HONEY BEES DO NOT LIKE BE(E)ING CONFINED

Dr. Jean-Christophe Lenoir, Cédric Pierru, Arnaud Zicot and Line Deslandes
SynTech Research France, 613 route du bois de loyse, 71750 La Chapelle de Guinchay, France; www.syntechresearch.com; jlenoir@syntechresearch.com

Introduction

The currently required setup for evaluation of side effects of plant protection products on honey bees under semi-field conditions is exposure of a hive to a treated crop for a specific duration in a confined environment (tunnel tents). As large as these tunnels can be, honey bees are enduring stressors challenging the reliability of the results of the studies. In addition to major stress of confinement, bees must deal with disturbances from multiple transports, visits, samplings and assessments in a very short period of time.

A honey bee colony represents a well-organized and effective group of insects, which implies an accurate regulation of conditions in the hive (temperature and moisture regulation, balance between colony needs, larval rearing, food consumption and food storage). In the experimental scenario of confinement and multiple assessments, such conditions are easily unbalanced (enhanced changes in environmental conditions, food supply, extensive hive visits, etc). The first observation of the consequence of these changes is the unusual escaping behavior: workers try to escape the tunnels instead of foraging, increasing the mortality rate of the caste dedicated to supplying the hive with food. It also represents the first step in the disturbance of the tested hives and their possible collapse.

In view of these recurrent situations, we assessed a possible way of reducing the main stressor of confinement by testing effects of hive positioning in the tunnels. Results of the study provide additional information on possible improvement of the experimental semi-field study designs in order to allow side effect tunnel studies to achieve their intended purposes.

Material and Methods

A semi-field testing design adapted from OECD 75 was used and the following parameters were considered in order to reduce as much as possible the stress of the honey bees: large tunnels (150 m²), sown with a nectar and pollen producing crop (*Phacelia tanacetifolia*), in a region and a period of the year providing good environmental conditions (Southern France, June 2018). Inside the tunnels, hives' entrances were oriented southwards and only the change of position of the hives into the tunnels was tested.

The study was a comparison between two modalities: hive positioning as recommended in OECD 75 (at the end of tunnel) and CEB 230 (central position).

5 replicate tunnels for each modality, all treated with water at 400 L/ha.
Linen sheets were placed according to the test design (see Figs. 1 and 2).

5 days before application, hives contained:

- about 9000 workers (mean = 8989 [4557-12867]; n=10)
- 3 to 4 brood combs with all larval stages from eggs to capped brood (mean = 24937 brood cells [10723-42065]; n=10)
- about 3 combs with reserves (honey and pollen) (mean = 17981 food cells [9623-28044]; n=10)
- 3 to 4 news waxed combs on the sides

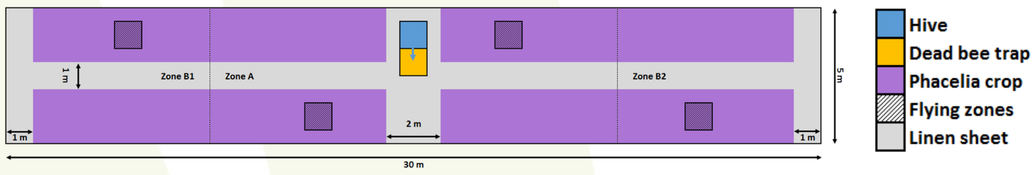

Figure 1: Centre hive design (CEB 230)

Assessments:

- Colony conditions assessments (CCA) at 5 days before application (DBA) then 3 and 11 days after application (DAA)
- Daily mortality assessments on bee traps from 6 DBA to 13 DAA, on linen sheets from 2 DBA to 8 DAA
- Daily flying activity assessments (15 sec.) from 2 DBA to 8 DAA

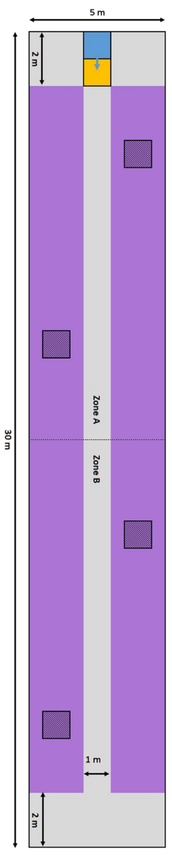

Figure 2: End hive design (OECD 75)

