INFLUENCE OF TWO DIFFERENT VARROA SUMMER TREATMENTS ON HONEY BEE HEMOLYMPH PROTEOME

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INTRODUCTION

One of the main biotic threat to honey bees all over the world is the ectoparasitic mite *Varroa destructor* (Acari: Mesostigmata). Without proper treatment, colonies are doomed and collapse within two years. Many treatments are used by beekeepers, with well-established acaricidal efficacy; however very little is known on the impact of the above-mentioned treatments on the well-being of the colony. Biochemical markers represent an interesting tool to assess the animal and human welfare. Particularly promising for our purposes seems to be the evaluation of Vitellogenin in the hemolymph of a pool of bees (van der Steen et al., 2015). The potential of this protein can be foreseen considering his pivotal role in the homeostasis of social insect colonies. In fact, the function of vitellogenin in these species is not limited to egg yolk constitution: trophic, antioxidant and hormonal function are also described. The aim of this research was to test the impact of two different *Varroa* control techniques with a proteomic approach.

MATERIALS AND METHODS

Brood interruption by queen caging (five colonies) and brood removal (five colonies) followed by trickling of Api-Bioxal (Oxalic acid based acaricide) were performed according to Figure 1. Hemolymph from 30 bees was sampled and pooled for each colony at four critical time-points: pre-manipulation (T1), post-manipulation (T2, 25th day), pre-wintering phase (T3, 67th day) and winter (T4, 148th day). Total proteins were determined and all samples underwent SDS-PAGE on 4-12% gels in MOPS buffer. The most abundant protein bands and spots were excised from the gels, trypsin-digested and analysed by mass spectrometry for protein identification. The *Varroa* infestation was assessed at T2 by counting the mites fallen on sticky boards after the treatment.

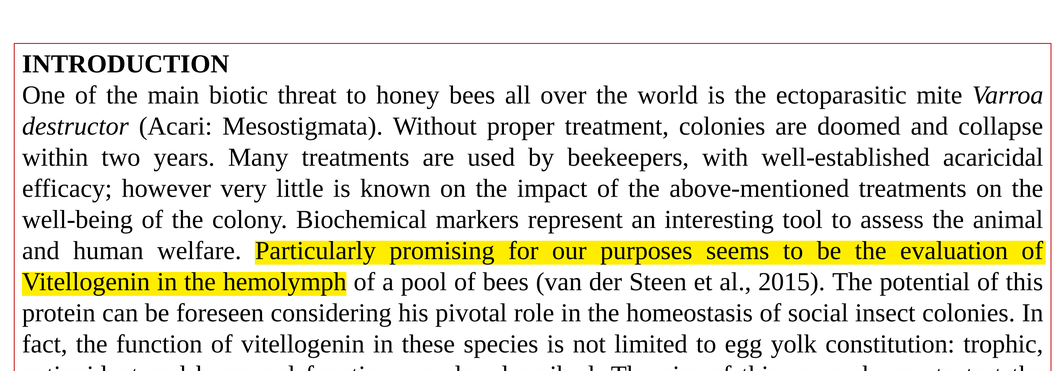

Figure 2. Representative SDS-PAGE and pherograms of honeybee hemolymph. A) Timepoint 1: pre-manipulation. B) Timepoint 2: post-manipulation.

