Karel Dušek, Pavel Krieg, Elena Dušková

Methodology for using insect pollinators in heterogamous vegetable species, medicinal, aromatic and culinary plants grown in technical isolation

METHODOLOGY FOR PRACTICE

Crop Research Institute, Prague

Bee Research Institute Ltd., Dol

2010

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Summary

The publication summarizes the knowledge verified in practice when regenerating heterogamous vegetables and medicinal, aromatic and culinary plants (MAPs) in technical isolators using honey-bee and bumble-bee as pollinators. Production of a sufficient quantity of the high-quality, healthy and germinative seeds is an assumption to maintain seeds as a genetic resource and initial material for potential users – plant breeders, research workers and educational institutions. Thereinafter, the methodology deals with and evaluates constructional elements of the isolators , utilization technology of the stationary and mobile technical isolators and agrotechnics during the plant regeneration in the technical isolators. It describes the technology of using the honey-bees and bumble-bees for pollination in the technical isolation.

Oponents:

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Methodology was approved by the Ministry of Agriculture CR, Department of Plant Commodities, under the Reference Number 7877/2010-17220.

Description of the methodology application

The methodology is intended for the institutions engaged in plant germplasms, for plant breeders and research workers in the field of research on vegetables, medicinal, aromatic and culinary plants.

The certification methodology will be passed on to the firm Moravoseed Ltd., Mikulov-Mušlov, engaged in the plant breeding and seed production in vegetables and medicinal plants, as well as to the others being from the abovementioned fields of interest.

English version of the methodology will be provided to the members of the international organization ECPGR (European Cooperative Programme for Plant Genetic Resources) where it will serve as a guide for the work on genetic resources in the working groups (Working Group Medicinal and Aromatic Plant and Vegetable Network).

Contents:

I. Objective of the metodology

An objective of the presented methodology is to provide experts and workplaces engaged in genetic resources, plant breeding and seed production in vegetables and medicinal, aromatic and culinary plants with information on a possibility of using pollinators (honey-bee and bumble-bees) in technical isolation.

II. Description of the methodology

1. Introduction

A world-wide biological diversity is an extensive and still undervalued source for a man. Ecosystems of plants and animals play the main role to ensure a biosphere stability. The loss of a key species can adversely affect ecological relationships being of a vital importance (Zaden, 1995).

Similarly under our conditions, the loss of a landrace variety is an irreplaceable genetic and cultural one, and disappearance of wild species causes depletion of our flora (Holubec, 2005).

1.1 Plant genetic resources

Genetic resources have an incalculable value for mankind whether they are used in traditional agriculture, conventional and modern plant breeding or in genetic engineering. They are unique and irreplaceable gene sources and gene complexes for a further genetic improvement of the biological and economic potential in production organisms (plants, microorganisms and animals) in agriculture and biotechnologies.

Therefore, genetic resources are a key for the further development in agriculture and biotechnologies to ensure a higher food safety and improve living conditions and life quality of the mankind (Palas, 2003).

Worldwide network of the genebanks and/or collections in national and international scale plays an important role for the maintenance of germplasm:

Two different approaches are used to maintain plant genetic resources:

- „*in situ*“ conservation aiming to maintain genetic resources as a part of ecosystems at the place of their original occurrence (for wild species in the nature it is an environment similar to conditions of their origin in landrace varieties). This type of conservation can be considered as a dynamic one because it enables a further population development in accordance with environmental conditions.
- „*ex situ*“ conservation, where the samples of genetic resources are conserved in collections away the place of their original occurrence, i. e. according to their biological nature in field collections (especially sustainable species), in seed genebanks, in „*in vitro*“ cultures, and in some selected species their „*in vitro*“ cultures are conserved in cryobank .

