# Plant insect interaction: linking herbivory and pollinators as selection agents in populations of *Eruca sativa*

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## Background

In Israel, plants of arugula (*Eruca sativa*) reveal a clear ecotypic differentiation in floral attraction traits: petal color that ranges from the yellow, being dominant in the Mediterranean habitat, to the cream being dominant in the desert habitat. Previous research confirmed that naïve honey bees are initially more attracted to the yellow flower morph than to the cream one. In addition, plants from desert and Mediterranean populations differ in their induced defenses against insects, and our observations indicate that herbivory caused modification in floral traits in the Mediterranean plants. In this study, we consider both processes, pollination and herbivory, with the aim of testing whether the yellow ecotype would have an advantage in the Mediterranean habitat.


*Figure 1: T*he distribution of *Eruca sativa* in Israel (green dots) including sampling areas, showing the yellow and cream morphs.

## Goals

- To test the behavior of honey bees when exposed simultaneously to the two morphs for a continuous period
- To understand the effect of flower morph on plant fitness
- To test the effects of the plant defense system on floral rewards (nectar volume, nectar sugar concentration)

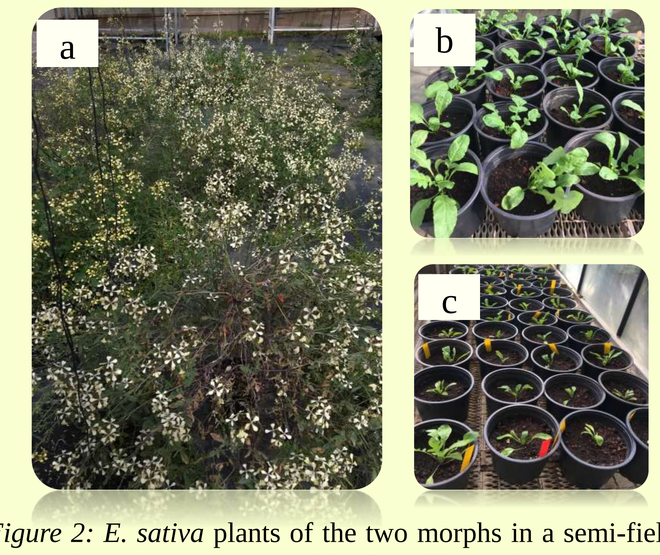



*Figure 2: E. sativa* plants of the two morphs in a semi-field honey bee preference experiment (a). *T*he effects of activating the plant defense system on vegetative state, before (b) and after (c) applying methyl jasmonate.

## Results

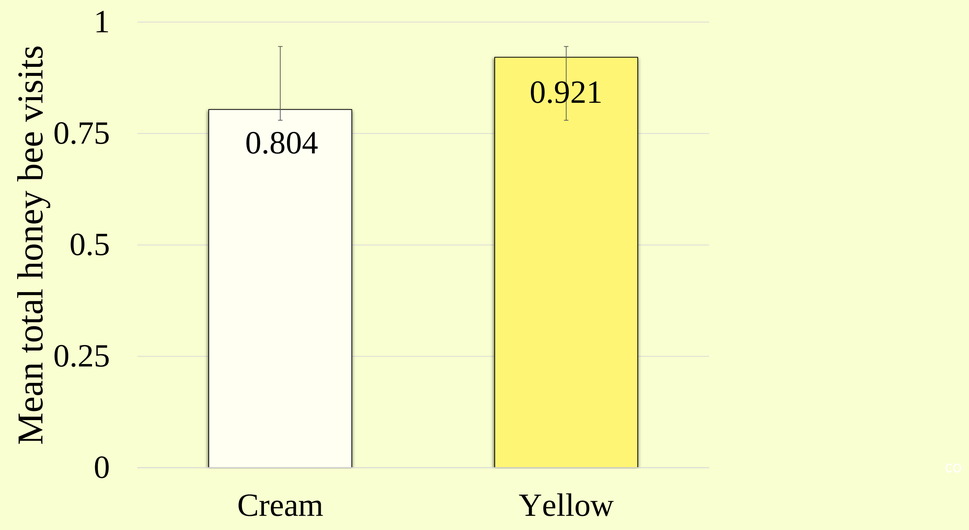
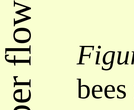



*Figure 3:* Mean (± SE) number of honey bee visits per plant. In a preference experiment we exposed a hive of honey bees to a population of both morphs (n=120) and recorded bee visits.

