen-model: consume lot of pollen in the first 3 days, mate, fill their stomachs and enter hibernation. Later they are able to start egg laying on a normal way (Ptacek 2002).

According to the invention of De Ruijter and Van Den Eijnde (2000), there is another method to rear bumblebees, wherein the queens are produced first, and wherein the average number of queens per colony is higher than occurs in nature. These advantages are achieved by the process which is characterised in that subadult and/or adult workers which themselves originate from at least one different colony are brought together with a young colony in the eusocial phase, consisting of a fertilised queen, brood and the first born workers. Furthermore, tests have shown that the best results are achieved when the workers originating from a different colony are first kept in a room without any queen and without brood for one day. According to the invention nearly four times as many queens were produced and this increased production took place within a period of time that was 14 days shorter in comparison to the control group.

The starter box

Queen starter boxes are small boxes in which mated queens are placed and induced to begin nesting. Those boxes can have two or one chamber. One design is a box approximate 23x12x5 cm with two chambers-a nest chamber and a feeding/defaecating chamber. Bees can pass between the nesting and defaecating chambers through a small circular opening. The floor of the defecating chamber can be of small-mesh hardware cloth or be lined with a square of corrugated cardboard that is replaced as needed. In the nest chamber it is helpful to provide a small plastic lid on which pollen will be given and the queen will build her brood clump. By encouraging the queen to nest on a plastic lid, it is easier to move the comb later when the incipient colony is graduating up to a finisher box. It is also helpful to attach a plastic honey bee queen grafting cup to the floor of the plastic lid with melted beeswax. These cups simulate the shape and size of a natural bumble bee honey pot and may encourage nesting (Delaplane). This design keeps the box interiors dark, but the nest chamber can be easily opened and bees observed through the clear inner cover.

The single-chamber starter box is about 12x5x10cm. It provides only a room to nest and to defecate. Simple “kitchen” plastic containers with sets of openings for ventilation, bottom covered with material absorbing moisture can be used for this purpose. It is simpler because it can be put into a bigger box from the beginning (the finisher box) and when the population is graduating we can remove the walls of the small inside box providing more space. The finisher boxes provide enough space in which the colony can grow to maturity. Ventilation is provided in both designs by holes drilled into the walls of the feeding/defecating chamber. Of course we have to supply sugar solution using feeders. Plastic tubes or bottles with tight cap and one tiny opening only, enabling access to the food.

The rearing room and ambient conditions

Because of the size of the finisher boxes (10-12lit) we don't need a very big room. In a 5x5m room we can rear about 500-700 colonies. This place should be totally dark and providing only a red light during observation because bees do not react to this range of the light spectrum. It is important to provide favourable climate conditions for starter and finisher boxes. For this purpose we need an air-condition with a thermostat capable of keeping constant level of temperature and air humidity. 29oC-30oC and 70% of humidity enable queens to lay eggs and incubate their first progeny (Ptacek 2002) or 28oC-30oC and 60-65% R.H. according to Duchateau (1988).

Colony initiation

Starting the first brood cell is the most important point during the bumblebees rearing process. This can be achieved by several procedures after hibernation or using the CO2 method:

- Keeping two queens together in the common rearing box. Most of them behave friendly for the first time and during the following week or 10 days at least one of them will start egg lying. Later, one of them becomes dominant (the egg laying) and kills the other. This one ought to be left alone and the other should be transferred into the society of another similar queen.
- Each queen can be given three or four young honey bees. The honey bee workers should be used only when they are less than 12h old. The presence of those bees stimulates the queen to begin brooding. Within approximately 10 next days most of the healthy queens will construct one cell and lay eggs on the bottom of the rearing box. Then the bees should be taken away (Ptacek 2006).
- Each queen can be given one newly emerged *Bombus terrestris* worker and its pupa. Using this method 57% of the queens founded colonies and 34%founded big colonies, which can be used in greenhouses (Goesterit 2005). It is necessary that one healthy queen and a minimum of 50-80 workers exist in the Bombus terrestris colonies used for pollination in the greenhouses.
- Providing a queen with just one young male pupa, fixed horizontally, was effective to stimulate colony initiation: 80% of the queens provided with such a male cocoon produced a colony, and most of them produced also young queens (Kwon and all. 2003). Similarly, the queen-as well as worker cocoons can be used. The emerged adults should be taken away immediately.
- Providing queens with four worker helpers and using frozen male old pupae were found to be sufficient, as they could produce 100% colony foundation and 91.46% first worker production colonies (Kwon and all. 2006).

