International Seed Federation
Seed is Life

Protocol for using pollinators in hybrid vegetable seed production

An outline for improving pollinator effectiveness

FEBRUARY 2018

APPROVED BY
ISF Working Group Vegetable Seed Production

International Seed Federation
Seed is Life

EDITTED BY
The listed pollination researchers :

Avi GABAI - Hazera, Israel

Bernard E. VAISSIÈRE - Institut National de la Recherche Agronomique, UR406 Abeilles et Environnement, 84914 Avignon cedex, France

Tjeerd BLACQUIÈRE - Wageningen Plant Research, Wageningen University & Research, Netherlands

Breno M. FREITAS - Departamento de Zootecnia, Universidade Federal do Ceará, Brazil

Mike ALLSOPP - Plant Protection Research, Agricultural Research Council, Stellenbosch, South Africa

Stan CHABERT - Association Nationale des Agriculteurs Multiplicateurs de Semences Oléagineuses, 17700 Saint Pierre d'Amilly, France

Arnon DAG - Plant Sciences, Agricultural Research Organization, Ministry of Agriculture, Israel

1. INTRODUCTION

Pollination in hybrid vegetable seed production is the transfer of pollen from the anthers of the male fertile flowers to the stigma of the male sterile (female) flowers. The pollination phase has a significant impact on final seed yield and quality. In many vegetable crops, such as onion (*Allium cepa*), carrot (*Daucus carota*), cabbage (*Brassica oleracea*), cauliflower (*B. oleracea*) and radish (*Raphanus sativus*), pollination is performed mainly by honey bees (*Apis mellifera*). However, although it is the main managed pollinator, there are other wild and managed pollinators that can be of significant commercial value.

Pollination quality is expressed as the quantity of pollen moved to the female flower; this depends on the pollinators' activity and their mobility between the flowers of the two lines.

2. OBJECTIVES

This document details the essential points in using pollinators for commercial hybrid seed production:

- Honey bee hive management and colony (populated beehive) strength regulations. These regulations are designed to assure seed producers that the colonies they rent meet the minimum standards needed for good pollination activity.
- Bumble bee hive management.
- Additional use of pollinators, such as flies and wild pollinators.

3. DEFINITIONS AND ABBREVIATIONS

Pollination – transfer of pollen grain from the stamen to the stigma

Cross-pollination – pollen transferred from the stamen of one flower to the stigma of another flower of a different genotype

Competitive flowering – flowering of either wild or domesticated plants close to the pollinated field that attracts pollinators away from the pollinated field

Beekeeper – the owner/manager of the bee colonies, who supplies them and is responsible for colony quality

Honey bee colony – population of honey bees that lives in a hive. The individual bee cannot survive on its own; it can only survive as part of a colony, which functions as one organism

Beehive – the box in which the colony lives

Healthy honey bee colony – colony with minimal infestation of *Varroa destructor* and neither American foulbrood nor European foulbrood, and minimum contamination of mites and other honey bee pathogens (i.e., at a level that does not reduce colony performance)

Populated frame – frame fully covered with adult bees. In Europe, Dadant hives are used and the dimensions of the body frame are 16.5 inches wide by 10.5 inches high. In the USA, Brazil, South Africa and Israel, Langstroth hives are used, with dimensions of 17.5 inches wide by 9.5 inches high. Thus, herein, the type of frame is indicated whenever applicable

Brood frame – a frame that contains the brood, which is the collective term for eggs, larvae, and pupae, the immature stages in the life cycle of bees

Bumble bee colony – population of bumble bees that lives in a managed hive (between 50 and 300 individuals). The most common bumble bee species used commercially is *Bombus terrestris*. To prevent insect invasion, use of a local bumble bee species is recommended (Inoue et al. 2008, Arbetman et al. 2012).
Wild pollinators – unmanaged native pollinators that provide pollination services and contribute substantially to crop pollination
Bee user – the hybrid seed producer/grower who rents the bee colonies for the season to pollinate the plants in his/her field
Pollination deficit – quantitatively or qualitatively inadequate pollen receipt which decreases the sexual reproductive output of plants (Wilcock and Neiland 2002).
Optimal pollination – pollination that leads to maximum sexual reproductive output given the concurrently available resources over the lifetime of the plant. To define pollination deficits, one must define (and understand) how to attain optimal pollination levels (Vaissière et al. 2011).

