Guidance document for the management of the Small Hive Beetle (*Aethina tumida*) infestation in soil

Second version (September 2024)

By alphabetical order:

Marie-Pierre CHAUZAT
Véronique DUQUESNE
Stéphanie FRANCO
Catherine GOURLAY-FRANCE
Per KRYGER
Marion LAURENT
Franco MUTINELLI
Marie-Pierre RIVIERE
Marc Oliver SCHÄFER

European Union Reference Laboratory for Bee health (EURL)
Anses
Honey bee pathology Unit
105 route des Chappes – CS 20 111
F-06 902 SOPHIA ANTIPOLIS
France
Tél: 00 33 4 92 94 37 00
Fax: 00 33 4 92 94 37 01
eurl.bee@anses.fr

Contents

1. Background and context

Based on documents of expertise (EFSA 2013, Anses 2023a) and literature reviews (Cuthbertson et al. 2013, Neumann et al. 2016, Sabella et al. 2022), we revised the present advisory document that provides a comprehensive guidance for managing small hive beetle outbreaks.

The small hive beetle is a free-living predator and scavenger of honey bees, native to sub-Saharan Africa. *Aethina tumida* has recently become an invasive species, and introductions have been recorded from America, Australia, Europe and Asia since 1996. The beetle and its larvae have been found to reproduce in association with honeybees in Europe (*Apis mellifera*) and in Asia (*A. cerana*), bumblebees in the United States, stingless bees which are not present in Europe and solitary bees under experimental conditions (EFSA 2015b). The beetle is capable of multiplying rapidly in the presence of bee brood, pollen and comb honey. Adult specimens can fly several kilometres to invade other such places. In addition to natural spread, human mediated movement of bees and beekeeping equipment contributes directly to large-scale geographical further spread. More generally, international trade can also play a role in its spread, through the accidental introduction of infested swarms on ships, for example. While the small hive beetle is usually considered a minor pest in Africa, it can cause significant damage to social bee colonies in its new ranges (Cuthbertson et al. 2013, Neumann et al. 2016). Furthermore, once the beetle widespread, it is extremely difficult to eradicate (WOAH 2018).

The life cycle of *A. tumida* is characterised by distinct phases. The adult life stage occurs in the natural environment, within the hive and within the soil. Eggs and larvae are rarely found outside bee colonies due to the oviposition of female beetles. Once the larvae are ready to pupate they exit the hive as wandering larvae and the pupal stage is only found in the soil. The measures adopted to eradicate/contain the beetle infestation should be applied according to each phase of the life cycle. The currently available methods can interfere with the beetle life cycle at either the hive or the soil stage, by trapping adults in the hive or by killing wandering larvae, pupae and young adults in the soil. Other approaches aim to prevent adult beetles from entering the hives, by trapping them outside the hives with lures or keeping them out with repellents (EFSA 2015a, Anses 2023a).

Pupation occurs in the soil near the colonies. The majority of wandering larvae and pupae are found within 0.90–1.80 m of the hive (Pettis and Shimanuki 2000) if suitable soil is present, but distance may increase drastically (to 200 m or more) if there is no suitable soil around the hive (Stedman 2006). Beetle larvae, pupae and newly emerged adults are mostly found in the top 20 cm of the soil (Pettis and Shimanuki 2000, deGuzman et al. 2010). Digging and sieving soil around infested hives is the only method available to screen for small hive beetle pupae. These investigations could give information

concerning the state of development of the infestation in the apiary. However, soil examination is time-consuming and not always easy to do, notably depending on the size of the apiary and the type of the soil (EFSA 2015a).

Within the framework of an eradication strategy, soil treatment appears to be a valuable tool for the small hive beetle eradication, following the complete destruction of an infested apiary. In addition, once a decision to change from eradication to management is made, this measure can also contribute to containing and controlling infestations in an area where the eradication is no longer the primary objective (EFSA 2015b). This document is not intended to be exhaustive but aims at synthesising a range of described soil treatments against small hive beetle infestation in accordance with the regulation for use of chemicals in the field.

2. Regulation on animal health

The infestation with the small hive beetle is a disease of honey bees listed by the World Organisation for Animal Health (WOAH Terrestrial Animal Code) and a listed disease in the Commission Implementing Decision (EU) 2023/110.

Four bee diseases have been already approved to be listed in Annex II of Regulation (EU) 2016/429 of European Parliament and of the Council "Animal Health Law". This legislation should be considered in terms of pest and disease controls since the impact of transmissible animal diseases and the measures necessary to control those diseases can be devastating for individual animals, animal populations, animal keepers and the economy. In addition, adverse interactive effects can be observed with regard to biodiversity, climate change and other environmental aspects. The Regulation (EU) 2016/429 provides an overarching legal framework, laying down harmonised principles across the sector. It consists of a series of linked and interrelated basic acts that lay down rules on animal health applying to intra–Union trade, entry into the Union of animals and products, disease eradication, veterinary controls and notification of diseases in relation to different animal species. There are five categories of listed diseases with different disease prevention and control rules applied (Regulation (EU) 2018/1882). The infestation with the small hive beetle has been recognised as an EU listed disease for *Apis* spp. and *Bombus* spp. and assigned to category D and E. A disease in category D and E refers respectively to Article 9(1)(d) and Article 9(1)(e) of the Regulation (EU) 2016/429. Category D disease means a listed disease for which measures are needed to prevent it from spreading on account of its entry into the Union or movements between Member States, as referred to in Article 9(1)(d) of Regulation (EU) 2016/429. Category E disease means a listed disease for which there is a need for surveillance within the Union.

In the framework of the management of an outbreak of a notifiable disease, according to Chapter III Section 3 of Regulation (EU) No 142/2011, the Competent Authority may authorise the disposal by burning or burial on site of bees and apiculture by-products. Necessary measures must be taken to ensure that the burning or burial endangers neither animal and human health, nor the environment depending on national legislation.

In view of the slow spread observed in recent years, *A. tumida* is still considered absent to the European Union, currently found only in the Calabria region in southern Italy as an exotic pest (Granato et al. 2016). Regular updates are pubished on the official Italian website - *Aethina tumida* in Italia: la situazione epidemiologica (izsvenezie.it). Measures directed to prevent the introduction of the small hive beetle into the European Union are given in the Commission Delegated Regulation (EU) 2020/692 of 30 January 2020 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for entry into the Union, and the movement and handling after entry of consignments of certain animals, germinal products and products of animal origin, in particular Title 4. Preventive measures concerning intra-EU movements are laid down in Commission Delegated Regulation (EU) 2020/688 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council, as regards animal health requirements for movements within the Union of terrestrial animals and hatching eggs.