Gretenkord (1997) compared several methods for stimulating the queen to oviposit, and found that the most successful method was the combined addition of workers and larvae to the nest box. For commercial rearing, this method is less practical because it is more laborious and when the larvae is detached from a colony, the cell covering of wax over the larvae is usually damaged, which results in the death of the larvae. For mass rearing, methods need to be both effective and economical. Supplementing a queen with one male pupa seems a promising method, because it was used successfully in several studies, it is less labour intensive and, in a mass rearing system, male pupae are always available.

Food supply

All colonies need fresh food that consists of carbohydrate and protein.
Syrup made of 1 part sugar to 1 part water can supply the necessary energy. Some authors recommend putting the antibiotic Fumidil into the syrup in order to control nosema disease. We can use syrup made of 1 part honey to 1 part water. Bees can locate it more easily because of its attractive odor. It is important to know that honey syrup ferments more rapidly than sugar syrup and must be replaced more frequently (at least every 2 days). Dr Ptacek uses feeders providing constantly approximately 60% sugar solution with the 10% of fructose and 90% of sucrose in the dry matter to prevent granulation. Solution should be stabilized against microbial processes by 0,1% Na-benzoate and/or Na-sorbate (Ptacek 2002). It is estimated that approximately one million kg of sugar syrup is used the whole bumblebee rearing facilities and that another two million kg of sugar syrup (approx. 65%, w/w) are used annually to feed all of the colonies used in the greenhouses. This amount is needed because the flowers of the main target crop, tomatoes, do not produce nectar. About 2 litres of the syrup is sufficient for the entire lifespan of the colony in the greenhouse, which is typically between 8 and 12 weeks. Finally, more than 200 tons of honeybee-collected pollen is used annually by all bumblebee producers worldwide (Velthuis et all 2006).

Pollen pellets collected by honey bees cover the need in proteins. For this reason it is useful to keep some colonies of honey bees with pollen traps. It is important to collect and freeze enough pollen to sustain the operation during winter if it is intended to grow bumble bees year-round. Fresh bee-collected pollen must be cleaned of visible debris and must be kept fresh-frozen in its bee-collected pellet form until it is needed. For single queens, just starting colonies, fresh or deeply frozen fresh pollen is advisable, whereas colonies having workers can consume the commercially available good quality dry pollen (which naturally must be moistened before use to the original stage, +20% of water) (Ptacek and all 2006). Not only the physical, but also the chemical properties play important role. The quantitative and qualitative variations of pollen proteins have considerable influence on the reproductive success of bumblebees (Genissel and all 2002)

The food components in pollen differ according to plant species. When pollen quality was tested for bumblebees, it was found that rearing success is better with pollen containing a high protein content (e.g., from *Brassica* and *Prunus*) than with pollen from other plants species (e.g., *Helianthus* and *Taraxacum*). Therefore companies buy tons of spring pollen for their rearing needs. Queens produced from colonies that are fed fresh-frozen pollen are larger, have lower mortality rate, and produce larger colonies than queens produced from dried-frozen pollen fed colonies do (Delaplane et all). We estimate that a colony needs about 300g of pollen for its entire life

Mating

From the 6th day onward queens fly out for mating. They should be caught and transferred into the mating room, to the presence of unrelated males. Ideally, there should be about twice as many males as females. As mating room a Plexiglas box can be used, sized about 40x40x60cm with an access of fresh air. Feeders must not be forgotten. *Bombus terrestris* can mate using artificial light. The connected couples should be removed and transferred into suitable boxes, several together. When mating has finished (10min–

1hr.), 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 (Ptacek 2002).

Storing queens

Because of the big number of produced queens and according to the demand for colonies we have to make a stock. We can use a normal refrigerator and put them all together in big boxes or place each one individually in a vial half-full of damp peat moss and store them at 5oC, maximum possible air humidity and quietness. The longest survival is 8 months. 70-80% of queens survived the period of 3-6 months of cold storage. Then concerning the sales or the need on colonies about 8 weeks later, young hibernated queens can be taken from the stock and by an increase of temperature become active again.

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Companies

Agriapi	www.agriapi.it
Agrobio	www.agrobio.es
Bio bee	www.seliyahu.org
Biobest	www.biobest.be
Bioplanet	www.bioplanet.it
Biopol	www.biopol.nl
Koppert	www.koppert.com
Stb Biocontrol	www.stb-control.de
Syngenta Bioline Bees BV	www.syngentaagro.es
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