4. ISOLATION DISTANCES

One major factor during the pollination of seed crops is the prevention of undesired cross-pollination between cross-compatible plots or fields. For insect-pollinated crops, this is mainly achieved using isolation by distance. Table 1 specifies the recommended isolation distances for vegetable crop seed production.

5. SAFETY RULES

5.2 Never open a populated beehive without being authorized to do so by the beekeeper, and always alert people working nearby.
5.3 Use safety equipment (suit, gloves, smoker, etc.) whenever dealing with a populated beehive.
5.4 Do not open a bumble bee hive unless under a red light as safe methods that work on honey bees do not work on bumble bee colony.
5.5 Avoid positioning honey bee colonies close to human-populated areas to decrease the chance of stings.
5.6 Position the beehive's entrance opposite the direction of human pathways.
5.7 It is highly recommended that people working in a field with bees, especially those who are allergic to bees, carry an antidote for injection in case of bee sting.

6. HONEY BEES

6.1 Colony quality

6.1.1 Before moving colonies to the field, the beekeeper must check that they meet the required standards.
6.1.2 In open fields, except for early flowering crops such as oilseed rape (*Brassica napus*) for which the requirements are less (in France, the standard is 7 frames of bees, 4 with brood), in each beehive, there should be at least 10 bee-populated frames, 7 of which should contain brood. As African bees are smaller than European strains, the requirements are 8 frames of bees and 5 frames of brood. If the crop blooms during the swarming season, the colony should not be too strong unless adequate monitoring and management can be performed to prevent swarming.

6.1.3 There should always be free space for honey storage to ensure nectar collection from the target crop.

6.1.4 A colony can be strengthened by advanced feeding or by shifting brood and bees from other populated beehives.

6.1.5 The beekeeper should ensure that the colony is healthy.

6.1.6 The beehive should contain a minimum of 2 frames with honey and pollen, and have empty storage space.

6.1.7 The beekeeper must ensure that there is a laying mated queen in the colony.

6.2 Colony quality inspection

6.2.1 It is the seed producer's responsibility to bring in an objective professional inspector to inspect the quality of the colonies brought to his/her field. The inspection must also be coordinated with the beekeeper.

6.2.2 The inspection should take place just a few days after positioning the colonies in the field.

6.2.3 In general, inspection should be performed when the colony is showing substantial activity. The inspector opens the beehive and inspects the frames one after the other, assessing the presence of brood and a substantial number of adult bees on the frame (there are some modifications to this procedure in accordance with beekeeping experts in different countries).

6.2.4 Another possible method, which is now being implemented on a large scale in France and is adapted from that used in Almond orchards in California, is as follows: the inspection consists of counting the number of spaces between adjacent frames (inter-frames) covered by bees on the top and bottom of the different stacked hive boxes to the nearest one-half frame, and then summing these numbers. The inter-frames counted on the two outermost edges of the boxes each count as half a frame, so that one cannot count more inter-frames than there are frames. Because the surface of the frames contained in the Dadant supers is equivalent to 0.56 times the surface of Dadant body frames, the sum of the inter-frames counted in the super chambers placed on top of the hive body is simply divided by two. This relationship can be transposed to Langstroth frames by multiplying the sum of the inter-frames obtained in the Langstroth body by 0.8—the ratio of the Langstroth frame surface to the Dadant frame surface.

6.2.5 Colonies that have been inspected will be marked in order to replace below-standard colonies or for reassessment in a second inspection, if necessary.

6.2.6 A report will be submitted no later than 2 days after the inspection to enable the beekeeper to correct the rejects, if necessary.

6.3 Moving and positioning the beehives

6.3.1 The grower should alert the beekeeper of the desired colony placement in advance so that the beekeeper can be ready on time.

6.3.2 The grower, after consulting with the beekeeper, should clearly mark the desired locations of the colonies in the field and give the beekeeper a map of the field marked with the places where the beehives should be positioned (locations and number of beehives per location).

6.3.3 The beekeeper will move the beehives to the field at night, or in the early morning just before sunrise or at dusk, when most of the bees are inside the beehive.

6.3.4 Suggested stocking rates in colonies per hectare are specified in Table 2.

6.3.5 The colony stocking rate will be increased by 30–50% when a significant area of competing flowers is present or if the field is smaller than 1–2 ha. In the case of increased colony numbers due to the presence of competing flowers, it is recommended to put more colonies on the field border away from the competing bloom.