Following the confirmation of the occurrences of the small hive beetle in Italy, the Commission Implementing Decision 2014/909/UE established also certain protective measures. According to the epidemiological situation of the infestation with *A. tumida* in Italy, this decision has been amended several times in 2015, 2017, 2019, 2021 and lastly in 2023 by the Commission Implementing Decision (EU) 2023/110 (this decision shall apply until 31 December 2024).

3. Soil treatment

Many documents reviewed the knowledge on this exotic pest of bees, including the measures adopted for its eradication, containment and control (Cuthbertson et al. 2013, Neumann et al. 2016, Schäfer et al. 2019). For further detailed information on the available treatments against *A. tumida,* we would refer readers to the above listed reviews. In this section, we will consider in detail only soil treatment against the beetle.

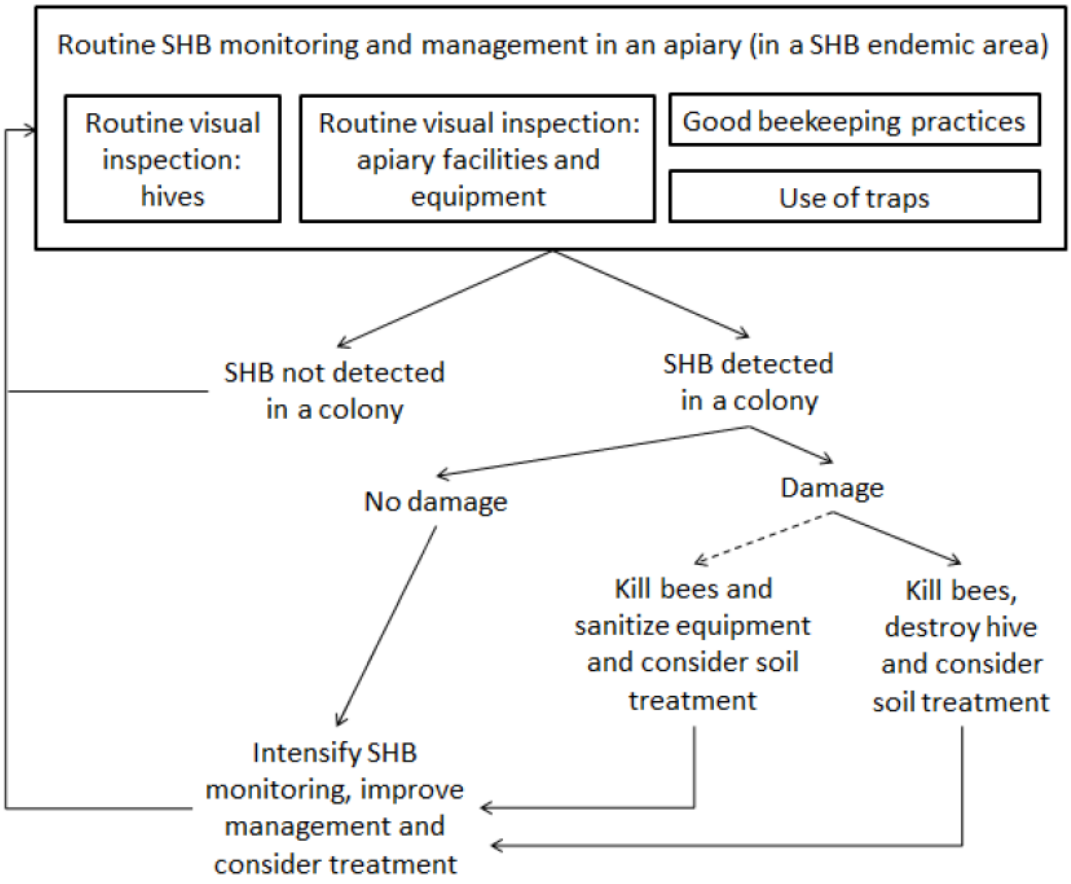

Figure 1: Overview of routine SHB monitoring and management in an apiary in an SHB-infested area where eradication is no longer the objective. ----> alternative route that takes into account damages from *A. tumida* in colonies, particularly egg and larvae observation and general state of the colony. This decision tree comes from EFSA report (EFSA 2015a).

If eradication is the primary control objective, for each new introduction of the beetle, the soil around infested colonies has to be treated, as the beetle might have reproduced at low levels without obvious damage to the colonies (Spiewok and Neumann 2006). In a small hive beetle infested area where eradication is no longer the control objective, soil treatment should be recommended only when colony damage by the beetle larvae is observed (Figure 1). A review of treatments against *A. tumida* other than soil treatment is available (EFSA 2015a, Neumann et al. 2016, Sabella et al. 2022).

3.1 Chemicals use

3.1.1 Context on chemicals use

Only treatments compliant with the Biocidal Products Regulation should be used in the Member States. This particular issue should be checked by the Competent Authorities, so that the treatment is applied according to the relevant EU and national rules and procedures.

Soil treatment with pyrethroids is intended to kill all stages of the small hive beetle possibly present. Examples of typical approaches are set out below. Based on experience in the US (Pettis and Shimanuki 2000), at least an area of 2 m radius around the hives should be treated to a depth of at least 20 cm. In Italy, a 1% solution of cypermethrin and tetramethrin was abundantly sprayed at high pressure (50 L/min) in order to drench the soil after soil ploughing (Granato et al. 2016) to ensure a higher probability of exposure of small hive beetle. In the United States, Gard Star® (40% EC permethrin) used at label rate of 0.05% (active ingredient) is used as soil drench, especially to treat the soil underneath colonies showing severe larval development, to prevent new emerging beetles entering nearby colonies.

Soil drenching with permethrin has been shown to be highly effective against small hive beetle larvae (2 ml of 0.05% solution per 6.45 cm^2, this means 0.31 ml/cm^2; therefore, 7.8 litres for 2 m radius and 0.2 m depth; and 19.5 litre for 0.5 m depth). Following administration of the solution, the soil should appear wet (Smith et al. 2008). As these pyrethroids are very toxic to all arthropods, including bees, beehives must not be sprayed (Hood 2011) and exposure of non-target species (e.g. wild bees) should be avoided. It is therefore recommended that soil treatment takes place after sunset, and other environmental considerations should be taken into account (for instance if colonies are located in a protected area such as natural park).

