SUSINCHAIN
SUSTAINABLE INSECT CHAIN

Technical report on resistance of different insect pathogens and pests

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SUMMARY

The management of pests and diseases is a crucial part of any mass production system. With the maturation of the edible insect industry comes the need to minimize the negative effects that both diseases and unwanted pests can have on productivity. Within the context of SUSINCHAIN, the aim was to describe the main pathogens and pests affecting insects produced for food and feed, and to develop potential methods to minimize their impact and/or improve the health and immune system of the insects in production. This was achieved using a variety of methods, from surveys with insect producers to scientific reviews of our current knowledge on pathogens of insects. Furthermore, methods to attempt to control pests in the production systems were assessed for their efficacy. Finally, the potential to improve insect health and increase the resilience of production systems was investigated by testing whether probiotics (both lactic acid bacteria indigenous to *T. molitor* and commercial probiotics) could confer some protection to the larvae when supplemented to the insects' diet.

Overall, progress is made evident by the number of publications linked to SUSINCHAIN in these areas. However, it is clear that, even though some methods are better than others at controlling pests, and that, the addition of probiotics did protect the insects against a fungal infection, no silver bullet was discovered and it will be necessary to continue research into new methods to improve insect health and control pests.

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1. Insect pathogens

1.1 Introduction

Due to a swift and continuous growth of the insect rearing industry during the last two decades, there is a need for a better understanding of insect diseases (caused by insect pathogens). In the insect production sector, insect diseases are a bottleneck for every type and scale of rearing system with different degrees of technology investment (i.e. semi-open rearing, closed rearing, industrial production, small-scale farming). A large body of our current knowledge on insect host-pathogens interactions is based on a limited number of studies on insect pathogens causing disease outbreaks in insects, either in wild or in captive insect populations. Usually, the discovery and description of pathogens took place because of striking epidemic disease outbreaks in insect populations or based on observations of a few diseased individuals.

Nevertheless, given the vast amount of insect and pathogen species in the world and the many different ways in which insects can be useful for humanity, there is still a lot to learn about insects and their pathogens. This is underlined by the challenge posed by the development of infectious diseases in the rearing systems of insects produced for food and feed. On the bright side, the widespread use of molecular techniques has increased the discovery of (insect) pathogens and the understanding of the microbiome of several insect species, including that of several edible insects. At the same time, new knowledge is continuously being gathered as more research is conducted on the impact of known pathogens on insect health in species commonly reared as food and/or feed.

The following sections outline the developments and new knowledge gathered during the SUSINCHAIN project.

1.2 Diseases in edible insect rearing systems

Viruses infecting insects comprise RNA-viruses as well as DNA-viruses and belong to a range of different virus families, all with the potential to cause problems in mass-production systems. Among these viruses, many are host-specific. An exception is the invertebrate iridescent virus 6 (IIV-6), known to infect several hosts in the orders Orthoptera and Blattodea (Just and Essbauer, 2001; Kleespies et al., 1999) including gryllids, locusts, and cockroaches. In addition, larvae of the great wax moth, *Galleria mellonella* have shown susceptibility to IIV-6 under experimental conditions (Jakob et al., 2002) as well as lepidopteran and dipteran cultured cell lines (Bronkhorst et al., 2014; Williams et al., 2009). Most entomopathogenic viruses known up to date are taxonomically distant from vertebrate viruses (Miller and Ball, 1998). Viruses have a high potential to cause epizootics in insect-rearing systems, and in some cases they pose a threat to whole production stocks. *Acheta domesticus* densovirus (AdDV), an important pathogen of the European house cricket *A. domesticus*, is well known to cause disease outbreaks, which in the worst case could lead to major losses and bankruptcy of cricket-rearing companies (Szelei et al., 2011; Weissman et al., 2012). Overt viral infections are initially identified by the symptoms displayed by infected insect hosts. For example, a disruption in moulting, reduced oviposition, or reduced weight gain may be symptoms. Other symptoms may be a translucent exoskeleton, swollen and/or translucent abdomen, enlarged brownish or milky midgut, or hindgut, watery faeces, and paralysis. The particular symptoms depend on the virus and the host. Viruses can be transmitted through horizontal transmission (between conspecifics), vertical transmission (from

parent to offspring), and sexual transmission. Often, viruses are transmitted through more than one of these transmission routes. Methods for viruses detection include molecular techniques, virus isolation, serological studies, histopathology, and electron microscopy (Eberle et al., 2012; Harrison and Hoover, 2012).

Entomopathogenic bacteria belong to various groups. They can belong to spore-forming (genera *Bacillus* and *Clostridium*) or non-spore-forming (genera *Pseudomonas, Serratia and Rickettsiella*) bacterial groups and they can be generalists or specialists. In most cases, they infect their hosts orally (Jurat-Fuentes and Jackson, 2012). For example, a specialist bacterium, *Bacillus popilliae* is infectious to a few selected species in the order Coleoptera. On the other hand, strains of *Bacillus thuringiensis var. kurstaki* have a broader host spectrum within the order Lepidoptera and can infect many species. Some generalist and opportunistic bacteria, such as non-spore-forming bacteria from the genera *Pseudomonas* and *Serratia*, can cause problems in insect colonies subjected to stress. As tested by artificially induced infection, a strain of the bacterium *Aeromonas hydrophila* has been reported to be pathogenic to the yellow mealworm *Tenebrio molitor* (Noonin et al., 2011). A change in coloration, flaccidity, bad odour, and the cease of (usual) movement of infected hosts are often the first signs of bacterial diseases. Diagnosis has to be followed by microscopy and molecular methods.

Insect pathogenic fungi can be specialists or generalists. Entomophthorales, an ancient order of fungi, is mostly comprised of specialists (Boomsma et al., 2014; Vega et al., 2012). The species *Entomophthora muscae* infects the house fly *Musca domestica*. The fungus discharges conidia from dead hosts, which increases the likelihood of the conidia to be spread effectively to new hosts (Bellini et al., 1992). Hypocreales (Ascomycota) is another order of fungi that includes genera like *Metarhizium* and *Beauveria*; species in these genera are mostly generalists and can cause diseases in a wide range of insect species. Fungal species belonging to the mentioned genera can infect mealworms (*T. molitor*, a coleopteran species), silkworms (*Bombyx mori*, a lepidopteran species), *M. domestica* (a dipteran species), and *Locusta migratoria* (an orthopteran species) (Bhattarai et al, 2018). Most fungi infect via penetrating the insect cuticle and growing in the haemolymph, and then sporulating externally upon host death. The first diagnosis of a fungal infection can be done by observing conidia or other external features on dead insects and by subsequent analysis using a microscope to identify the fungal genus. Molecular methods such as DNA sequencing help to identify the fungal species in most of the cases.

