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Harmful and beneficial symbionts of *Tenebrio molitor* and their implications for disease management

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REVIEW ARTICLE

Abstract

The yellow mealworm, *Tenebrio molitor*, is currently one of the most important insect species produced for livestock feed and human consumption. High-density rearing conditions make the risk of disease and infections by parasitic symbionts a challenge in the mass production of these insects. However, certain symbionts are beneficial and should be favoured in order to promote healthy insect populations. Knowledge of parasitic symbionts and their management is essential for the insect rearing industry and its associated research. Here we review the documented microbial infectious agents, invertebrate parasites, and beneficial symbionts occurring in *T. molitor*. Furthermore, we discuss detection, prevention, and treatment methods for disease management in *T. molitor* production systems to inform future management and decision making in *T. molitor* rearing.

Keywords: mass-rearing, insect diseases, beneficial microorganisms, entomopathogens, probiotics, yellow mealworm

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1. Introduction

The insect rearing industry has grown rapidly in recent years to meet the global demand for alternative and sustainable sources of feed and food (Francuski and Beukeboom, 2020). In 2017, 6,000 tons of insects were produced for animal feed in Europe alone (Derrien and Boccuni, 2018) and the global production of insects for food and feed is estimated to reach up to 500,000 tons by 2030 (De Jong, 2021). The yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), is one of the major insect species produced on a large scale due to its high protein and fat content, its efficient feed conversion rate and its comparatively simple rearing process (Costa *et al.*, 2020; Grau *et al.*, 2017b). Such characteristics make *T. molitor* an ideal candidate for addressing societal issues like sustainable food production and hunger. Consequently, the European Food Safety Authority (EFSA) (Reg EU 2021/882, Reg EU 2015/2083) permitted in 2021 the processing and commercialisation of dried *T. molitor* larvae for human consumption in Europe (European Commission, 2021).

One important challenge in the mass-production of insects is the risk of diseases and infections in these systems (Eilenberg *et al.*, 2015, 2018; Van Huis, 2017). Mass-produced insects are generally kept at high densities. This favours the spread of microbial infectious agents and invertebrate parasites (either endo- or ectoparasites) between individuals if environmental conditions are conducive and no preventive measures are taken (Eilenberg *et al.*, 2018). Pathogens can be a major barrier in scaling up insect production, as well as an economic obstacle causing significant losses for insect production companies (Bhat *et al.*, 2009; Liu *et al.*, 2011). Furthermore, the ecological risk of pathogen spillover from mass-rearing facilities into natural insect populations further emphasises the importance of disease management (Bang and Courchamp, 2021).

Like in other animals, infectious diseases in insects are diverse and can be enigmatic, manifesting as lethal or sublethal, and as single or co-infections. Moreover, mutualistic and commensal symbionts can appear as etiological agents if their ecology is unknown, but in actuality are beneficial to the insect host, and in certain cases can protect the insects from disease (Lecocq *et al.*, 2021). Beneficial symbionts are therefore candidates for introduction as probiotics in mass-reared insects (Savio *et al.*, 2022).

To date, harmful and beneficial symbionts of *T. molitor* and their management have not been comprehensively reviewed. Understanding the variety of symbiotic interactions and their implications for disease outcomes is critical to the success of insect farming. Here we review the symbionts known to occur in *T. molitor*, and document the symptoms they cause, present detection methods, and discuss potential innovative treatment and prevention strategies.

2. Harmful symbionts

In this section, parasitic symbionts harmful to *T. molitor* are addressed, although it is worth mentioning diseased states can be induced by a range of non-infectious factors, *e.g.* malnutrition (Kaya and Vega, 2012). Here, we distinguish between 'microbial infectious agents' (bacteria, fungi, protists, viruses) and 'invertebrate parasites' (mites, cestodes, nematodes, and parasitoids). Microbial infectious agents that infect insects are often also referred to as 'entomopathogens' or 'insect pathogens' in the literature (Eilenberg *et al.*, 2015; Hajek and Shapiro-Ilan, 2018).

Infections in insects manifest as acute or chronic and covert or overt. The most obvious and easily observable are acute infections, which are of short duration and may result in sudden death of the host. Chronic infections, on the other hand, are less apparent and can often be overlooked due to sublethal effects and the long incubation period before death. Covert or latent infections, primarily observed in viral infections, are a dormant form of disease, presenting no visible symptoms in the host. These have the potential to become overt or acute infections when triggered by appropriate abiotic or biotic factors, e.g. environmental effects (Hajek and Shapiro-Ilan, 2018) or transfer from one host to another (Martin and Brettell, 2019).