3.1.2 The Biocidal Products Regulation

Soil chemical treatments to protect bees notably against the small hive beetle fall under the scope of the Biocidal Products Regulation. Biocidal products are used to protect humans, animals, materials or articles against harmful organisms like pests or bacteria. They contain one or more active substances that can be a chemical (synthetic or natural) or a microorganism. Biocidal products can pose risks to humans, animals and the environment owing to their intrinsic properties and associated use patterns. Therefore, biocidal products should only be made available on the market and used provided proper authorisation has been given in accordance with the Biocidal Products Regulation (EU) No 528/2012.

The Biocidal Products Regulation is currently being implemented in the European Union. This applies directly to all European Member States, European Economic Area countries and Switzerland, and concerns the placing on the market and use of biocidal products. This regulation aims at improving the functioning of the biocidal product market in the EU, while ensuring a high level of protection for

humans and the environment. There are different biocidal product types (PT). The class PT 18 corresponds to insecticides, acaricides and products used to control other arthropods. The process illustrating the placing on the market and the use of biocidal products in accordance with the Biocidal Products Regulation is detailed below and illustrated in Figure 2.

A biocidal product can only be authorised if all the active substances that are included in its composition are present in the European Union list of approved active substances, regularly updated by the Commission or in Annex I to that Regulation (the so-called "low risk" active substances). The work program undertaken to establish an exhaustive list of authorised active substances is ongoing. The website of the European Chemicals Agency (ECHA) lists active substances approved at European level or under evaluation[1]. For example, permethrin is approved for biocidal products of class PT 8 (wood preservative) and of class PT 18 (insecticide). Deltamethrin is also approved for insecticide use (class PT 18). Tetramethrin is currently being evaluated for class PT 18. Late 2023, 43 active substances of class PT 18 have been approved and 9 are still under assessment.

A biocidal product containing active substances under evaluation is made available on the market and used according to national procedures. This period is referred to as the transitional period and is defined by Article 89 (2) of the Regulation (EU) No 528/2012. Only products containing existing active substances under evaluation for the specific type of use may be subject to this transitional period. During the transitional period, a Member State may continue to apply its current system or practice of making available on the market or using of a given biocidal product. The practice or system applied among Member States may differ.

In case the biocidal product contains new active substances not formally approved yet but with a recommendation for approval by the evaluating authority, it can be used for a period not exceeding three years according to the derogation "provisional authorisation" defined by Article 55 (2) of Regulation (EU) No 528/2012. **As soon as active substances are included in the list of approved biocidal active substances, the biocidal product must be subject to a marketing authorisation process**. The marketing authorisation process can take up to three years after the date of approval of the last of the active substances to be approved in that biocidal product. The transitional period no longer applies once the biocidal product is authorised. For a biocidal product to be authorised, it must be demonstrated that the product is efficacious against the target organisms and safe for humans, animals and the environment. Authorised biocidal products can only be used according to their specific

[1] https://echa.europa.eu/en/information-on-chemicals/biocidal-active-substances

authorisation, any other use is illegal. The website of the European Chemicals Agency (ECHA) also lists biocidal products authorised at European level[2].

There are several types of authorisation procedures depending on the product and the number of countries where the product may be sold:

- National authorisation and mutual recognition: if the product is placed only on a single market, authorisation from that country is sufficient. If a company wishes to place the product on the market in several countries with national authorisations, it applies for authorisations in those countries, and chooses one of them as the reference Member State. The reference Member State performs the assessment and proposes authorisation conditions to other Member States for agreement. At the end of the procedure, Member States have an agreement on the assessment and conditions of authorisation unless justified by national specificities. The authorisation granted by a Member State is applicable on its territory only.

- Union authorisation: the Biocidal Products Regulation introduces a new alternative for companies that wish to apply for an EU-wide authorisation in one go. In this case, the authorisation is granted by the European Commission.

- Simplified authorisation: for low risk products that meet certain criteria specified in Article 25 of the Regulation, e.g. active substance(s) appear in Annex I of the Biocidal Products Regulation or the biocidal product does not contain any substances of concern (no need for personal protection equipment, etc…). This type of authorisation is granted by one Member State. However, once authorised, the product can be placed on the market in all Member States subject to a simple notification procedure.

- Same biocidal product authorisation: there is the possibility to apply for an authorisation of a biocidal product (either at the national or EU level), which is identical to an already authorised biocidal product.

Having an authorised biocidal product depends on the willingness of companies to go through approval and marketing authorisation process for a specific use. This would roughly take two years. The overall cost for application for marketing authorisation is considerable. Another eventuality is that an organisation (e.g. beekeeper association) applies for authorisation. The producer of the biocidal

[2] https://echa.europa.eu/information-on-chemicals/biocidal-products

product has to be informed and the organisation will have to support the cost of extension of the biocidal product use to the specific use.

In case the application for authorisation of a biocidal product is neither rejected nor granted, the biocidal product is prohibited and off the market with effect for up to 180 days after the date of decision of the Competent Authority according to Article 89 (4) of Regulation (EU) No 528/2012. The use of existing stocks of the biocidal product may continue for up to 365 days after the date of the decision of the Competent Authority.

If an active substance is not included in Annex I of Regulation (EU) No 528/2012, neither under evaluation nor approved, and thus absent from the EU list of approved active substances, the biocidal products containing the active substance are prohibited and off the market.

Where under exceptional circumstances those biocidal products are needed, the Biocidal Products Regulation provides for four types of derogations:

- ✓ Two of them are defined by Article 55 of Regulation (EU) No 528/2012:

- Derogation of 180 days: it is the permission to make a product available on the market or to use a biocidal product that does not fulfil the requirements in the Biocidal Products Regulation. The national Competent Authority may permit the limited and controlled use of a biocidal product for a period not exceeding 180 days. The national Competent Authority shall inform the Competent Authorities of the other member states, as well as the Commission of its actions, concerning their justification for derogation and the revocation of such action. The use may be extended for a period not exceeding 550 days upon request to the Commission.