Microsporidia are unicellular parasitic organisms closely related to fungi. In order to infect their hosts, the spores must be orally ingested (Solter et al., 2012a). Most known microsporidian species are specialists, although some species have been reported to 'jump' to another host. Microsporidian infections are classified as chronic and rarely as acute (Becnel and Andreadis, 2014). Their presence is not necessarily immediately lethal to an insect population, although they can cause harm upon reaching a critical mass. The most studied microsporidian species have been found in honey bees and locusts. Another group of unicellular insect pathogens are gregarines (Lange and Lord, 2012), which occur in the insect gut. Gregarines are only known to be parasitic to insects and mostly non-lethal, but can lower the insects' fitness. They can be present in insect populations without being immediately noticed. The reported effects of gregarines in adult fall field crickets (*Gryllus pennsylvanicus*) are decreased longevity and weight loss under nutritional stress (Zuk, 1987). In addition, a *Gregarina* sp. isolated from the German cockroach *Blattella germanica* was reported as being highly pathogenic, and furthermore as being able to increase the susceptibility of its host to microbial and chemical challenges (Lopes

and Alves, 2005). High prevalence of gregarines was found in a survey of protozoan parasites in edible insect species including *Gromphadorhina portentosa* (Madagascar hissing cockroach), *T. molitor*, *A. domesticus*, and *L. migratoria* (Gałęcki and Sokół, 2019). Gregarines have also been reported to occur in tenebrionids *Zophobas morio* (Jahnke, 2005) and *Alphitobius diaperinus* (Bala et al., 1990). To our knowledge, there is very limited information on the effect of gregarines to edible insects in rearing systems. Conducting more comprehensive research might give insight into the role of gregarines in insect production. Insects that are heavily infected with gregarines can exhibit symptoms such as swollen abdomen and lethargy (Lopes and Alves, 2005). As for microsporidia, gregarines can be detected by examination of gut samples under the microscope, and quantification can be achieved by staining gut fluid (Solter et al., 2012b).

1.3 Measures to control diseases and pests on rearing systems

The main focus of the SUSINCHAIN project was to find ways to minimise the effects of diseases in insect production systems. This was mainly achieved by focusing on the potential for probiotics to play a role as a preventive measure in the production.

Gut microbiota modulate insect immune response, enhancing the resiliency of insects against pathogens (Muhammad et al., 2019) or assisting the pathogens to overcome the immune system of their host (Jakubowska et al., 2013). From the perspective of insect rearing, modifying the diet would also modify the microbial composition of insect guts, a feature that could promote higher disease resistance of insects reared under mass-production schemes.

During the SUSINCHAIN project, we focused on investigating the potential for probiotics to improve *T. molitor* health and development (Lecocq et al, 2021). Overall we found that:

- *Pediococcus pentosaceus*, a species of lactic acid bacteria indigenous to a local population of *T. molitor* was successful at inhibiting the growth of known pathogens, *in vitro* (Figure 1).
- *Pediococcus pentosaceus* increased larval development rate when supplemented to *T. molitor* feed during the whole development. This effect was not observed (or not as pronounced) when other commercial probiotics were used (Figure 2).
- *Pediococcus pentosaceus* appeared to confer some degree of protection to *T. molitor* larvae following exposure to a known fungal pathogen, *Metarhizium brunneum* (Figure 3).

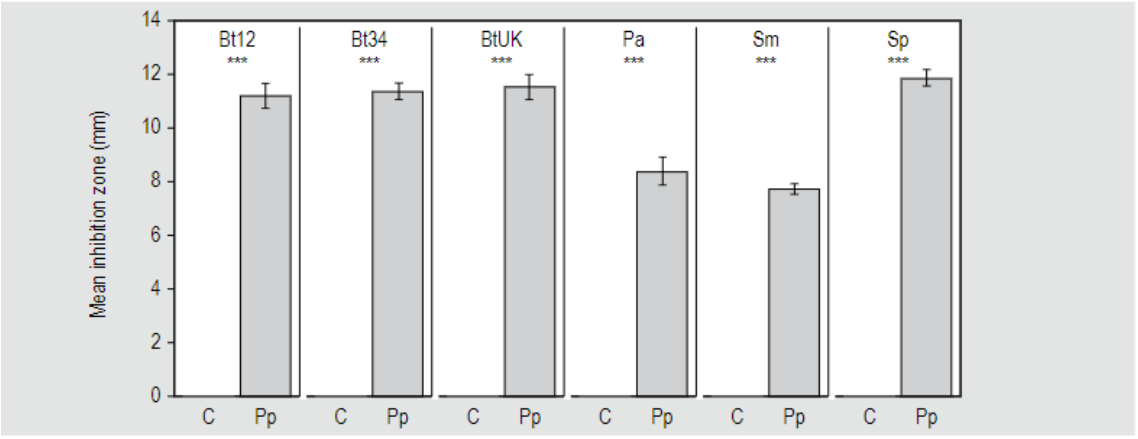

Figure 1. Dual overlay test of the *Pediococcus pentosaceus* (Pp) isolate against six pathogenic bacteria: *Bacillus thuringiensis* (Bt12, Bt34 and BtUK), *Pseudomonas aeruginosa* (Pa), *Serratia marcescens* (Sm) and *Serratia plymuthica* (Sp). Growth inhibition scores (in mm) for all three replicates are based on the average of two measurements, per replicate, of the radius of the inhibition zone. The final inhibition score (Mean) was defined as the average from the measurements taken from the replicates. A control (C) using sterile water instead of *P. pentosaceus*, confirmed growth potential with no inhibition, of each the pathogenic bacteria in the absence of *P. pentosaceus*. *P. pentosaceus* was able to inhibit the growth of all of the tested pathogens to some degree. Error bars represent ±1 S.E. from the mean. * Significant at *P*<0.001.**

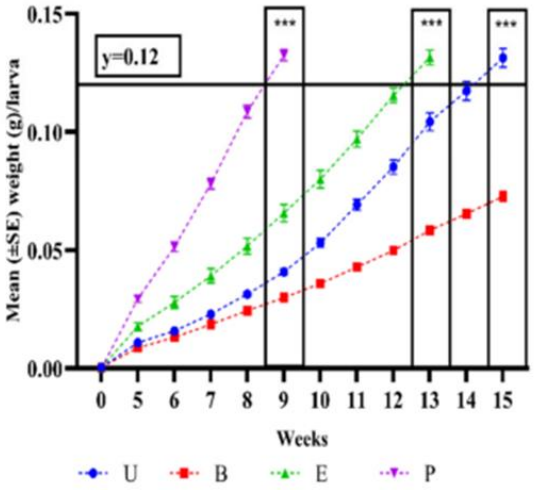

Figure 2. Average body weight (g) of individual *Tenebrio molitor* larvae in the four probiotic treatment groups during the experimental period of 15 weeks. Horizontal reference line at Y = 0.12 g indicates the threshold weight of the larvae to harvest. Blue: control (C); red: *Bacillus subtilis* (B); green: *Enterococcus faecium* (E); and violet: *Pediococcus pentosacceus* (P). Each treatment group consisted of 10 subgroups, each with 100 larvae. Larvae were harvested at three time points: P larvae at week 9, E larvae at week 13, and C larvae at week 15, as indicated by final data points. The bars in each curve represent the standard error (±SE) from the mean weight. Significant differences ($p < 0.0001$) in larval weight at weeks 9, 13, and 15 are indicated by ***.