Parasites can occur in different life stages of *T. molitor* given the vast difference in body composition, behaviour, and environmental requirements throughout the insect lifecycle. For this reason, we specify the life stage in which each pathogen or parasite has been identified in *T. molitor* (Supplementary Table S1). This classification should not be considered exhaustive, as bioassays may not have been performed on every life stage, rather it is the sum of current knowledge on *T. molitor* diseases.

Infections in insects can both be naturally occurring or experimentally induced. Given that the artificial environments in which *T. molitor* is mass-produced are ecologically novel relative to the evolutionary time scale on which symbiotic relationships have been formed, it is possible some parasites might be able to extend their ecological host ranges under these new conditions. We

Definitions of terms used in this review

Symbiosis	Refers to any ecological relationship between two species, whether beneficial or detrimental to either partner, and is further subdivided into different forms.
Mutualism	A symbiotic relationship in which both partners benefit from each other.
Commensalism	A symbiotic relationship in which one partner benefits and the other partner is neither harmed nor benefits.
Parasitism	A symbiotic relationship in which one partner causes harm to the other partner (Martin and Schwab, 2012).
Symbiont	An organism living in some form of symbiosis with another species.
Parasite	An organism living on or in a host organism, deriving nutrition at the expense of the host's health (Hajek and Shapiro-Ilan, 2018).
Pathogen	Microorganisms, including viruses, that have the potential to cause disease (Pirofski and Casadevall, 2012).
Facultative pathogen or parasite	Pathogenic or parasitic symbionts capable of surviving and reproducing outside of a host organism.
Obligate pathogen or parasite	Pathogenic or parasitic symbionts reliant on a host organism for survival and reproduction.
Ecological host range	The sum of all host species a parasite is capable of encountering and infecting in the natural environment; also refers to natural infection.
Physiological host range	The sum of all host species a parasite has been found to infect under laboratory conditions; also refers to experimental infection.

therefore include parasites that have *T. molitor* in their ecological as well as physiological host range to give a complete overview (Supplementary Table S1).

Microbial infectious agents

Bacteria

The microbial communities of *T. molitor* are characterised by the presence of a resident microbiota, mainly composed of the bacterial phyla of Proteobacteria and Firmicutes, which can be shaped by feed and environmental conditions (Urbanek *et al.*, 2020). Bacteria can exploit different relationships with insects from mutualistic to pathogenic interactions (Vallet-Gely *et al.*, 2008). Assurance of safety for food and feed necessarily raises several questions regarding bacterial pathogens, including susceptibility, persistence, and transmission of pathogens in the host organism. This is especially relevant in mass-rearing systems characterised by high-density conditions and the practice of using organic side streams as insect feed (Jensen *et al.*, 2020; Maciel-Vergara *et al.*, 2021; Urbanek *et al.*, 2020; Wynants *et al.*, 2019). *T. molitor* is not naturally associated with food-borne and environmental bacterial pathogens considered infectious to humans, reducing their risk of acting as a biological vector between different trophic levels (Urbanek *et al.*, 2020).

Infection mainly occurs via oral ingestion of bacteria, although insect haemocoel can be directly infected when exposed through injuries or damage from fungi or nematodes (Maciel-Vergara *et al.*, 2018; Vallet-Gely *et al.*, 2008). A pathogenic bacterial species of *T. molitor, Bacillus thuringiensis* var. *tenebrionis,* was first isolated from *T. molitor* in 1982 (Krieg *et al.*, 1983) and is commercially used in biocontrol of certain pest species from Coleoptera. This Gram-positive spore-forming bacterium belonging to the phylum Bacillota is well known for causing death of the larval stages of many insects. Mortality occurs via sepsis-related organ failure (Nielsen-Leroux *et al.*, 2012), when the insect gut is perforated through the action of bacterial pore forming toxins, followed by infection of the whole body. Bacterial spores then germinate and proliferate throughout the haemocoel. In the case of *T. molitor* larvae, the extensive gut leakage has not been correlated to the killing mechanism of *B. thuringiensis,* but reduced feeding behaviour has been observed in infected individuals (Zanchi *et al.*, 2020). Other bacteria, such as *Serratia marcescens* (phylum Pseudomonadota), act mainly as opportunistic pathogens, when the insects are already physiologically weakened (Dupriez *et al.*, 2022).