- Derogation "cultural heritage": it concerns a biocidal product containing a non-approved active substance that is essential for the protection of cultural heritage. It is used for furniture, buildings or museum specimens of insects.

- ✓ Article 56 of Regulation (EU) No 528/2012 mentions the derogation "R&D" that concerns experiments or tests for research and development purposes involving the use of an unauthorised biocidal product containing approved active substances or a non-approved active substance intended to be used in a biocidal product.

- ✓ The derogation "essential use" from Article 22 of Delegated Regulation No 1062/2014 concerns the use of a non-approved active substance that a Member State considers as essential within 18 months of the date of decision of non-approval.

Where no biocidal product containing pyrethroids is authorised in a given Member State to control the small hive beetle via soil treatment, the provisions in Article 55 of Regulation (EU) No 528/2012 or, where relevant, Article 56 of the Regulation, could apply especially for a short period of use and/or in case of emergency.

It is necessary for a Member State wishing to set up protocols for soil chemical treatments to know the regulatory status of the relevant biocidal products in their countries. However, application of such biocidal product on the ground raises environmental questions about the toxicity of these products to non-target species (e.g. bees), as well as about contamination of the soil and groundwater compartment.

There are particular cases where soil chemical treatments could not be applied: organic farms or apiaries located in a groundwater protection zone, a national park or inside a forest.

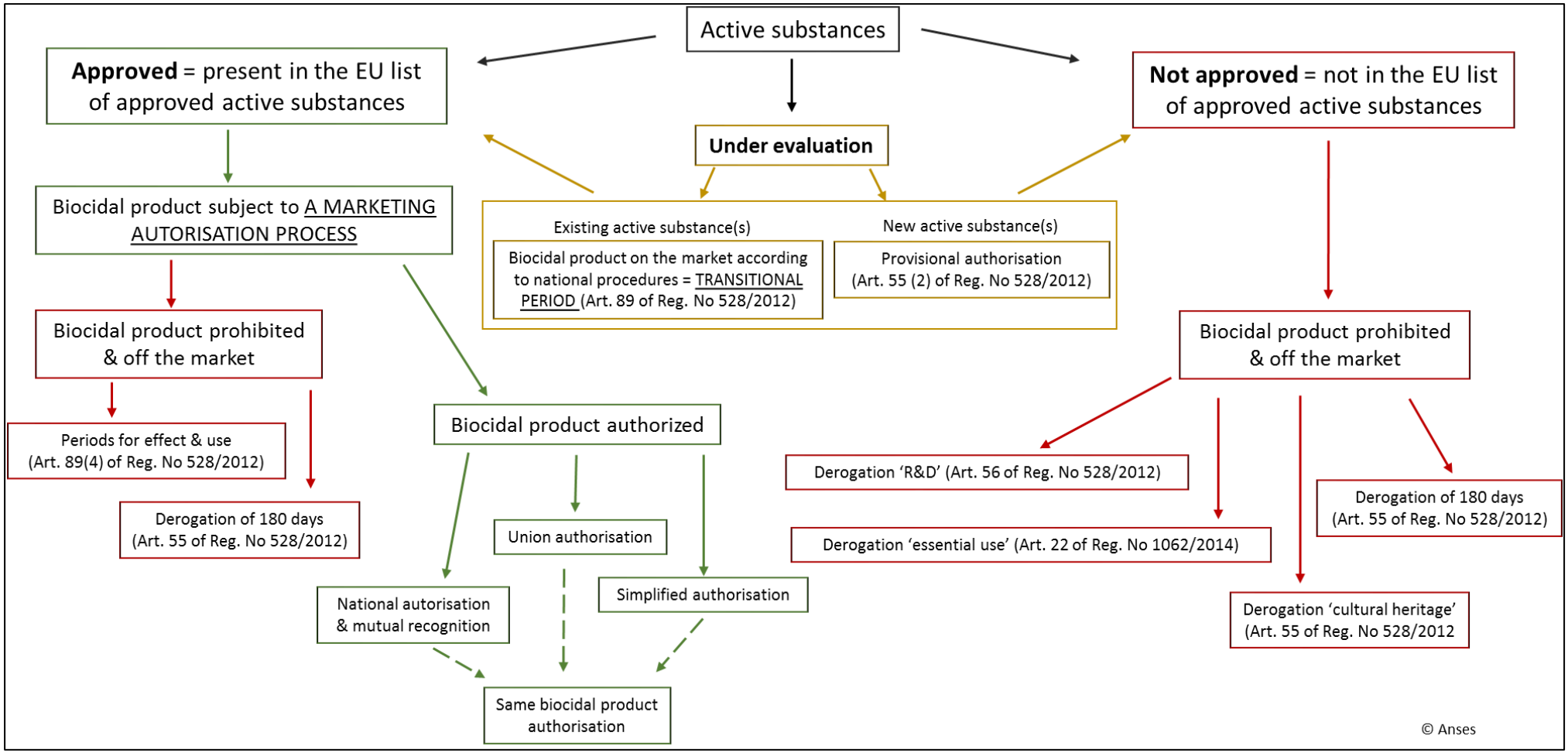

Figure 2: Diagram outlining the process of the Biocidal Products Regulation No 528/2012 on the placing on the market and use of biocidal products

3.2 Application of nematodes use

Soil can be treated with nematodes to stop *A. tumida* development (Anses 2023b). The nematodes target the pupal stage and wandering larvae of the small hive beetle (Hill et al. 2015, Hill et al. 2016, Sabella et al. 2022). The success of their use depends on several factors such as humidity, temperature, soil cover and sun exposure (Sanchez et al. 2021). It has to be noted that nematode use is still experimental. However, they can be a promising option in the European context. More studies are needed to use *Steinernema kraussei* and *Steinernema carpocapsae* for instance, that are currently commercially available to fight against vegetal pests in integrated pest management (IPM) programs.

Recently, the lethal effects of entomopathogenic nematodes on honey bees have been investigated by (Rüfenacht et al. 2023).

The European Commission does not consider nematodes as biocidal active substances.

