

INTRODUCTION

Aethina tumida (Small Hive Beetle - SHB), order Coleoptera, Nitidulidae family, is an exotic bee pest, responsible for a notifiable disease in the EU for which specific veterinary policies are in place. It is a beetle native to sub-Saharan Africa that feeds on pollen, brood and honey, causing damages both directly to hives and honey, and indirectly to the environment due to the reduction of the pollination services provided by bees. In September 2014, SHB has been detected in Southern Italy (Calabria and Sicily region) and nowadays it is endemic in Reggio Calabria and Vibo Valentia province.

Diagnostic and surveillance methods, previously adopted in other countries, have been implemented, but they resulted very demanding in terms of time and energy.

The aim of this project was to improve the early diagnosis (both on field and in the laboratory, also with the use of innovative matrices), prevention and control of aethinosis. Outputs of the project were reported in 3 peer reviewed publications.



Fig1. *Aethina tumida* (Small Hive Beetle - SHB)

METHODS

Comparative study to identify new hive matrices for early diagnosis of SHB

Between 2016-2017 we compared sensitivity of different hive matrices for early diagnosis of SHB using molecular analysis (Real Time PCR).

More in details, N. 66 swab samples (both from frames and hive bottom boards) and 51 debris samples were taken from 3 apiaries with different infestation levels (low, medium and high).

The sampling method is describe in figure 2.