A first indication of the presence of pathogenic bacteria is the observation of reduced feeding behaviour and decreased movement. In the case of bacterial proliferation and sepsis-related organ failure, the insect cadavers change colour, presenting a flaccid consistency and a foul odour (Maciel-Vergara *et al.*, 2021). Specific insect colorations can sometimes be related to the presence of bacterial infection such as pink or red for *S. marcescens,* and dark colours for other bacterial species (Dupriez *et al.*, 2022; Eilenberg *et al.*, 2015). The application of molecular techniques such as Next Generation Sequencing (NGS) are recommended for identifying bacterial pathogens to the species level (Verma *et al.*, 2017).

Fungi

Several fungal species of the orders Hypocreales, Eurotiales, Capnodiales and Saccharomycetales have been shown to affect *T. molitor*. Most of the studies are based on experimental infections and focus on the application of entomopathogenic fungi as biocontrol agents for tenebrionid pest species. Fungal infections either lead to mortality of the insects or induce sublethal effects, such as a modification of the lipid composition (Gołębiowski *et al.*, 2020) or a negative effect on the number of offspring (Pedrini *et al.*, 2010) as shown in other species of Tenebrionidae. It is important to note that different fungal isolates from the same species can have highly variable virulence (Praprotnik *et al.*, 2021).

In insects, infections by fungi are typically transmitted when a spore from their surrounding environment encounters an appropriate insect host cuticle, and there is sufficient humidity and temperature for the spore to adhere and germinate (Vega *et al.*, 2012). The germinating spores penetrate the host cuticle by producing chitinase and induce mortality as fungal structures are produced throughout the host body (Vega *et al.*, 2012).

Within the order Hypocreales, members of the genera *Metarhizium* (Barnes and Siva-Jothy, 2000; Bharadwaj and Stafford, 2011; Keyser *et al.*, 2014, 2016; Korosi *et al.*, 2019; Mathulwe *et al.*, 2021; Moret and Siva-Jothy, 2003; Praprotnik *et al.*, 2021) and *Beauveria* (Korosi *et al.*, 2019; Lee *et al.*, 2014; Maistrou *et al.*, 2018) have been described to infect *T. molitor*. Many members of these two genera are generalist pathogens having many different host species (Maciel-Vergara *et al.*, 2021). They are highly relevant in production systems of *T. molitor* because they can be found in stored grains (Wakil *et al.*, 2014). Stored grains are not only a natural habitat of *T. molitor*, but they are also frequently used to feed *T. molitor* larvae in production facilities (Cortes Ortiz *et al.*, 2016). A typical symptom of *T. molitor* infected with fungi from the order Hypocreales is white fungal outgrowth when cadavers are kept at high humidity. After mycosis, the fungi start to produce conidia (green conidia: *Metarhizium* spp.; white conidia: *Beauveria bassiana*). The genera can often be determined based on characteristics of the conidia using light microscopy (Maciel-Vergara *et al.*, 2021), whereas molecular methods are needed to identify the species (Castrillo and Humber, 2009).

Several fungal species not classified as entomopathogens have been shown to affect *T. molitor* when they were ingested together with the feed. Guo *et al.* (2014) found that *T. molitor* larvae fed with *Fusarium avenaceum*- and *Fusarium culmorum*-colonised wheat kernels had an increased mortality compared to the control. This was despite having found none of the tested *Fusarium* species multiplied inside the insect haemocoel, indicating that mycotoxins produced by the fungi are responsible for the mortality of the larvae (Guo *et al.*, 2014). Other fungal species growing on grains (*Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum*, *Cladosporium herbarum*, *Fusarium nivale*, *Fusarium equiseti*, *Fusarium roseum* and *Fusarium tricinctum*) have been shown to inhibit the growth of *T. molitor* larvae (Davis *et al.*, 1975; Reiss, 1973). The growth inhibition by mycotoxins might be a combination of the effect of the toxins inside the insect and deterrence in feeding behaviour of the contaminated feed (Davis *et al.*, 1975).

