

Short Communication

# MONITORING OF SMALL HIVE BEETLE (*AETHINA TUMIDA* MURRAY) IN CALABRIA (ITALY) FROM 2014 TO 2016: PRACTICAL IDENTIFICATION METHODS

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## Abstract

The Small Hive Beetle (SHB), *Aethina tumida*, is an invasive pest of honey bee colonies that causes significant damage to the beekeeping sector. SHB was detected in southern Italy (EU) in 2014 and despite adopted eradication measures, is still present there. After three years of observations of SHB in Calabria (2014-2016), we provide here some practical tips for improving control measures. A new time-saving colony examination method, including the use of an internal divider reduced the time needed for hive inspections by 31.86 % on average. Prioritizing the inspection of pollen and honey combs rather than brood combs is advised. Sentinel apiaries with no more than five colonies without supers are suggested for each beekeeping location in order to attract and to monitor the early appearance of SHB. The use of these methods will enable early detection and prompt control measures application before this destructive pest can spread in the region.

**Keywords:** *Aethina tumida*, behaviour, monitoring, sentinel apiary, small hive beetle

## MANUSCRIPT BODY

In September 2014, the presence of the Small Hive Beetle (SHB), *Aethina tumida* Murray, was officially confirmed in the Calabria and Sicily Regions (Southern Italy) (European Commission, 2014). Through December 2016, SHBs have been found in a total of 137 infested apiaries: 136 in Calabria and 1 in Sicily (INRC, 2017). Prevalence of the *Aethina tumida* infestation in the Calabria region was 3.59%, 1.89% and 2.80%, respectively, for the years 2014, 2015 and 2016. In Sicily, where eradication measures were effective, the prevalence was 0.04%, 0.00% and 0.00%, respectively,

for the years 2014, 2015 and 2016<sup>1</sup>.

Eradication measures have been applied since 2014, including the destruction of all colonies at apiary sites (8502 destructed colonies as of 08/10/2016) whenever a single infested colony was found. These measures, while resulting in SHB eradication only in the Sicily region (European Commission, 2017), maintained a low prevalence and slow spread of the pest in the Calabria region.

<sup>1</sup> Prevalence was calculated considering the number of outbreaks recorded (Italian National Reference Centre for Apiculture, 2017) in the populated apiaries registered in the Italian National Bee Registry (Italian Ministry of Health, 2010) on 31/12/2016



Fig. 1. Divider to be placed on the external side of the nest to act like a hiding place for the SHB (Photograph by Francesco Artese, FAI Calabria).

The official procedure to examine hives for SHB presence is effective but time consuming. As recommended by the Italian National Reference Centre for Apiculture, and adapted from Neumann & Hoffmann (2008), it includes detailed colony examination. More recent examination methods (Neumann et al., 2013; OIE, 2013) were not applicable in the field during routine colony inspections due to the high

risk of robbing and honey bee aggressiveness (Mutinelli et al., 2014).

Due to its dark body colour and fast movements, SHB are not easily identified within a colony, particularly when at low population densities. Moreover, SHBs avoid light, hide in crevices or cavities of the hives or fly away from combs (Neumann, Pettis, & Schafer, 2016).

After three years of observing SHB colonization behaviour since its appearance in Calabria (2014–2016), we are now able to share our practical experience to neighbouring EU member states that are in danger of SHB introduction. In order to improve hive inspections, these practical tips should be followed:

1. A divider made of wood, felt, cardboard or a similar material should be placed laterally between the hive wall and the external comb (Fig. 1), to act as a refuge for SHB. This divider should be installed at least 48 hours before the examination, following recommendations for traps with a similar mechanism of action (Neumann et al., 2013). A similar trap is currently in use in Australia to detect the presence of SHB (Annand, 2008). In fact, we developed a new “time-saving protocol” (Tab. 1) recommending the inspection of the nest to be started on the opposite side from the divider, transferring combs one by one into an empty hive or nuc box. When three combs and the divider are

