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ABSTRACT

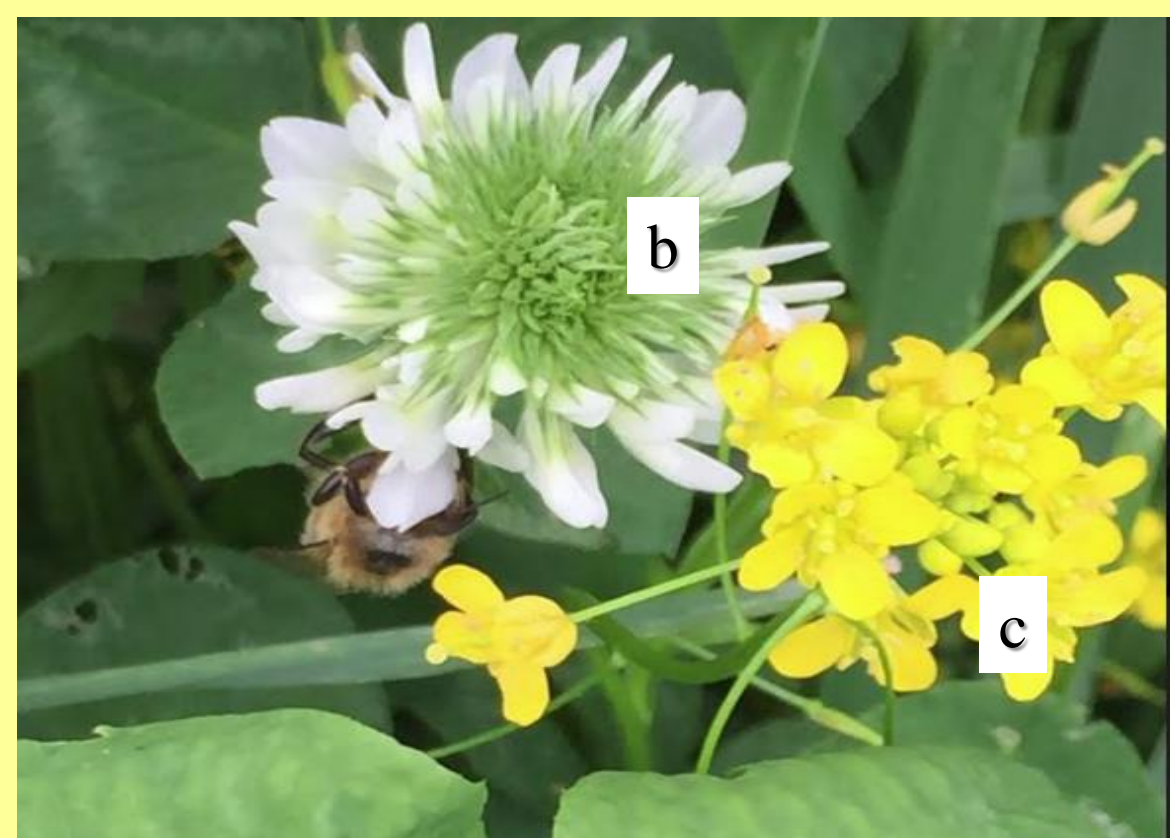
In summer 2018, twelve test tunnels were put up at Le Centre de Recherche en Sciences Animales de Deschambault (CRSAD), a para-governmental experimental farm in Quebec, Canada. Two groups 1. Control (e.i. water) and 2. Treatment (BioCeres *e.i. Beauveria Bassiana*) of six hives were placed in the twelve tunnels covering a red and white clover surface areas of 41,8m² (first trial from 21 June to 1 July 2018) to 50,6 m² (second trail from 13 to 23 August 2018). Water feeders were placed near hives in each tunnel. To assess acute bee mortality, dead bee traps were positioned on hives and vegetation mats were placed on tunnel floors. The bioinsecticide was applied, according to the label, when honeybees were not active, after sunset. Colonies were initially exempt of clinical symptoms of disease. Daily data collected included honeybee, drone and larvae mortality and flight and drinking activity. In hive periodic data collection included number of food stock cells (honey and pollen), number of brood cells and adults bees on frames. Meteorological data was collected hourly inside and outside tunnels during the trials. Using T-test comparisons, no statistical difference was found between treatments for all recorded data. On rare occasions where dead bee counts were greater than average (although very low), specimens from the BioCeres tunnels were sent for laboratory incubation and biotic analysis. Results did not show the presence of the fungal infection to *B. bassiana*. Hives were thereafter wintered inside at de CRSAD and after wintering data were registered in April 2019. Since no statistical differences between colonies exposed to BioCeres treatments au controls were found, we conclude that BioCeres WP, containing the active ingredient of conidiospores of the strain *B. bassiana* ANT-03, at the highest recommended doses did not infect, cause sub lethal behavioural abnormalities, nor kill the honey bees under tested conditions. The study results indicate that BioCeres WP is safe to honeybees under field conditions and can be applied against field pest insects