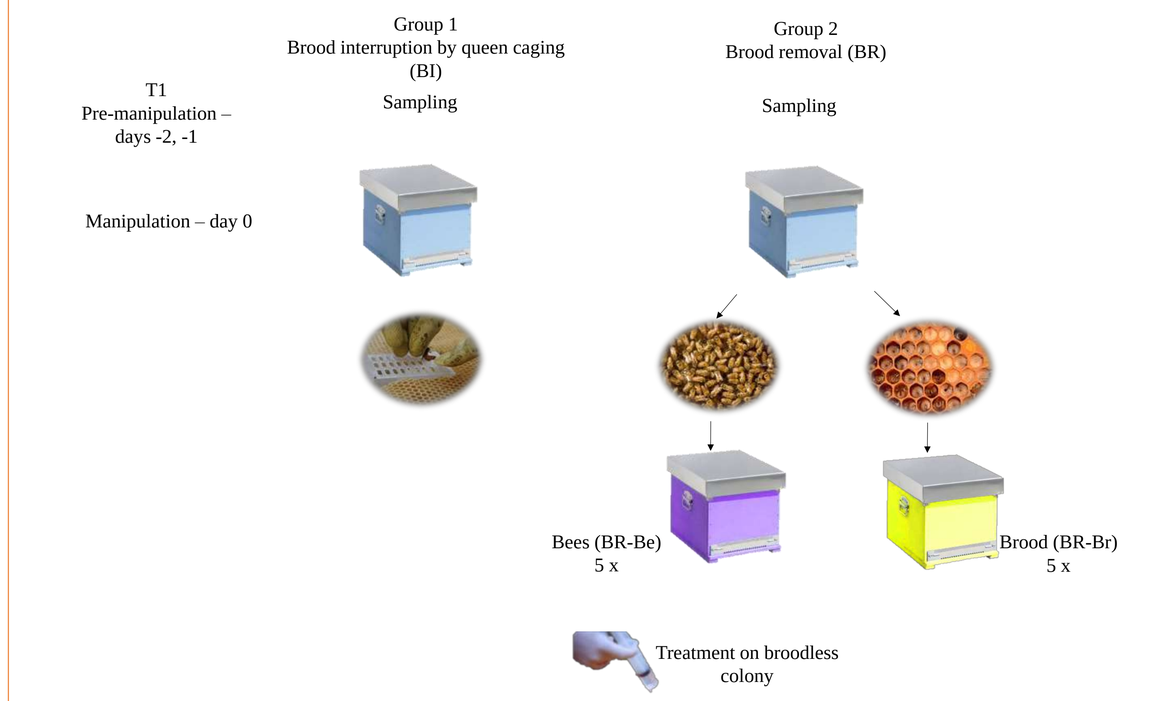

Figure 1. Schematic representation of the manipulations, treatments and sampling at each timepoint.