Studies investigating the effect of the mycotoxin deoxynivalenol (DON) on *T. molitor* larvae report contradicting results. Jankovic-Tomanic *et al.* (2019) found growth-inhibiting effects of larvae reared on wheat containing 4.9 to 25 mg/kg DON (Janković-Tomanić *et al.*, 2019). Other studies, however, describe no effect on weight gain when the larvae of *T. molitor* were fed with wheat flour containing up to 8 mg/kg (Van Broekhoven *et al.*, 2017) or even up to 12 mg/kg DON (Ochoa Sanabria *et al.*, 2019). This indicates that mycotoxins from different fungal species or strains might have different effects on the larvae of *T. molitor*. Additionally, the human pathogenic yeast species *Candida albicans* and *Candida neoformans* have been found to cause mortality in *T. molitor* when directly injected into the haemocoel of the larvae (De Souza *et al.*, 2015).

Microsporidia

Microsporidia are obligate, intracellular, spore-forming parasites, considered to be most closely related to fungi based on recent phylogenetic studies (Capella-Gutiérrez *et al.*, 2012; Strassert and Monaghan, 2022) and are common parasites of insects: 93 of the 200 described genera of microsporidia have an insect as a host (Becnel and Andreadis, 2014). The most common pathway of microsporidia transmission to a new insect host is through direct oral ingestion of infectious spores, which are found in food, faeces, or liquids within the host's immediate environment (e.g. soil, water, plant, insect cadaver). Vertical transmission, where infection is transferred directly from parent to progeny, can also occur in the case of transovarial transmission (Becnel and Andreadis, 2014). Microsporidian infection in *T. molitor* thus far appears to be rare, with the only account of natural infection reported by Armitage and Siva-Jothy (2005), who identified unnamed microsporidians in a *T. molitor* lineage. In total, 87% of the beetles in the infected culture carried microsporidia, although they determined the infections were not harmful for the insect (Armitage and Siva-Jothy, 2005). Fisher and Sanborn (1962) experimentally induced infection in *T. molitor* using the microsporidium *Paranosema whitei* (natural host: *Tribolium* spp.) with infected feed, but susceptibility to infection was limited to second and third-instar larvae immediately post-moult (Fisher and Sanborn, 1962). Moreover, Milner also found that first instar *T. molitor* larvae were not susceptible to *P. whitei* infection (Milner, 1973). Based on the limited evidence of the ability of microsporidians to induce diseased states in *T. molitor*, further research is required to evaluate the disease-risk of microsporidians in mass-rearing systems.

Protists

Protists, historically called protozoans, are an informal group describing free-living or parasitic single-celled eukaryotes other than fungi, animals, and plants. They are found in a myriad of cellular forms with diverse biochemistries, which allow them to colonise every biome and many different types of hosts. Multiple protistan groups have the capacity to infect animals and may cause serious disease (Kolářová *et al.*, 2022). However, compared to prokaryotic microbes, fungi, and viruses, protists are often overlooked as potential pathogens of mass-reared insects (Bessette and Williams, 2022; Garofalo *et al.*, 2019; Maciel-Vergara *et al.*, 2021).

In insects, protist parasites typically start their life cycle when their environmental cysts or spores are ingested by a susceptible host (Lange and Lord, 2012). All protists identified as symbionts of *T. molitor* are typically transmitted via this route, causing infection after oral ingestion of the infectious stages of the protist. Infection by protistans is not typically obvious, as it is generally chronic with no external indications of disease. An infection of high intensity with neogregarines or coccidians can cause insects to become lethargic with a swollen, whitish appearance (Lange and Lord, 2012). The detection and identification of protist parasites has historically relied on microscopy and morphological identification in combination with knowledge of biological parameters, such as host specificity, tissues tropism, and route of infection (Solter *et al.*, 2012). Presently, the use of polymerase chain reaction (PCR) and, more recently, NGS, are being widely applied for discovering novel protist lineages and to understand their contribution to microbiomes (Bass and del Campo, 2020). The 18S (small subunit) ribosomal RNA gene (18S) is the most extensively used genetic barcode for protist surveys (Vaulot *et al.*, 2022).