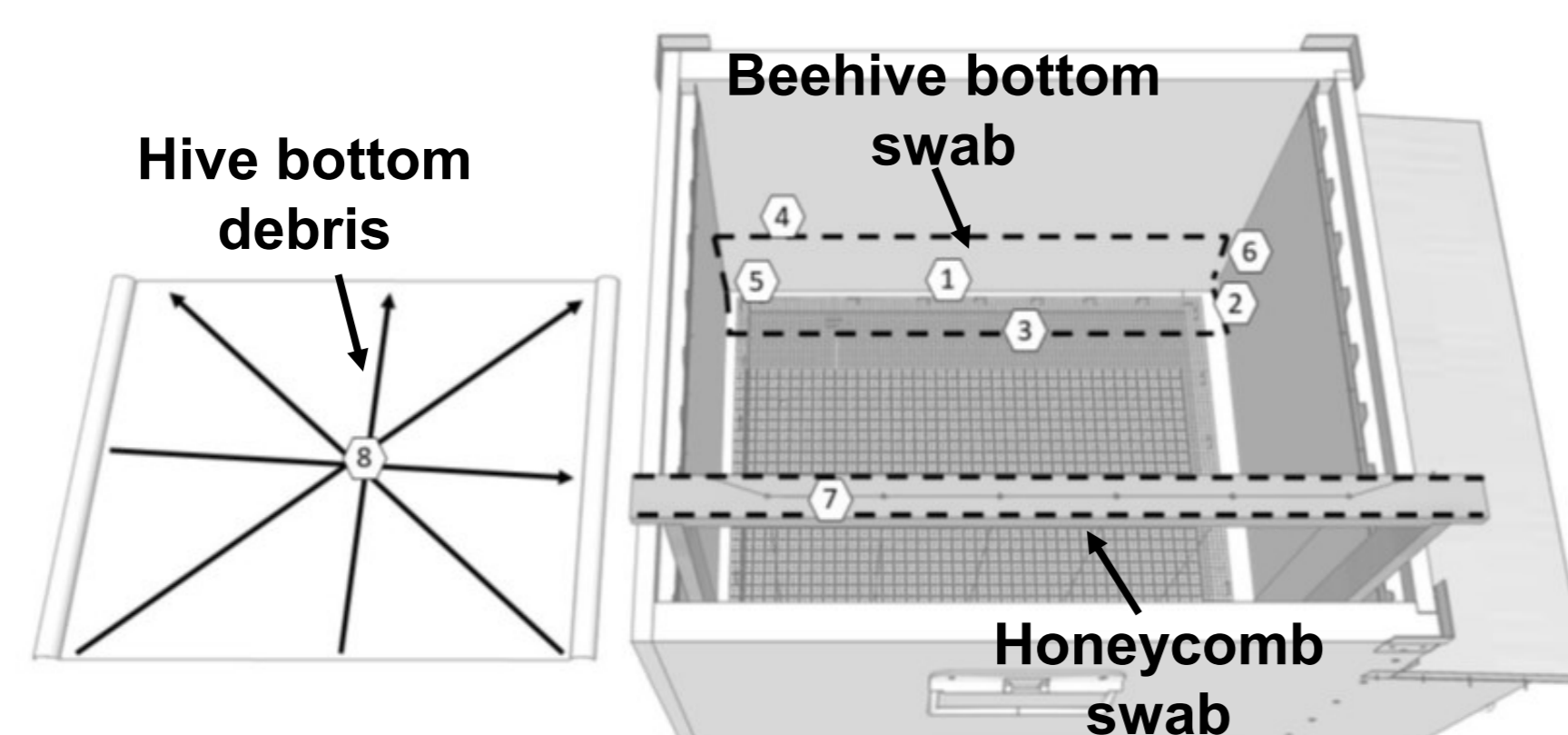


Fig2. Sampling method

Definition of a Protocol to Manage and Officially Confirm SHB Presence in Sentinel Apiaries

In order to improve Italy's national surveillance program for the control of the beetle, a pair of sentinel bee colonies supervised by the Local Veterinary Services were established in infested areas of Calabria and Sicily (southern Italy). The protocol used in Calabria in 2018 to manage the positive sentinels for SHB was describes in the paper "Definition of a Protocol to Manage and Officially Confirm SHB Presence in Sentinel Honeybee Colonies".

It defines the biosafety conditions of packing and transportation, from the field to the official laboratory, of the infested sentinel bee colonies with subsequent inspection procedures.

Evaluation of the biocides compounds to control adults and larvae of *Aethina tumida*.

Biocides such as Azadiractina, Neem Oil, and Garlic Macerate were tested to control their toxicity on both adults and larvae of SHB.

Trials were performed at the Istituto Zooprofilattico of Reggio Calabria, where we established the first laboratory dedicated to SHB in EU, under the coordination of Dr. Giovanni Federico.

For each substance and for the negative control (no treatment), substrates consisting of treated combs were prepared and placed inside insect cages as shown in Figure 4 and 5.

Each test was monitored after 1, 4 and 9 days (last inspection), and the presence/absence of dead individuals and indicators of vitality were reported at each inspection.



Fig 4-5. Setting up the treated combs for the execution of the trial.

Improving inspection visits using the "mobile divider"

Field trials were carried out in collaboration with FAI Calabria beekeepers in two apiaries located in *Aethina tumida*-infested areas of Reggio Calabria. We compared the official Ministry of Health method and the Mobile divider method, both already described in the article "Monitoring of small hive beetle (*Aethina Tumida* Murray) in Calabria (Italy) from 2014 to 2016: Practical identification methods" published by Rivera-Gomis, J. et al. in 2017.



The Mobile divider is a trap to easily detect the beetle. It is placed between the last frame and the lateral wall of the hive, creating a dark zone, where SHB goes to hide to escape from the light.

Fig3. The Mobile divider

RESULTS

Comparative study to identify new hive matrices for early diagnosis of SHB

Considering different infestation levels, debris resulted the more sensitive matrix to use for early diagnosis.