MATERIALS AND EXPERIMENTAL DESIGN

Tunnels were shielded with FIINTEX knitted insect exclusion netting (1,9mmx0.095mm mesh; 60gr/m²) covering a crop area of 41,8m² (25ft X 18ft) for the first series of trials 40 m² and were enlarged to 50,6 m² (20ft X 30ft) for the second series of trials, taking into account the colony development. Tunnels were (2,74m in height) were placed in two groups or six, sited 30 meters apart to avoid interference between treatment groups.



Plant species foraged in tunnels



Trifolium pretense (a)
Trifolium repens (b)
Brassica sp. (c)
Water feeder placed in each tunnel (10cm)(d)



Rationals

This study reports the results of the Semi-Field study trials to evaluate the short and long term impacts of BioCeres WP treatments on honeybee's, *Apis Mellifera*, heath and behaviour.

This study has been requested by the PMRA for the registration of the BioCeres wettable formulations, F WP, D WP, and BioCeres granular formulation F GR and D GR against field pest insects. The study will provide information to evaluate the effect of the active ingredient of the BioCeres, the strain of the *Beauveria bassiana* ANT-03 on the health of the colonies and the risk assessment for the pollinator (CODO M9.5.1).

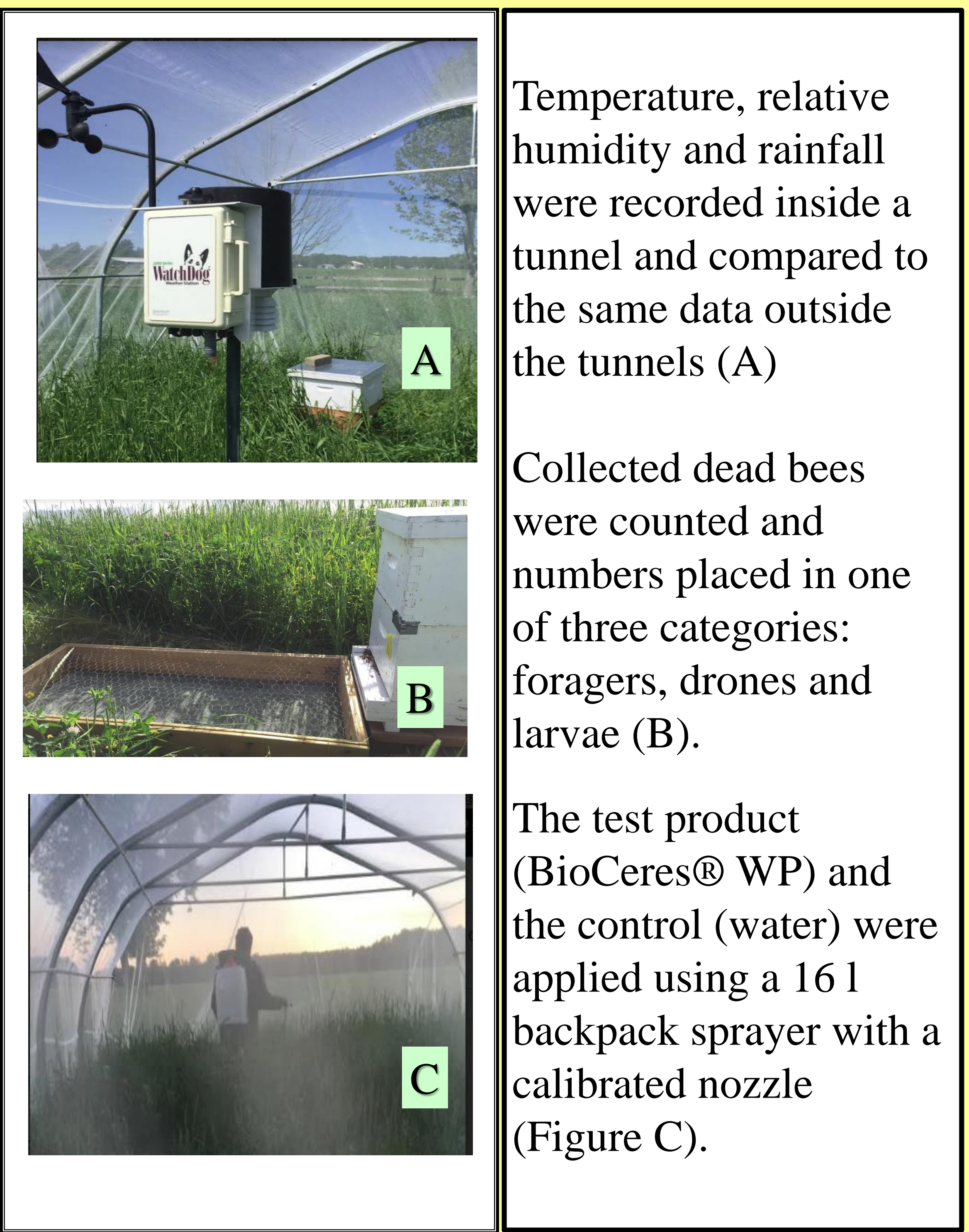
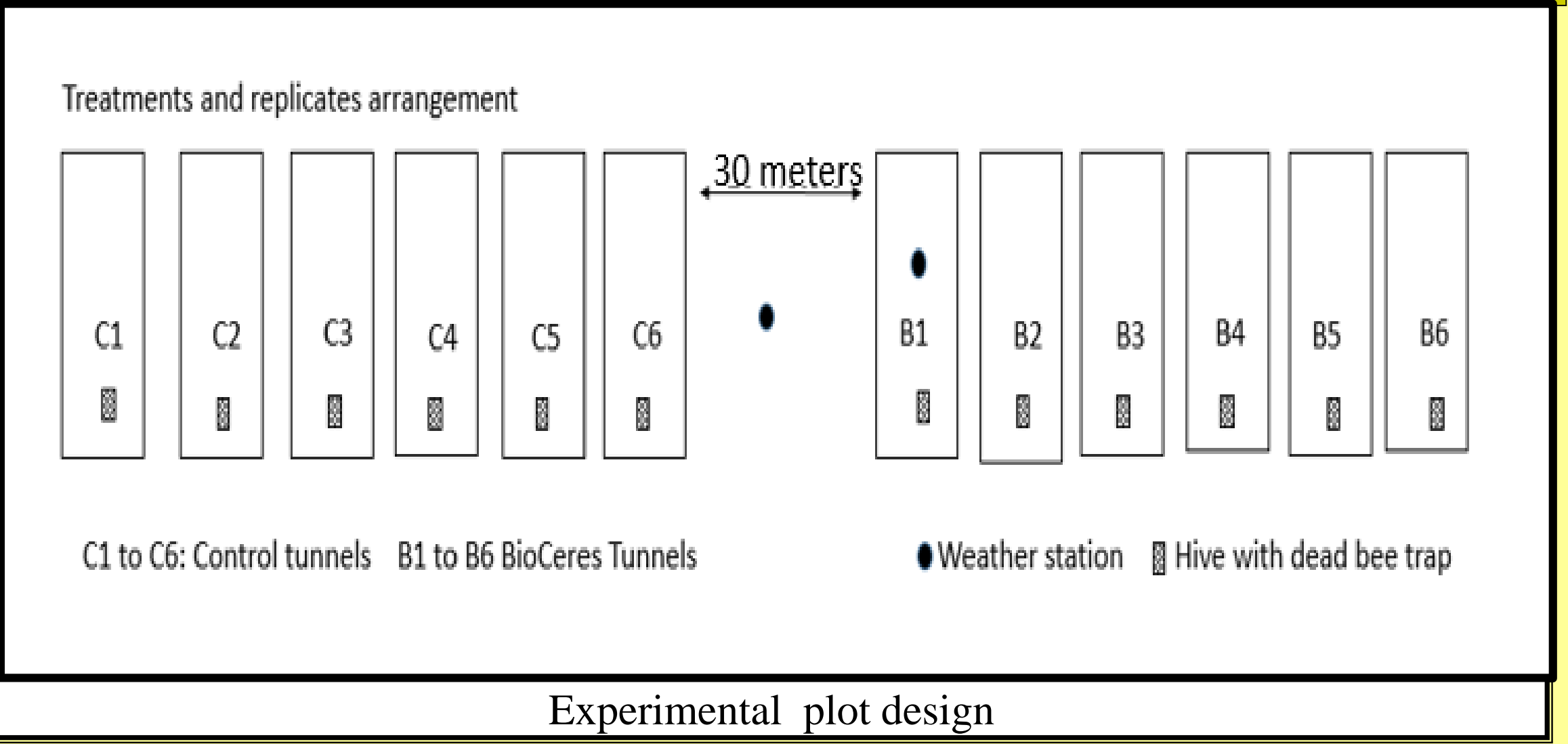
METHODOLOGY