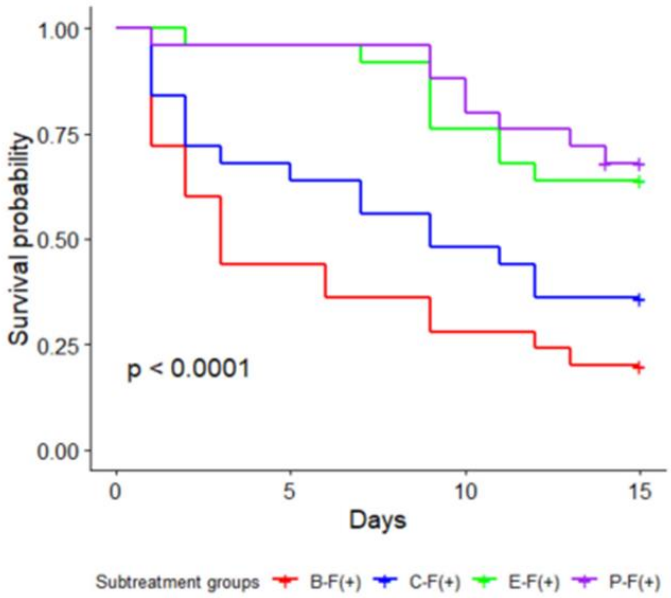

Figure 3. Kaplan–Meier curve showing the proportion of alive Tenebrio molitor larvae recorded over two weeks in four probiotic treatment groups. Blue: control (C); red: *Bacillus subtilis* (B); green: *Enterococcus faecium* (E); and violet: *Pediococcus pentosacceus* (P). Larvae were exposed to the fungus *Metarhizium brunneum* strain KVL 12–37 and observed for 14 days as a part of the fungal pathogen challenge experiment. Highest mortality was observed for fungus-treated larvae fed with *B. subtilis* bacterium, which was significantly different from the two probiotic treatment groups but not from the control group. The least mortality was observed for larvae supplemented with *P. pentosacceus* strains, which was significantly different from *B. subtilis* treatment groups but not from *E. faecium* and control groups. Different letters at the right end of the curves indicate significant differences ($p < 0.05$, Bonferroni adjustment for multiple comparisons) between the corresponding treatment groups.

Those results remain a starting point for the development of novel disease preventive measures in insect mass production systems. Ongoing work has confirmed that *P. pentosaceus* can confer a level of protection in *T. molitor* larvae exposed to both fungal and bacterial pathogens, but our understanding of the mechanisms by which this is achieved remain obscure (Dahal et al, 2022). Furthermore, research found that *P. pentosaceus* did not confer any advantage or protection in another species, namely, the black soldier fly, *H. illucens* (Gorrens et al, 2023). Finally, it is too early to predict which pathogens will cause most problems in insect facilities and whether probiotics will become an economically viable solution in the future. Other avenues of research such as breeding for enhanced disease resistance or the development of techniques such as immune priming should continue to be considered and evaluated in future projects.

2. Insect pests

2.1 Introduction

At the start of the SUSINCHAIN project a survey was conducted to assess the problem of pest species in the insect farming sector. The results indicated that 70 % of the farmers had problems with pest species in their farm. When the results are assessed in more detail it is clear that there are 2 major pest species. From the farmers that had issues with pest species, 44 % had problems with flies either *Musca Domestica* or Drosophila sp. and 44 % had issues with meal moths (Ephestia or Plodia). Although in 60 % of the cases the pest species were described as nothing more than a nuisance, the other 40 % did indicate a loss of productivity or loss of product quality.

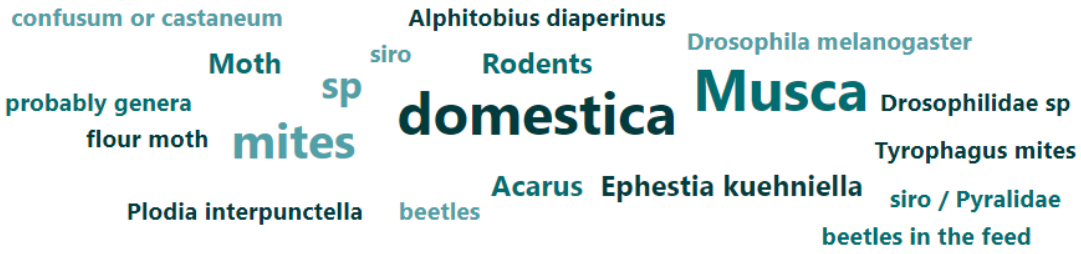

Figure 1. The results on pest species from the SUSINCHAIN Survey.

2.2 BSF farmers

As mentioned above, housefly and fruit fly infestations are the main problem for BSF farms. Co-rearing the flies with BSF will result in feed competition and degraded quality of the final product. Besides that adult flies are a huge nuisance and, especially houseflies, are known that they can transmit diseases (Khamesipour et al. 2018). An comprehensive review has been written on the general control of *M. domestica* by Malik et al. (2007).

As they are all Diptera, it is difficult or impossible to use chemical treatments. Prevention of the infestation is, obviously, the best strategy. Although total prevention may be difficult, with proper management the pest species can be kept under control. Those can include:

1. Location of BSF farm not within 500m of a housefly hotspot (e.g. landfill).
2. All entrances (doors and windows) covered with a mesh or air curtain (Carlson et al., 2006).
3. High hygienic standards inside the facility to avoid proliferation in e.g. spilled feed or garbage bins

Finally, good rearing practices can prevent unwanted fly intrusions. Previous research indicated that BSF can outcompete the other flies, yet from personal experience at the Insect Research Centre (IRC) of Inagro it is clear that this competition is only successful when they reach a certain age. Therefore, the neonates (0-5 days old larvae) are placed in a separate cage with a fine mesh ensuring that both the house fly and fruit fly cannot lay any eggs in the neonate crates. Only after 5 days the neonates are moved to the main rearing chamber without protection. Since implementing this method at the IRC, no intrusions of either fly were observed in the last 3 years. This indicates that the prevention of intrusion at the neonate stage is a suitable way to control the fly population.

