

OP-049 [Bee Biology]

Effect of vitamin A and nutraceuticals on learning, memory retention, and foraging behavior of honey bees (*Apis mellifera*)

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The Western honey bee is the most beneficial insect to societies and ecosystems due to their global pollination services to many flowering plant species. Unfortunately, bee colony losses have increased significantly over the past decade, particularly in North America. One of the factors associated with these losses is colony stress and malnutrition because of the intensive management to which beekeepers subject bees, including the transportation and rental of colonies for crop pollination services. Bees depend on their learning and memory abilities to efficiently collect pollen and nectar from flowers and to return to their hives after foraging trips. Additionally, bees collect pollen for brood rearing and colony growth, which is also beneficial for efficient pollination services when hives are rented for this purpose. Therefore, it would be desirable to supplement honey bee colonies with diets and additives that could improve their learning, memory retention, and foraging efficiency. Consequently, this study investigated the effects of β -carotene (pro-vitamin A) and the nutraceuticals daucosterol (a phytosterol from walnuts and other plants) and terpinen-4-ol (a terpene from tea tree oil and pine oil), incorporated into a commercial protein supplement on learning, memory retention, and foraging behavior of honey bees. These antioxidants and their doses were selected from preliminary screening experiments indicating no toxicity to the bees. Bees fed with β -carotene and/or the two nutraceuticals contained in a commercial protein supplement during seven days were compared to those fed the supplement alone. Proboscis extension reflex (PER) tests and foraging observations in hives were carried out with the experimental bees. Results showed that terpinen-4-ol, as well as the combination of daucosterol and β -carotene, significantly increased both memory retention and pollen foraging of honey bees relative to bees fed only the supplement ($P < 0.01$). Therefore, these compounds have the potential to be used as additives in artificial protein supplements to feed honey bee colonies. This could bring multiple benefits to bee health such as improving learning and memory retention, as well as to increase pollen collection and crop pollination efficiency. These benefits would positively impact both beekeepers' and growers' economies, as well as potentially increase pollinated food production.



Effect of provitamin-A and selected phytochemicals on memory retention and foraging behavior of honey bees

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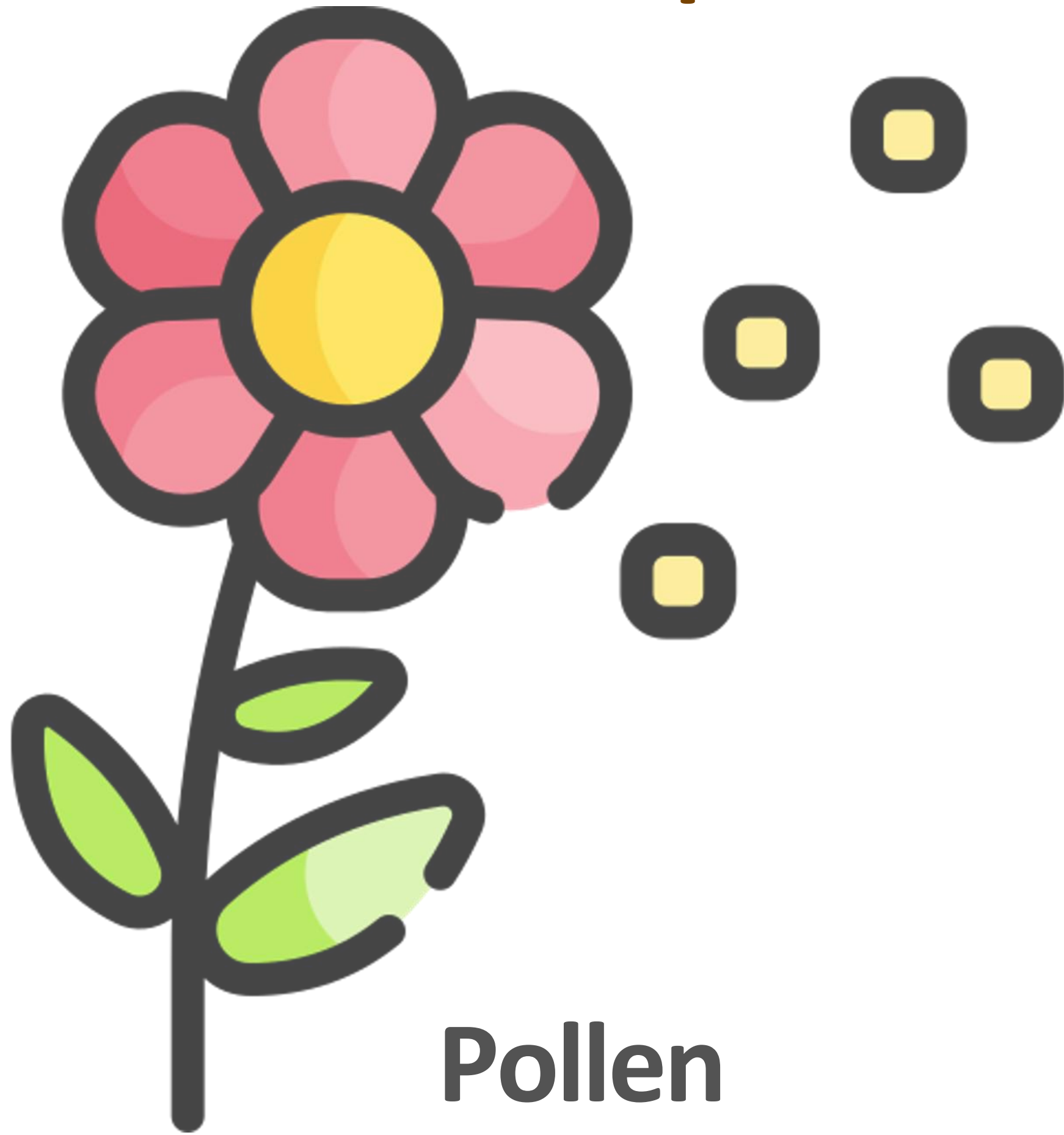
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Macronutrients