6.3.6 The beehives will be positioned equally around the field. It is highly recommended to position beehives inside the field as well. When positioning the beehives, make sure that no plants in the pollinated field are more than 150 m from a beehive. In certain cases, this distance can be increased to 240 m (Vaissière et al. 1984). When using defensive honey bee strains (such as Africanized bees), it is recommended that the hive be placed outside the field to reduce the risk of bee stings.

6.3.7 Beehives in the field can be distributed in groups of 4 to facilitate the beekeeper's job of delivering and checking the beehives.

6.3.8 In the open field, the position of the hive entrance does not affect the flying direction of the honey bees. But to prevent pesticides from entering the hive, the hive entrance should be directed away from the field to a position that will minimize this risk.

6.3.9 The colonies should be positioned in the field only when both parental lines are flowering. Positioning too early will motivate the bees to look for flowers outside the target field.

6.3.10 Initially, only about 30% of the colonies should be placed. Completion to full stocking rate should be done a few days later as flowering progresses. In the case of crops or cultivars with an Effective Pollination Period as short or shorter than 7 days, all of the colonies should be placed during the initial introduction.

6.3.11 The practice of several colony deliveries may boost pollination by increasing mobility between flowers due to the presence of new scouting bees (Stern et al. 2004).

6.3.12 The beehives will be moved off the field immediately after the grower decides to terminate the pollination period. This is done to avoid possible damage to the bees from pesticide applications and at night to avoid leaving foraging bees behind.

6.4 Treating the colonies during the pollination period

6.4.1 The beekeeper should avoid any risk of stings by notifying the growers regarding hive-maintenance checks.

6.4.2 The beekeeper should manage his/her colonies so as to avoid swarming at all cost during the pollination period, especially in the spring. This can be achieved by adding supers, removing queen cells, and having young queens. Colonies that swarm lose most of their foragers and are then of little value for pollination.

6.4.3 The beekeeper should avoid using pollen traps or any device that interferes with free movement of the bees at the entrance of the beehives during the pollination period.

6.4.4 The bees should not be fed sugar syrup during the pollination period unless it is to prevent colony starvation.

6.4.5 There should always be free space for honey storage in the beehives.

6.4.6 There should always be drinking water available for the bees.

6.5 Pollination in greenhouses/net houses

6.5.1 A small colony (nucleus) may be introduced in closed environments with a surface area of less than 500 m^2. The adult and brood populations of such beehives should be lower than those in open fields (around 5 bee frames, 3 containing brood).

6.5.2 Colonies should be moved during the day as the old foragers will not get accustomed to their new environment and will die outside the hive following its installation. By leaving the majority of foragers at the original apiary, new foragers will start to forage in the confined environment while old ones will return to the colonies left in place by the beekeeper to host them.

6.5.3 With some crops, such as onion, cucumber (*Cucumis sativus*) and melon (*Cucumis melo*), honey bees generally not collect pollen. Therefore, the pollen stores inside the beehives should be checked frequently (at least every 2 weeks). In the case of pollen deficiency, pollen supplements or substitutes should be supplied.

6.5.4 There should always be free space for honey gathering.

6.5.5 There should always be drinking water available for the bees. The most highly recommended method is a vessel with water containing floating elements or a clean burlap that is dipped in the water so that bees can stand while drinking.

6.5.6 Avoid using greenhouse covers that absorb or diffuse the UV part of the spectrum (wavelengths ~320–380 nm).

7. BUMBLE BEES (*BOMBUS TERRESTRIS*)

In some crops, *Bombus* workers can be the main pollinator or can be used to complement honey bees. Bumble bees are active at temperatures from 10°C, and even below, to 30°C. They function best at temperatures between 15 and 25°C and are extensively used for pollination in greenhouses. Bumble bee colonies can be supplied by Biobest (http://www.biobest.com), Koppert (http://www.koppert.com) and Biobee (http://www.biobee.com). General instructions for optimal pollination management of bumble bee colonies for vegetable seed crops can be obtained from bumble bee suppliers. Prior to using bumblebees, one needs to check the local registration requirements and use only products that are permitted in the country/state where the field is grown.

8. FLIES

8.1 General

8.1.1 Flies can only be used as managed pollinators in enclosures. In some hybrid seed production, where one or both parental lines produce too little pollen or nectar or if the parental lines are not attractive to bees, flies may provide a valuable alternative as pollinators.

8.1.2 Another way to use flies as pollinators is in very small cages that contain a few flowers or a limited surface for colonies of managed eusocial pollinators such as honey bees or bumble bees. The main dispersal method for flies is at the pupal stage when the flies emerge, following an incubation period in the field.