When flies are present during insect larvare production, there is a need to control the population. There are a few options but the electrocuting insect trap is not one of them. It is fairly ineffective and produces airborne particles due to the high voltage disintegration. The resulting fly dust may impose some health risks. Sticky tapes do not pose this problem and the proper use can reduce the house fly population strongly, certainly in combination with an insecticide on the glue (Geden, 2006; Kaufman et al., 2005). A downside is that they need to be replaced frequently in order to stay effective (Kaufman et al., 2001). A last measure is the use of fly swatters, although this may be effective it is, very time-consuming. Other, biological, control agents could be possible but have not been assessed as they are not commercially available. More on those agents can be read in annex 1.

2.3 Mealworm farmers

In mealworm farms, the main pest species are meal moths. The most infamous are *Ephestia kuehniella* (Mediterranean meal moth) and *Plodia interpunctella* (Indian meal moth). In both literature and experience at the IRC there seems to be no direct negative effect on the mealworm population and based on the biology this is also not expected. Yet there are many indirect effects on the mealworm farm as meal moths can:

1. Be a general nuisance;
2. Potentially carry entomopathogens;
3. Decrease the substrate (e.g. wheat bran) quality;
4. Disrupt automation (e.g. sieving) with as larvae produce a lot of webbing;
5. Be a potential health hazard towards allergens

Similar to the house flies, prevention is important. This means that all doors and windows should have either a mesh or an air curtain. Ensuring high hygienic standards in order to prevent the proliferation of the meal moth in spilled feedstock, garbage or other places.

Based on a **literature review made for SUSINCHAIN: "Meal moth pest management in an insect farm"** (annex 2) several promising control techniques were suggested:

1. Climate
2. Chemical
3. Biological
4. Mechanical

At the IRC different methods to control or eradicate the meal moth (both *Plodia* and *Ephestia*) were assessed on a pilot scale. Yet none of the methods resulted in a complete eradication of the pest species.

2.3.1.1 Control via photoperiod

Cymborowski and Giebułtowicz (1976) indicated that *E. kuehniella* males were unable to successfully fertilize the females when they were exposed to 18h light in their 6^{th}-7^{th} day of pupal development and similar effects have been seen in other moths. Mbata (1985) saw a 50 % decrease in oviposition with continuous light compared to the control (12-12 or dark). At the IRC we have implemented continuous light in two of our climate rooms but at pilot scale no change in the moth population density was observed with or without light. This may be due to the fact that the feed layers are quite thick which ensures darkness inside the feed and the vertical farming system also provides many shaded areas.

2.3.1.2 Control via pheromones

Pheromones can be used to lure adult male meal moths to a trap or disrupt mating when used in a high concentration. In both cases the commercially available (Z,E)-9,12-tetradecadienyl acetate (ZETA) is used. At the IRC both techniques were tested on a large scale. However, in contrast to what was expected, this yielded limited results with only a handful of moths captured in the sticky traps. After discussing this with the manufacturer, the most likely cause of this inefficiency is the high ventilation rate in our facility compared to warehouses which reduces the ZETA concentration and renders it useless.

2.3.1.3 Control via active mechanical removal

To date, the active mechanical removal of the moths is the most effective method to use at the IRC. It consists of 2 actions. Firstly, there is a daily round of vacuuming. Each working day the newly hatched moths are vacuumed away from walls and ceilings. For this we use the highly portable Makita VC4210M although any portable vacuum will do. Although this reduces greatly the infestation, it is not 100 % effective as moths may remain between boxes. Secondly, every 1 or 2 weeks all crates are sieved to remove the webbing reducing the number of caterpillars and finally adult moths.

Although this technique works, it is not very efficient. It demands a lot of time and a great deal of dedication. Any reduction in the counter measurements (e.g. during Christmas break) enviably results in a massive outbreak that again takes weeks to control.

2.3.1.4 Control via biological agents

Based on the literature search, it is clear that some parasitoid wasps are commercially available that specifically target pyralid moths. Based on this information, two species were assessed at the IRC: a *Trichogramma sp.* and *Habrobracon hebetor*. *Trichogramma* wasp larvae feed on the eggs of pyralid species and although promising, this species was less useful than expected due to its limited flying ability. This means that when added to a crate, they did not disperse well into the farm and the population died off quickly. *H. hebetor* is a strong flyer which can easily spread throughout the climate room. It did reduce the meal moth population and remained present in our climate room for several months. However, when used in combination with the active mechanical removal, the moths were kept at such low concentrations that our wasp population disappeared after a few generations and new wasps needed to be introduced. Although *H. hebetor* is not able to eradicate the moth population it is able to slow the growth and as no manual labour is involved this is a good method to implement at the facility.

Finally, the use of *Bacillus thuringiensis* (BT) was assessed. Certain BT strains are known to affect only very specific groups of insects and do no (or limited) harm to other species. Because they are currently not authorized to be used in insect farming, this experiment was not done on the full pilot scale but on a smaller experimental scale. Within the experiment, we assessed the impact of *E. kuehniella* and *P. interpunctella* on the growth of the mealworm.

The results indicate that the BT toxin was not totally harmless to the mealworm larvae as a small decrease in growth was observed at the highest assessed concentrations (3 % at 500 ppm and 10 % at 1000 ppm). When both meal moth and mealworms were present simultaneously, none of the moths survived even in the control hinting at least that mealworms can predate on the meal moth and so that actively growing larvae are able to outcompete the moths. Both *P. interpunctella* and *E. kuehniella* were significantly negatively affected by the BT toxin with a near total eradication at 125ppm (1 surviving *E. keuhniella*). With eradication at 125 ppm and adverse effects on mealworms starting only at 500 ppm it is certainly a promising contender to be used

in the meal moth eradication. Yet, further work is needed if different application methods may reduce the lethal concentration and if the larvae do not have adverse but sublethal effects.

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SUSINCHAIN
SUSTAINABLE INSECT CHAIN

Annex I: Musca domestica pest management in an insect farm

Literature review: Inagro

1. Introduction

The housefly, both larvae and adults, can feed on a myriad of different feedstuff including, but not limited to, manure, decaying feed and carcasses. The lifecycle has 4 distinct stages: egg, larvae, pupae and adult and the time it takes from egg to adult is very short in a hot environment (10 days at 28°C).

It is best to avoid or minimize the occurrence of *M. domestica* in a BSF farm. Not only are houseflies known to transmit numerous diseases to humans (Khamesipour *et al.* 2018), but they may also transmit diseases, parasites, fungi, etc. to the black soldier fly colony. Besides the transfer of diseases, they may also co-breed in the BSF rearing crates, especially with the first instar BSF larvae and thereby degrade the quality of the final product.