Proteins & lipids



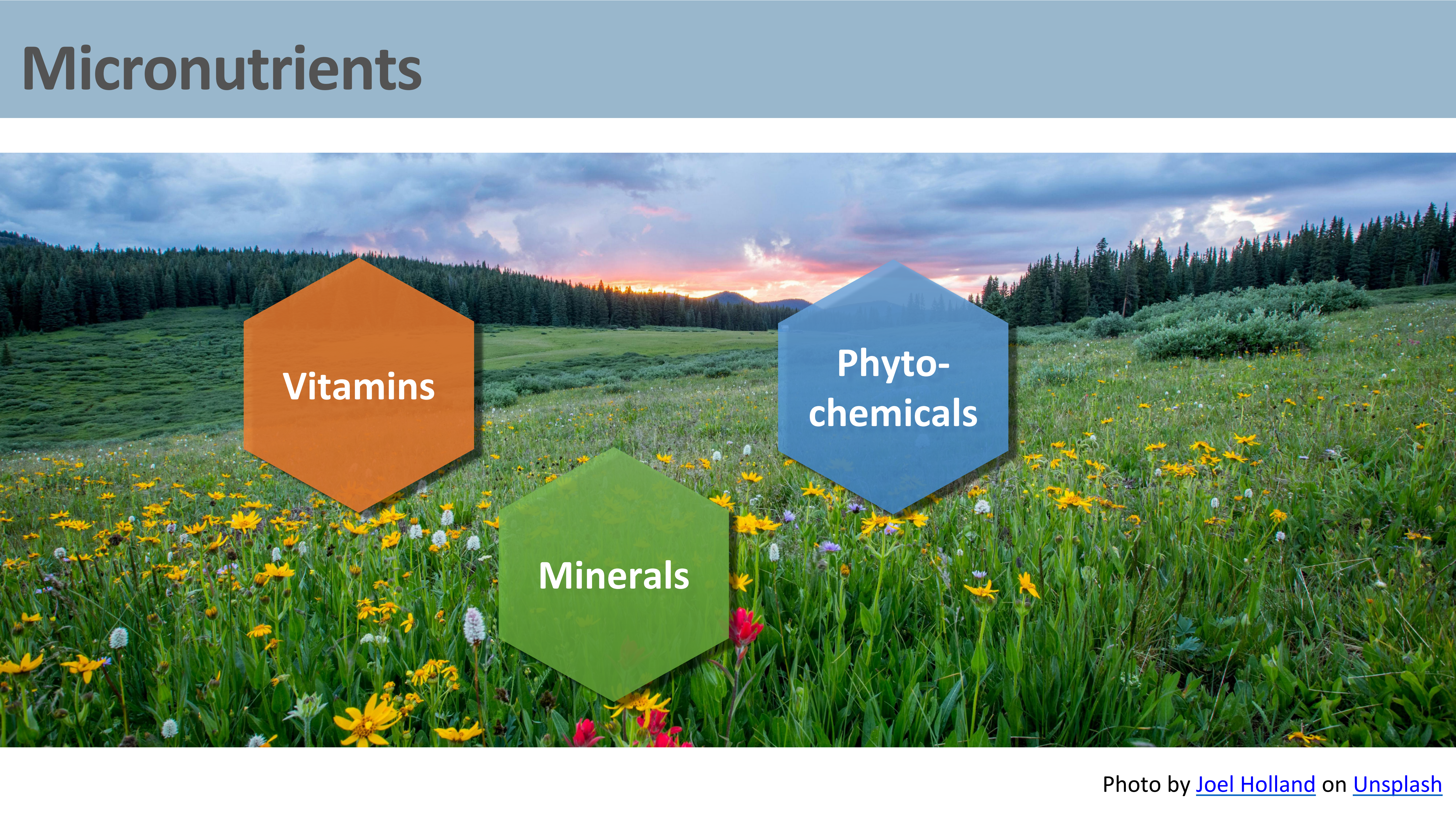
Pollen

Carbohydrates



Nectar

Micronutrients

A wide-angle landscape photograph of a meadow at sunset. The foreground is filled with green grass and numerous yellow wildflowers. In the middle ground, there are patches of low-lying green shrubs. The background features a dense forest of evergreen trees under a dramatic sky with orange, pink, and blue hues. Three semi-transparent hexagonal callouts are overlaid on the image: an orange one on the left, a blue one on the right, and a green one in the center foreground.

Vitamins

**Phyto-
chemicals**

Minerals



Optimal nutrition

- Under conditions of abundant multi-floral pollen with high protein content:
- Favors colony growth
- Increases honey bee resilience
- Increases colony productivity

Optimal nutritional conditions not year-round



- Not always present
- Floral resources not abundant and diverse
- Times of scarcity (eg. fall, winter, early spring)
- Apiaries located in agricultural eco-systems
- Pre-pollination season

Mono-floral pollen, deficient in nutrients



Mora azul, Nova Escocia (Foto credito: Kayla Hounsell/CBC News)

Hives rented for pollination



Foto credito: [Wikimedia](#)/Pollinator, [CC-BY-2.5](#))

Beekeepers' strategy to minimize malnutrition

- Provide sucrose syrup to colonies
- Provide pollen patties to colonies
- Provide patties of protein supplements
- Supplements work when there is little or no pollen in the field



Studies on honey bee nutrition

- Most studies focused on carbohydrates, proteins, amino-acids and lipids (fats)
- Little information on micronutrients added to protein supplements
- There is some knowledge on the B complex and vitamin C, But little is known about vitamin A and



Vitamin A

- Pigments: retinol, β -carotene (provitamin-A)
- β -carotene in artificial diets increased the lifespan of ladybird beetles (Sun et al. 2018)
- β -carotene improved memory in frogs (McInerney 2024)
- Antioxidant and neuro-stimulant
- What about its effects on honey bees??????