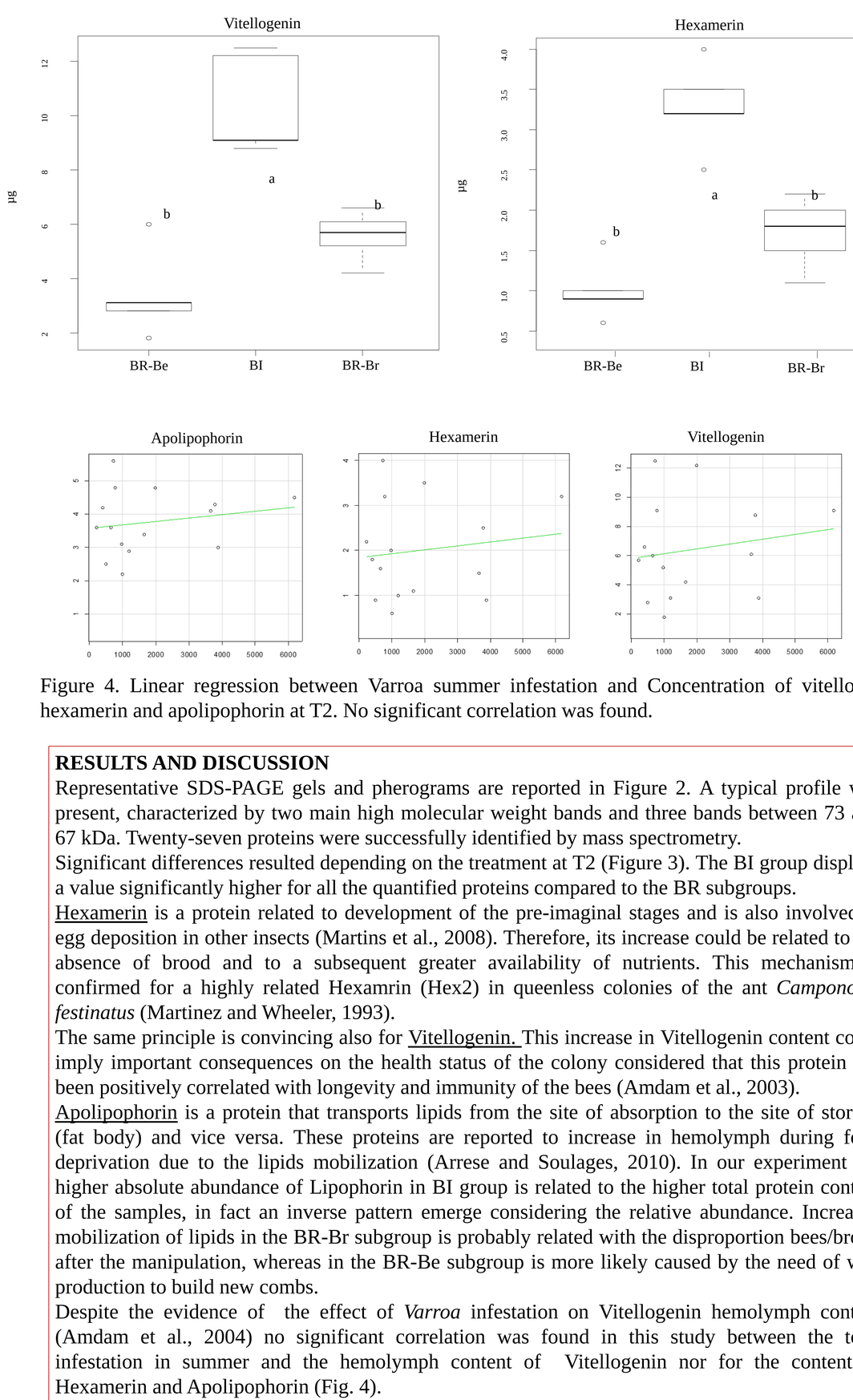

Figure 4. Linear regression between Varroa summer infestation and Concentration of vitellogenin, hexamerin and apolipophorin at T2. No significant correlation was found.

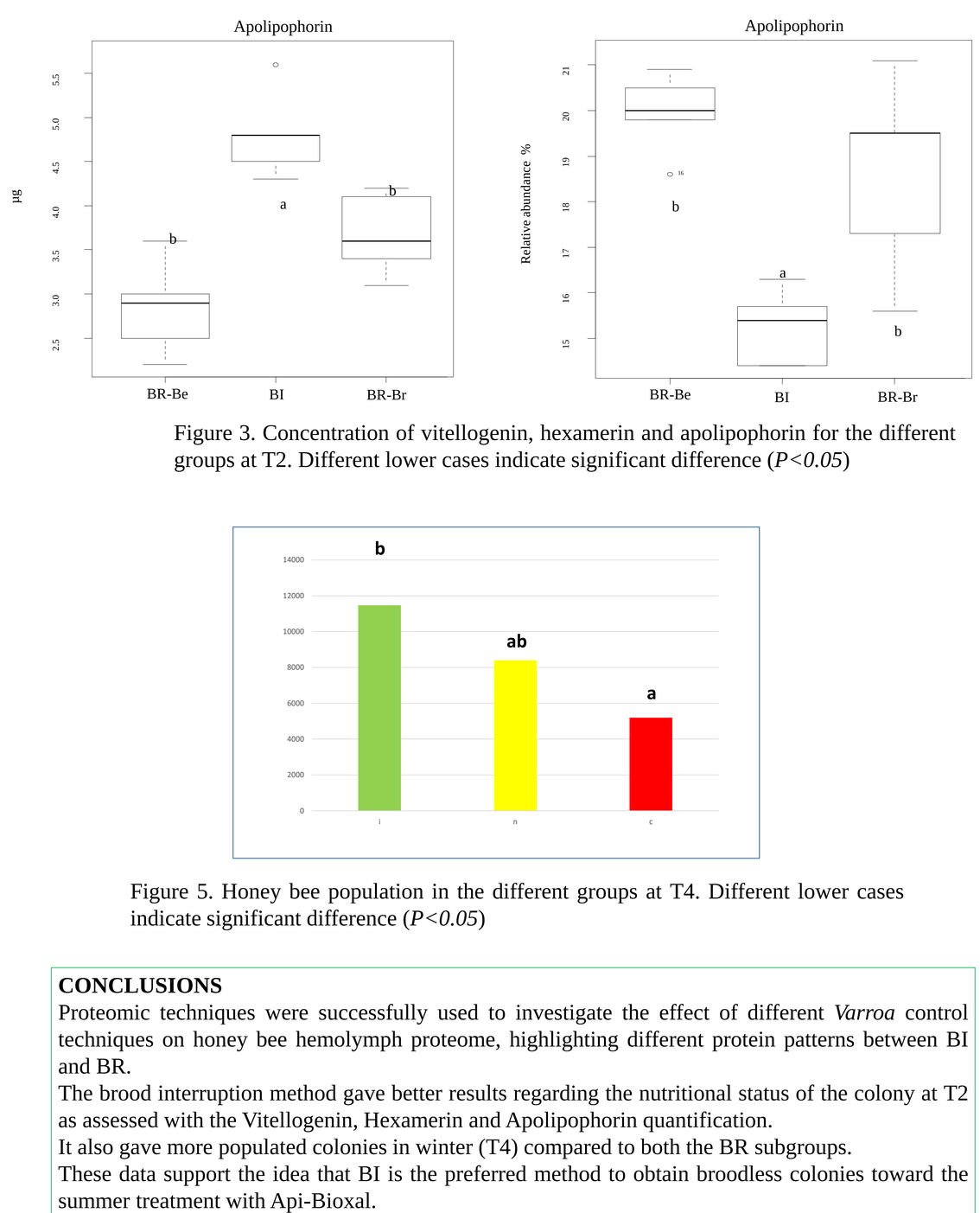

Figure 3. Concentration of vitellogenin, hexamerin and apolipophorin for the different groups at T2. Different lower cases indicate significant difference ($P<0.05$)