Amoebozoa

The Amoebozoa group includes unicellular eukaryotes that possess pseudopodia for their motility and ingestion (Anderson, 2017). Six amoebae species are reported to be parasitic to insects. Moreover, it is possible for insects to act as mechanical vectors for amoebae pathogenic to humans and other animal hosts (Lange and Lord, 2012). Entomopathogenic amoebae are known to form resilient uninucleate cysts in the environment that will excyst within a suitable host and release trophozoites (i.e. active feeding stages) found either in the midgut or Malpighian tubules (Lange and Lord, 2012). Newly formed cysts are then released in the environment through the insect frass. Specific parasitic amoebae of the mealworm have yet not been described, but *T. molitor* has been found to carry *Entamoeba* spp., with the amoebae species *Entamoeba histolytica* known to cause dysentery to humans (Gałęcki and Sokół, 2019) (Supplementary Table S1).

Coccidia

Coccidians, also called haemogregarines, are similar to neogregarines. These endoparasites primarily infect vertebrates but are also found in invertebrates, with less than 1% of the described species infecting insect hosts (Lange and Lord, 2012). Few studies have examined coccidians within *T. molitor*, but some species are known to infect other Tenebrionidae, such as *Adelina castana* and *Adelina picei,* parasites of *Tribolium castaneum* and *Alphitobius piceus*, respectively (Ghosh *et al.*, 2000). *Adelina* spp. are not well studied in *T. molitor,* and only one reference from 1930 has reported a natural infection from reared *T. molitor* with *Adelina tenebriosis* (Sautet, 1930). The potential effects of coccidian infections on *T. molitor* are not studied, but lag in development time has been reported in another Tenebrionidae, which could impact insect production (Park and Frank, 1950).

Cryptosporidia

Recently, *Cryptosporidium* has been proposed as a gregarine (Adl *et al.*, 2019) but later this was abandoned to place *Cryptosporidium* as a basal group of apicomplexans (Salomaki *et al.*, 2021). Interestingly, *Cryptosporidium* spp., a vertebrate parasite, can be found on the surface or in the intestines of different insects, which could serve as mechanical vectors. A parasitological evaluation undertaken in European farms (with insect stock from all over the world) has reported that *T. molitor* could be a vector of this pathogen, presenting a risk for human health (Gałęcki and Sokół, 2019). The same study also found *Isospora* spp. in *T. molitor*, a coccidian pathogen that can induce gastrointestinal symptoms in humans, livestock, and exotic animals (Gałęcki and Sokół, 2019).

Gregarines

The most well studied protist group infecting *T. molitor* is Gregarinasina, known commonly as gregarines. Gregarines are the most abundant group of Apicomplexa that infect invertebrates (Desportes and Schrével, 2013; Votýpka *et al.*, 2017). Gregarines are mainly extracellular parasites and attach to the host via anchoring structures which allow them to feed on host cell cytoplasm (Lange and Lord, 2012). Two main orders compose the subclass Gregarinasina, the Eugregarinorida and the Neogregarinorida. In contrast to the eugregarines, the neogregarines (syn. schizogregarines) can develop intracellularly in the host tissues. This development induces more serious disease than eugregarines, as the intensity of infection of the eugregarines is limited to the number of oocysts that are ingested by the host (Lange and Lord, 2012).

Five species are known to infect *T. molitor*: *Gregarina polymorpha*, *Gregarina niphandrodes*, *Gregarina cuneata*, *Gregarina steini* and *Mattesia* spp. (Berndt, 1902; Clopton *et al.*, 1991; Hammerschmidt, 1838; Harry, 1967; Kleespies *et al.*, 2008; Koura and Kamel, 1993; Lipa, 1967; Rodriguez *et al.*, 2007; Schawang and Janovy, 2001; Stein, 1848; Valigurova, 2012). Gregarines are naturally present in *T. molitor* populations with controversial effects on their host. Rueckert *et al.*, 2019 did an extensive review on the effects of gregarines and how they transgress the symbiosis spectrum (Rueckert *et al.*, 2019). Two references showed positive effects on *T. molitor* infected by *Gregarina* spp. with enlarged host growth and positive impact on host development, fitness and longevity (Sumner, 1936; Valigurova, 2012). Other studies showed negative or no effect on the development and fitness of *Tenebrio* hosts. They can include a destruction of the gut cells (Lipa, 1967) and a decrease of the longevity of highly infected hosts (Rodriguez *et al.*, 2007) (Supplementary Table S1).