An obtainment of high-quality and healthy samples of genetic resources characterized by the high viability, suitable for a long-term maintenance, is a basic assumption for successful „*ex situ*“ conservation. If such material is not at disposal, the regeneration of genetic resources available is neccessary aiming to provide with samples of an extent and quality needed. At present, according to estimation, at about 13 percent of all accessions in collections require to be regenerated. Seed samples from collections of all generatively propagated crops in the Czech Republic are maintained in the genebank of CRI in Prague where 36.6 thousand of accessions in total are now stored, i. e. more than 91 percent of all the seed-propagated genetic resources in collections. Their regeneration (if there is no greater quantity of viable seeds or plants available), and subsequent conservation, are a basic assumption for safety maintenance of genetic resources (Dotlačil, 2004).

Among others, the gathering of genetic resources in genebanks brings along another task, namely the regeneration of respective samples to get a sufficient quantity of high-quality, healthy and germinative seeds which are then added to genetic resource collections, of those being passed on to potential users.

1.2 Regeneration of genetic resources

With respect to the fact that heterogamous plants make up a substantial part of the collections, it is necessary to ensure a high-quality space or technical isolation to obtain identical seed material under conditions of work on genetic resources.

1.3 Space isolation

The use of space isolation when regenerating heterogamous plants is very problematic in practice.

To keep a minimum distance between reproduction stands (100-1000 m), as required under the valid regulations (Promulgation of MA no. 175/2004), it is very space-demanding. In this way it becomes possible to regenerate only a very few genotypes of one species during a year.

The making use of a barrier crop is another method of space isolation used in seed production (e.g. *x Triticosecale, Secale cereale*). Nevertheless, it appears to be also very labourious, and low efficient for the needs of vegetable and MAPs regeneration.

1.4 Technical isolation

Since 1955 the problematics of technical isolation by constructing stationary isolation cages has been solved at the Vegetable Research and Breeding Institute (VRBI) in Olomouc.

The cages were originally intended for regenerating the vegetable collections, afterwards successfully utilized for seed production in heterogamous species of medicinal, aromatic and culinary plants (MAPs) as well as in plants growing wild in nature being then transferred to be cultured (the utilization for flower meadows setting-up, for maintenance of endangered or disappearing populations, etc.). Stationary constructions, partly glassed-in, having ventilation apertures covered by insect-proof netting, served as the basis.

Since 1980 mobile isolation cages have been used thereby representing a turning point in the use of technical isolators. These cages enabled the isolation of plants grown directly in field stands.

At present, about 150 stationary isolation cages and 50 portable ones are successfully used every year (Dušková, Chytilová, 2003). A problem of the isolation is subsequently connected to that of pollination. Hand pollination carried out in practice is labourious, time-consuming and skilled labour-demanding, therefore the utilization of pollinators to obtain seed material is a very actual idea, not only for the work concerned with germplasm, but in basic research and plant breeding practice as well.

Since 2003 the programme of using incomplete honey-bee colonies (*Apis mellifera* L.) and bumble-bee (*Bombus terestris* L.) nests for pollination (Dušek, 2003, Dušková, Chytilová, 2003) has been developed in cooperation with the Bee Research Institute Ltd. in Dol (the station in Prostějov and Přerov-Žeravice) which is also engaged , among others, in laboratory rearing the bumble-bees (Krieg et al., 2009).

1.5 Pollinators

The use of isolation cages brings a problem with plant pollination. Hand pollination with the brush and/or hand-knocking in the stand (Apiaceae, Liliaceae, etc.) are difficult to carry out in a great number of cages.

Utilization of incomplete honey-bee colonies (*Apis mellifera* L.) or bumble-bee (*Bombus terestris* L.) nests for plant pollination appears to be the solution. (Dušek, 2003, Dušková, Chytilová, 2003).

2. Methodology

2.1 Technical isolators

Based on the contemporary knowledge, long-term verified, it is possible to recommend the isolators as follows:

A. Stationary glassed-in isolation cages

- **dimension 5150 x 2850, height 1500-2000 mm**
- **material well proven for the construction: profile T 30/30/3 mm and closed profile (jäckel) 40/20/2 mm**

Galvanized cage construction is placed on a concrete socle where it is fix anchored. The roof, faces and a part of the sidewalls are glassed-in. The ventilation is provided through four side windows and triangles in the face of isolator equipped with the netting.