Phytochemicals as micronutrients

- Phenols and terpenes modulate cognitive functions and memory in humans (Kennedy 2019): eg., terpinen-4-Ol
- Sterols (similar to cholesterol) improve memory in humans and increase brood production in honey bee colonies (Chakrabarti et al. 2020): eg., eleutherosids
- Have antioxidant, immunostimulant, anti inflammatory activity

β -carotene

- Provitamin A
- Improves memory in other organisms
- Reduces cost of immunity in other insects
- Antioxidant

Terpinen-4-Ol

- Terpenoid from tea tree oil & other EOs
- Antioxidant, antimicrobial, anti cancer activity
- Reduces mortality of caged bees

Daucosterol

- Phytosterol from walnuts
- Type of cholesterol
- Antioxidant, anti-inflammatory, immunostimulant, neurostimulant
- First time tested in bees

Memory and learning in bees



- Bees rely on their learning ability and memory to forage
- Important for navigation and to remember how to return to the hive

Supplementation with β -carotene & phytochemicals



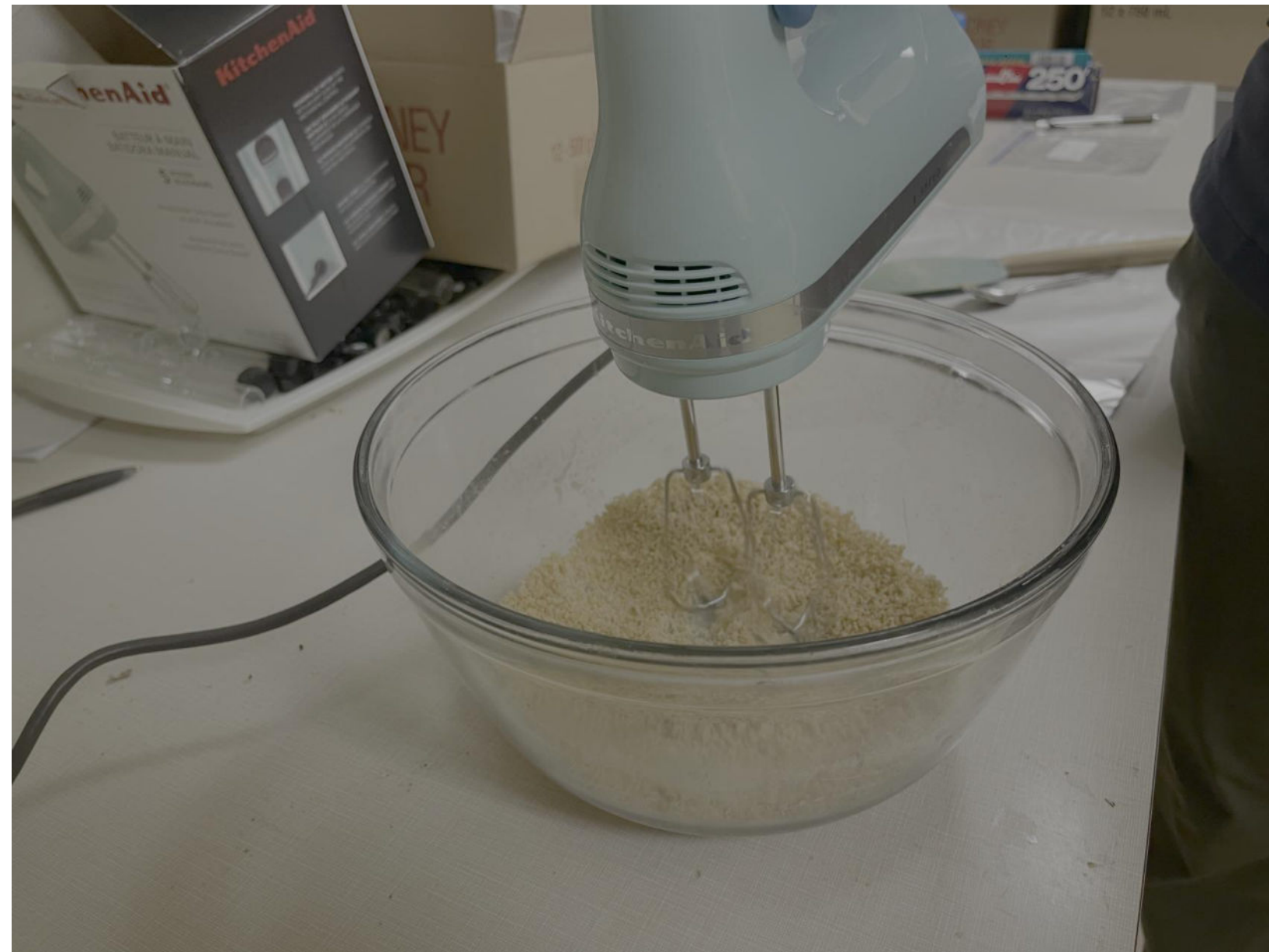
- Could they improve bees' memory and foraging?
- Could they improve bee health?
- Could they be used as additives in protein supplements?
- Useful in times of scarcity or to prepare colonies for pollination services or pollen harvesting?



Objective

- To assess the potential of selected micronutrients (provitamin A and two phytochemicals) to improve memory retention and foraging behavior of honey bees

Experimental treatments



- Supplement alone: Feedbee®
- Supplement + one or more micronutrients (additives)
- Provided to newly-emerged bees, caged in the lab

Micronutrients added to a protein supplement

Micronutrient	Dose based on study	Dose
β-carotene	Abdu-Allah et al., 2020	0.05 mg/g
Daucosterol (Eleuth. A)	Lee et al., 2007	1.157 µg/g
Terpinen-4-Ol	Klassen, 2018	1.52 µl/g