3.3 Application of entomopathogenic fungi

Entomopathogenic fungi could be an alternative (Ellis et al. 2004, Richards et al. 2005, Muerrle et al. 2006, Leemon and McMahon 2009). Lundie (1940) first reported a potential unidentified fungal control agent when noticing high mortality of adult beetles during laboratory rearing. Similarly, Ellis et al. (2004) found a 32% pupae mortality rate after contact of post-feeding larvae with pupae killed by a pathogen(s). Five fungal species were identified in a complex isolated from the pathogen-killed pupae: two of these were *Aspergillus niger* van Tieghem (Eurotiaceae) and *A. flavus* Link: Grey (Eurotiaceae). Both species are cosmopolitan soil fungi that appear to infect *A. tumida* pupal stage when post-feeding larvae exit the host honey bee colony and burrow into the surrounding soil for pupation. A recent publication lists *Aspergillus flavus* as a common bee bread contaminant (Bush et al. 2024). *Aspergillus* fungi can cause disease in honey bees (stonebrood) and in humans, so their application is not recommended. Mortality of adult small hive beetles caused by an unidentified fungus was also observed during mass rearing of beetles (Murrle and Neumann 2004). A study by Leemon and McMahon (2009) demonstrated that various isolates of both *Metarhizium* and *Beauveria* had good efficacy against larvae and adult of *A. tumida* in laboratory assays. Generally, the *Metarhizium* isolates performed best against larvae while the *Beauveria* isolates performed best against adult beetles. Three isolates of *Metarhizium* killed more than 70% of larvae by day 7, while two individual *Beauveria* isolates produced 99% and 100% mortality of adult beetles respectively 14 days after treatment. Muerrle et al. (2006) report promising results of the effects of several species of entomopathogenic fungi against

A. tumida and recommended screening of further species to continue the development of an efficient mycoinsecticide.

Entomopathogenic fungi application to the soil also falls within the scope of the Biocidal Products Regulation.

3.4 Thermal treatment

Heat or freezing treatments are physical treatments so they do not fall within the Biocidal Products Regulation.

In early studies on the beetle, it was reported that no adults were hatching from the ground at temperatures below 10°C and that the survival rates of larva and pupa were very low, when water content of the soil was lower than 5% or higher than 25% (Somerville 2003). Further studies showed high mortalities at 35°C soil temperature and also calculated that below 10°C pupa of small hive beetle will be unable to develop further (Meikle and Patt 2011, Meikle and Diaz 2012, Meikle et al. 2012). Bernier et al. (2014) calculated the minimum temperature for development to 10.2-13.2°C, depending on the water content of the soil and showed that high water content is damaging for the pupation process (Bernier et al. 2014)(Bernier et al. 2014)(Bernier et al. 2014)(Bernier et al. 2014)(Bernier et al. 2014)(Bernier et al. 2014). Noor-ul-Ane & Jung (2020) investigated the effects of temperature on survival of wandering larvae and pupae, they found that both did not complete development and died at 39°C. They further calculated the optimum temperatures with maximum survival for wandering larvae (26.7°C with 100%) and pupae (26.5°C with 100%), the optimum temperature for the development of wandering larvae (35.5°C) and pupae (34.8°C) and the lower and higher threshold temperatures wandering larvae (11.7°C and 42.5°C) and pupae (12.5°C and 41.5°C) (Noor-ul-Ane and Jung 2021b). The same authors also investigated this plasticity of cold tolerance in wandering larvae and pupae of SHB. The supercooling points occurred at lower temperatures (- 19.4°C) in wandering larvae than in the other stages (pupae: - 12.5°C, and feeding larvae: - 10.7°C). A lethal temperature (LT50) of feeding larvae was achieved earlier at 4.9°C after 7 h exposure than the wandering larvae (3.7°C after 48 h) and pupae (5.6°C after 48 h) (Noor-Ul-Ane and Jung 2021a).

The WOAH Terrestrial Animal Health Code indicates 50°C or higher and -12°C or lower, each for 24 hours to kill all small hive beetle live stages (WOAH 2018).

Therefore, to avoid the use of chemicals in apiaries, alternative soil treatments, such as using heating or freezing systems or increasing the water content of the infested soil to a high level (> 25%), should be considered as potential options.

Several methodologies for heating the soil surface exist:

- Flaming of the soil surface or systems using hot air is commonly applied as weed control methods. However, when using flames or hot air, most of the heat is transported to the atmosphere and the heating-effect will not reach the larvae and pupae that are deeply buried in the soil. Therefore, the flaming technology and the use of hot air are not appropriate to use to kill deeply buried small hive beetle larvae and pupae.

- Electrically driven systems, using heating blankets or heating electrodes that are pierced into the soil, would directly heat the soil and such systems could be in place as long as they reached the temperatures required to kill all small hive beetle stages.

- High-pressure steam has been used in strawberry fields in California for sterilisation of soil to control soil weeds, pathogens and pests. Temperatures above 50°C were maintained for 30 minutes at a depth of 25 cm (Fennimore et al. 2014). However, there are many options for the use of steam, e.g. superheated steam, including methods to reach greater soil depth using heating lances, and as with the electrical systems approach, the treatment applications would need to be applied over a longer time period to reach temperature levels that kill all stages of small hive beetles.

- Irrigation with hot water would combine high heat conduction from water to the soil and high specific heat content. This might be useful for a shallow application but the depth effect and the finally reached temperatures in the soil would relate to the area-specific mass of water. Using hot water, the larvae or pupae not only could be killed by heat but also the water content would be detrimental for survival and development of the small hive beetle.

- All the above mentioned heating applications could possibly be combined with soil solarisation to increase their efficacy (Samtani et al. 2012). In the case of solarisation, the surface is covered with a black tarp and the soil is heated under the tarp by solar radiation. However, if only the energy of the sun is used, the heating process requires very long periods of sunshine and is therefore only applicable on free surfaces and in particular climates. Furthermore, one has to consider that if the resulting increases in temperature do not reach levels that kill *A. tumida* larvae or pupae, they might inadvertently even boost their development, which would be of course counter-productive and this must be strictly prevented. Solarisation seems therefore not to be an option for beetle control in the soil, as rapid temperature elevation and accurate monitoring is required when heating soil and as slightly higher temperatures will increase small hive beetle development.

Only heating systems should be used that are able to reach 50°C and that are able to hold this level for 24 hours in the depth where it is needed, thereby fulfilling the WOAH standards.