These cages are mainly suited to the regeneration of early-flowering vegetable species such as vegetable brassicas, lettuces, radish, etc., and the species susceptible to air humidity during flowering time and seed maturing (lettuce, caraway, etc.).

Fig. 1 Stationary isolation cages

B. Stationary isolation cages with the net hood

- dimension 5150 x 2850, height 1500 mm

- material is the same as in the stationary isolation glassed-in cages

The metal construction with fixed anchorage in a concrete socle is covered up with net hood just before plant flowering. It is suited to the most of vegetables and MAPs. A better ventilation and temperature regulation inside the cage appears to be the advantage of this construction. However, in rainy weather the plant stand inside the cage is more subject to a pathogen damage, mainly to fungal diseases.

Fig. 2 Stationary isolation cage covered with the net hood

C. Mobile isolation cages

- **dimension 2000 x 3000 x 1700 mm**
- **material well-proven for the construction: closed profille (jäckel) 40/20/2 mm and ¾“ tube with strength 2 mm**

These isolation cages consist of demountable metal construction covered with the net hood which equipped with a wooden frame for its anchorage. The isolation due to the cages is suitable for field stands, especially for biennial and perennial cultures. When using portable isolation cages the good results were observed mainly in MAPs regeneration (lavender, thyme, caraway, coriander, digitalis, mallow, fringed rue, savory, etc.). In addition to the simple installation there is also an advantage of possible multiple use of the constructions in the course of vegetation period when successive flowering of various plant species takes place from April until July.

Polyamide monofil textile is successfully used to make the parts of isolation cages (the type no. 737968, filament diameter 0.3 mm, mesh size 0.6-0.8 mm, manufactured by TECHNOLEN, technical textile Ltd., Lomnice nad Popelkou).

Fig. 3 Mobile isolation cages

2.2 Growing technology in technical isolators

Soil preparation before sowing and plantation in the stationary isolators consists in the hand digging after crop harvest in autumn and the hand raking to smooth out surface in spring. The covering of soil surface with a black non-woven fabric just before plantation in stationary isolation cages proved good as it creates a favourable microclimate for plants grown, prevents from weed penetration, maintains suitable humid conditions, enables plant manipulation within vegetation period (plant training to supporting constructions, protection against diseases and pests, pollinator location and treatment, seed harvest, etc.) and reduces a frequency of cultivation activities inside the cage during vegetation.

Installation of the drop irrigation directly to the plants has also proved good. It ensures the most suitable water regime for plant growth and additional fertilizing, respectively. Due to regulation of water supply it is possible to partly influence the time of seed setting and maturing so that a manual labour on plant treatment can be reduced at the same time. Financial savings in water consumption are also indispensable. We recommend PE tubes with connection to the capillary hose and irrigation needle for installing the drop irrigation system.

A commonly available mechanization (small tractor with additional cultivating tools) is used under the field conditions until the beginning of plant flowering. Portable isolators are placed above plant stand just before the start of flowering, and further stand treatment is similar to that carried out in stationary isolators (plant training to supporting construction, protection against diseases and pests, location and treatment of pollinators). After the end of flowering the isolators can be removed.

Fig. 4 Layout inside the isolator and pollinator location

Plant protection against diseases and pests plays an important role in using the isolators. The plants grown in technical isolation are more often attacked, especially by fungal diseases, under unfavourable climatic conditions (long-term rain, cold weather). An increased occurrence of animal pests such as aphids, whiteflies, thrips, chiggers, webworm moths (Depressaria) and moths is also indispensable. To make a good choice of chemical protective agents it is necessary to take into account their selective and considerate impact on pollinators.

Fig. 5 Plantation in the isolators

Fig. 6 Detail of drop irrigation

2.3 The use of pollinators – general conditions

Wooden small hives with pollinators are located in isolation cages where installed on the top of a metal stake in the height of 60-70 cm above the ground. Base of the stake is protective-coated (e.g. Chemstop, etc.) to prevent undesirable penetration of another insect (mainly ants) into the hives. When placing the stakes in the isolator any contact of the hives with plant stand (there is a threat of insect penetration into the hives), and also the hive falling-down due to the net hood during a wind gust must be eliminated. The hive fastening to the stake with elastic material (wider elastic band) and/or binding wire has been proven well.