DATES AND DOSES : First series of exposures started on 21 June 2018 and were continued until 1 July 2018 while the second series were from 13 August to 23 August 2018. At the end of days 0, 3 and 6 of the experimental periods, the vegetation inside each of the six the BioCeres® treatment tunnels (containing one of the experimental hives) was sprayed with BioCeres® WP at the maximum label recommended application rate (6 g/L) and a volume rate of 200-400 L/ha. In the six control tunnels, the vegetation was treated with tap water at a volume rate of 200-400 L/ha (B).

The test product (BioCeres® WP) and the control (water) were applied using a 16 l backpack sprayer with a calibrated nozzle. Treatments started at sunset to avoid honeybee foragers and spraying of the insect net was also avoided. The bioinsecticide was applied, according to the label, when honeybees were not active, after sunset. Both test product and water were applied during the same time period. Honeybees foraged the treated vegetation on the next morning (a, b, c) Following the full first honeybee exposure period in the tunnels, the hives were placed outside their respective tunnel and were free to forage in the field.

The second series of treatments started on 13 August 2018 and were continued until 23 August 2018. Experimental design was identical to that of the first series of treatments. The hive identification numbers and treatment codes are listed in Table 1. The same hives were attributed to the same tunnel/treatments for the two series.

After the final November 2018 data collection on hives from both treatments (BioCeres and Control (water)), colonies were wintered in a heated and ventilated indoor wintering facility at le Centre de Recherche en Sciences Animaux de Deschambault (CRSAD). In April 2019, hives were taken out of hibernation for final evaluations.