To freeze the infested soil *in situ*, a cryogen, like liquid nitrogen, might be introduced into the soil via lances or drilled holes. Anyway, for physical reasons the cooling process might hinder itself, as ice and the expansion of frozen water will seal the pores in the soil, thereby limiting the conduction of the cold.
Heating or freezing approaches are not selective and could therefore have a strong environmental impact. However, after the application, the soil will likely recover quickly from the surrounding ground fauna and no chemical residues become concentrated.

Alternatively to on field applications, another option is to remove the upper level (20-50 cm) of the affected soil and subject it to heat or freezing treatment or desiccation in appropriate facilities (if available) to kill all small hive beetle pupae. Nevertheless, this option could pose safety risk for the duration of the transport of the potentially infested soil to the treatment facility. To avoid this risk, Dietemann and Lerch (2015) proposed an on-site application of low temperature by means of freezer-equipped trucks to treat beekeeping equipment following the occurrence of a small hive beetle outbreak. Alternatively, the soil around the hives could be removed and deep buried (1 to 2 meters depth) in the ground as done in Portugal (Murilhas 2004, Valerio Da Silva 2014).

All these non-chemical examples (excluding solarisation) are potentially new control avenues to be considered. They have not yet been analysed and no data are available so far, but these approaches would avoid environmental contamination (EFSA 2015b, Sabella et al. 2022). Therefore, further research is needed to determine the optimum use of heating or freezing systems in order to control the beetle in the soil, including their energy-use efficiencies to determine the cost of such treatments.

3.5 Other alternative treatments

Alternative treatments, such as treating the ground with powdered limestone, have been found to be ineffective, while slaked lime produced inconsistent results (Buchholz et al. 2009). As rain would probably reduce the efficacy of the slaked lime treatment, more field experiments are needed to test its impact on all soil-dwelling life stages of the small hive beetle.

Anaerobic soil disinfestation, which consists in using the by-products of anaerobic metabolism, obtained by covering organic matter with air-tight plastic, has been recently considered as an alternative to soil chemical sterilisation to control potato cyst nematode (Streminska et al. 2014). Similarly, in Portugal at the site of the suspected small hive beetle infested beehives, greenhouse plastic polyethylene films were used to cover the places where hives had been placed, and from where the soil was removed (Murilhas 2004, Valerio Da Silva 2014). However, anaerobic soil disinfestation has not yet been tested against small hive beetle or any other *Coleoptera* pest. As with solarisation it has to be considered that if the increases in temperature do not reach levels that kill small hive beetle larvae or pupae, they might even boost their development.

4. Procedures to use pesticides to control small hive beetle in the soil: the Italian experience

In the operating experience described below, the regulatory management is not discussed regarding the different processes and products used.

In case of suspicion of small hive beetle infestation, the apiary is placed under Standstill Notice by the Competent Authority responsible for applying national pest and disease control measures in apiaries. Once the suspicion is confirmed by the national Competent Authority and/or the EU Reference Laboratory, the local Competent Authority can proceed with the killing of the colonies and the destruction of the apiary according to the specific control orders applied on the affected apiary. The Standstill is lifted after the destruction is carried out.

No EU rule is available concerning the killing and destruction of bee colonies in relation to small hive beetle outbreak management (Mutinelli 2023). However, according to Section 3 of the Regulation (EU) No 142/2011, the Competent Authority may authorise the disposal by burning or burial on site of bees and apiculture by-products, as referred to Article 19(1)(f) of the Regulation (EU) No 1069/2009, provided that all necessary measures are taken to ensure that the burning or burial does not endanger animal or human health or the environment.

As an example of the eradication policy for the small hive beetle, in Italy, the within apiary stamping-out control policy is carried out according with a routine protocol based on closure of the hives in the evening, killing the honey bees (e.g. by using sulphur dioxide), and then burning the dead colonies on site. The protocol is applied and supervised by the Competent Authority. In Italy, the burning of the hives is carried out under the supervision of the firefighter brigade that guarantees from any residual fire and under the presence of the Municipal Police.

The soil is rotated for a depth of at least 20 cm in a radius of at least 2 m around the beehives by means of an excavator. The disinfestation team of the Competent Authority carries out the soil treatment. The soil is abundantly drenched with a pyrethroid solution using a pump mounted on a truck to facilitate the approach to the apiary or using lighter alternatives such as small rotator machines or back-pack systems. This operation is carried out only once.

In Italy, a 1% solution of a commercially available biocide containing two synthetic pyrethroids, cypermethrin and tetramethrin, ensuring both optimal persistence and very fast lethal capacity, is applied. The disinfestation team of the Local Bee Health Unit was already using this biocide for other control purposes at time of the first detection of *A. tumida* (Mutinelli et al. 2014).

The availability of other active ingredients and/or commercially available products classified as pesticides rather than biocides with more environment friendly characteristics is now under investigation.

A commercially available broad-spectrum pyrethroid insecticide (with deltamethrin as the active ingredient), that can be used on a wide range of crops, that is effective on larvae of Coleopteran species and that is administered to the soil as a liquid solution (spray), could also be a possible candidate (Mutinelli and Maroni Ponti 2017).

5. Conclusion

Despite a spreading trend observed in recent years, the small hive beetle is still exotic to European Union, except Calabria region in southern Italy. In this document, the authors considered in detail only soil treatment against the small hive beetle, a key treatment in the small hive beetle control arsenal. If eradication is an objective in case of introduction, the soil around infested colonies has to be treated, as the small hive beetle might have reproduced in low levels without obvious damage to the colonies. In Italy, for the small hive beetle outbreak response, pyrethroids are used as an integral part of the response to treat soil infestations. Soil treatments fall within the Biocidal Products Regulation. It is necessary for Member States wishing to set up protocols for soil chemical treatments to know the regulatory status of the biocidal products in their countries. However, application of biocidal products to the soil raises environmental questions about the toxicity of these products to non-target species and contamination of the soil and groundwater compartment. There is an ongoing discussion at the EU level about the consideration of nematodes as biocidal active substances.

Heat or freezing treatments are physical treatments so they do not fall within the Biocidal Products Regulation. These non-chemical examples are potentially new control avenues to be considered

against small hive beetle spread. They have not yet been evaluated and no data are available so far for small hive beetle control, but these approaches would avoid environmental contamination. Therefore, further research is needed to determine their applicability and optimum use of heating or freezing systems in order to control small hive beetle in the soil, including their energy-use efficiencies to determine the cost of such treatments.