Fig. 7 Location of the small hive on the stand in mobile isolator

The combination of several species (minimum 2 – 4), characterized by a gradual flowering, appeared to be advantageous for plantation in the isolation cages.Thus pollinators are offered more various flowers as well as the pollinator utilization is enlarged during the period of their viability. In monoculture, after its flowering, a labourious move out of the hives is carried out and then „cleaning" of the pollinators by placing them in another unrelated species for minimum 3 up to 5 days.

2.3.1 Honey bee (*Apis mellifera L.*)

To prepare honey bee pollination units we use the portable wooden hives, outside dimensions to be 270x130x220 mm, consisting of one compartment, their roof being metal-plated and with PVC-spacer insulated, which prevents a water leakage to the hive and its overheating. In the front wall there is a ventilation aperture 25 mm in diameter. The hive entrance, dimension 40x8 mm, is situated at the level of hive bottom. Inside the hive there is a frame with strip of comp foundation, ceiling feeder and the dish with a pheromone.

About 150-200 g of narcotised worker bees (about 1500 of individuals) are put into the hive. The bees are narcotized using N_2O released from NH_4NO_3 combustion in apicultural smoker. The pheromone is an ethanol extract of fertilized honey-bee queens. It is applied in plastic small bottle the volume of which is 80-100 ml and equipped with the wick in order to evaporate active agents. As a bee feeding the honey dough (the mixture of powder suggar with honey in the ratio 3:1), confectionary fondant and/or currently free available special fondant for additional feeding the bees (APIFONDA, manufactured by Südzucker AG, Mannheim/Ochsenfurt, Postfach 1164, D-97195 Ochsenfurt) are used in the dosage of 400 g per feeder. It is important to keep the correct consistency of the feeding because a diluted dough consistency causes the bee death owing to a sticky layer all over their body.

Figs. 8, 9 The hive for bees – feeder, dish with a pheromone and frame with comb foundation

The closed hives filled with bees, having sufficient ventilation netting apertures, are neccessary to be placed in a cold room for 2-3 days. Within this time the worker bees form the cluster around a pheromone steamer. The coherence and working activity of such incomplete bee colonies was tested and repeatedly confirmed in previous research conducted under similar conditions (Krieg, 1994). After transferring the beehives to technical isolators they are equipped with a water feeder.

Figs. 10, 11   Preparation of the honey-bee pollination unit

According to our experience a viability of the pollination units is ranging from 30 to 80 days. The greatest bee loss and death occur within the first days after beehive placing in the technical isolators where the bees, seeking an escape way from the closed space, fly towards the glass filler or into the net hood. To prevent the loss of all the bees due to their flying off the cage we must perfectly seal it up. During the exposure a gradual bee death is observed. Towards the end of the season (based on the weather conditions it is the end of August or beginning of September) only a few tens of bees, in optimal case, remain in the hive.

Fig. 12   Honey-bees seeking an escape way

Fig. 13 Frame at the end of pollination

Fig. 14 Bee hive at the end of pollination period

A treatment of the pollinators in technical isolators consists in a regular supplying the water feeder, bee feeding and pheromone (as a rule once per viability period), in the check of health state and number of bee individuals in the hive.

2.3.2 Evaluation of the honey-bees used as pollinators

Resulting from a long-term experience of our workplace the costs to set up one pollination unit depends on the date required. The bees used for preparation of early pollination units (the end of April, the beginning of May) are usually four times more expensive than those used in June or July. The advantage is a possibility of the timing – to apply a sufficient pollinator quantity for the required time of stand flowering.

A significant negative fact is that the bees, getting into the closed space, are exposed to a strong stress so that about 40 percent of the bees die during the first days after the hive installation. Considering a further maintenance and plant treatment in the technical isolators the bees working in the closed space appear to be more aggressive.