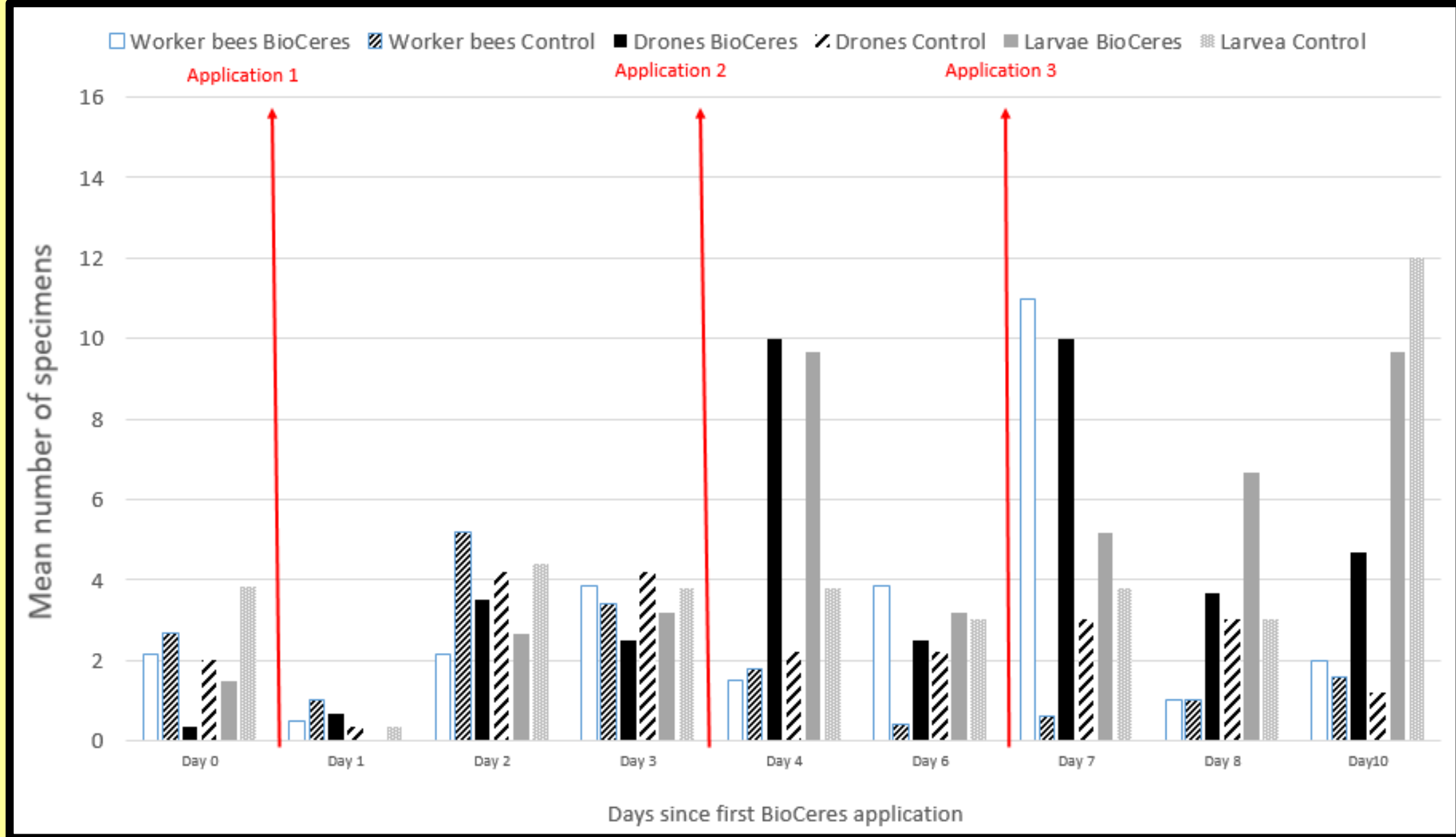


Temperature, relative humidity and rainfall were recorded inside a tunnel and compared to the same data outside the tunnels (A)