2. Prevention

Preventing an infestation or explosion of houseflies is always preferred. Although it may not be always possible to prevent a single fly from entering the facility, proper management will prevent an all-out infestation or explosion of the housefly population.

The first consideration is your location. According to the WHO, house flies tend to remain within a radius of 100 to 500 m from their breeding site. Therefore, if no location is chosen yet for the BSF farm, it is advised to choose one more than 500 m away from a possible housefly hotspot (e.g. a landfill).

The infrastructure itself should be adapted to block the entrance for the house fly as much as possible by using tight-fitting windows and (self-closing) doors. Screens with a maximum mesh size of 2.3 mm can be used for windows or doors that open frequently, antifly curtains can be used if they are heavy enough and overlap sufficiently (WHO 1991). When doors remain open or any screens are inconvenient, a well-designed air curtain can be used (Carlson *et al*., 2006).

Inside the facility, all measures should be taken to improve environmental sanitation and hygiene to avoid proliferation of the houseflies in or near the facility. This can include (but is not limited to) tightly closed garbage containers inside and outside, removing any spilled BSF feed or organic material, avoiding any decaying material close to the facility, ensuring a good sewer system, etc. Those same measures should be taken for the feedstock as they (could) enter the facility as eggs/larvae in the feedstock for the BSF. Either try to avoid infestation between collection and use (e.g. use closed containers for transport) and/or ensure a proper treatment of the feed before it enters the farm.

Finally, the general health of the BSF colony and good rearing practices already reduce the risk of an infestation as female house flies are less likely to oviposit in a substrate with a high density of BSF larvae (Bradley and Sheppard, 1984). Based on personal experience, the first few days the BSF larvae crates are still susceptible to infestation but this is rare after 5 days in a healthy and densely populated crate. Provide vulnerable stages, e.g. the eggs and neonates, with extra protection by placing them within a cage or in a separate room.

These preventative measures have the added benefit that also other insect species are locked out or minimized in the facility (e.g. cockroaches, mice, …).

3. Control

There are several options to control pest species described in the literature:

- via unfavourable climate conditions

- via mechanical control: light, electricity, swatting,…

- via biological control: viruses, bacteria, parasites,…

- via chemical control: insecticides, aromatic oils, …

3.1 Climate control

The housefly is able to develop from egg to fly (at least) between 16 °C and 34 °C with 12.4 °C as estimated temperature threshold (Fletcher et al, 1990; Wang et al., 2018). As expected, the time it takes to develop is significantly longer at lower temperatures. At 34 °C it takes a mere 6 days while it takes 24 days at 16 °C. This indicates that the housefly is perfectly capable of reproducing (fast) in the standard temperatures at a BSF facility and controlling the housefly population via temperature is unlikely.

3.2 Mechanical control

Controlling the house fly population can be accomplished through various physical means. One of the most frequently used methods is an electrocuting insect trap (EIT, combination of an attractive light source and an electrocuting grid). According to the WHO (1991), electrocuting insect traps (EIT) are not effective against *Musca domestica*. Furthermore, this method produces airborne particles when the insects are disintegrated by the high voltage and these particles may contain pathogens (Urban and Broce, 2000). Sticky tapes do not pose this problem and the proper use can reduce the house fly population strongly, certainly in combination with an insecticide (Geden, 2006; Kaufman *et al.*, 2005). A downside is that they need to be replaced frequently in order to stay effective (Kaufman *et al*., 2001). A last measure is the use of fly swats, although this may be effective, it is very time consuming.

A main advantage of using mechanical control is that many products are already on the market and it does not produce any resistance in the fly's body as observed in the case of chemical insecticides because these methods are used to kill or trap the flies directly.

3.3 Biological

There are several biological ways to address housefly larvae or adult flies with viruses, bacteria, fungi, parasites and predators. However, most of these biological control agents are, or may be, also harmful to other Diptera including the BSF. Because the two species are fairly closely related, research is needed to assess any influence on the BSF fly or larvae before it can be used in a BSF farm to combat the house fly.

Important: ensure that the release of any, non-indigenous, species is legal in your country.

3.3.1 Viral control

The house fly is prone to MdSGHV or Salivary Gland Hypertrophy virus (Coler *et al*., 1993). Infection results in a shorter life span and reduced mating success (Lietze *et al*., 2010). Currently, this is not commercially on the market.

3.3.2 Bacterial control

Bacillus thuringiensis is a widely used biopesticide. The specific strain and application method is very important if it has any effects on the flies, larvae or neither one of them (Lonc *et al*. 2001, Indrasith, 1992) . For example, the pathogenicity of *B. thuringiensis kurstaki* H3a3b3c was found to be substantially high for house fly larvae, as it results in 50 to 80 % mortality of house fly larvae.

3.3.3 Fungal control

Fungal control can be achieved by using baits infected with the fungus and in theory, this would prevent the direct contact between the pathogenic fungi and the BSF. However, one has to ensure that the infected (house)flies cannot transfer the fungi to the BSF flies or larvae. There are several pathogenic fungi known that can infect and kill houseflies:

Entomophora Muscae has optimal temperature of 21 °C and less effective at higher temperatures (max between 26.7 and 32.2 °C, Carruthers and Haynes, 1986) although deadly it may not be effective as biocontrol agent as the infection may not be able to self-propagate effectively. *Metarhizium anisopliae* can be used in the form of a bait. The data of Renn *et al.* (1999) indicates that 80-90 % of the flies die after 6 days and 90 to 100 % after 10 days (150 flies in 10 m^3). When used on the larvae, only 16 % emergence was observed at 10^5 conidia/mL and 0 % emergence at 10^7 conidia/mL (Barson *et al.*, 1994). A positive property is that this fungus thrives at high humidity and temperature (25 °C-30 °C), therefore *M. anisopliae* may be more suited to use than *E. Muscae*. (Carswell *et al.* 1998). *Tolypocladium clindrosporum* can be used to kill larvae as there was 0 % emergence at 10^5 conidia/mL (Barson *et al*., 1994). Finally, *Beauveria bassiana* could be used to control flies (Watson *et al*., 1995, Geden *et al*., 1995) and is a cheap fungus to produce with a long shelf life. However, it is known to have a very broad host spectrum and therefore also affects BSF (Lecocq *et al*., 2020).

A general downside for using fungi on adult flies is the time it takes between infection and death (frequently 6 to 9 days). Female flies could be able to lay most of their eggs before they die. Furthermore, no commercial mycoinsecticide is available at this time.

3.3.4 Parasites, parasitoids and predators

The use of parasites or predators is already a common practice in, greenhouse, agriculture (e.g. ladybugs to control aphids). As with most biological control agents, we will need to find a very species-specific parasite or predator that makes the distinction between housefly and BSF and is unlikely to switch. When it exists it would greatly reduce the need for toxins (both chemical and biological) and reduce the workload compared to the mechanical control measures. To our knowledge, the species currently on the market for houseflies (or any other species) have not yet been assessed for adverse effects on BSF.