Temperature conditions are a limiting factor for the bee pollination as they start working only at the temperatures above 15^0 C that is why their use in early-flowering plant species represents a problem. On the contrary, the bees stop flying again at the temperatures above 35^0 C, they form clusters on the hive and/or inside it, thereby no plant pollination effect takes place.

They are strong dependent on the sunshine intensity. The bees do not fly out from the hives during a longer cloudy weather, which also results in no pollination. Under unfavourable weather conditions the utilization of the small bee colonies for seed production in some plant species (especially vegetable brassicas, radish, cauliflower, marigold, basil, caraway, etc.) during their flowering period is therefore problematic.

The honey-bee turned out to be suitable for the pollination in root vegetables (carrot, parsley, parsnip, celery, etc.), vegetable brassicas (cabbage, pak-choi and Chinese cabbage, Savoy cabbage, kohlrabi, broccoli, etc.), radish, turnip, also cucurbit vegetables (pumpkin, cucumber, squash, melons, etc.) and in majority of MAPs and wild-growing species transferred to be cultured.

2.3.3 The bumble-bees

In recent years the bumble-bees of which mainly the buff-tailed bumble-bee (*Bombus terrestris* L.) and the red-tailed bumble-bee (*Pyrobombus lapidarius* L.), in a limited quantity, have been successfully utilized for pollination in technical isolators. The hives with bumble-bee colonies are placed on the top of stakes in the technical isolators just in the flowering time of plant stands. These wooden hives consist of two compartments with inner dimensions to be 170x210x130 mm. Using a division board the hive is divided into the feeding part (1/3 of hive interior) and nest one (2/3 of hive interior), the hive entrance is 25 mm in diameter. The nest compartment is lined with teased tailor wadding and dry moss. Mother, several bumble-bee workers (minimum number to be about 15 pcs according to the stand condition) and the brood at various stage of development form a young colony. To provide the beginning feeding for the bumble-bee brood we place a dish with pollen (the beebread taken from the honey bee colonies during their pollen collection) near the brood. After a gradual hatching of young female workers the number of pollinators in the isolator increases.

The nest viability in accordance with a natural colony development is ranging from 70 to 120 days under conditions in closed isolators and it is possible for healthy nest to reach a maximum number of adults about 50-150, it can be exceptionally more. At the end of vegetation period a gradual nest degradation and death of individuals occur.

Figs. 15, 16 The hive for bumble-bees

Similar to the honey-bees, we recommend 2 to 5 gradually flowering species to plant out in isolators. Regarding that the number of individuals in the isolators increases without having a possibility of further food choice it is neccessary to provide the bumble-bees with additional feeding. For this purpose we use the bird feeders adapted, filled with a solution of honey, suggar and water in the rate of 1:2:1. Refilling of the feeders is carried out once in two days and/or every day according to need and a nest size. The regular check-up of solution quality in the feeder is important to prevent its fermentation, mostly in the rainy weather.

Fig. 17 Bumble-bee nest

Fig. 18 Feeder for bumble-bees

Fig. 19 The hive located in the isolator

2.3.4 Evaluation of the bumble-bees used as pollinators

The advantage of using bumble-bees for plant regeneration in technical isolators is above all the fact that bumble-bees are not liable to suffer from stress in a closed space, they quickly orientate there and after being familiar with the surroundings they start working immediately.

They are already able to work at temperatures 6-8^{0} C (maximum to be 35^{0} C). In contrast to the honey-bees they also fly during cloudy and rainy days. This ability can be, in particular, utilized in spring for the pollination of early-flowering vegetables such as cabbage, Savoy cabbage, radish and for that of MAPs, mainly in the species such as basil, sage, marigold, milk thistle, etc.). In some species, especially in the mint family (Lamiaceae), the use of bumble-bees is necessary to obtain high-quality seeds (lavender, officinal betony, hyssop, thyme, origanum) as well as they are often used in black-eyed Susan, digitalis, borage, etc..

The bumble-bees do not behave so aggressively in a closed space as the honey-bees do, which makes easier a subsequent plant treatment in the isolators.