Viruses

The first report of a virus naturally infecting *T. molitor* dates back to 1969 (Huger, 1969). Viral particles similar to densovirus were identified in diseased larvae using an electron microscope, however, no further molecular analysis was carried out (Huger, 1969). These larvae presented a grey discoloration and cytopathic modifications on diverse tissues such as the epidermis, or the fat body. Moreover, *T. molitor* may act as a mechanical vector for *Acheta domesticus* densovirus (AdDV). AdDV-positive *T. molitor* individuals were detected in a colony reared together with infected house crickets (*A. domesticus*) within the same facility (Szelei *et al.*, 2011). These results indicate the possibility of horizontal transmission of densovirus between insect species. Apart from densovirus, viruses of the family Iridoviridae were found and demonstrated to be capable of infecting *T. molitor*. Particles of small iridescent virus (type 29) were identified in *T. molitor* larvae using electron microscopy (Black *et al.*, 1981; Kelly *et al.*, 1979). Wild type and recombinant invertebrate iridescent virus 6 (IIV6) have also been shown to cause disease in *T. molitor* via injection. Symptoms of infection include paralysis of larvae and darkening of cadaver three days after infection (Gencer *et al.*, 2020).

Regarding detection, molecular techniques are the most suitable method to correlate viral infection with the disease symptoms. Moreover, the advent of high-throughput sequencing has spurred the discovery of covert viruses in insects (Käfer *et al.*, 2019; Shi *et al.*, 2016; Wu *et al.*, 2020), including mass-reared edible species (Bertola and Mutinelli, 2021). Most of these viruses infect the host in a covert state with no visible biological costs. Therefore, it is likely that the number of viruses described for *T. molitor* and related species increases in the near future. In this scenario, analysing the risk of a potential viral outbreak and the sublethal effects caused by covert infections will be of high value to assess the level of risk for the mass-rearing industry.

Invertebrate parasites

Acari

Acari are ectoparasites capable of colonising many orders of insects. A parasitological evaluation of farmed insects found that *T. molitor* can carry mites belonging to the Acaridae (Gałęcki and Sokół, 2019). However, further research is needed to determine whether mites are parasites of *T. molitor*. Furthermore, mite debris should be considered as a potential hazard in insects produced for human consumption due to dust mite allergies. Other Tenebrionidae, such as *Alphitobius diaperinus,* are known to be parasitised by *Acarophenax mahunkai*, which feed on the eggs of this species (Steinkraus and Cross, 1993).

Cestodes

Cestodes are a group of intestinal endoparasites. In their adult stage, cestodes mainly affect vertebrates. However, the larval stages (cysticercoids) can infect invertebrates via oral ingestion as intermediate hosts while they develop their infective capacity on the definitive host (Saari *et al.*, 2019). Most literature concerning cestodes focuses on the family Hymenolepididae. Natural infections of this cestode have been reported in *T. molitor* and other species of Tenebrionidae such as *T. castaneum* and *Tribolium confusum* (Heyneman and Voge, 1971; Hurd *et al.*, 1990; Makki *et al.*, 2017) but most of the studies report experimental infections.

Although cestodes are not considered direct insect parasites, some studies have shown sublethal effects of cestode infection in the insect host. For instance, infection with *Hymenolepis diminuta* reduced the locomotion of *T. molitor* larvae in comparison to healthy individuals (Hurd *et al.*, 1990; Hurd and Parry, 1991; Sheiman *et al.*, 2006). Moreover, infection with *H. diminuta* was related to a decrease in the reproductive vigour of infected males and the fertility of females (Cole *et al.*, 2003; Hurd and Parry, 1991; Maema, 1986). In addition, we should avoid the presence of cestodes in mass-reared insects for assuring the food safety of the final product (Boelaert *et al.*, 2021).

To detect the infective stage of cestodes in insects, the use of a light microscope is recommended, while the application of molecular techniques may be required for characterisation at the species level.

Nematodes

No natural nematode infections have been described in *T. molitor* to date. However, diverse studies have assessed the physiological host range and pathogenicity of nematodes through experimental infection. These studies, which aim to unravel the potential of nematodes as biocontrol agents, concluded that several nematode species can infect *T. molitor* when added to the diet (de Carvalho Barbosa Negrisoli *et al.*, 2013; Ramos-Rodríguez *et al.*, 2006; Shapiro-Ilan *et al.*, 2008) (Supplementary Table S1). Species belonging to the genera *Steinernema* and *Heterorhabditis* are the main entomopathogenic nematodes described in *T. molitor*. These nematodes require high moisture conditions for infection (Eilenberg *et al.*, 2015) and desiccation tolerance is strain dependent (Shapiro-Ilan *et al.*, 2014). Similarly, the heat tolerance and the virulence of nematodes are influenced by the behavioural and physiological characteristics of the specific isolates and the environmental conditions (Lulamba and Serepa-Dlamini, 2020; Ramakuwela *et al.*, 2018). Members of the *Oscheius* spp. are also capable of infecting *T. molitor* both through experimental infection and using *T. molitor* as a bait (Foelkel *et al.*, 2017; Torrini *et al.*, 2015).