Caged bees fed with the diets

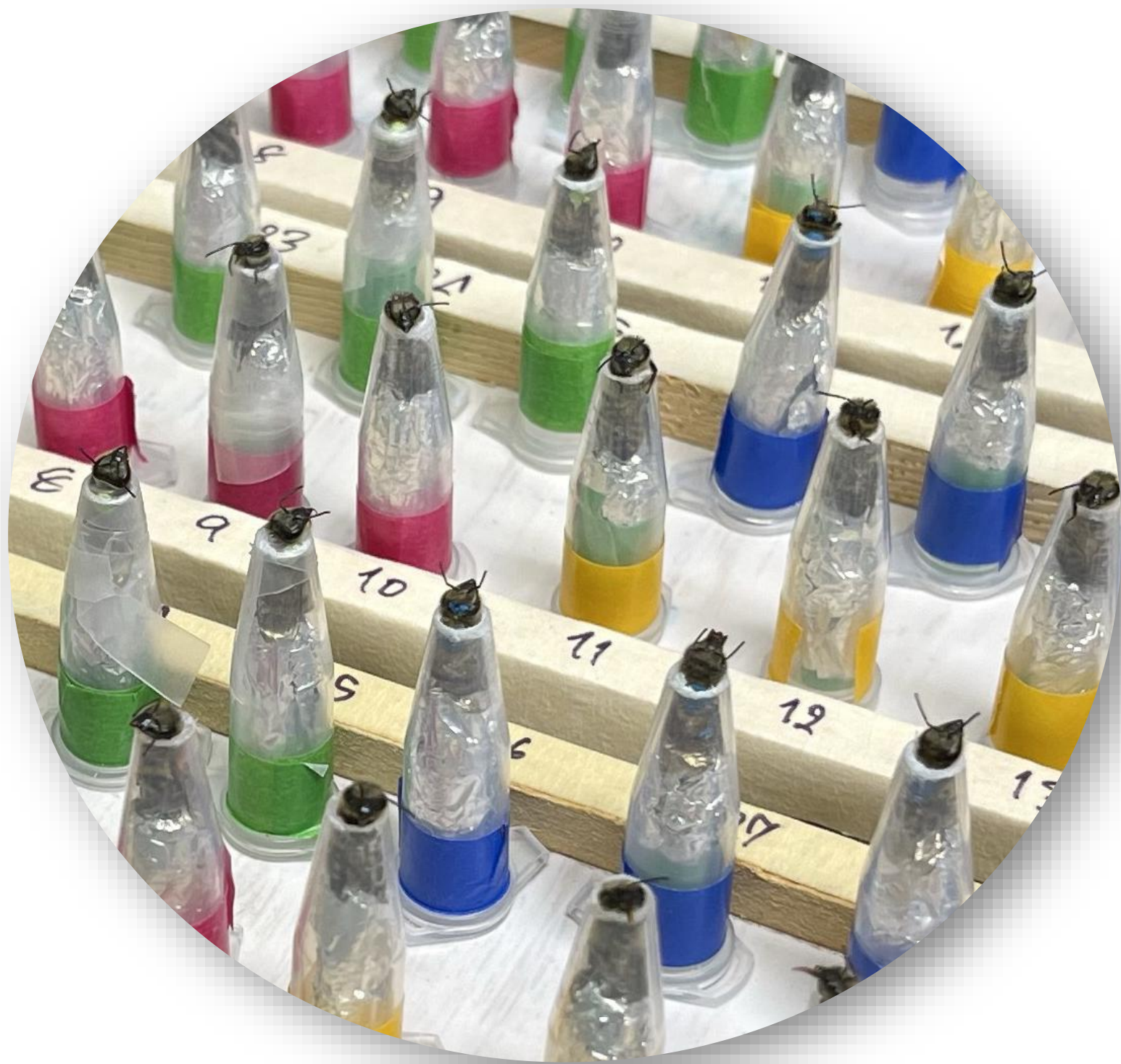


- Newly emerged bees were marked with different colors and fed with the treatments in incubators for either 14 or 7 days
- At 14 days tested for memory retention with PER tests
- At 7 days, introduced to field colonies to assess foraging behavior