Some entomopathogenic nematodes are known to infect and kill the housefly within a few days and may infect both the larval and adult stage. The nematodes can be added to a bait, but the type of bait is important (Renn *et al*. 1998 and 2000). Several parasitoids (species that lay their eggs inside the host) are known to infect the housefly. For example, in Denmark they found *Spalangia cameroni* and *Muscidifurax raptor* (Skovgard and Jespersen, 2000). However, the natural parasitism rate remains low (5-13%). The results after release into a farm are not consistent (Mckay and Galloway, 1999; Skovgard and Nachman, 2004) and may depend on the species used but also the local climate. Finally, predators (species that eat the house fly) could be used in the future, but many predators are not species-specific. One of these species is the black dump fly (*Hydrotaea aenescens*) which can consume up to 17 *M. domestica* larvae.

3.4 Pesticides

3.4.1 Botanical

Botanical extracts can be used as a natural insecticide or just repel the insect. In general botanical pesticides are more species-specific and therefore less harmful to non-target organisms compared to chemical pesticides. Similar to chemical pesticides, the effect of plant oils on the flies varies with the sex and the developmental stage of the house fly as well as with the mode of application. Some examples that are published are: Eucalyptol (EC50: 118-177 µg/fly; Sukontason et al., 2004), *Matricaria chamomilla* oil (EC50: 76 µg/fly), *Clerodendron inerme* oil (EC50: 84 µg/fly; Shoukry, 1997), and Neem leaf extract (EC50: 8.4 µg/fly; Khan and Ahmed, 2000). *Ocimum gratissimum*, *Thymus serpyllum* L., *Illicium verum* Hooks.f., *Myristica fragrans* Houtt. and *Curcuma amada* Roxb. show 100 % repellency (Dwijendra and Sucheta, 1998). As with most control measures, the impact on BSF is unknown.

3.4.2 Chemical

Obviously there are many chemical insecticides on the market that can kill larvae, pupae or adult houseflies. As most or all of these are expected to affect the BSF population and there is a tendency to avoid insecticides in the insect industry we will not go into detail on which products are available on the market. Besides the major threat to BSF production when chemicals are (mis)used, resistance to the chemicals may be(come) a major problem (Shad and Akram, 2013; Tomberlin et al., 2002)

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SUSINCHAIN
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Annex II: Meal moth pest management in an insect farm

Literature study: Inagro

1. Introduction

Several species of 'Meal' moths are known as pest species in the stored food/feed industry such as *Plodia interpunctella* or the Indian meal moth and *Ephestia kuehniella* or the Mediterranean flour moth. A questionnaire prepared by Inagro and filled in by several EU insect companies within the framework of SUSINCHAIN indicated that meal moths were seen as the most prevalent pest species in mealworm farms. Ironically, mealworms are historically seen as a pest species in stored products but are currently also seen as possible feed or food sources.

In this emerging industry, the moth population is able (and known) to explode fast as the climate conditions are near optimal with high-quality feed presented in thin layers resulting in a huge surface area for the moths. Yet, currently, there is no scientific information on the (economic) effects of meal moths in a mealworm facility.

This literature study intends to gather the existing information from the stored product industry on the control of *P. interpunctella* and *E. kuehniella* and to assess whether or not they are feasible to implement in the mealworm industry.

2. Problems related to meal moths

There is little to no data available on the direct effect of the coexistence of moth larvae and mealworm larvae. Based on the biology of the two species, one would not expect a direct negative effect of the moth larvae on the mealworm larvae (via predation, parasitism, etc.). This does not mean there are no potential issues as they do compete for the same feed (e.g. wheat bran) and it is not inconceivable that they may serve as a vector of entomopathogens.

From the stored product industry we do know that, besides degrading the quality of the end product (e.g. polluting the mealworm protein with moth protein), the webbing of the larvae and pupae may disrupt sieving and processing lines as they clog the machinery and are a general nuisance in the factory. Furthermore, they may be a health hazard to the employees as it is possible to become allergic to the meal moth and although the prevalence is unknown it should not be underestimated. For example, Haahtela (2003) confirmed a case of IgE-mediated occupational respiratory allergy to *Ephestia kuehniella* and Armentia *et al.* (2009) indicated that wheat infested with meal moth had a higher allergenic potential than uncontaminated flour. Finally, it is possible that people with an allergic response to other arthropod species (e.g. dust mite) or shellfish may be more prone to meal moths (in this case *P. interpunctella*) or vice versa due to the cross reactivity of the allergens (Binder *et al.* 2001).

3. General life cycle

Similar to the mealworm, meal moths have a lifecycle with 4 distinct stages: egg, larva, pupa and adult. As with all insects, the climate and feed influence the development time and fecundity (Kurtulus *et al.*, 2020; Mbata, 1985; Mohandass *et al.*, 2007). Therefore the numbers below are only an indication and may be different at your location.

Brindley (1930) assessed the life cycle of *E. keuhniella* in depth (29.7°C, 60-70 % RH). Adult moths emerge from their pupa and the next night females start laying eggs with two-thirds on the first

2 days and a total average of 167 eggs (range: 25-352 (Brindley, 1930) or 176-293 (Kurtulus *et al.*, 2020)). Adults moths do not feed and live only 6-7 days when mated and 10-11 days when unmated. Eggs are preferably laid on the substrate, but if this is not possible due to an obstruction they will lay eggs near the feed. The eggs hatch in just 4 days. Freshly hatched *P. interpunctella* caterpillars can walk 38 cm to the nearest feed (Mohandass *et al.*, 2007). The newborn larvae feed and grow for 29 days. When fully grown, the larva crawls to the surface and spins a cocoon of silk and feed particles to pupate. After 8 days a new moth emerges from the pupa with a total generation time of 41 days (± 2.4 days, egg to adult). Similar to *E. keuhniella*, *P. interpunctella* can also reproduce in as brief as 22.6 days (on bran at 28.3°C) up to 60 days (20°C) and lay on average 212 eggs (Johnson *et al.,* 1992; Mohandass *et al.,* 2007; Perez-Mendoza and Aguilera-Pena, 2004).

Good growing mealworms will be harvested the earliest 64 days after the eggs were laid, but more likely in the range of 77 days. The generation time of both moth species is short enough to complete at least one generation during the larval period of the mealworm. This happens certainly because mealworms are generally bred at 26-27°C and the temperature inside a densely populated crate can easily go a few degrees higher (Inagro, unpublished results).

4. Prevention

Preventing an infestation is always preferred, although it may not always be possible to prevent a single (female) moth from entering the facility. Proper management may prevent an all-out infestation or explosion of the population or continuous/yearly reinfestation from outside.