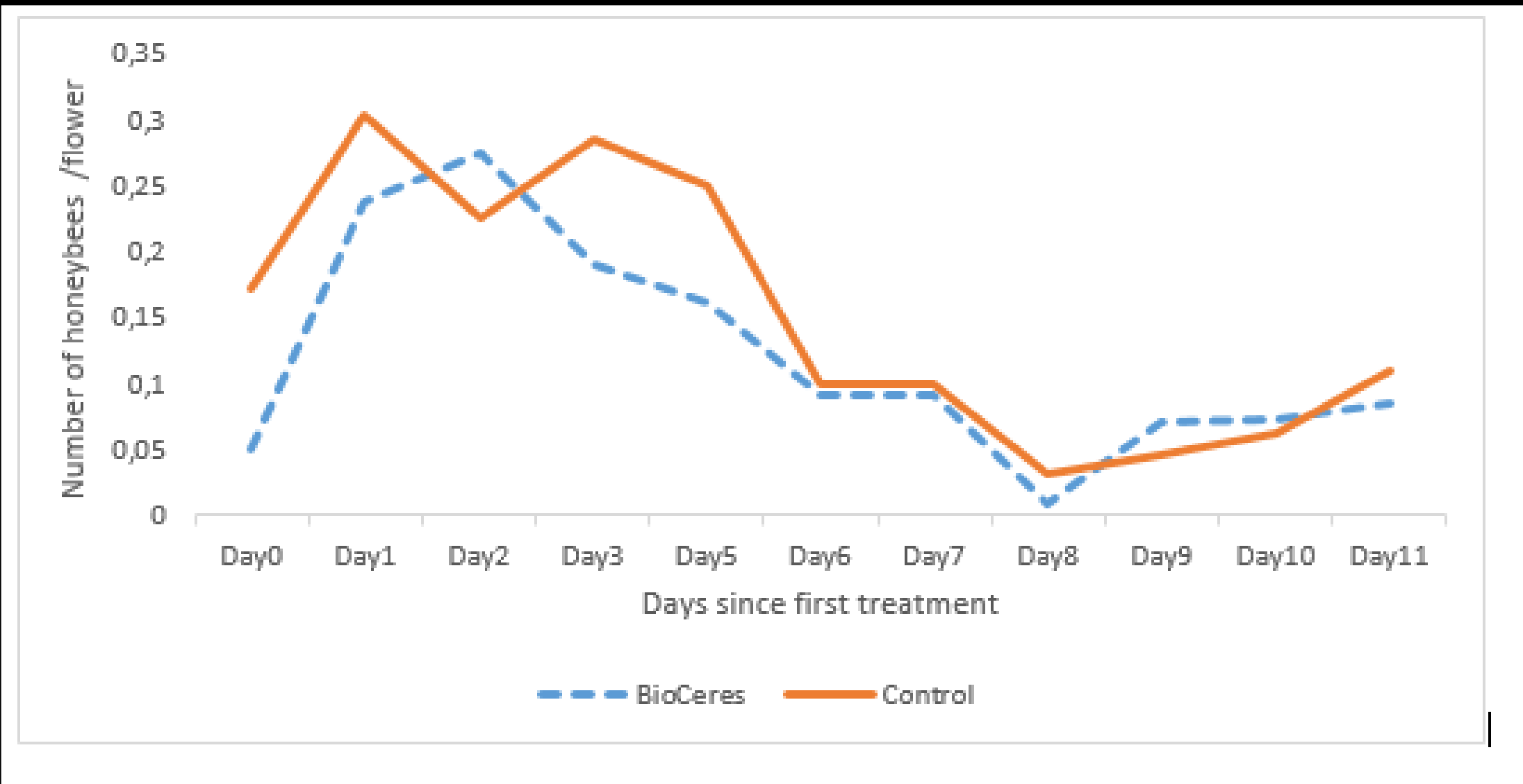
Collected dead bees were counted and numbers placed in one of three categories: foragers, drones and larvae (B).

The test product (BioCeres® WP) and the control (water) were applied using a 16 l backpack sprayer with a calibrated nozzle (Figure C).