A limiting factor for the bumble-bee use there are especially high costs to acquire the bumble-bee nest, being, according to our experience, 5 to 10 times higher compared to those on the honey-bee pollination „packets“.

The timing and obtaining of a sufficient nest quantity in period of the start-flowering of the stands are also difficult to carry out. When rapid start and end of plant flowering (influenced by the weather) occur, a small number of individuals at the beginning period after the hive installation is also a limiting factor, which can lead to a decrease in seed set.

The check-up of bumble-bee health state is also very important as the nest destruction and damage due to pests, primarily the bumble-bee moth (*Aphomia sociella*), is often found out.

Figs. 20, 21, 22 Nest destruction caused by bumble-bee moth

The subsequent use of overwintering pollinators (the nesting of bumble-bee mothers in nature) enables their utilization for seed production in the species grown without isolations under field conditions. From the viewpoint of nature protection it is possible to positively appreciate the release of young mothers and males into the open air after finishing the pollination, thereby giving a support to bumble-bee occurrence in a given landscape and locality.

2.3.5 Conclusion

The results of long-term reserch on the use of technical isolators and pollinators when regenerating heterogamous vegetable and MAPs species are summarized in the publication.

The methodology provides a survey on technical parameters of the isolators, it presents knowledge on new elements in agrotechnics of growing, on technical and material equipment used in regeneration, and on pollination service using small artificcially-created honey-bee colonies and bumble-bee nests.

When keeping the principles described the sufficiency of high-quality, healthy and germinative seeds from regenerated vegetable and MAP species can be obtained.

The knowledge can be utilized not only when regenerating plant material in genebanks, but in seed production, plant breeding and research practice as well.

III. Comparison of a novelty of the procedures

The presented methodology provides with new knowledge on the use of technical isolators, agrotechnics, technical and material equipment utilized in growing heterogamous vegetable and MAPs species. A pollination service based on the use of small artificcially-created honey-bee colonies and bumble-bee nests is described. The utilization of pollination units represent a special pollination system which can be mobile installed, with minimum costs, in respective technical isolators without a need of complete bee colonies. In practice, the complete bee colony is not possible to use in a small technical isolator, and the pollination would have to be carried out manually.

A great biological diversity and species representation of heterogamous vegetables and MAPs require for seed production the high-quality pollination service with the lowest costs and demands for skilled manpower. The described system of technical isolations and pollination service can meet these requirements.

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IV. Description of the methodology application

The methodology is intended for the workers engaged in plant germplasm, for plant breeders and research workers in the field of research on vegetables, medicinal, aromatic and culinary plants.

The certification methodology will be passed on to the firm Moravoseed Ltd., Mikulov-Mušlov, engaged in the plant breeding and seed production in vegetables and medicinal plants, as well as to the others being from the abovementioned fields of interest.

English version of the methodology will be provided to the members of the international organization ECPGR (European Cooperative Programme for Plant Genetic Resources) where it will serve as a guide for the work on genetic resources in the working groups (Working Group Medicinal and Aromatic Plant and Vegetable Network).

V. References to related literature

V.1 References cited in the text

Dotlačil, L., Stehno, Z., Faberová, I., Holubec, V.: 2004. Rámcová metodika Národního programu konzervace a využívání genetických zdrojů rostlin a agro-biodiversity. VÚRV Praha.

Dušek, K.: 2003.: Řešení kolekce GZ aromatických, kořeninových a léčivých rostlin na pracovišti VÚRV v Olomouci. In: Benediková, D. (ed.) Hodnotenie genetických zdrojov rastlín. Zborník z 3. odborného seminára 27-28. máj 2003, Piešťany, Slovenská republika. Str. 18. ISBN80-88790-27-12

Dušková, E., Chytilová, V.: 2003. Využití opylovačů při přemnožování GZ zelenin, aromatických, kořeninových a léčivých rostlin. In:Benediková, D. (ed.) Hodnotenie genetických zdrojov rastlín. Zborník z 3. odborného seminára 27-28. máj 2003, Piešťany, Slovenská republika. Str. 26. ISBN80-88790-27-12