8.1.3 The adult flies will search mainly for nectar in the flower and thus come into contact with the pollen. This pollen is not packed or collected by the flies, and therefore remains available for pollination when the fly visits another flower to collect its nectar. Prior to using flies, one needs to check the local registration requirements and use only products that are permitted in the country/state where the crop is grown.

8.2 *Lucilia* spp. blow flies (Calliphoridae)

Blow flies have been used with the following crops: cauliflower, cabbage, oilseed rape (*Brassica napus*), carrot, onion, leek (*Allium porrum*), and asparagus (*Asparagus officinalis*). Their optimal temperature range for foraging is 18–28°C. Koppert (http://www.koppert.com) commercializes *Lucilia sericata* blow flies.

8.3 *Musca domestica* house fly (Muscidae)

Where *Lucilia* blow flies are not endemic and their importation is not permitted, an alternative is the house fly, which is raised in many countries for its maggots, used as bait for fishing. Where there is no commercial production of house flies, a box with chicken manure can be placed under a source of water (irrigation drip for example) that keeps the substrate wet but not flooded. Flies will propagate and increase the fly population.

9. WILD POLLINATORS

The most important wild pollinators are wild bees. Insects other than bees are often recorded visiting flowers of commercial crops, but in general they lack the necessary behavioral pattern for efficient and consistent pollen transfer (such as switching between male fertile and male sterile lines). Nevertheless, the existence of wild bees can contribute to pollination service by increasing the number of efficient pollinators visiting flowers and by enhancing the pollination effectiveness of managed pollinators (Greenleaf & Kremen 2006). Improving the conditions for wild pollinators, such as keeping the wild bloom next to the fields and reducing pesticide use, may contribute to improved pollination service for seed crops. Care must, however, be taken not to create an abundance of competitive forage during the pollination period, as this can limit the effective pollination.

10. PEST-CONTROL MANAGEMENT

10.1 Use of pesticides prior to bee placement should be done after consulting with the beekeeper so as not to use pesticides with a long residual lifetime.

10.2 No pesticides shall be applied during the pollination period unless there is a catastrophe and pesticide use is obligatory. Consulting with the beekeeper as to which pesticide to use is a must!

10.3 In any case of pesticide use, the application (spray) must be coordinated with the beekeeper and should be done in the evening after dusk when as many of the bees as possible are inside the beehives. Advanced precautionary steps must be taken if needed, such as covering the beehives, closing the beehives or even moving them off the field if at all possible.

10.4 The grower should make the effort to coordinate with neighbors regarding pest control in their fields, to avoid damage to the bees that may forage in those fields.

11. POLLINATOR POPULATION AND POLLINATION ASSESSMENTS

There are various ways of estimating the size of the pollinator population in a crop:

11.1 One method is to demarcate strips along the field and count the number of pollinators per unit surface (Vansel & Todd 1947)

11.2 Another method that may be suitable for seed crops is counting the number of easily visible flowers and recording the number of visits they receive during a given period of time (Levin et al. 1968).

11.3 Scan sampling, in which one counts the number of flowers with bees in them over a standard number of flowers, is also commonly practiced. These are instantaneous counts so that time is not an issue (Levin et al. 1968, Vaissière et al. 2011).

11.4 The number of pollen grains deposited onto a sample of stigmas has been found to be well correlated with the final seed yield and to give a significant indication of pollinator effectiveness.

11.5 It is recommended that potential pollination deficit be evaluated by comparing hand pollination of a small cohort of flowers to assess the seed yield potential under the field's conditions with the results obtained from a similar cohort of flowers left for open pollination under commercial conditions (Delaplane et al. 2013).

12. CROPS IN SEED PRODUCTION USING INSECT POLLINATION

12.1 General

12.1.1 In general, irrigation of the pollinated crop impacts nectar production. Adequate watering during pollination period is important for pollinator visitation. It is recommended to use ground irrigation since top irrigation (sprinkler, pivot etc.) interferes to pollinators' activity and might decrease pollen and stigma viability.

12.2 Brassica spp

12.2.1 Flowers of *Brassica* species usually produce ample nectar, and therefore the hives should be supplied without sugar syrup.

12.2.2 *Brassica spp*. can be pollinated by honey bees, bumble bees and flies.