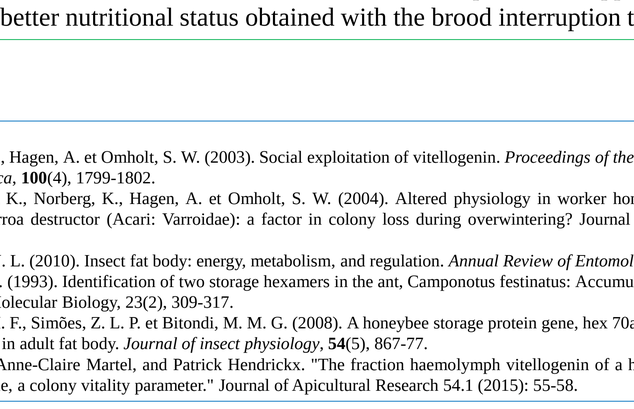

Figure 5. Honey bee population in the different groups at T4. Different lower cases indicate significant difference ($P<0.05$)

RESULTS AND DISCUSSION

Representative SDS-PAGE gels and pherograms are reported in Figure 2. A typical profile was present, characterized by two main high molecular weight bands and three bands between 73 and 67 kDa. Twenty-seven proteins were successfully identified by mass spectrometry.

Significant differences resulted depending on the treatment at T2 (Figure 3). The BI group displays a value significantly higher for all the quantified proteins compared to the BR subgroups.

Hexamerin is a protein related to development of the pre-imaginal stages and is also involved in egg deposition in other insects (Martins et al., 2008). Therefore, its increase could be related to the absence of brood and to a subsequent greater availability of nutrients. This mechanism is confirmed for a highly related Hexamrin (Hex2) in queenless colonies of the ant *Camponotus festinatus* (Martinez and Wheeler, 1993).

The same principle is convincing also for Vitellogenin. This increase in Vitellogenin content could imply important consequences on the health status of the colony considered that this protein has been positively correlated with longevity and immunity of the bees (Amdam et al., 2003).

Apolipophorin is a protein that transports lipids from the site of absorption to the site of storage (fat body) and vice versa. These proteins are reported to increase in hemolymph during food deprivation due to the lipids mobilization (Arrese and Soulages, 2010). In our experiment the higher absolute abundance of Lipophorin in BI group is related to the higher total protein content of the samples, in fact an inverse pattern emerge considering the relative abundance. Increased mobilization of lipids in the BR-Br subgroup is probably related with the disproportion bees/brood after the manipulation, whereas in the BR-Be subgroup is more likely caused by the need of wax production to build new combs.

Despite the evidence of the effect of *Varroa* infestation on Vitellogenin hemolymph content (Amdam et al., 2004) no significant correlation was found in this study between the total infestation in summer and the hemolymph content of Vitellogenin nor for the content of Hexamerin and Apolipophorin (Fig. 4).

The reason of this disagreement could be found in the experimental setup (caged bees for Amdam et al. *versus* free flying colonies in our study) and in the relatively low infestation of our colonies (2564±1907 mites).

No significant differences at the proteomic level emerged at T3 nor at T4 despite the great variations in bee population found among groups at T4 (Fig. 5).

CONCLUSIONS

Proteomic techniques were successfully used to investigate the effect of different *Varroa* control techniques on honey bee hemolymph proteome, highlighting different protein patterns between BI and BR.

The brood interruption method gave better results regarding the nutritional status of the colony at T2 as assessed with the Vitellogenin, Hexamerin and Apolipophorin quantification.

It also gave more populated colonies in winter (T4) compared to both the BR subgroups.

These data support the idea that BI is the preferred method to obtain broodless colonies toward the summer treatment with Api-Bioxal.

It is worth mentioning the unexpected presence of multiple contaminants in wax foundations used for the BR-Be sub-group, that may have altered some parameters of these colonies.

Further studies are needed to confirm these results and to explore the supposed beneficial effects consequent to the better nutritional status obtained with the brood interruption technique.

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