Holubec, V.: 2007. Strategie a metodika sběrů s ohledem na efektivnost a využití materiálů. In: Faberová, I. (ed.) Aktuální problémy práce s genofondy rostlin v ČR. Sborník referátů ze seminářů 23. listopadu 2005, Hradec Králové a 22. listopadu 2006, Kostelany. Str. 5. ISBN 978-80-87011-04-1

Krieg,P.: 1994. Queen substitutes for small pollination colonies of the honey bee, *Apis mellifera* (Hymenoptera:Apidae). Eur. J. Entomol. 91:205-212

Krieg, P., Hofbauer, J. ,Komzáková,O.: 2009. Čmeláci a jejich podpora v zemědělské krajině. VÚVč Dol, ISBN 978-80-97196-01-4. 80 stran.

Národní program konzervace a využívání genetických zdrojů rostlin, zvířat a mikroorganizmů významných pro výživu, zemědělství a lesní hospodářství. Mze Praha, 2003, Č.j. 33 083/03-3000.

Národní program konzervace a využívání genetických zdrojů rostlin, zvířat a mikroorganizmů významných pro výživu a zemědělství. Mze Praha, 2006, Č.j.: 20139/2006-13020.

Palas, J.: 2003. Národní program konzervace a využívání genetických zdrojů rostlin, zvířat a mikroorganizmů významných pro výživu, zemědělství a lesní hospodářství. Mze Praha, 2003 Č.j.: 33 083/03-3000

Zedan, H.: Loos of plant diversity: a call for action. In: Guarino L., Ramatha Rao V., Reid V., Reid R. Collecting of plant genetic diversity. Technical guidlines. IPGRI, FAO, IUCN, UNEPRome, p. IX-XIV.

Vyhláška č. 175/2004, kterou se stanoví podrobnosti o uvádění do oběhu osiva a sadby pěstovaných rostlin. Sbírka zákonů České republiky, částka 58, z 21. dubna 2004.

V.2 Further references non-cited in the text

Pavelka, M., Smetana, V.: 2003. Čmeláci. Metodika ČSOP číslo 28, 2. vydání, Valašské Meziříčí 2003, ISBN 80-239-1023-X, 105 stran.

Přidal, A.: 2005. Ekologie opalovatelů, Brno, LYNX, ISBN 80-86787-4, 112 stran.

VI. List of publications preceded the methodology

Dušek, K.: 2004. Current status of the collection of aromatic, culinary and medicinal plants in the Czech Gene bank in Olomouc. In: Baričevič, D., Bernáth, J., Maggioni, L. and Lipman, E., compilers, 2004. Report of Working Group on Medicinal and Aromatic Plants, First Meeting, 12-14 September 2002, Gozd Martuljek, Slovenia. International Plant Genetic Resources Institute, Rome, Italy. Str. 42. ISBN 92-9043-633-6.

Dušek, K. a kol.: 2007. Metody techniky regenerace cizosprašných zelenin a léčivých, aromatických a kořeninových rostlin. In: Faberová, I. (ed.): Aktuální problémy s genofondy rostlin v ČR. Sborník referátů ze seminářů, 23. listopadu 2005 VŠÚO Holovousy s.r.o. v Hradci Králové a 22. listopadu 2006 ZVÚ Kroměříž s.r.o. v Kostelanech. VÚRV, v.v.i.. Str. 45. ISBN-978-80-87011-04-1.