**Summary – no preference between cream and yellow.**



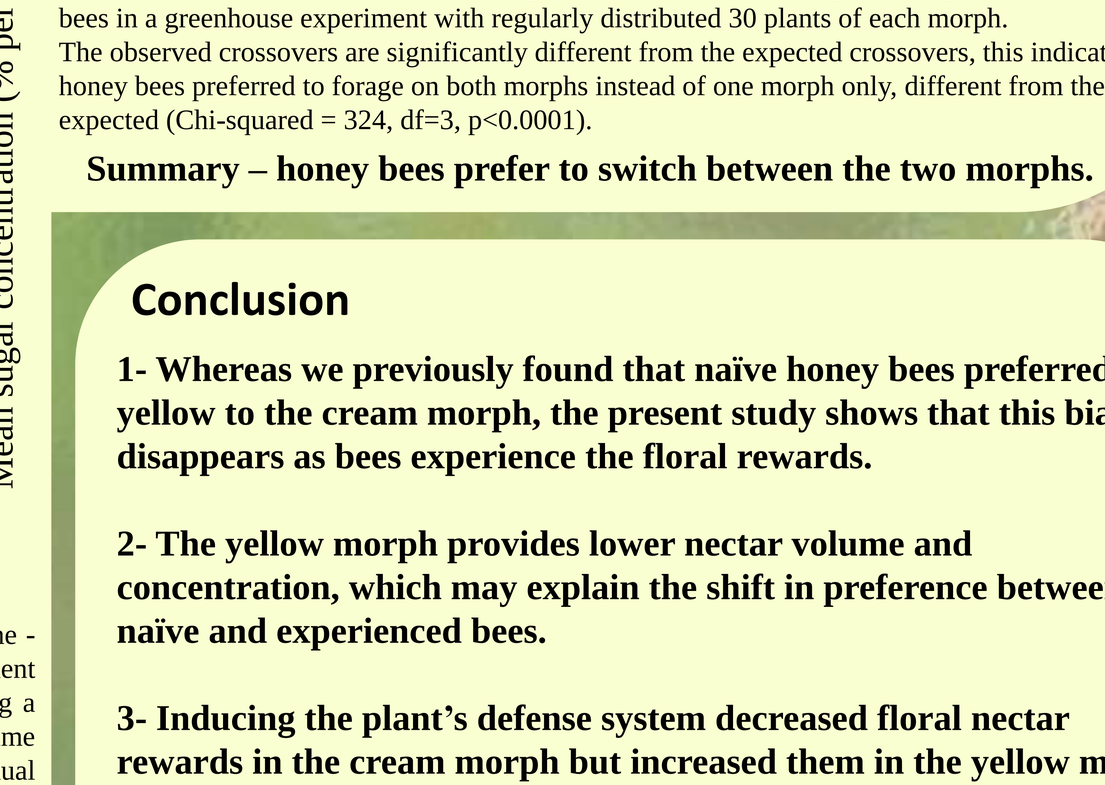



*Figure 4:* Observed (blue) and expected (green) crossovers between plant morphs of individual bees in a greenhouse experiment with regularly distributed 30 plants of each morph.
The observed crossovers are significantly different from the expected crossovers, this indicates that honey bees preferred to forage on both morphs instead of one morph only, different from the expected (Chi-squared = 324, df=3, p<0.0001).