6. Documentation

7.1 Legal basis

Commission Delegated Regulation (EU) No 1062/2014 of 4 August 2014 on the work programme for the systematic examination of all existing active substances contained in biocidal products referred to in Regulation (EU) No 528/2012 of the European Parliament and of the Council. Official Journal of the European Union L 294, 10.10.2014, p. 1-34. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R1062&from=EN

Commission Delegated Regulation (EU) 2018/1629 of 25 July 2018 amending the list of diseases set out in Annex II to Regulation (EU) 2016/429 of the European Parliament and of the Council on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law') (Text with EEA relevance.) OJ L 272, 31.10.2018, p. 11–15. https://eur-lex.europa.eu/eli/reg_del/2018/1629/oj

Commission Delegated Regulation (EU) 2020/688 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council, as regards animal health requirements for movements within the Union of terrestrial animals and hatching eggs. OJ L 174, 3.6.2020, p. 140–210. https://eur-lex.europa.eu/eli/reg_del/2020/688/oj

Commission Delegated Regulation (EU) 2020/692 of 30 January 2020 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for entry into the Union, and the movement and handling after entry of consignments of certain animals, germinal products and products of animal origin. OJ L 174, 03/06/2020, p. 379–520. https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32020R0692

Commission Implementing Decision of 12 December 2014 concerning certain protective measures with regard to confirmed occurrences of the small hive beetle in Italy. (2014/909/EU). Official Journal of the European Union L 359, 16.12.2014, p. 161-163. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014D0909&from=EN

Commission Implementing Decision (EU) 2023/110 of 12 January 2023 laying down emergency measures as regards confirmed cases of infestation with small hive beetle (Aethina tumida) in Italy and France and repealing Implementing Decision (EU) 2021/597. OJ L 13, 16.1.2023, p. 5–8. https://eur-lex.europa.eu/eli/dec_impl/2023/110/oj

Commission Implementing Regulation (EU) 2018/1882 of 3 December 2018 on the application of certain disease prevention and control rules to categories of listed diseases and establishing a list of species and groups of species posing a considerable risk for the spread of those listed diseases. Official Journal of the European Union L 308, 4.12.2018, 21-29. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R1882&from=IT

Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. Official Journal of the European Union L 54, 26.02.2011, 1-242. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0142&from=EN

Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). Official Journal of the European Union L 300, 14.11.2009, 1-33. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1069&from=EN

Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. Official Journal of the European Union L 167, 27.6.2012, 1-123. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R0528&from=EN

Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law'). Official Journal of the European Union L 84, 31.3.2016, 1-208. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.L_.2016.084.01.0001.01.ENG

7.2 Bibliography

Anses. 2023a. Avis relatif une évaluation de la pertinence de la poursuite de la stratégie d'éradication du petit coléoptère des ruches Aethina tumida à La Réunion et de ses évolutions possibles suite à la contamination du réservoir sauvage (Saisine 2023-SA-0122). Anses, Maisons-Alfort.

Anses. 2023b. Note d'appui scientifique et technique relatif à « la gestion du petit coléoptère des ruches (Aethina tumida), suite à sa détection sur l'île de La Réunion en juillet 2022 », Réponse à la question concernant la prévention. Saisine 2022-SA-0141, Maisons-Alfort

Bernier, M., V. Fournier, and P. Giovenazzo. 2014. Pupal development of *Aethina tumida* (Coleoptera: Nitidulidae) in thermo-hygrometric soil conditions encountered in temperate climates. Journal of Economic Entomology 107.

Buchholz, S., K. Merkel, S. Spiewok, J. Pettis, M. Duncan, R. Spooner-Hart, C. Ulrichs, W. Ritter, and P. Neumann. 2009. Alternative control of Aethina tumida Murray (Coleoptera: Nitidulidae) with lime and diatomaceous earth. Apidologie 40:535-548.

Bush, D. S., B. Calla, and M. R. Berenbaum. 2024. An Aspergillus flavus strain from bee bread of the Western honey bee (Apis mellifera) displays adaptations to distinctive features of the hive environment. Ecology and Evolution 14:e10918.

Cuthbertson, A. G., M. E. Wakefield, M. E. Powell, G. Marris, H. Anderson, G. Budge, J. J. Mathers, L. F. Blackburn, and M. A. Brown. 2013. The small hive beetle Aethina tumida : A review of its biology and control measures Current Zoology 59:644-653.

deGuzman, L., A. M. Frake, and T. Rinderer. 2010. Seasonal population dynamics of small hive beetles, Aethina tumida Murray, in the south-eastern USA. Journal of Apicultural Research and Bee World 49:6.

EFSA. 2015a. EFSA scientific opinion on the survival, spread and establishment of the small hive beetle (Aethina tumida). EFSA journal 13:4328.

EFSA. 2015b. Small hive beetle diagnosis and risk management options. EFSA journal 13:4048.

EFSA, P. o. A. H. a. W. A. 2013. The risk of introduction and spread of the small hive beetle (Aethina tumida) and Tropilaelaps in the EU. EFSA. EFSA Journal 11:3128-.

Ellis, J. D. J., I. H. Rong, M. P. Hill, H. R. Hepburn, and P. J. Elzen. 2004. The susceptibility of small hive beetle (*Aethina tumida* Murray) pupae to fungal pathogens. American Bee Journal 144:486-488.

Fennimore, S. A., F. N. Martin, T. C. Miller, J. C. Broome, N. Dorn, and I. Greene. 2014. Evaluation of a mobile steam applicator for soil disinfestation in California strawberry. HortScience 49:1542-1549.

Granato, A., B. Zecchin, C. Baratto, V. Duquesne, E. Negrisolo, M. P. Chauzat, M. Ribière-Chabert, G. Cattoli, and F. Mutinelli. 2016. Introduction of Aethina tumida (Coleoptera: Nitidulidae) in the regions of Calabria and Sicily (southern Italy). Apidologie 48:194-203.

Hill, E. S., A. B. Smythe, and D. A. Delaney. 2015. Assessing the role of environmental conditions on efficacy rates of Heterorhabditis indica (Nematoda: Heterorhabditidae) for controlling Aethina tumida (Coleoptera: Nitidulidae) in honey bee (Hymenoptera: Apidae) colonies: a citizen science approach. Journal of Economic Entomology:1-7.