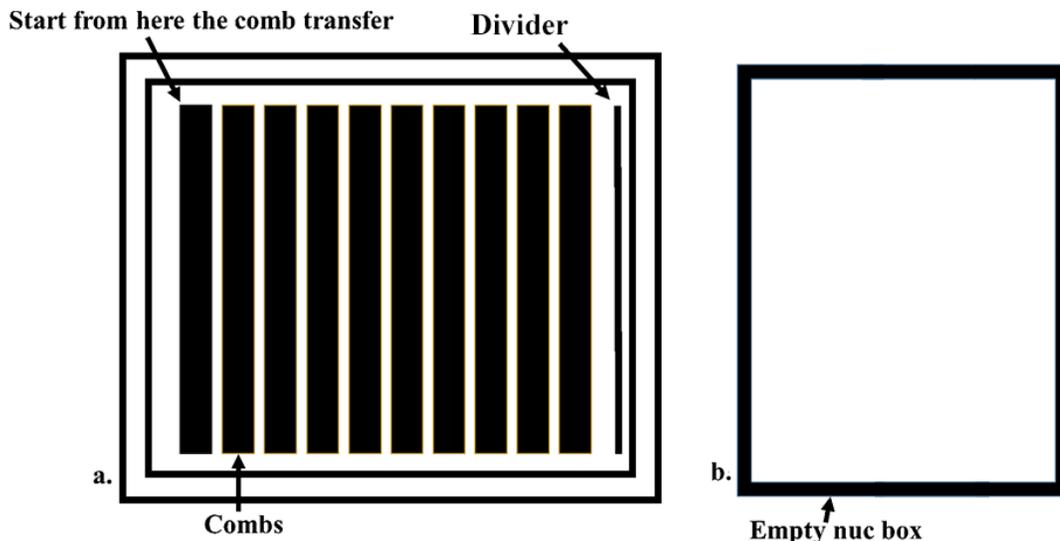


Fig. 2. Hive provided with divider placed at one extremity of the box to create a hiding place for SHB (a) and empty nuc box for transferring frames from the nest during the hive inspection (b).

Table 1

Comparison between the two hive inspection methods tested

Italian Ministry of Health (MoH) inspection protocol	Time-saving Protocol
<ol style="list-style-type: none"> <li>1. Remove the outer cover. Examine the external surface of the inner cover, remove it and examine the internal surface. After that, put the outer cover on the hive supports or on the ground.</li> <li>2. To inspect the nest, remove the first lateral comb and set it outside the hive. Then, inspect all the combs of the hive one by one observing both surfaces after removal of the first lateral comb. Alternatively, use an empty hive where inspected combs could be temporarily placed.</li> <li>3. Once the comb inspection is finished, return the combs to their original position.</li> <li>4. If a honey super is present, examine all combs one by one. After that, remove the super and set it on the outer cover.</li> <li>5. Observe the content of the bottom board if present.</li> </ol>	<ol style="list-style-type: none"> <li>1. A divider made of wood, felt or cardboard (Fig. 1) should be placed between the last comb and the lateral wall of the hive, at least 48 hours before the inspection.</li> <li>2. Remove the outer cover. Examine the external surface of the inner cover, remove it and examine the internal surface. After that, put the outer cover on the hive supports or on the ground.</li> <li>3. Then, inspect the nest starting at the first lateral comb that is on the opposite side to the divider. The inspection of the combs with pollen and honey should be more diligent, while it can be much quicker for the other brood combs. Place the inspected combs one by one into an empty hive or into a nuc box.</li> <li>4. In general, when combs are removed, always proceed with slow movements, in order to allow SHBs to move towards the remaining, not inspected, frames.</li> <li>5. When three combs and the divider are left to be inspected, slowly move the combs to the opposite (empty) side of the hive.</li> <li>6. After moving the last comb, carefully inspect the surface of the lateral divider and the space behind it, searching for the SHB. Carefully inspect also the corners, walls and bottom of the hive.</li> <li>7. If the honey super is present, remove it and inspect the surface where it was placed carefully. Then, inspect the super combs quickly and, the lateral walls more carefully.</li> <li>8. Observe the content of the bottom board if present.</li> </ol>

left, the combs are moved to the opposite side of the hive. Removing the combs causes SHBs to move progressively towards the divider, where there will be a higher probability of finding them (Fig. 2).

2. Areas with higher probability of finding SHB's (e.g., corners and inner side of the walls of the hive, behind dividers, combs containing pollen and honey, etc.) should be inspected more thoroughly, while brood combs should only be quickly scanned due to the lower

probability of finding SHBs there (Pietropao-li et al., 2015, Spiewok et al., 2007) (Tab. 1).

3. While inspecting combs, avoid removing the bees by shaking the comb as SHB could be dislodged with the bees; observe the frame at a distance further than normal, with arms fully extended, to guarantee a vision of the entire comb surface and facilitate identification of SHB movements across the comb; SHB is much easier to detect on the lighter wax of newly built combs, so more

