Evaluating the Impact of the Varroacide Formic Acid on Honeybee Foraging Performance

Background and Introduction

Managed honeybees (Apis mellifera) are essential to food security and agriculture by providing pollination services to a variety of crops. They also produce honey, beeswax, and other products that are valued for their nutrition and medicinal applications and contribute to economic growth.

Beekeepers have been facing a 35-45% annual colony loss rate. The varroa mite, as the host of the lethal Deformed Wing Virus and several other pathogens, is considered the most dangerous threat to honeybee health. If left untreated, infected hives collapse within 1-3 years.

Adequate nutrition from natural foraging is critical to the growth and survival of colonies, and high foraging input during nectar flow correlates with low winter colony loss.

Formic acid, particularly in slow-release form, is a leading varroacide for spring treatment when major nectar sources start to bloom and create nectar flow.

It is very important to understand whether the field application of formic acid affects foraging activity. Currently, there is limited information on this topic.

Radio frequency identification (RFID) is a technique that can continuously track the movements of a large number of animals and has gained popularity in bee research after the emergence of miniature transponders. Foraging activities and life-long foraging performance of bees can be quantitatively measured through the recording of the movements of honeybees as they enter and exit the beehive.

Methods

RFID:
RFID readers with an open tunnel and fitted with two tandemly arranged antennae were placed in front of the hive entrance. Detection at each antenna was recorded using a data logger.

Tagging of foragers:
Foragers leaving the hive were captured in glass vials at the hive entrance and placed on ice until they were chilled to a state where they did not actively fly but still actively walked. RFID transponders were attached with cyanoacrylate adhesive to the center of the thorax of each bee.

Tagging of newly emerged workers:
Frames with sealed brood cells were placed in an incubator at 94ºF and 55-70% relative humidity. Newly emerged workers (4-24hr) were collected and placed in a box containing a honey-filled mini frame harvested from the designated receiving hives. The boxes were kept in the incubator unless taken out for tagging.

RFID Data analysis:
RFID data was analyzed by a Python algorithm to define departures and arrivals and calculate trip durations and trip numbers.

Onset of foraging was defined using a separate Python algorithm calculating changes of daily cumulative flight time.

Histogram plots of trip time were generated using the Seaborn Python data visualization library based on Matplotlib.

Statistical analysis was conducted in GraphPad Prism 9.

Existing Foraging Capacity

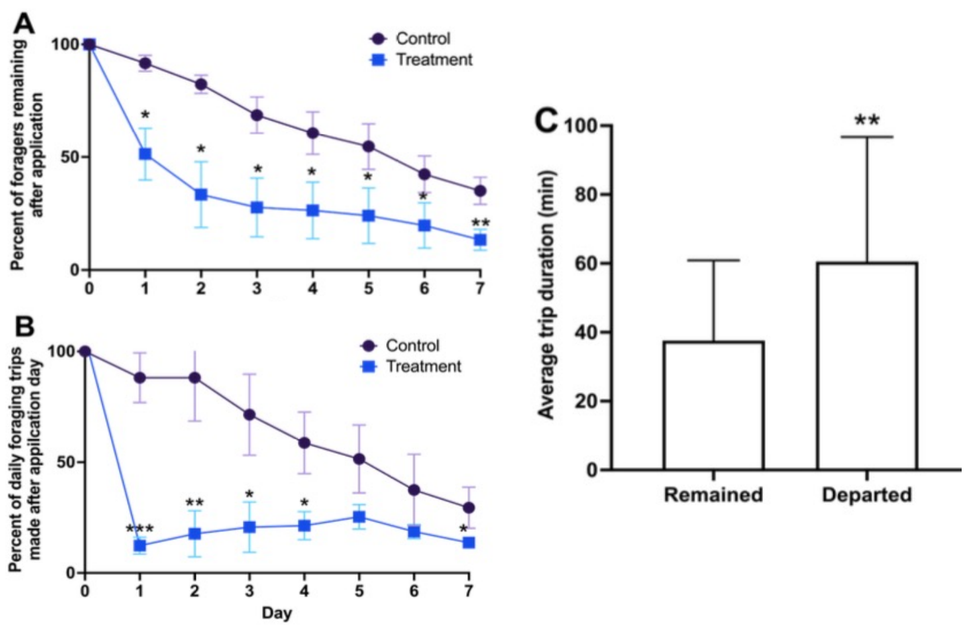

Figure 1: Changes induced by formic acid treatment in established foragers

(A) Percent of foragers remaining in the control (n=3) and the treatment hives (n=3), 1-7 days after application of Formic Pro
(B) Percent of total number of foraging trips made by the tagged foragers in the control (n=3) and the treatment hives (n=3), 1-7 days after application of Formic Pro.
(C) Comparison of pretreatment trip durations between foragers that have remained (n=47) or departed (n=145). *p<0.05, **p<0.01, ***p<0.001 by t test.