Hill, E. S., A. B. Smythe, and D. A. Delaney. 2016. Erratum: Assessing the role of environmental conditions on efficacy rates of Heterorhabditis indica (Nematoda: Heterorhabditidae) for controlling Aethina tumida (Coleoptera: Nitidulidae) in honey bee (Hymenoptera: Apidae) colonies: A citizen science approach (Journal of Economic Entomology (2015) 109:1 (106-112) DOI: 10.1093/jee/tov296). Journal of Economic Entomology 109:2247.

Hood, M. 2011. Handbook of small hive beetle. Clemson University.

Leemon, D., and J. McMahon. 2009. Feasability study into in-hive fungal bio-control of small hive beetle. RIRDC.

Meikle, W. G., and R. Diaz. 2012. Factors affecting pupation success of the small hive beetle, *Aethina tumida*. Journal of Insect Science 12:1-9.

Meikle, W. G., and J. M. Patt. 2011. The effects of temperature, diet, and other factors on development, survivorship, and oviposition of *Aethina tumida* (Coleoptera: Nitidulidae). Journal of Economic Entomology 104:753-763.

Meikle, W. G., J. M. Patt, and D. Sammataro. 2012. Intraspecific competition effects on Aethina tumida (Coleoptera: Nitidulidae). J.Econ.Entomol. 105:26-33.

Muerrle, T. M., P. Neumann, J. F. Dames, H. R. Hepburn, and M. P. Hill. 2006. Susceptibility of adult *Aethina tumida* (Coleoptera: Nitidulidae) to entomopathogenic fungi. J.Econ.Entomol 99:1-6.

Murilhas, A. M. 2004. *Aethina tumida* arrives in Portugal. Will it be eradicated? EurBee Newsletter 2:7-9.

Murrle, T., and P. Neumann. 2004. Mass production of small hive beetles (Aethina tumida, Coleoptera: Nitidulidae). Journal of Apicultural Research 43:144-145.

Mutinelli, F. 2023. Euthanasia and welfare of managed honey bee colonies. Journal of Apicultural Research 62:2-10.

Mutinelli, F., and A. Maroni Ponti. 2017. Update on the occurence of small hive beetle, *Aethina tumida* Murray, in Italy. Technical dossier Italian Ministry of Health 0017392-24/07/2017-DGSAF-MDS-P.

Mutinelli, F., F. Montarsi, G. Federico, A. Granato, A. MaroniPonti, G. Grandinetti, N. Ferrè, S. Franco, V. Duquesne, M. P. Rivière, R. Thiéry, P. Hendrikx, M. Ribière, and M. P. Chauzat. 2014. Detection of Aethina tumida Murray (Coleoptera: Nitidulidae.) in Italy: outbreaks and early reaction measures. Journal of Apicultural Research 53:7.

Neumann, P., J. S. Pettis, and M. O. Schäfer. 2016. Quo vadis Aethina tumida? Biology and control of small hive beetles. Apidologie 47:427-466.

Noor-Ul-Ane, M., and C. Jung. 2021a. Characterization of Cold Tolerance of Immature Stages of Small Hive Beetle (SHB) Aethina tumida Murray (Coleoptera: Nitidulidae). Insects 12.

Noor-ul-Ane, M., and C. Jung. 2021b. Temperature-dependent modelling of adult performance of small hive beetle Aethina tumida Murray (Coleoptera: Nitidulidae). Journal of Apicultural Research 61:284-293.

Pettis, J. S., and H. Shimanuki. 2000. Observations on the Small HIve Beetle, Aethina tumida Murray, in the United States. American Bee Journal:152-155.

Richards, C. J., M. P. Hill, and J. F. Dames. 2005. The susceptibility of small hive beetle (*Aethina tumida* Murray) pupae to *Aspergillus niger* (van Tieghem) and *A. flavus* (Link: Grey). American Bee Journal 145:748-751.

Rüfenacht, A., L. Straub, and D. Grossar. 2023. Underestimated adverse effects of entomopathogenic nematodes on honey bees.*in* I. P.-M. 141, editor. Setac Europe 33rd annual meeting, Dublin, Ireland.

Sabella, G., R. Mule, L. Robba, A. Agro, and B. Manachini. 2022. Could Europe Apply a Suitable Control Method for the Small Hive Beetle (Coleoptera: Nitidulidae)? J Econ Entomol.

Samtani, J. B., C. Gilbert, J. B. Weber, K. V. Subbarao, R. E. Goodhue, and S. A. Fennimore. 2012. Effect of steam and solarization treatments on pest control, strawberry yield, and economic returns relative to methyl bromide fumigation. HortScience 47.

Sanchez, W., D. Shapiro, G. Williams, and K. Lawrence. 2021. Entomopathogenic nematode management of small hive beetles (Aethina tumida) in three native Alabama soils under low moisture conditions. Journal of Nematology 53.

Schäfer, M. O., I. Cardaio, G. Cilia, B. Cornelissen, K. Crailsheim, G. Formato, A. K. Lawrence, Y. Le Conte, F. Mutinelli, A. Nanetti, J. Rivera-Gomis, A. Teepe, and P. Neumann. 2019. How to slow the global spread of small hive beetles, Aethina tumida. Biological Invasions 21:1451-1459.

Smith, M., L. Goodrum, N. Chinneck, and M. Stedman. 2008. The importance of hive health in apiculture from a veterinary perspective. UK Vet: Companion Animal 13:65-69.

Somerville, D. 2003. Study of the small hive beetle in the USA. Rural industries research and development corporation.

Spiewok, S., and P. Neumann. 2006. Cryptic low-level reproduction of small hive beetles in honey bee colonies. Journal of Apicultural Research 45:47-48.
Stedman, M. 2006. Small hive beetle (SHB): *Aethina tumida* Murray (Coleoptera: Nitidulidae). Government of South Australia, Primary Industries and Resources SA.
Streminska, M. A., W. T. Runia, A. J. Termorshuizen, H. Feil, and A. W. G. Van der Wurff. 2014. Anaerobic soil disinfestation in microcosms of two sandy soils. Communications in Agricultural and Applied Biological Sciences 79:15-19.
Valerio Da Silva, M. J. 2014. The first report of Aethina tumida in the European Union, Portugal, 2004. Bee World 91.
WOAH. 2018. Infestation of honey bees with Aethina tumida (small hive beetle). WOAH Terrestrial Manual. WOAH, Paris.