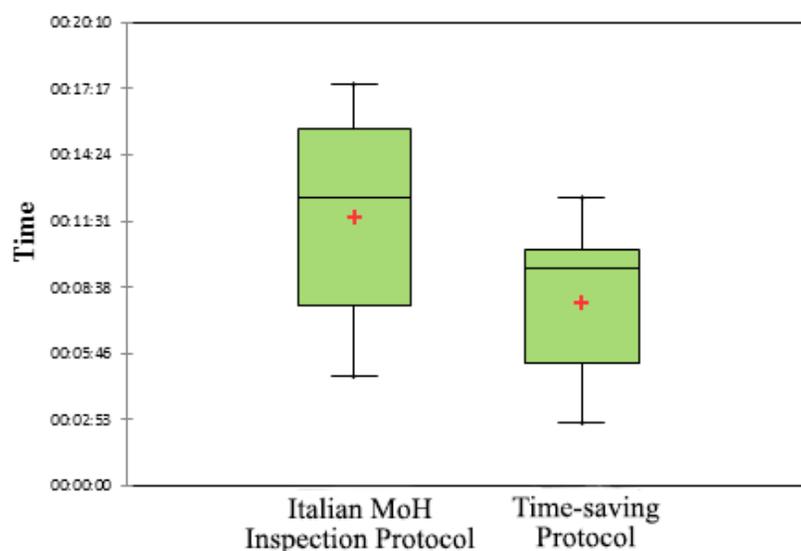


Fig. 3. Box plots of time needed for the hive inspection with the two different methods.

care should be used when inspecting older, darker combs.

- Once SHB infestation is suspected, sampling is needed for ID confirmation. The body shield is small and hard, making them difficult to capture. While standard leather beekeeper's gloves are not useful for SHB sampling, tight fitting latex gloves are more convenient for examination, handling and sampling of beetles.

At the beginning of June 2017, a field trial was begun comparing this new protocol with the Italian MoH inspection protocol (Italian Ministry of Health, 2014) (Tab. 1), recording the time needed to inspect thirty potentially SHB-infested colonies in Calabria. Each inspection protocol was used on fifteen beehives and time needed for the inspection was recorded. The average time of application for the Italian MoH inspection protocol was 11 minutes and 43 seconds per hive, while our "time saving protocol" required only 7 minutes and 59 seconds per hive (standard deviation of 00:04:18 and 00:03:09 respectively). This was equivalent to a 3 minutes and 44 seconds (31.86 %) reduction in inspection time. Using the Mann-Whitney test (Mann & Whitney, 1947) with XLSTAT™ software (Addinsoft & S.A.R.L., 2010) we observed a statistically significant ( $P = 0.014$ ) difference between the two methods (Fig. 3).

This time saving is indeed economically important, as the personnel expenses represent the greatest cost for SHB control measures. Moreover, this monitoring time reduction would favour beekeepers' compliance in collaborating with the authorities and could represent a key factor for the success of SHB management strategies. As the most efficient strategy, the person in charge of the apiary is supposed to place the divider at least 48 hours before the inspection is carried out (Neumann et al., 2013). As an alternative, the beekeeper is envisaged to

keep the divider placed in the beehive as a good beekeeping practice to ease SHB detection, being always ready for the official controls and minimizing the workload of placing the dividers. In order to improve monitoring activities, since 2014 Italian MoH has been using its own "sentinel apiaries" placed in areas potentially affected by SHB (Mutinelli et al., 2014; Italian Ministry of Health, 2015a; Italian Ministry of Health, 2015b; INRC, 2016). Due to the lower number and size of hives inspected per site, these sentinel apiaries have demonstrated advantages compared to the use of beekeeper's apiaries, including an easier and time-saving monitoring procedure. Moreover, sentinel apiaries can allow an easier and more accurate diagnosis compared with conventional apiaries, where a beekeeper may delay diagnosis and eradication procedures. In conclusion, sentinel apiaries ensure higher examination efficiency by revealing new infested areas more quickly.

According to our experience, these sentinel apiaries should be established using two to five colonies, to increase SHB attraction while limiting the time needed by inspectors for accurate inspection. Moreover, the colonies should be strong, healthy, queen right, as these are more attractive to the parasite (Annand & Spooner-Hart unpubl. data). Some final consid-



Fig. 4. Marks on the protein candy made by SHB feeding (Photograph by Francesco Artese, FAI Calabria).

erations are that, to ease inspections, sentinel colonies should never be provided with supers, and colonies should be placed in sunny and windy locations. In fact, we have observed SHBs to invade more often strong colonies placed in these conditions, as they facilitate propagation of attractive volatile compounds. Another useful tip for locating SHB in sentinel apiaries could be to insert protein candy or protein substrates into the hives to feed the bees, as both adult and immature stages of the SHB are attracted to protein substrates (Buchholz et al., 2008), and the presence of small holes in the candy are feeding signs of this parasite (Artese unpubl. data) (Fig. 4).

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#### REFERENCES

Addinsoft, S. A. R. L. (2010). XLSTAT-software user's manual, version 10. Addinsoft, Paris, France.