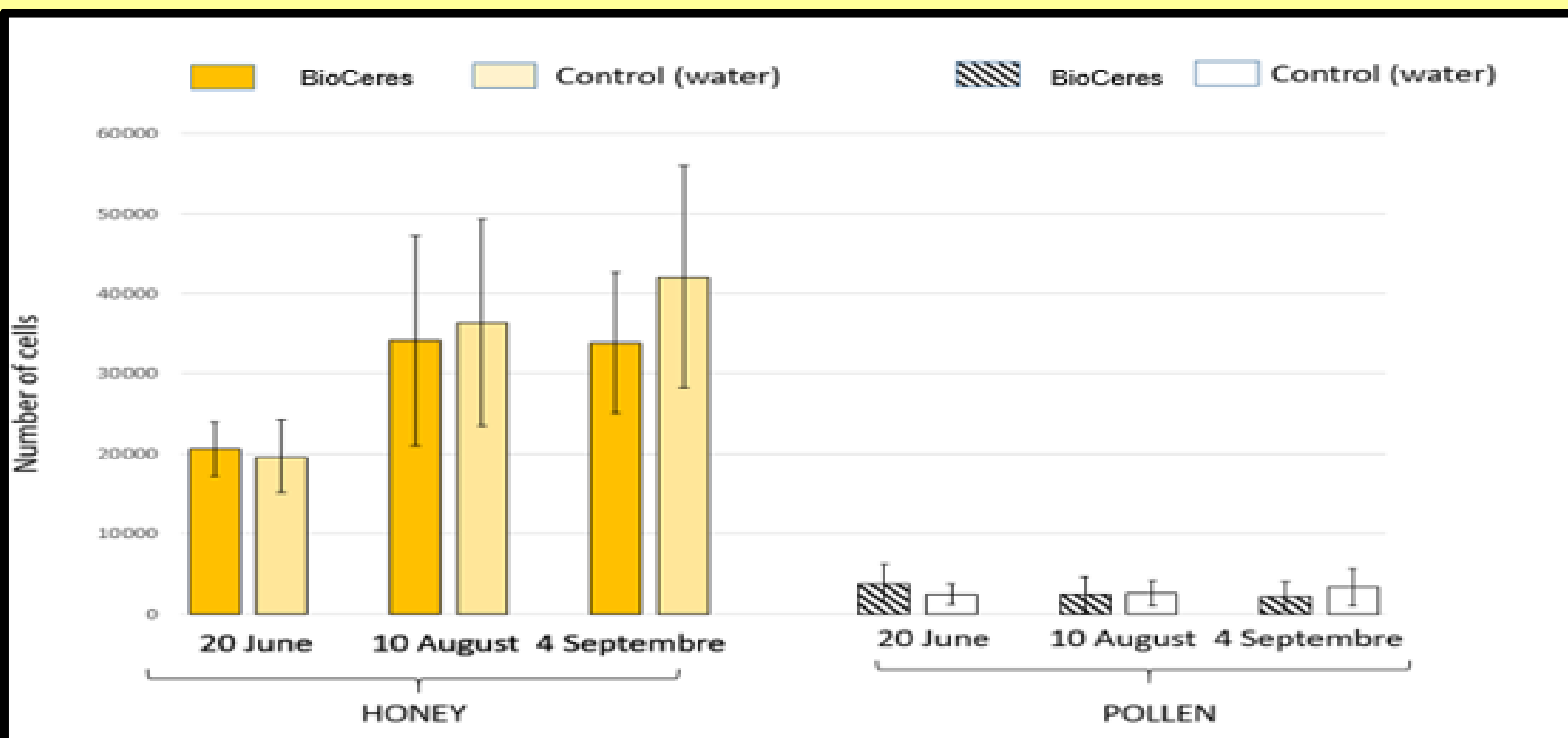
RESULTS



Daily mean number of dead specimens (Worker bees, drones, larvae) found in dead bee traps on hives of BioCeres and Control Tunnels from 13 to 23 August 2018 (ABOVE).



Mean number of honeybees per flower per m² at three different places in the BioCeres and Control tunnels. 21 June to 1 July 2018. (ABOVE)



Mean number of honey and pollen cells in hives from BioCeres and Control (water) tunnels on 20 June, 10 August and 4 September. Deschambault, Québec, Canada.(ABOVE)

DISCUSSION AND CONCLUSIONS

Under similar weather conditions, honeybees in tunnels of both control and BioCeres treatments were foraging, drinking and flying in a similar fashions. For adult bees, the number of specimens collected in the dead bee traps from each treatment were low and not statically different. On rare occasions, more drones and larvae were collected in dead bee traps of the BioCeres tunnels during hive seclusion.

After wintering, colonies treated with BioCeres were similar to those treated with water as to surviving adult bees, brood cell numbers, food supplies and syrup consumption. Honeybee colonies exposed to the two series of BioCeres treatments were therefore not affected, on the short nor, the long term, by treatments. the BioCeres, containing the active ingredient of conidiospores of the strain *B. bassiana* ANT-03, at the highest recommended doses did not infect, cause sub lethal behavioural abnormalities, nor kill the honey bees under field conditions. *B. bassiana* strain ANT-03 is safe to honeybees under field conditions

ACKNOWLEDGMENTS

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