The infrastructure itself should be adapted to block the entering as much as possible by using tight-fitting windows and (self-closing) doors. Screens or curtains can be used if they are heavy enough and overlap sufficiently (WHO 1991). When doors remain open or any screens are inconvenient, a well-designed air curtain can be used (Carlson *et al*, 2006) although the efficiency has not yet been assessed for the meal moth.

Inside the facility all measures should be taken to improve environmental sanitation and hygiene to avoid proliferation of pest species in or near the facility. This can include (but is not limited to) tightly closed garbage containers inside and outside and removing any spilled feed or organic material. Those same measures should be taken for the feedstock as the meal moths (could) enter the facility as eggs/larvae in the feedstock. Either try to avoid infestation between collection and use (e.g. use closed containers for transport) and/or ensure a proper treatment of the feed.

These preventative measures have the added benefit that also other pest species are locked out or minimized in the facility (e.g. cockroaches, mice, …).

5. Control

Based on decades of experience and research into pest species in grain mills, bakeries and storage sites for grain products it is clear that complete prevention is, in the long run, near impossible. However, several measures could and should be taken to control the moth

population. Because of the potentially large economic losses in stored products due to a meal moth infestation, there is already plenty of research to control the population of those species:

via climate control

via biological control: viruses, bacteria, parasites,…

via pesticidal control: insecticides, aromatic oils, …

However, certainly not all described techniques are applicable in the insect industry as some may also harm mealworms and can only be used for the storage of the feed for the mealworms or the dried insect flour.

5.1 Climate control

Controlling the moth population via high and/or low temperatures, has been frequently investigated (Lewthwaite *et al*., 1998). However, it seems unlikely that this is a viable strategy within an insect farm except for the storage of feed or potentially insect flour. For example, killing the eggs of *P. interpunctella* required more than 10 hours at 42°C and almost 20 days at 0.5°C (Lewthwaite *et al*., 1998). Another study indicated that the eggs of *P. interpunctella* should be more prone to low temperatures with 95 % mortality after 'only' 12 days at 10 °C (Johnson *et al.* 1997). In the case of *Ephestia keuhniella*, 10 weeks at 10°C were needed for a complete eradication of the population (Ayvaz and Karabörklü, 2008). Nevertheless, the recommended temperatures and times would be detrimental to the mealworm production. On the one hand, mealworm larvae die at 42°C after 22.5 to 104 minutes, on the other hand at 10°C they do not die but growth will be severely reduced (Punzo and Mutchmor, 1978 and 1980). A more practical method for killing incoming moths via the feed could be radiofrequency treatment for 5 minutes at 52 °C (Johnson *et al*., 2003).

Besides the temperature, it may be possible to partially control the population of moths by changing the photoperiod (Cymborowski and Giebułtowicz, 1976). It has been observed that male of *E. keuhniella* were unable to fertilize females when they were exposed to 18 h light : 6 h dark on their 6th to 7th day of pupal development. Similar effects have been seen in the gypsy moth (*Lymantria dispar*, Giebultowicz and Ridgway, 1990). This method is very specific towards timing and life stage and more research is needed to know why this happens and assess if this is a viable option in insect rearing. A method that could be implemented rapidly is using continuous light as this would reduce oviposition by 50 % by *P. interpunctella* compared to darkness or a 12-12 cycle (Mbata, 1985) but the effect should be assessed on an industrial scale and on mealworm production.

5.2 Biological

There are several biological ways to control moths with viruses, bacteria, fungi, parasites and predators. However, some of these biological control agents are, or may be, also harmful to the mealworm.

Important note: ensure that the release of any, non-indigenous, species is legal in your country.

5.2.1 Pheromones

Pheromones are semiochemicals and are generally considered safer than more conventional techniques as they are specific with a low toxicity to other species and due to the volatility they do not leave behind residues. It is one of the major ways to combat the different meal moth species (Burks *et al*. 2011; Trematerra 2012; Trematerra *et al*. 2013). Research indicated that *P. interpunctella* produces a blend of pheromones (Z9,E12–14 : OAc, Z9,E12–14 : OH, Z9,E12–14 : Ald, and Z9–14 : Oac). However, the use of a single component, (Z,E)-9,12-tetradecadienyl acetate commercially known as TDA or ZETA, works just as good as the mixture (Ryne *et al*. 2001). Furthermore, this is a multi-pheromone that is produced by several *Pyralidae* like *E. kuehniella* (Anderbrant *et al*., 2009).

There are two main, not mutually exclusive, ways to use pheromones, but treatment may take months before a decrease in the population is observed (Trematerra *et al*. 2013). Therefore pheromones are more suited to control low levels of infestation. For a thorough review read Trematerra (2012).

5.2.1.1 (mass)Trapping

The mass trapping is a combination of mechanical and biological control. The male moths are lured to the trap via the pheromones and then trapped in various ways such as sticky traps (sometimes in combination with an insecticide). Several studies have reported success in the control of E. *kuehniella* (Athanassiou *et al*., 2003; Trematerra and Gentile, 2010) and *P. interpunctella* (Campos-Figueroa, 2009).

Because this technique only affects the males and a single male (*P. interpunctella*) can mate with up to 10 females in its lifetime (Brower, 1975) this means that 90 % of the male population can be trapped without affecting the population size. A well-designed layout of the traps is therefore necessary.

5.2.1.2 Mating disruption

The limitations and theoretical bases of mating disruption are similar to those for mass trapping: a substantial proportion of the male population has to fail to locate females, and therefore success of this method is more likely under relatively low population levels. Several studies have been able to indicate a clear decline in matings/population. A 90 to 95 % reduction in mating of *P. interpunctella* was observed in a small room with low densities by Sower *et al*. (1975) and Fadamiro and Baker (2002) indicated that pheromone puffs suppressed the mating in an infested corn store. Similar results were found for *E. keuhniella* (Sieminska *et al.*, 2009, Trematerra *et al.*, 2010, Trematerra *et al.*, 2013). Fortunately, Svensson *et al.* (2002) indicated that the selection to resistance to the pheromone is weak.

5.2.2 Viral control

The use of the *P. interpunctella* granulovirus (PiGV) was described in 1968 by Arnott and Smith and is currently registered as biopesticide in the USA. The virus is capable of reducing the number of moths when applied to the stored product (Johnson *et al*., 2002). However, no information was found on the efficiency in a large-scale industrial setting.