12.3 Onion

12.3.1 In onion, the stamens in each floret dehisce a few days before the stigmas become receptive. Florets of onion produce nectar and pollen. Bees collect onion nectar, but very rarely collect onion pollen under Israeli conditions, and therefore the hive should be supplied without sugar solution, and in enclosures they should be provided with additional pollen supply.

12.3.2 Onion can be pollinated by honey bees and flies.

12.4 Carrot

12.4.1 In carrot, the stamens in each flower and umbel ripen a few days before the stigma becomes receptive, such that cross-pollination occurs more often than self-pollination.

12.4.2 The flower produces nectar as well as pollen. Nevertheless, the nectar and pollen produced by the carrot flower is not always sufficient for optimal colony development. For this reason, sugar syrup and pollen are supplied for the development of bumble bee colonies.

12.4.3 Carrots can be pollinated by honey bees, bumble bees and flies.

12.5 Cucumber, melon, watermelon and squash

12.5.1 Cucumber, melon and squash have male and female/hermaphrodite flowers on the same plant.

12.5.2 Both flower types produce nectar, while only the male and hermaphrodite flowers produce pollen. Visits by bees can be determined by the presence of pollen grains on the stigma of the female/hermaphrodite flower.

12.5.3 Because the cucumber and especially the squash flower produce ample nectar, the beehives are provided without sugar syrup.

12.5.4 These crops can be pollinated by honey bees and bumble bees.

13. DATA TABLES

Table 1: Recommended isolation distances for seed crops

Crop species	Isolation distance
	(x 1000 m)
Onion (hybrid) (*Allium cepa*) [1]	3–5
Cotton (hybrid) (*Gossypium hirsutum*) [2]	2
Crucifers (*Brassica* spp.) [1]	1–1.5
Cucumber (*Cucumis sativus*) [1]	1–1.5
Carrot (*Daucus carota*) [1]	1–1.6
Sunflower (*Helianthus annuus*) [3]	2

[1] George, R.A.T. 2009. Vegetable seed production, 3rd ed. CABI, Wallingford.

[2] Lee JA.1980. Cotton p.313-325 inFehr WR, Hadley HH (19680). Hybridization of crop plants. ASA-CSSA, Madison, WI.

[3] Internal data.

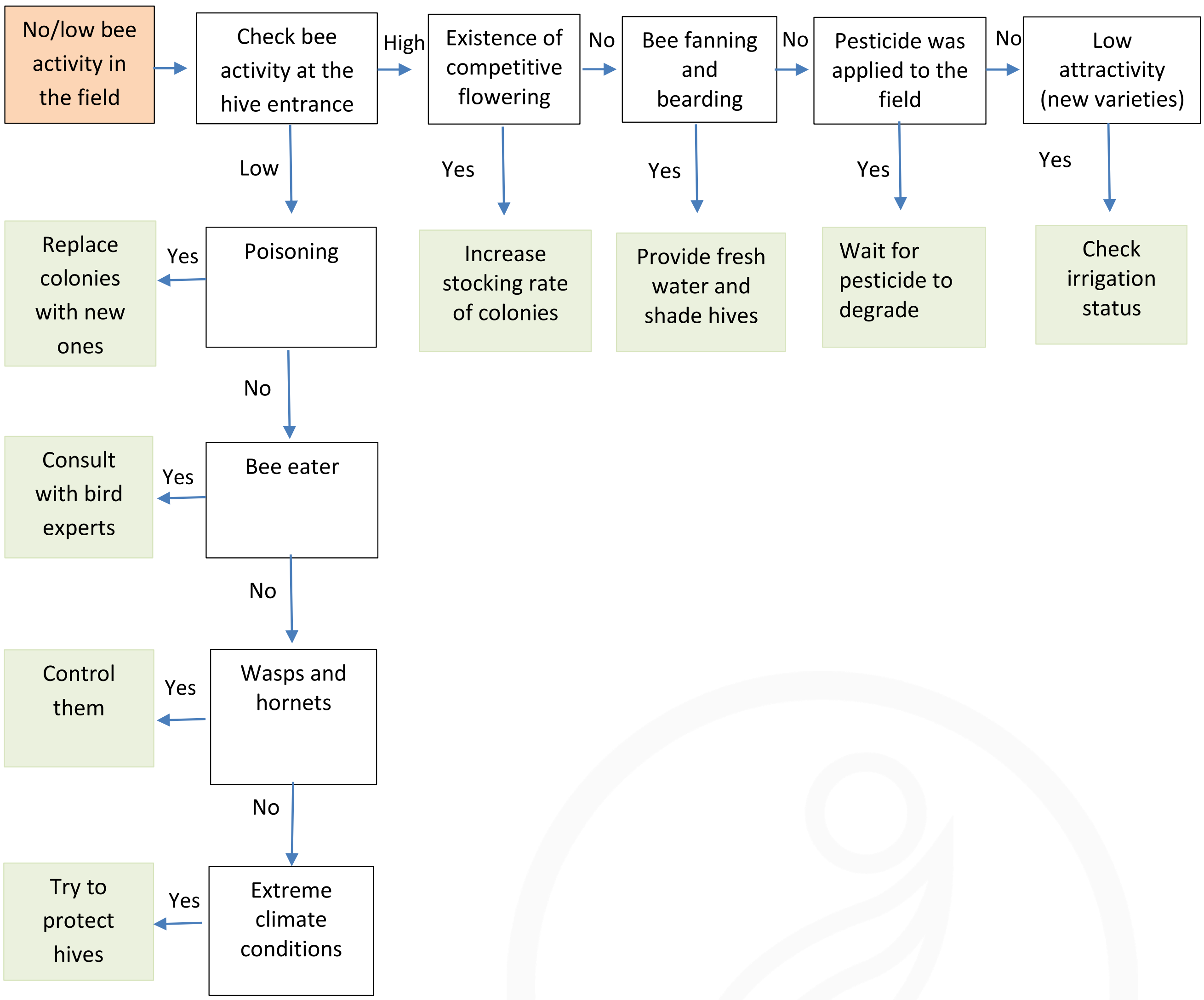
Table 2: Suggested stocking rates of honey bee colonies for seed production