Immobilizing bees for Proboscis Extension Reflex (PER) tests

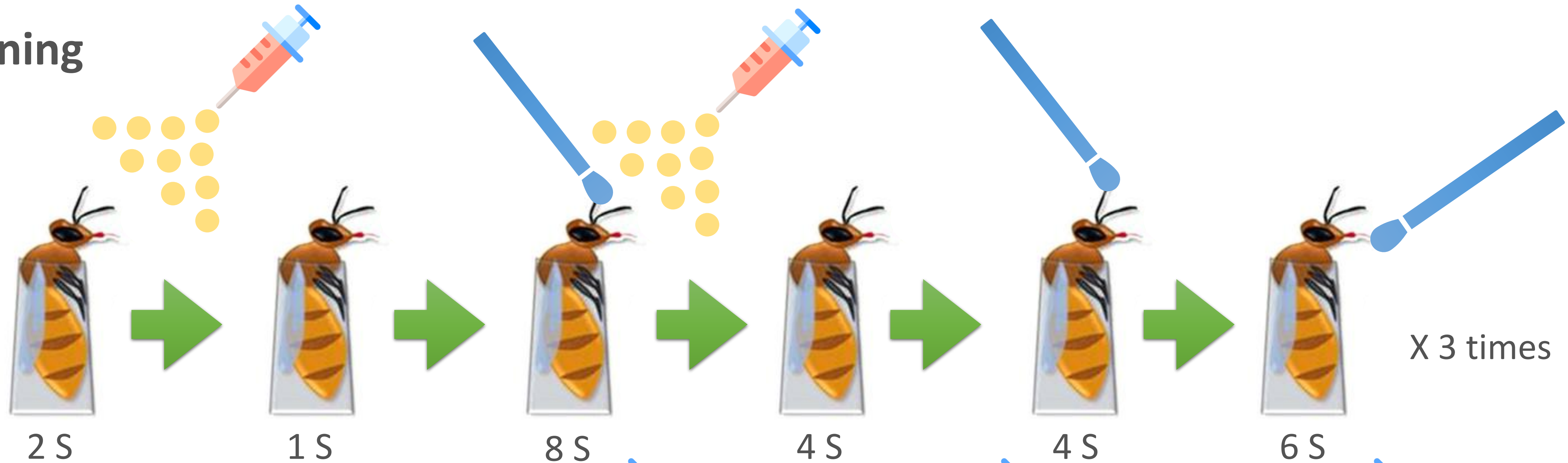


Proboscis Extension Reflex (PER) tests

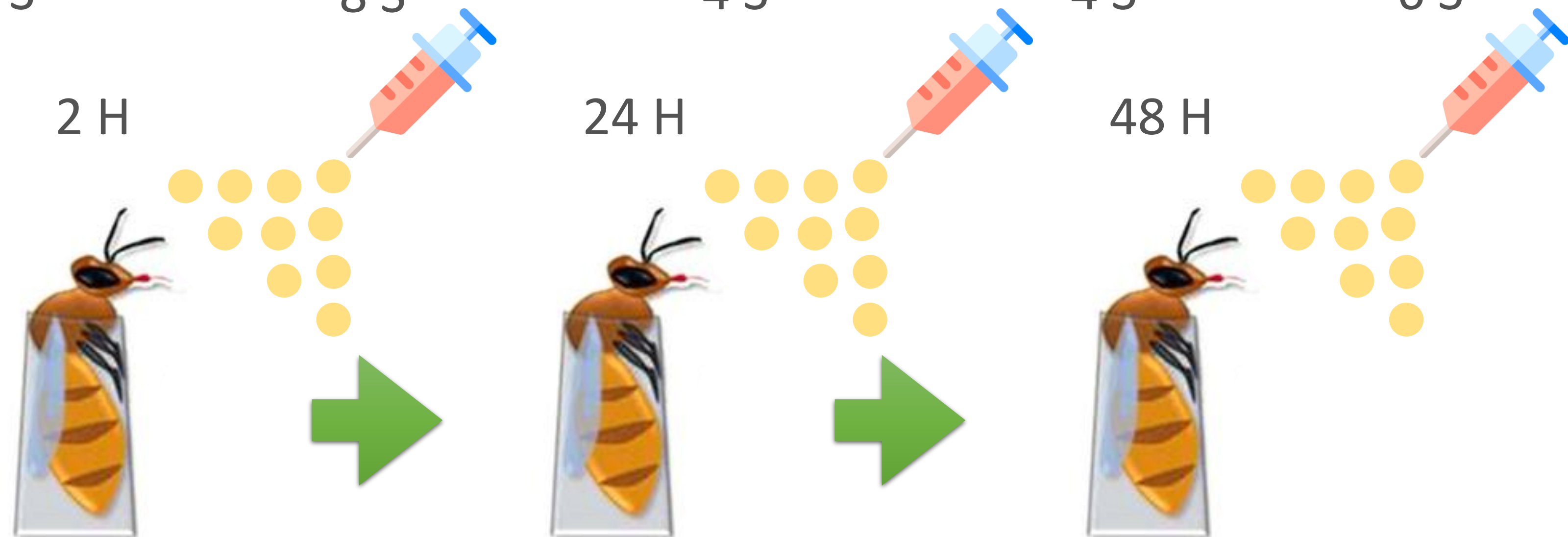


Proboscis Extension Reflex (PER) tests

Training

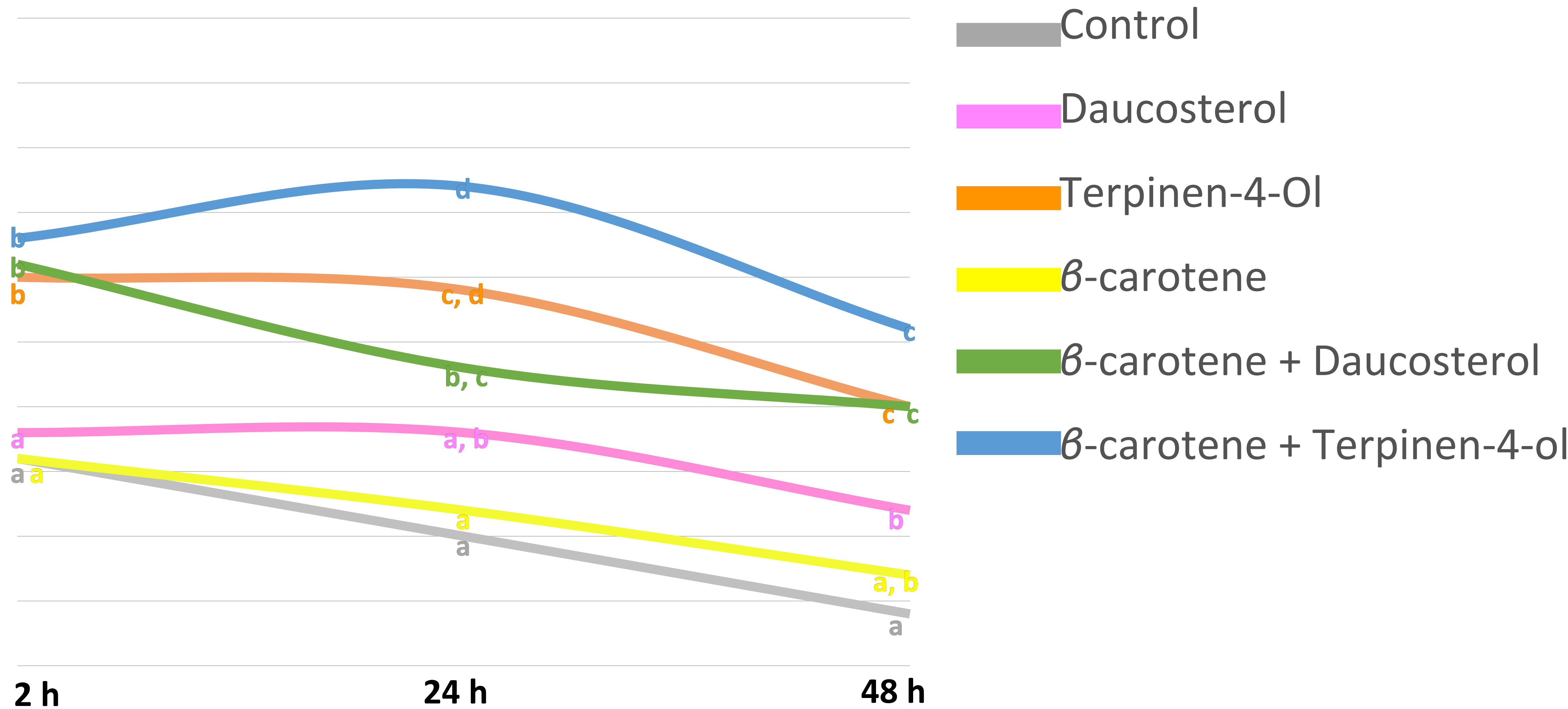


PER test



Memory of worker bees evaluated with the PER assay

(Proportion of bees that memorized the associated stimuli)



Foraging behavior experiment



Newly emerged bees were inoculated and painted with different colours according to treatments