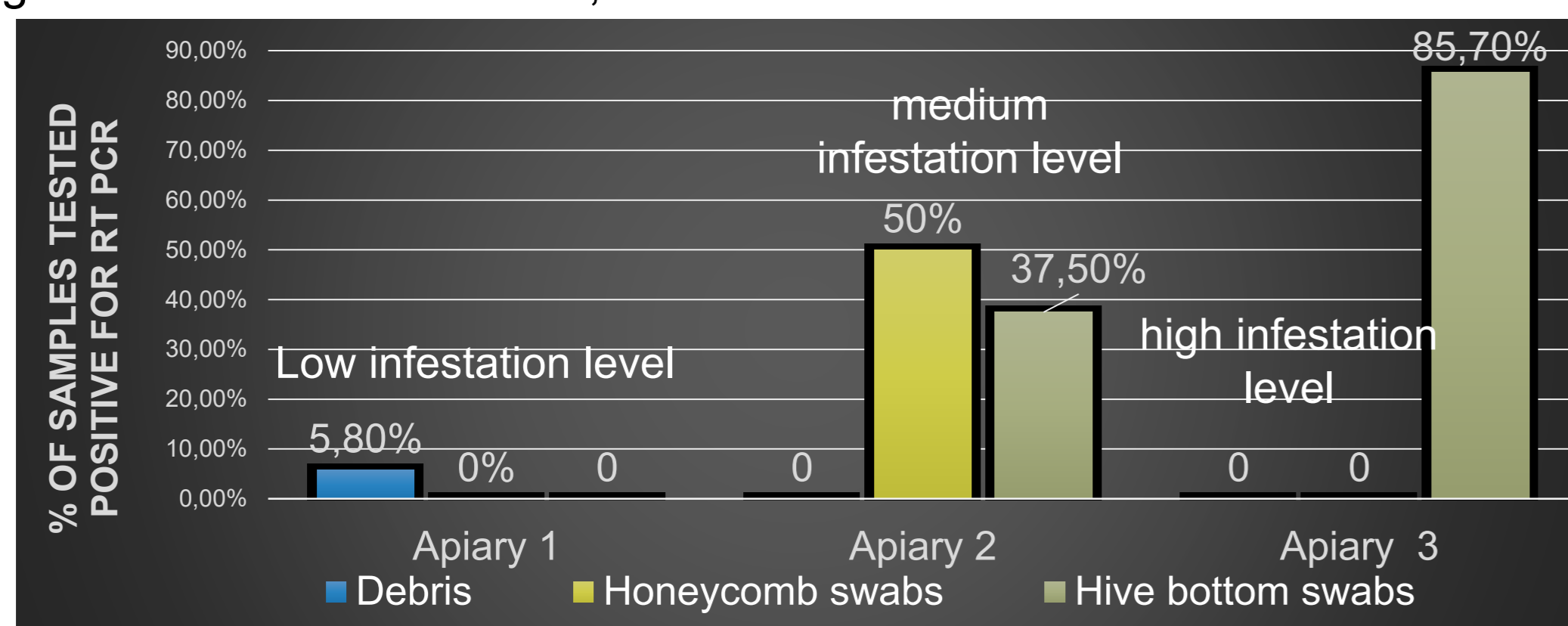


Fig6. Percentage of positive samples for RT-PCR taken from 3 different apiaries.

Publication (output): Cersini, A.; Pietropaoli, M.; Pietrella, G.; Rivera-Gomis, J.; Federico, G.; Tofani, S.; Conti, R.; Rubino, R.C.; Di Ruggiero, C.; Formato G. "New matrixes to diagnose *Aethina tumida* presence at Apiary level" J. Apic. Sci. 2021, 65

Definition of a Protocol to Manage and Officially Confirm SHB Presence in Sentinel Apiaries

Cheaper than a regular colony Efficient in attracting and detecting the presence of SHBs Short inspection time Ease in management by official veterinarians Veterinary Services can access it freely.

Publication (output): Formato, G.; Federico, G.; Di Ruggiero, C.; Pietropaoli, M.; Milito, M.; Mutinelli, F. "Definition of a Protocol to Manage and Officially Confirm SHB Presence in Sentinel Honeybee Colonies". Appl. Sci. 2021, 11, 8260.

Improving inspection visits using the "mobile divider"

The inspection time and efficacy are improved using the Mobile divider.

	N. of SHB		Inspection Time (in minutes)	
	Mobile Divider	Official Method	Mobile Divider	Official Method
min	0	0	00:03:00	00:02:50
Median	2	1	00:05:12	00:06:00
Mean	2.95	2.05	00:05:37	00:06:02
Max	20	11	00:10:21	00:09:55

Fig8. Comparison of the two hive inspection methods (Mobile divider and Official method).

Publication (output): Di Ruggiero, C.; Mezher, Z.; Mutinelli, F.; De Carolis, A.; Poggi, N.; Formato, G. "Updates on the Mobile Divider and Its Use in Calabria Region to Monitor and Control *Aethina tumida* Infestation". Appl. Sci. 2021, 11, 10637

Evaluation of the biocides compounds to control adults and larvae of *Aethina tumida*.

Azadiractina is the biocide compound which acts more on the SHB reproductive capacity. It slowed down the development of larvae born from treated adults.