VII. Enclosures:

Fig. 23 The use of technical isolators with pollinators

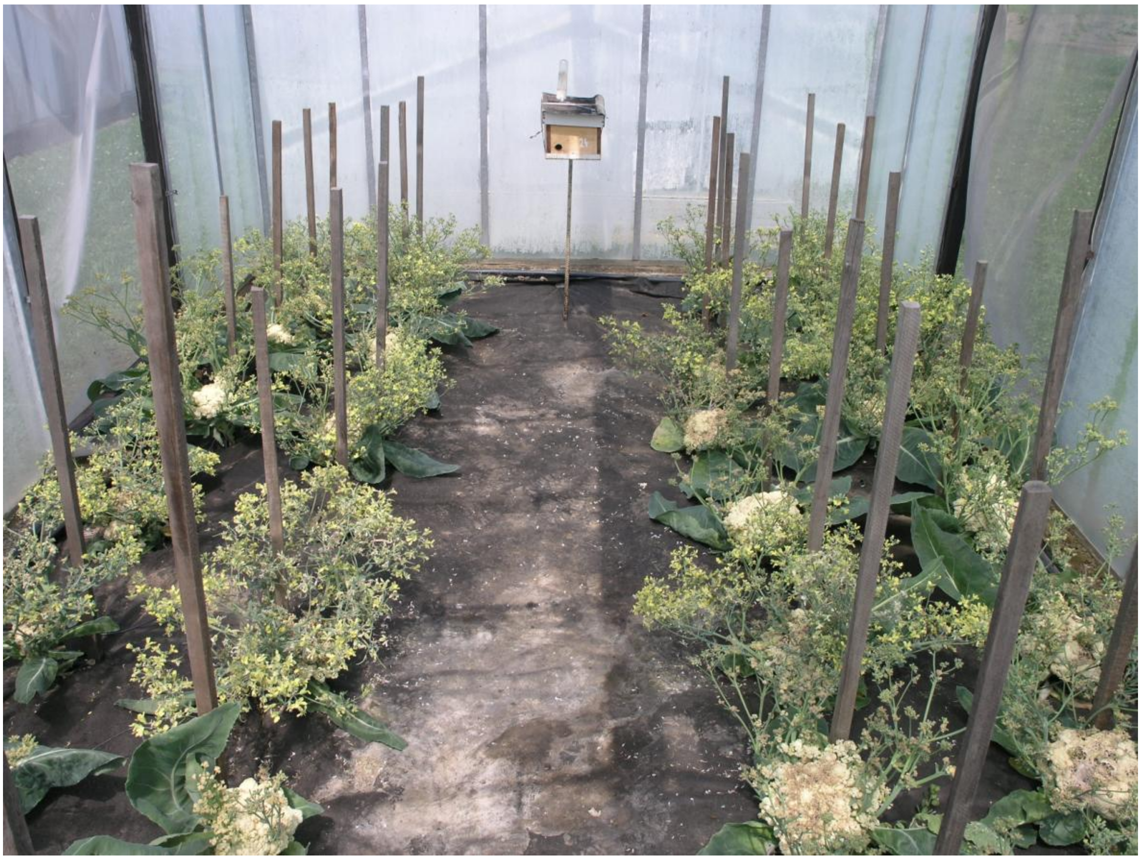

Fig. 24 Bumble-bees pollinating cauliflower stand

Fig. 25 Start-up of the bumble-bee nest in laboratory

Fig. 26 Young red-tailed bumble-bee colony in laboratory rearing

Fig. 27 Red-tailed bumble-bee nest at the top of its development in the isolator

Fig. 28 Buff-tailed bumble-bee nest before its destruction in the isolator

Fig. 29 Buff-tailed bumble-bee in phacelia stand

Fig. 30 Honey-bee on the mullein

Fig. 31 Buff-tailed bumble-bee on the black-eyed Susan

Fig. 32 Buff-tailed bumble-bee on the officinal betony

Fig. 33, 34 Bumble-bees and honey-bees pollinating early-flowering brassicas

Fig. 35 Buff-tailed bumble-bee pollinating the buckwheat

Fig. 36 Buff-tailed bumble-bee pollinating the carrot seed stand

Fig. 37 Honey-bee pollinating the garlic

Fig. 38 The pollinators on the chives

Fig. 39   Cucumber pollination in the technical isolation

Fig. 40 The use of pollinators in melon

Fig. 41 Bumble-bee on the garlic

Fig. 42 Red-tailed bumble-bee pollinating the cinquefoil

Fig. 43 Buff-tailed bumble-bee pollinating the levander

Fig. 44   MAPs assortment - the source of the food for pollinators

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