5.2.3 Bacterial control

Both *E. kuehniella* and *P. Interpunctella* are susceptible to *Bacillus thuringiensis* if the appropriate strain is used (Azizoglu *et al*., 2011), but care should be taken as there is a possibility for increased resistance (Mahbubur Rahman *et al*. 2003, McGaughey and Johnson, 1992). Using the inappropriate strain may result in adverse effects on the mealworm population (Oppert *et al*., 2011 and 2012)

5.2.4 Fungal control

Several entomopathogenic fungal species can infect meal moths such as *Beauveria bassiana*, *Lecanicillium lecannii*, *Metarhizium anisopliae*, *Isaria fumosorosea* and *Paecilomycers farinosus* (Būda and Pečiulytė, 2008; Sedehi *et al*., 2014; Sabbour *et al.*, 2012). However, a laboratory experiment by Būda and Pečiulytė (2008) indicated that the time to kill half the adults is at least two days. If this holds true in an industrial setting, this would mean that most females would be able to lay most of their eggs before succumbing to the fungus. Furthermore, this technique would require a very species-specific entomopathogenic fungus species/strain. Several strains of *B. bassiana* and *M. Anisopliae* are known to be able to infect *T. molitor* (Rodríguez-Gómez *et al.*, 2009; Orste *et al.*, 2012).

5.2.5 Parasites, parasitoids and predators

5.2.5.1 Parasites

Some entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) are used frequently to combat stored product pest species and are known to infect *P. interpunctella* and or *E. kuehiella* (Athanassiou *et al.*, 2008; Mbata and Shapiro-Ilan, 2005; Ramos-Rodriguez *et al.*, 2006 and 2007). However, nematodes are not species-specific enough and can also infect beetles such as *Tribolium* (Athanassiou *et al.*, 2008; Ramos-Rodriguez *et al.*, 2007) and/or *Tenebrio* (Ramos-Rodriguez *et al.*, 2006).

5.2.5.2 Parasitoid

5.2.5.2.1 Trichogramma sp.

Egg parasitoids of the genus *Trichogramma* are known to parasitise the eggs of pyralid pest species and to successfully control the population. For example: the effect of *Trichgramma cacoeciae* Marchall, *T. Evanescens* Westwood, *T. turkestanica* and *T. Brassicae* Bezdenko on E. *Kuehniella* Zeller has been assessed (Hansen and Jensen, 2002; Özder and kara, 2010, Steidle *et al.*, 2001). The number of parasitized eggs varied between 79 and 109 with a generation time of approximately 8 days (at 25 and 30 °C). Also for *P. interpunctella* a reduction in population size was observed after release of *Trichgramma sp.* (Brower, 1988; Brower and Press, 1990; Grieshop *et al.,* 2006, Grieshop *et al*. 2014). *Trichogramma sp.* are already commercially available today and used in a large scale. However, research is needed to assess the potential sublethal effects of *Trichogramma sp.* on the eggs of *Tenebrio molitor*.

5.2.5.2.2 Habrobracon hebetor

H. hebetor is a larvae parasitoid that can parasitise many species within the *Pyralidae* family. It has a short life cycle of only 13 days on both *E. kuehniella* and *P. interpunctella* (Eliopoulos and Stathas, 2008) at 25 °C but can be as low as 9 days at 32 °C (Kim *et al.* 2000). The research of Dabhi *et al.* (2012) indicated that the intrinsic rate of natural increase, in a laboratory

environment, was always higher for *H. hebetor* compared to *E. kuehniella* and *P. interpunctella* and is, therefore, able to control the moth population. Reinert and King (1971) indicated that even a 97 % mortality of *P. interpunctella* was possible at the ideal climate for rearing *T. molitor* (27 °C and 60 % humidity), albeit at a very high ratio of 1 wasp for every 7 moth larvae and in laboratory conditions.

5.2.5.3 Predator

According to LeCato and Flaherty (1973), the inclusion of dead eggs or adults of *P. interpunctella* increased the development rate and progeny production of *Tribolium castaneum* when the feed was substandard. However, it is currently unknown what the influence is of *T. molitor* on a meal moth infestation. Is it, for example, possible to partially suppress the moth population by using high-density mealworm crates?

5.3 Pesticides

5.3.1 Botanical

Similar to the entomopathogenic nematodes, etherical oils (EO) (e.g. Eucalyptus) have been shown effective against meal moths via fumigation killing the moths or reducing the fecundity (Jesser *et al.*, 2007). However, as nematodes, they may also influence other insect species. Several studies indicate that *Tribolium confusum* is more sensitive than *E. kuehniella* and *P. interpunctella* (Erler, 2005; Isikber *et al*., 2009; Salaheddine, 2013) but not always (Lee and Lee, 2016). More research is needed, to find an EO that does affect meal moths but not *Tenebrio molitor*.

5.3.2 Chemical

Obviously there are several chemicals that have been or could be used but this is outside the scope of this study.

6. Integrated pest management

Although the different options to control the meal moths are presented in different separate parts, they are not always mutually exclusive, and multiple solutions can be utilized to ensure an optimal result. Several studies already investigated the use of different combinations in stored products, for example, the combination of the oil of *Salvia officinalis* and the release of *H. hebetor* (Asadi *et al.* 2018) or entomopathogenic nematodes and *H. hebetor* (Mbata and Shapiro-Ilan, 2010). The latter combination resulted in a mortality of more than 98 %. However, there is a high chance that *S. officinalis* or the nematode have an adverse effect on the mealworm colony. A potentially more readily applicable option was assessed by Schöller and Prozell (2001) by using the combination of *T. Evanescens* and a phereomone baited trap. Although not assessed on a large scale, their research suggests that *T. Evanescens* is not attracted to the pheromone and could therefore be used in parallel. Another option is the combined use of an egg parasitoid (*Trichogramma spp.*) and a larval parasitoid (*H. hebetor*). Two studies assessed this combination. Brower and Press (1990) saw a 37 or 66 % population reduction when the species were used separately and 84 % when the combination was used.

Grieshop *et al.* (2006) observed a mortality rate of up to 97 % when both species were present in bagged cornmeal compared to 87 % or 71 % when present individually.

7. Conclusion

There are several ways currently in use to control *P. interpunctella* and *E. kuehniella* in stored products. However, some such as nematodes and essential oils are very likely to also be detrimental to the *T. molitor* population. The use of pheromones and/or lepidopteran parasitoids seems a promising option based on the current available literature as it is unlikely to affect the mealworm population due to the specificity. All these viable options are best used at low infestation levels. Yet, low-level infestations can quickly explode in a mealworm farm due to the favourable climate and abundance of feed. Therefore it is advised to ensure prevention as much as possible and to monitor any potential infestation (e.g. via a pheromone trap). Start with controlling the population as soon as they are observed.

8. Future research

It is clear from the review above that there are many options to control the meal moth population. Yet the effect of most currently used options on the mealworm is unknown at this point. Based on the available literature the ZETA pheromone, *Trichogramma sp.* and *H. hebetor* all seem very specific towards pyralid moths. Therefore, future research should focus first on one of these options (or a combination).

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