Crop species	Stocking rate
	Honey bee colonies/ha
Sunflower	2–4 [1]
Onion (hybrid)	5–10 [1]
Cotton (hybrid)	7–10 [1]
Crucifers	5–10 [1]
Cucumber	2–8 [2]
Carrot	8–10 [2]

[1] Internal data.

[2] George (2009).

14. TROUBLESHOOTING FLOWCHART FOR POLLINATION USING HONEY BEE COLONIES



15. REFERENCES

Arbetman M. P., I. Meeus, C. L. Morales, M. A. Aizen & G. Smagghe. 2012. Alien parasite hitchhikes to Patagonia on invasive bumblebee. Biol. Invasions 15:489-494.

Delaplane K. S., A. Dag, R. G. Danka, B. M. Freitas, L. A. Garibaldi, R. M. Goodwin & J. I. Hormaza. 2013. Standard methods for pollination research with Apis mellifera. J. Apic. Res. 52:1 -28.

George, R.A.T. 2009. Vegetable seed production, 3rd ed. CABI, Wallingford.

Greenleaf, S. S & C. Kremen. 2006. Wild bees enhance honey bees' pollination of hybrid sunflower. Proc. Natl. Acad. Sci. USA 103:13890-13895.

Inoue M. K., J. Yokoyama & I. Washitani. 2008. Displacement of Japanese native bumblebees by the recently introduced *Bombus terrestris* (L.) (Hymenoptera: Apidae). J. Insect Conserv. 12:135-146.

Levin M. D., R. O. Kuehl & R. V. Carr. 1968. Comparison of three sampling methods of estimating honey bee visitation to flowers of cucumbers. J. Econ. Entomol. 61:1487-1489.

Stern RA, Goldway M, Zisovich AH, Shafir S, Dag A. 2004. Sequential introduction of honeybee colonies increases cross-pollination, fruit-set and yield of 'Spadona' pear (*/Pyrus /communis* L.). J. Hort. Sci. Biotechnol. 79 :652-658.

Vaissière, B. E., B. M. Freitas & B. Gemmill-Herren. 2011. Protocol to detect and assess pollination deficits in crops: a handbook for its use. FAO, Rome.

Vaissière, B. E., J. O. Moffett & G. M. Loper. 1984. Honey bees (*Apis mellifera* L.) as pollinators for hybrid cotton seed production on the Texas High Plains. Agron. J. 76:1005-1010.

Vansel, G. H. & F. E. Todd. 1947. Alfalfa tripping by insects. J. Am. Soc. Agron. 38:470-488.

Wilcock, C. C. & M. R. M. Neiland. 2002. Pollination failure in plants: why it happens and when it matters. Trends Plant Sci. 7:271-277.