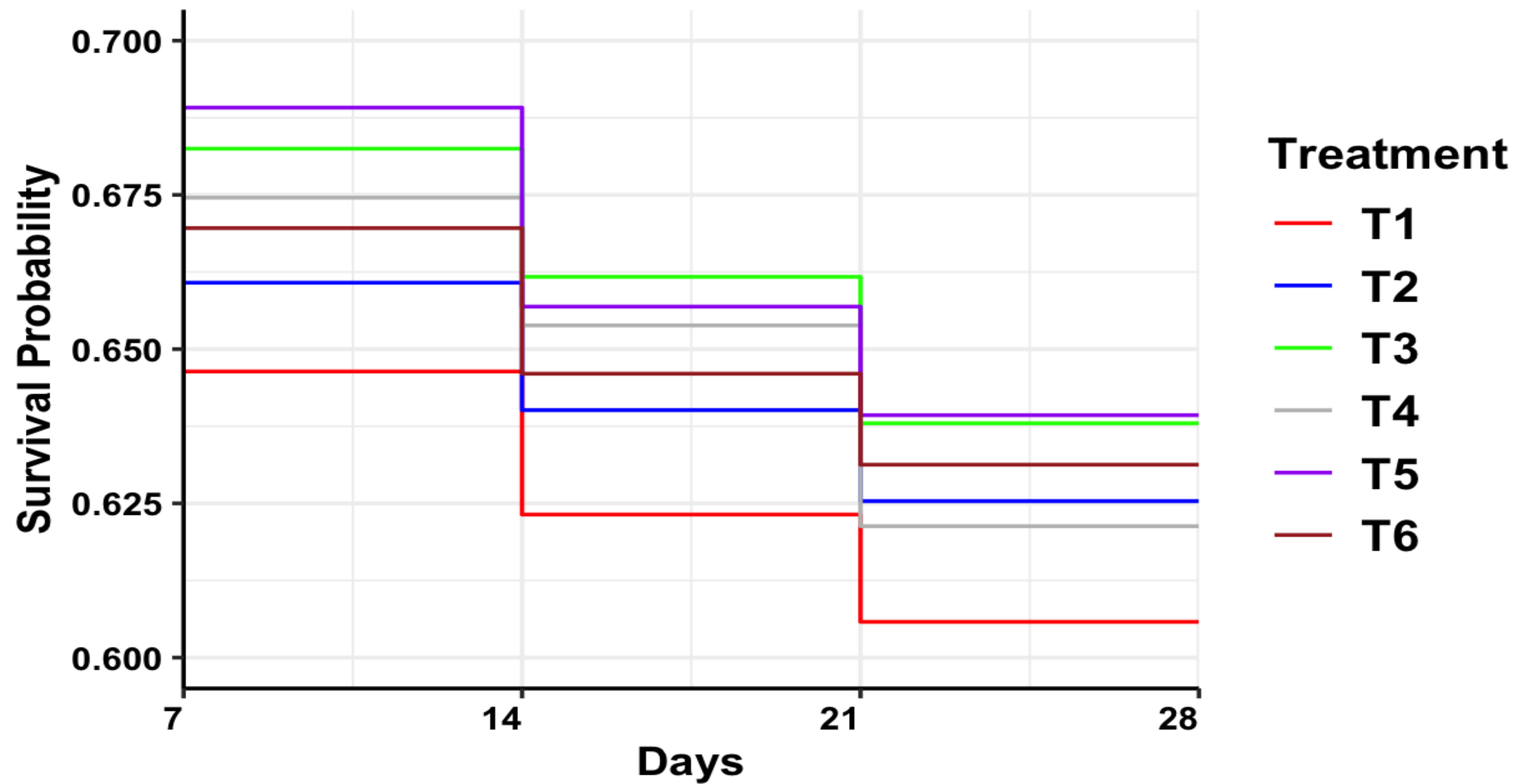
Bees were caged, incubated, and fed with diets for 7 days