Annand, N. (2008). Small hive beetle management options, Primefacts, NSW DPI, 764, 1-7. Retrieved Au-

gust 10, 2017, from [https://www.dpi.nsw.gov.au/\\_\\_data/assets/pdf\\_file/0010/220240/small-hive-beetle-management-options.pdf](https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0010/220240/small-hive-beetle-management-options.pdf)

Buchholz, S., Schäfer, M. O., Spiewok, S., Pettis, J. S., Duncan, M., Ritter, W., Spooner-Hart, R., Neumann, P. (2008). Alternative food sources of *Aethina tumida* (Coleoptera: Nititilidae). *Journal of Apicultural Research and Bee World*, 47(3), 202-209. <http://dx.doi.org/10.1080/00218839.2008.11101460>

Commission Implementing Decision 2014/909/EU of 12 December 2014 concerning certain protective measures with regard to confirmed occurrences of the small hive beetle in Italy. (2014). *Official Journal L*, 359, 161-163.

Commission implementing decision (EU) 2017/370 of 1 March 2017 amending Commission Implementing Decision 2014/909/EU by extending the period of application of certain protective measures and amending the list of areas subject to protective measures in relation to small hive beetle in Italy. (2017). *Official Journal L*, 56, 213-214.

Italian Ministry of Health (MoH). Nota Ministeriale 0020069-01/10/2014-DGSAF-COD\_UO-P. (2014).

Italian Ministry of Health (MoH). Nota Ministeriale 0022693-07/09/2015-DGSAF-COD\_UO-P. (2015a).

Italian Ministry of Health (MoH). Nota Ministeriale 0022698-07/09/2015-DGSAF-COD\_UO-P. (2015b).

Italian Ministry of Health (MoH). Decreto 4 dicembre 2009, Disposizioni per l'anagrafe apistica nazionale (10A04712). (2010). *Gazzeta Ufficiale*, 93, 44-46.

Italian National Reference Centre for Apiculture (INRC). *Aethina tumida* - piano di sorveglianza - porti. Nota 009852-17/11/2016. (2016).

Italian National Reference Centre for Apiculture (INRC). (2017). Retrieved March 30, 2017, <http://www.izsvenezie.it/aethina-tumida-in-italia/>

- Mann, H.B., & Whitney, D.R. (1947). On a test of whether one of two random variables is stochastically larger than the other. *The annals of mathematical statistics*, 50-60.
- Mutinelli, F., Montarsi, F., Federico, G., Granato, A., Ponti, A. M., Grandinetti, G., ... Chauzat, M. P. (2014). Detection of *Aethina tumida* Murray (Coleoptera: Nitidulidae) in Italy: outbreaks and early reaction measures. *Journal of Apicultural Research*, 53(5), 569-575. <http://dx.doi.org/10.3896/IBRA.1.53.5.13>
- Neumann, P., & Hoffmann, D. (2008). Small hive beetle diagnosis and control in naturally infested honeybee colonies using bottom board traps and CheckMite + strips. *Journal of Pest Science*, 81, 43-48. <http://dx.doi.org/10.1007/s10340-007-0183-8>
- Neumann, P., Evans, J. D., Pettis, J. S., Pirk, C. W., Schäfer, M. O., Tanner, G., Ellis, J. D. (2013). Standard methods for small hive beetle research. *Journal of Apicultural Research*, 52(4), 1-32. <http://dx.doi.org/10.3896/IBRA.1.52.4.19>
- Neumann, P., Pettis, J. S., & Schafer, M. O. (2016). Quo vadis *Aethina tumida*? Biology and control of small hive beetles. *Apidologie*, 47(3), 427-466. <http://dx.doi.org/10.1007/s13592-016-0426-x>
- OIE. Terrestrial Manual Chapter 2.2.5. – Small hive beetle infestation (*Aethina tumida*). (2017). Retrieved August 3, 2017, from [http://www.oie.int/fileadmin/Home/eng/Health\\_standards/tahm/2.02.05\\_SMALL\\_HIVE\\_BEETLE.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.02.05_SMALL_HIVE_BEETLE.pdf)
- Pietropaoli, M., Maroni Ponti, A., Artese, F., & Formato, G. (2015). Practical tips for the inspection of the hives in case of low levels *Aethina tumida* infestation: the Italian case. In *Proceedings of COLOSS Workshop "A European strategy for small hive beetles *Aethina tumida*"* (p. 24). Bologna – Italy.
- Spiewok, S., Pettis, J. S., Duncan, M., Spooner-Hart, R., Westervelt, D., Neumann, P. (2007). Small hive beetle, *Aethina tumida*, populations I: Infestation levels of honeybee colonies, apiaries and regions. *Apidologie*, 38, 595-605. <http://dx.doi.org/10.1051/apido:2007042>