Defining Onset of Foraging

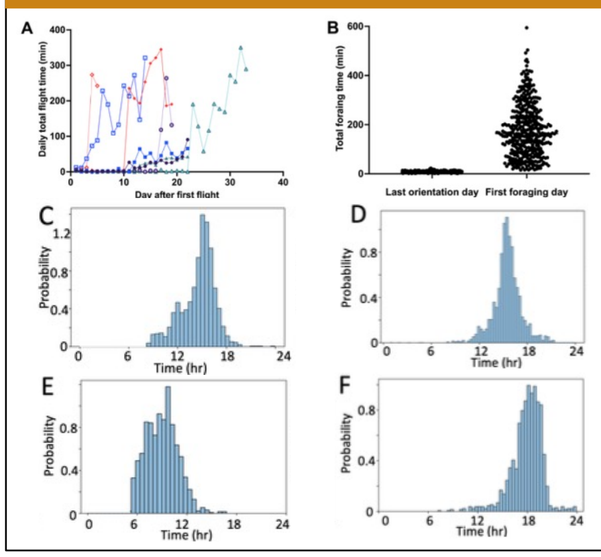

Figure 2: Define onset of foraging

(A) Life-long flight activities of 8 workers to demonstrate the sharp increase in daily total flight time at the transition.
(B) Total fight time on the identified last day of orientation and the first day of foraging (n=416).
(C) Time of the first departure during the orientation phase.
(D) Time of the last arrival during the orientation phase.
(E) Time of the first departure during the foraging phase.
(F) Time of the last arrival during the foraging phase.

Forthcoming Foraging Capacity – Newborn Bees

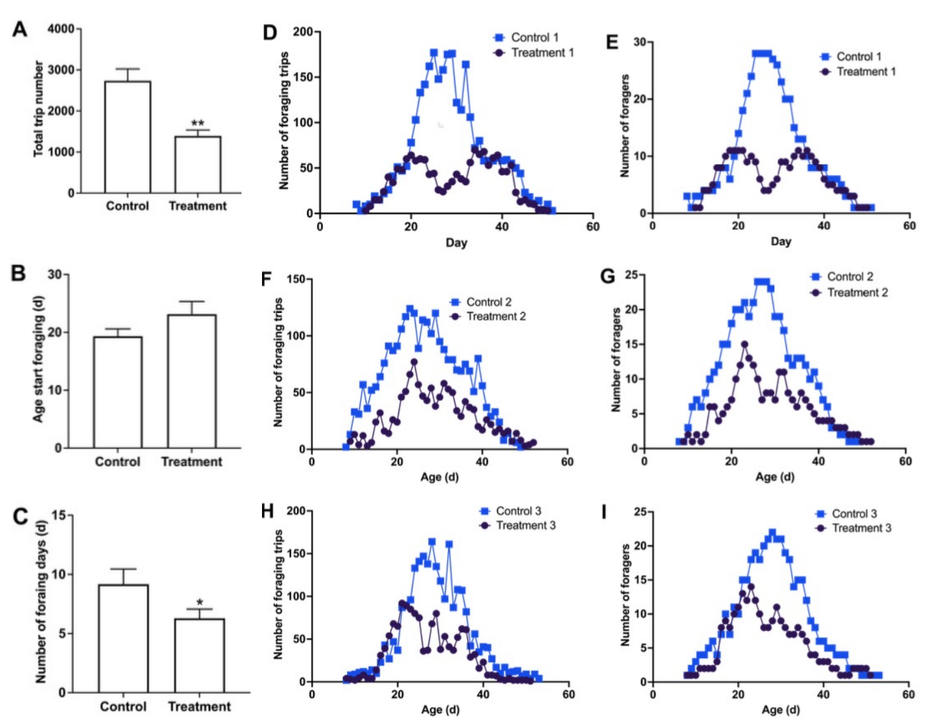

Figure 3: Changes induced by formic acid treatment in young workers.

(A) Average total foraging trips made by the tagged workers in the control (n=3) and the treatment hives (n=3).
(B) Average ages of foraging onset of the workers in the treatment hives (n=3) in comparison to that of the control hives (n=3).
(C) Average foraging span of the workers in the treatment hives (n=3) in comparison to that of the control hives (n=3).
(D, F, H) Daily total foraging trips made by tagged workers in each pair of control and treatment hives.
(E, G, I) Daily total numbers of detected foragers in each pair of control and treatment hives. *p<0.05, **p<0.01 by t test