Introduced to nucleus colony in experimental site

Censuses and foraging observations

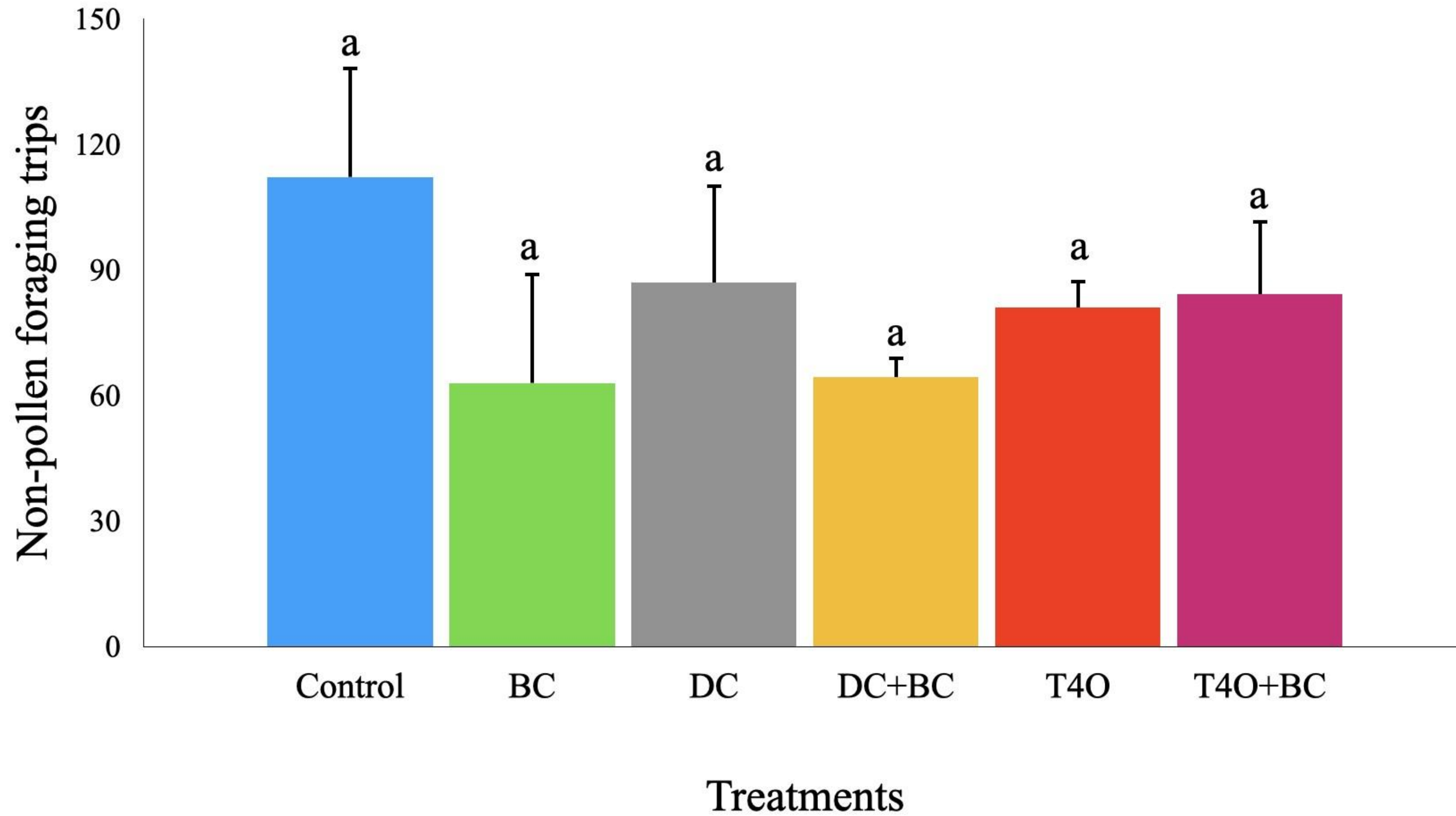




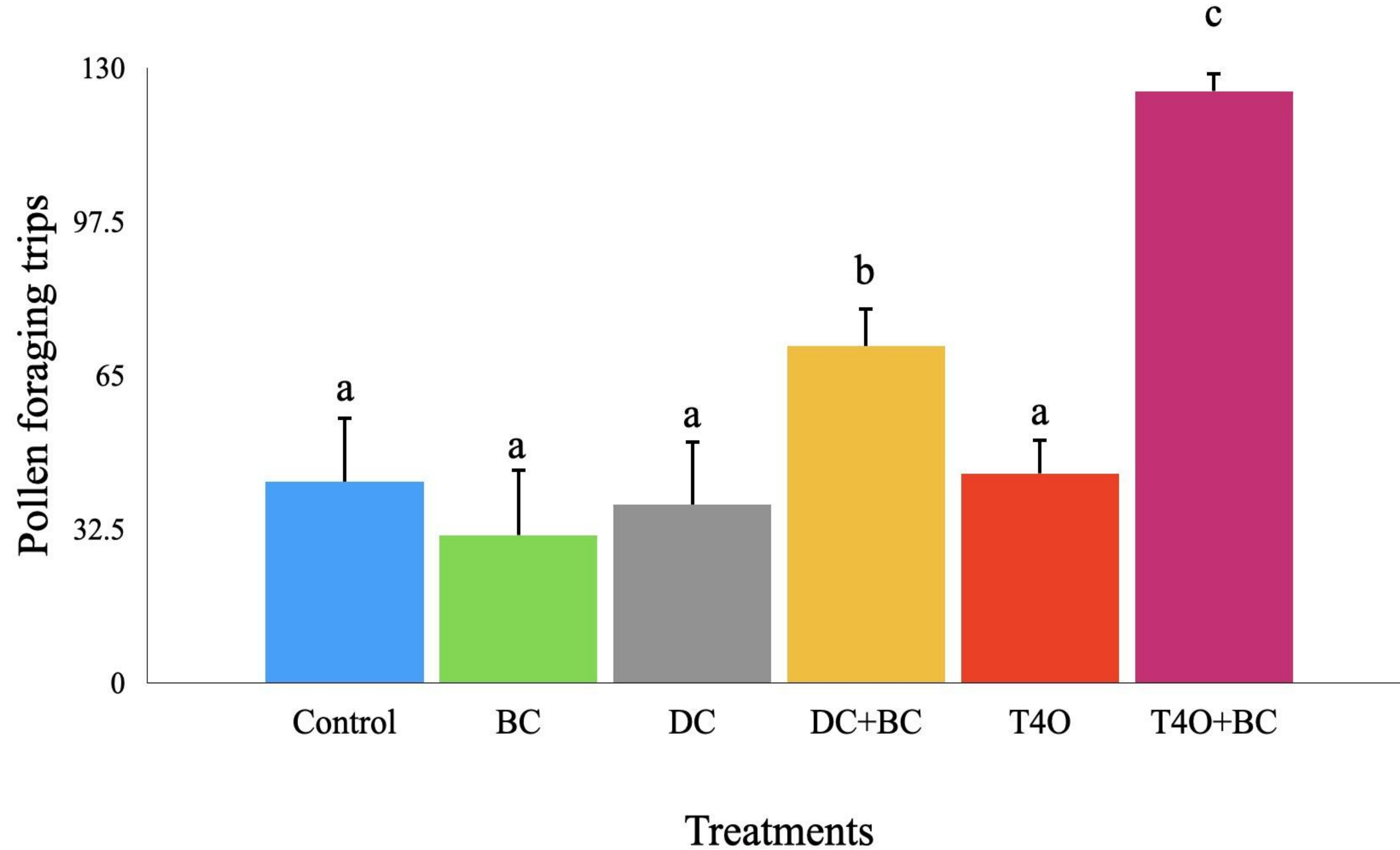
**Survival
probability of
honey bees
(Kaplan-
Meier
survival
curves)**