Results

Statistical analysis: Wilcoxon rank sum tests were used to compare assessed values between each modality.
(Center vs End design; ZoneA vs ZoneB; North vs South; East vs West)

DAA	CCA	Mortality							Flying			Walls		
	Nb bees Brood cells Food cells	Bee traps + Linen sheets (W,L,P)	Bee traps	Linen sheets					Mean nb of foragers/m² (15 sec.)			Nb of foragers flying on net walls		
				Zone A	Zone B	Total (W,L,P)	Center Zone A vs Zone B	End Zone A vs Zone B	Zone A	Zone B	Total	Center East/West	End North/South	Total
-6 -5 -4 -3		NA	NA	NA			NA		NA			NA		
-2	Tunnel phase						W (A < B)	W (A < B)						
-1							W (A < B)	W (A < B)	Center < End					
0														
1							W (A < B)	W (A < B)						
2									Center < End		Center < End			
3														
4														
5														
6				W (Center < End)			W (A < B)							
7		P (Center > End)	P (Center > End)	W (Center < End)			W (A < B)						North < South	
8		P (Center > End)	P (Center > End)				W (A < B)						North < South	
9 10 11 12 13		NA	NA	NA			NA		NA			NA		

W = Workers; L= Larvae; P= Pupae; DAA = Days After Application; CCA = Colony Condition Assessment; NA = Not Applicable; only statistically significant results are indicated (all other results not significant)

Table 1: Summary of statistical results

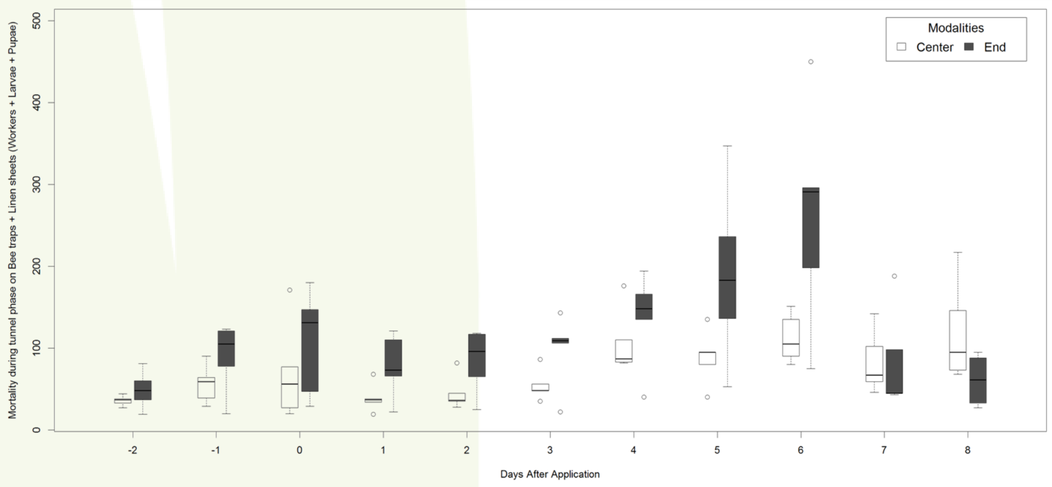

Figure 3: Total of mortality (workers+larvae+pupae on bee traps+linen sheets) according to time (DAA) for the "Center" and "End" modalities

Conclusions

Even if the honey bee colonies were placed in the best achievable conditions (no impact of the tunnel phase on the CCA), the position of the hives into the tunnels appears to play a role on the behavior and survival of colony members. After some days of containment, the hives positioned centrally show a lower and more stable mortality rate than the hives positioned at the end of tunnels (especially after the 5 DAA CCA disturbance). At the end of the tunnel phase, workers of the hives positioned at the end of tunnels try to escape to the south.

Using a central position of the hives in semi-field studies can be a key component in reducing the impact of major disturbance while testing side effects of plant protection products on honey bees.

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