Nematode infections can be directly detected using a magnifying lens, while the species characterisation requires the application of molecular techniques.

Parasitoids

Like nematodes, no natural parasitoid infection of *T. molitor* has been described to date. However, due to its high accessibility and low production costs, *T. molitor* has been used as an alternate factitious host to rear multiple parasitoid species for biocontrol. Several studies were conducted to investigate the use of *T. molitor* as a host for rearing parasitoids at various life stages, including different pupal ages and eggs (Supplementary Table S1). *T. molitor* was demonstrated to be a highly suitable host for rearing parasitoids of lepidopteran species, with the level of parasitism in *T. molitor* reaching 100% of efficacy and an emergence rate above 90% (Andrade *et al.*, 2012; Favero *et al.*, 2014; Zanuncio *et al.*, 2008).

3. Beneficial symbionts

Mutualistic associations between hosts and their microbiota are well-known in the animal kingdom. Several microorganisms, especially prokaryotes, have been shown to have beneficial effects on *T. molitor,* all of which increased the growth of the larvae (Table 1). Increased larval survival and adult emergence was conferred by *Pediococcus pentosaceus* when provided in both vital and inactivated form (Lecocq *et al.*, 2021). *Bacillus subtilis*, *Bacillus toyonensis*, and *Enterococcus faecalis* had effects on the nutritional contents of the larvae (Rizou *et al.*, 2022), all of which increased crude protein content. Additionally, it has been shown that the gut biome of *T. molitor* larvae affects the parasite establishment of the tapeworm *Hymenolepis diminuta* (Fredensborg *et al.*, 2020). Controversial effects have been recorded on Gregarines impact on host development, fitness and longevity. Sumner (1933, 1936) and Valigurova (2012) observed an increased larval growth and longevity in *T. molitor* larvae infected with *Gregarina* spp. In addition, the exposure of *T. molitor* larvae to the fungal species *Neurospora sitophila* (Reiss, 1973) and *Pithomyces chartarum* (Davis *et al.*, 1975) resulted in beneficial effects on larval weight gain of individuals fed with contaminated products (Table 1).

Bacteria also present the possibility for use as probiotics to prevent diseases in reared insects, as is practiced in other livestock populations (Grau *et al.*, 2017b; Savio *et al.*, 2022). Probiotics are usually bacteria that either inhibit parasites (*e.g.* via inhibition of the expression of virulence genes or the increased production of antimicrobial substances) or increase the resistance of the insects by the stimulation of the host immune response (Grau *et al.*, 2017b). The *in vivo* application of probiotics to make *T. molitor* more resistant to parasites has not been demonstrated thus far. However, in the red flour beetle *T. castaneum*, the feeding of a probiotic (*Enterococcus mundtii*) increased the survival of larvae after exposure to *B. thuringiensis* (Grau *et al.*, 2017a), and *in vitro* studies of *P. pentosaceus* demonstrated growth-inhibiting effects on different entomopathogenic bacteria (*B. thuringiensis*, *S. marcescens*, *Serratia plymuthica* and *Pseudomonas aeruginosa*) (Lecocq *et al.*, 2021).

4. Implications for mass-rearing systems

Methods for detection and isolation

Diagnostic techniques used in detection, identification, and characterisation of parasites in diseased insects have evolved considerably over the past decades. Conventional methods to examine these parasites include microscopic analysis, observation of the respective signs and symptoms, and isolation using specific selective media (Bing *et al.*, 2021; Vandeweyer *et al.*, 2021). Gałęcki and Sokół (2019) demonstrated the use of microscopic analysis and Ziehl-Neelsen application of staining methods (Carter and Cole Jr, 2012) in identifying various parasites in *T. molitor* production facilities (Gałęcki and Sokół, 2019).