**Summary – honey bees prefer to switch between the two morphs.**

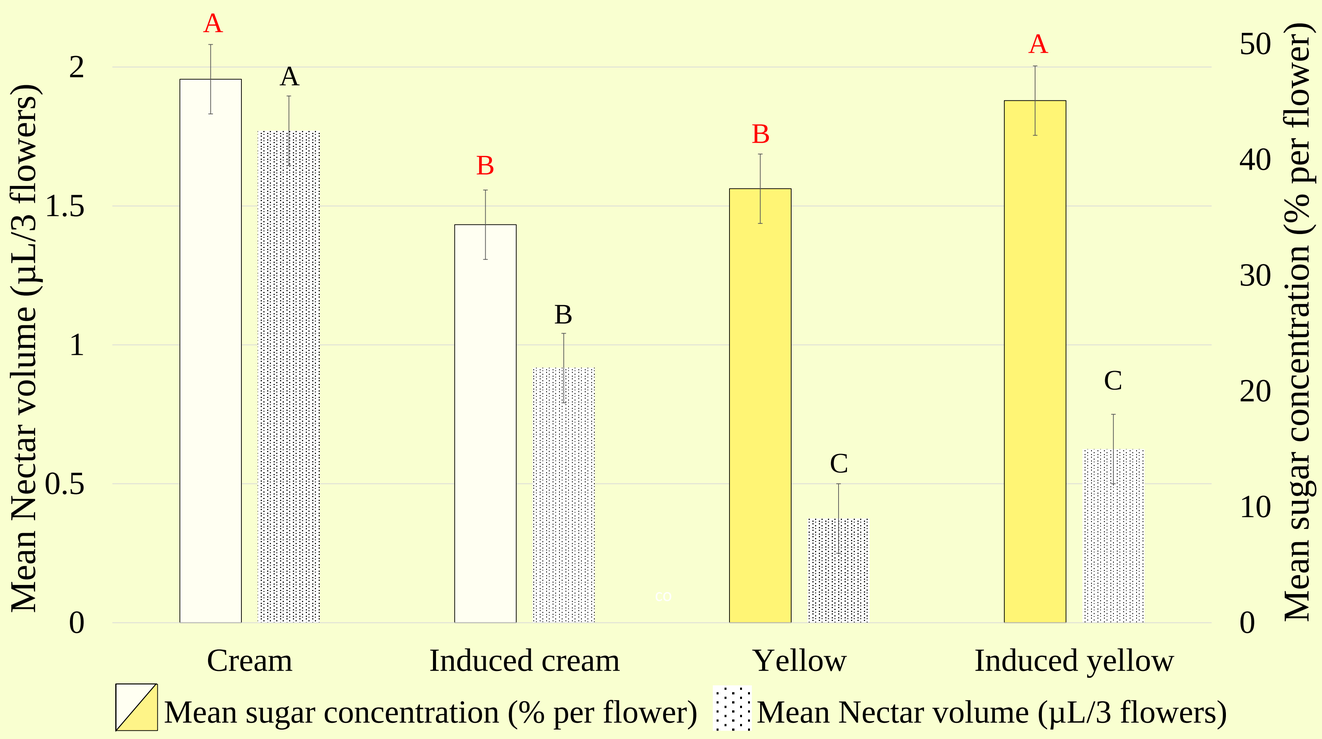



*Figure 5:* The effects of methyl jasmonate on nectar characteristics (Sugar concentraion – smooth, nectar volume - dotted). We activated the plant defense system by applying methyl jasmonate to a 2-week old plants. Each treatment included 40 plants, from each plant flowers were selected (top 3-4 flowers) and the nectar was gathered using a micropipette. For the **nectar volume test**, we pooled nectar from three flowers at a time and measured the volume (n=1041). For the **sugar concentration test**, we measured the total dissolved solids in the nectar of each individual selected flower (n=127) using a manual refractometer. Different letters indicate significant differences between treatments (Tukey test, P<0.05).

**Summary – the yellow morph provides lower reward (nectar volume and concentration), but methyl jasmonate increases reward in yellow morph while reducing it in the cream morph.**

## Conclusion

**1- Whereas we previously found that naïve honey bees preferred the yellow to the cream morph, the present study shows that this bias disappears as bees experience the floral rewards.**

**2- The yellow morph provides lower nectar volume and concentration, which may explain the shift in preference between naïve and experienced bees.**

**3- Inducing the plant's defense system decreased floral nectar rewards in the cream morph but increased them in the yellow morph.**

**4- The bias of bees towards alternating between visits to the two morphs might be related to reward in nutritional composition and is subject to further investigation.**