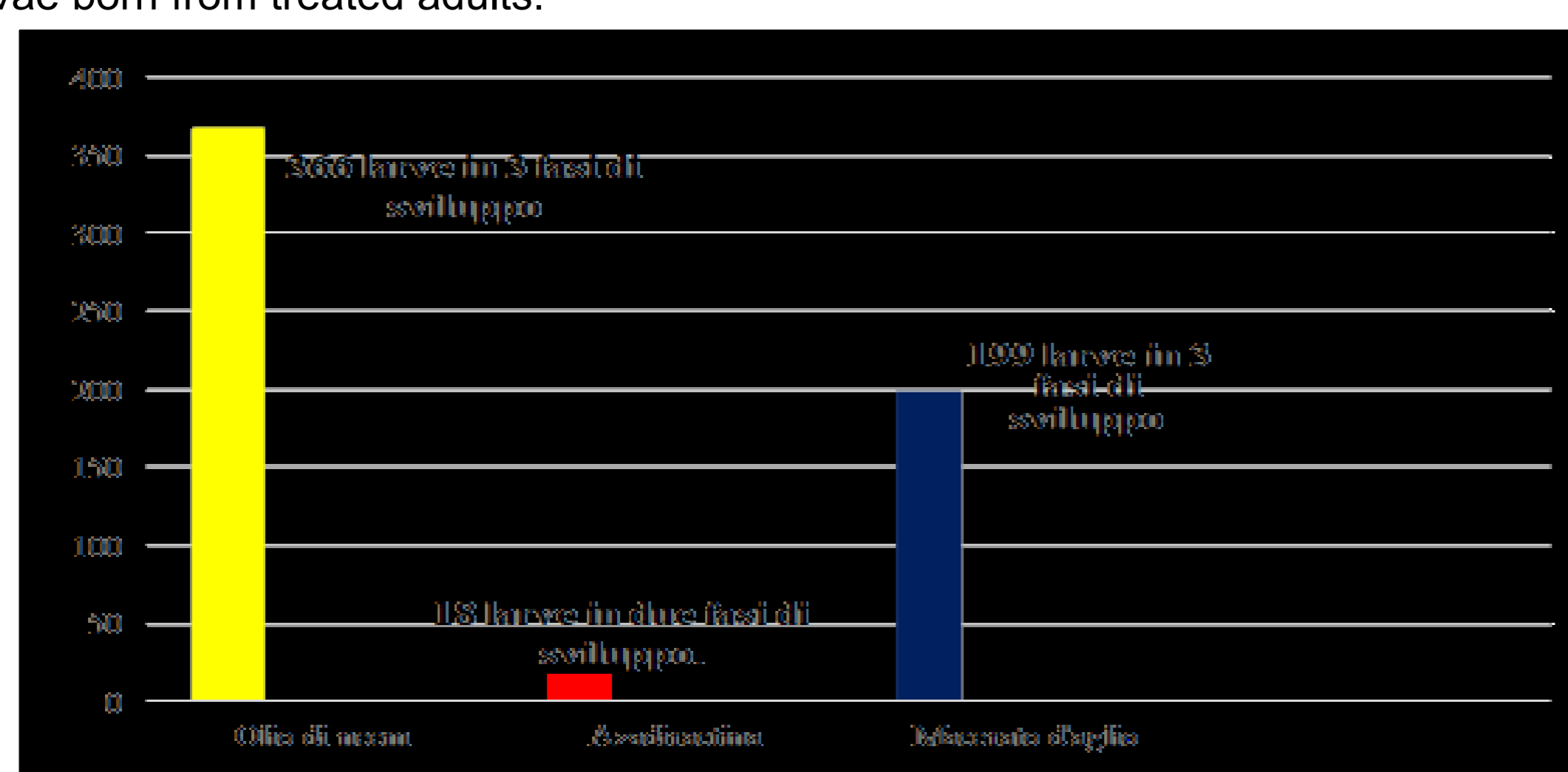


Fig 7. Larvae hatched from breeding adults in combs treated with different biocides.

Acknowledgements

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Thanks are also extended to all project partners for their collaboration.

Bibliography

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Neumann, P., et al. (2016). Quo vadis *Aethina tumida*. Apidologie, 47(3), 427-466.