Existing and Forthcoming Foraging Capacity - 22-Day Old Workers

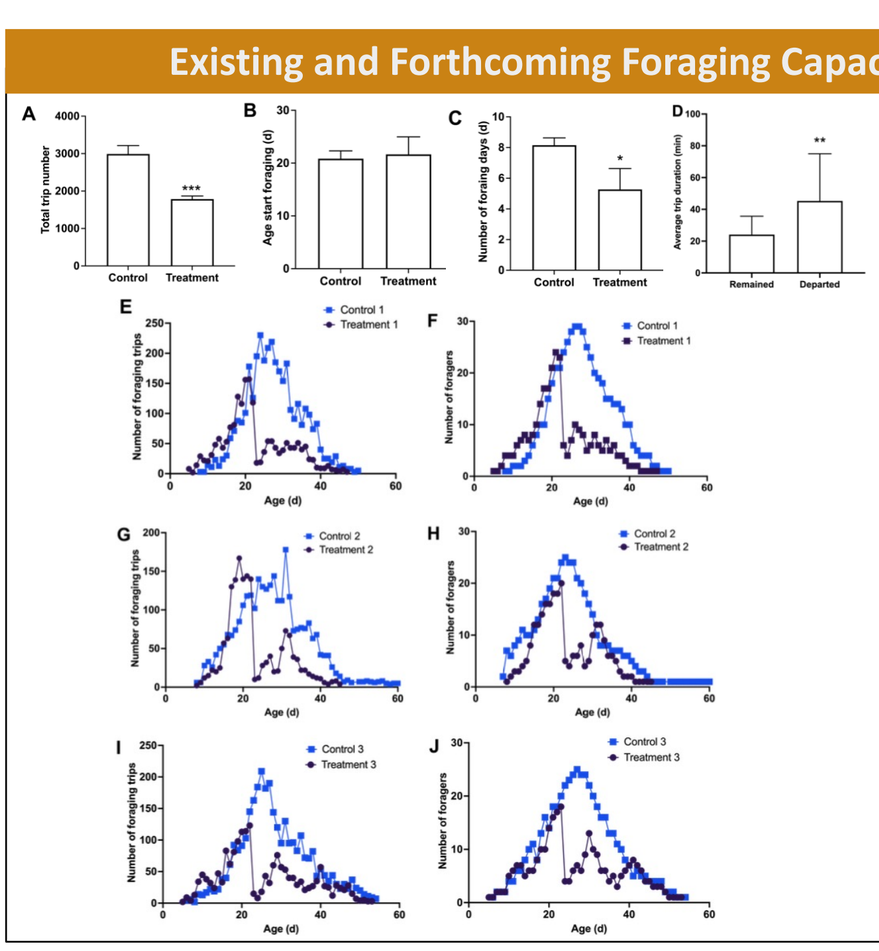

Figure 4: Changes induced by formic acid treatment in 22-d old workers

(A) Average total foraging trips made by the tagged workers in the control (n=3) and the treatment hives (n=3).
(B) Average ages of foraging onset of the workers in the treatment hives (n=3) in comparison to that of the control hives (n=3).
(C) Average foraging span of the workers in the treatment hives (n=3) in comparison to that of the control hives (n=3).
(**D)** Comparison of pretreatment trip durations between foragers that have remained (n=14) or departed (n=59) the day after Formic Pro application.
(E, G, I) Daily total foraging trips made by all tagged workers in each pair of control and treatment hives.
(F, H, J) Daily numbers of detected foragers in each pair of control and treatment hives. *p<0.05, **p<0.01, ***p<0.001 by t test.

Conclusion

The effect of the field application of formic acid on foraging performance was evaluated in three different age groups of honeybee workers

Formic acid significantly suppressed the existing foraging capacity of the colony, indicated by the response of the established foragers. The effect mostly resulted from many foragers departing the hive without returning and appeared to be more selective for foragers that made longer foraging trips prior to the treatment.

Formic acid delayed the onset of foraging in workers that were treated when they first emerged and also reduced the number of foraging trips and number of foragers and decreased average foraging span.

In the group of workers treated at 22-d old, when 25-50% of the workers performed foraging tasks, while foraging onset was not delayed, foraging span, number of foraging trips, and number of foragers decreased without ever rebounding to previous levels. The treatment was also more selective for foragers making longer foraging trips, reinforcing the plausibility of an energy- based mechanism.

Future Directions

As foragers who made longer trips were more impacted by the treatment, this suggests that affected energy expenditure is likely a contributing factor, which is consistent with previous reports that exposure to formic acid in the laboratory setting affected gene expression levels and protein levels of molecules involved in mitochondrial respiration. Additional studies to evaluate gene expression and protein levels after field application could shed light on the potential mechanism of action of the negative impact of formic acid on foraging performance.

The results of this study warrant an investigation into safer treatment options during nectar flow and suggest that chemical treatments with unknown mechanisms of action are not the permanent solution to treating mite infestations. Biological control methods employing RNAi carrying bacteria could lead to safe and effective long-term solutions.
Following the first recently approved vaccine for bees against American Foulbrood disease, we could look into developing a vaccine for bees against Deformed Wing Virus.

*All images and figures created by the researcher unless otherwise noted