**No
differences**

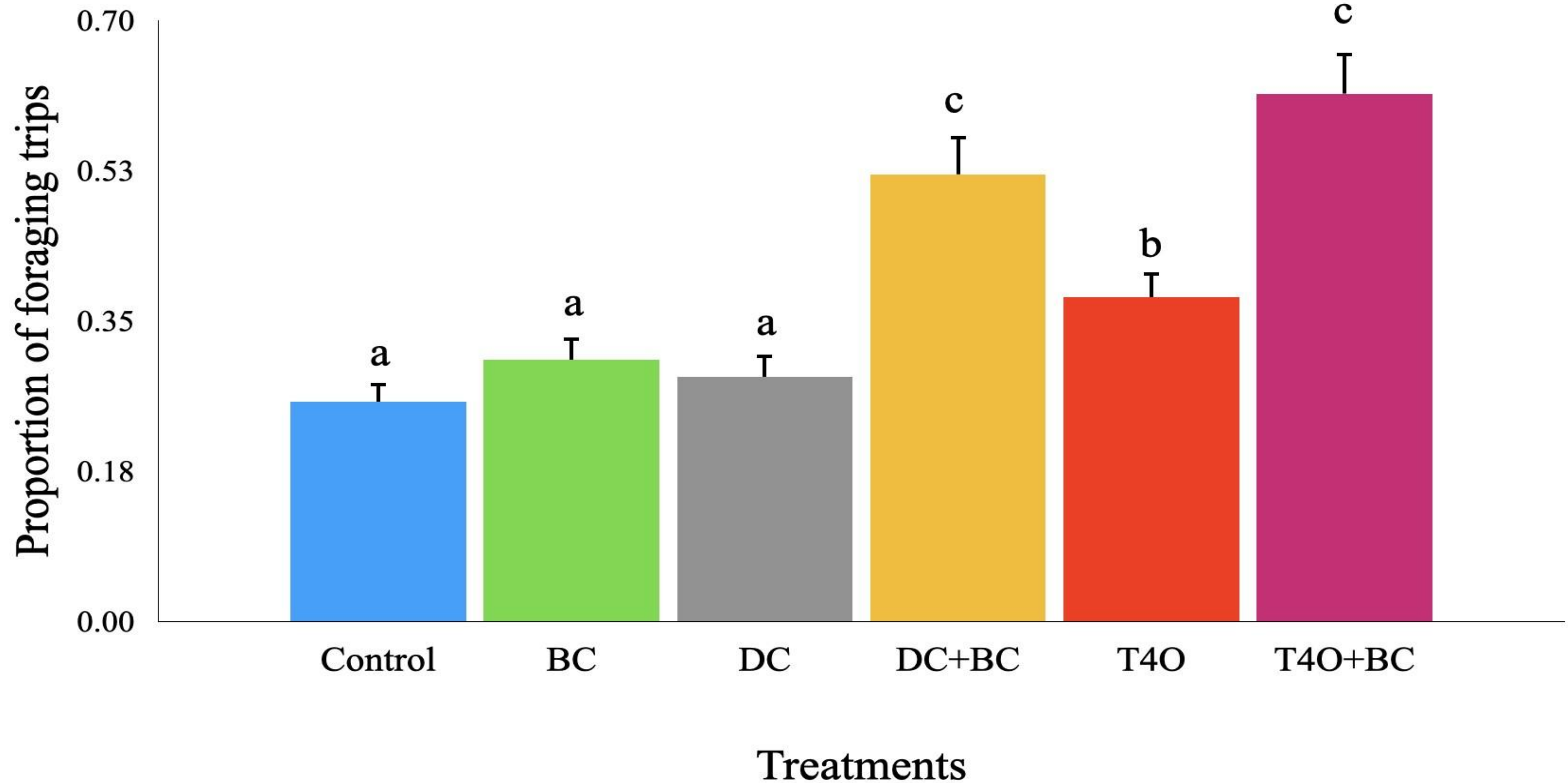
Non-pollen trips in 3 h (No. $\pm$ EE)



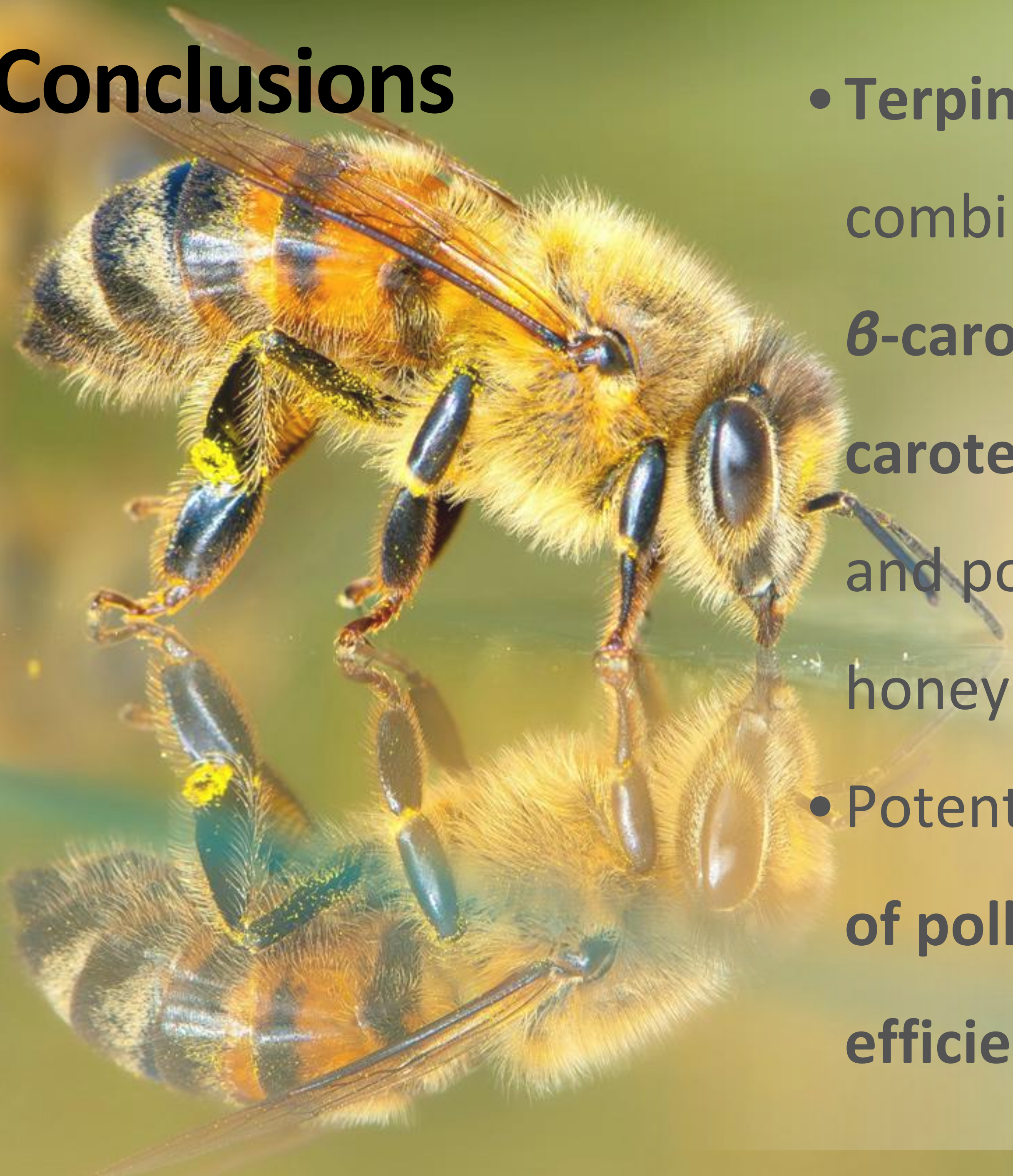
Pollen trips in 3 h (No. $\pm$ EE)



Proportion of pollen trips in 3 h ($\pm$ EE)



Conclusions



- **Terpinen-4-Ol** and the combination of **Terpinen-4-Ol & β -carotene** or **daucosterol & β -carotene** increased the memory and pollen foraging ability of honey bees
- Potential for **increased collection of pollen** and **pollination efficiency**

Thanks