However, many of these parasites are unculturable (Masson and Lemaitre, 2020) on artificial media (e.g. *Ichthyosporea* spp.). In addition to that, parasites like protists and viruses may be present in covert states, presenting no observable signs or symptoms. The advent of new technologies in

Table 1. Overview of beneficial symbiont species and their effects on *Tenebrio molitor*.

Classification	Species	Effect on *T. molitor*	References
Bacteria	*Enterococcus faecalis*	Increased larval growth, reduced larval development time, increased crude protein content of larvae	Rizou *et al.*, 2022
	Bacillus subtilis	Increased larval growth, increased crude protein content of larvae, decreased crude fat content of larvae, decreased microbial counts of *Enterobacteriaceae*	Rizou *et al.*, 2022
	Bacillus toyonensis	Increased larval growth, increased crude protein content of larvae, decreased microbial counts of *Enterobacteriaceae*	Rizou *et al.*, 2022
	Pediococcus pentosaceus	Increased larval survival and growth, increased adult emergence	Lecocq *et al.*, 2021
	Mixed culture of *Bifidobacterium bifidum*, *Clostridium butyricum*, *Bacillus subtilis* and *Bacillus licheniformis*	Increased larval survival and growth, increased crude protein content of larvae, decreased calcium and phosphorus contents of larvae	Zhong *et al.*, 2017
Fungi	*Neurospora sitophila*	Increased larval growth	Reiss, 1973
	Pithomyces chartarum	Increased larval growth	Davis *et al.*, 1975
Gregarinasina	*Gregarina* spp.	Increased larval growth and longevity	Sumner, 1933; 1936; Valigurova, 2012

the field of molecular biology allows for the identification of these parasites via PCR by targeting parasite-specific genome regions. For example, by amplifying the non-structural protein 1 (NS1) coding region in densovirus, it was possible to identify AdDV positive *T. molitor* colonies reared together with *A. domesticus* within the same facility (Szelei *et al.*, 2011). In another case, confirmation of the presence of IIV6 in *T. molitor* larvae was determined using PCR in larvae showing symptoms of paralysis (Gencer *et al.*, 2020).

The rapid development of NGS technology in recent years allows for the detection of unsuspected and novel parasites via a metagenomics approach, as well as providing the possibility to simultaneously assess the microbiome and macrobiome of species of interest (Gibson *et al.*, 2014). A general estimate of the relative abundance of particular organisms within a sample is also possible using metagenomic approaches, which can help determine the clinical significance of a parasite of interest. The initial culturing procedure or preliminary knowledge of signs and symptoms of parasites are not necessary in this technique (Frey and Bishop-Lilly, 2015). The use of metagenomics has already revealed novel pathogens in several commonly reared insect species, such as the presence of a new iflavirus in *A. domesticus* colonies (de Miranda *et al.*, 2021). Reference databases of parasite genomic sequences are crucial for untargeted metagenomic screening approaches (de Miranda *et al.*, 2021). Regardless of the detection method, storage conditions such as temperature (-80 °C) prior to analysis are essential to maintain the stability of the genetic material for long periods (Bing *et al.*, 2021; Yang *et al.*, 2021).

In many cases, conventional detection methods are sufficient to identify common parasites with well-described signs and symptoms. Routine surveillance can be performed for the detection of previously recorded parasites in insect farming with standard PCR assays. In scenarios where unknown or suspected chronic diseases covertly reduce the fitness of the insects, the metagenomics approach is helpful in discovering the potential causative agent. In the future, it is possible that NGS techniques could be used to detect diseases even before infection, for example in the feed or in the circulating air (Sikorowski and Lawrence, 1994; Szelei *et al.*, 2011).

Management of harmful symbionts

Previous reviews and protocols of measures and good practice used in insect mass-rearing systems provide a useful framework for prevention and management of diseases in insects (Eilenberg *et al.*, 2015; IPIFF, 2022; Maciel-Vergara and Ros, 2017; Maciel-Vergara *et al.*, 2021). These general guidelines include hygiene and facility design, and are largely applicable to the production of *T. molitor.* While diligent hygienic practices are the most important aspect of disease prevention in insect production, research into new prevention methods is continuously ongoing. Here we focus on research with future potential for innovative methods in the context of *T. molitor* disease management.

Insights in insect ecology offer promising potential for managing disease in the future. For example, it has recently been discovered that insects have a form of innate immune memory called 'immune priming', which protects them from pathogens when previously exposed to a pathogen or a pathogen-derived compound (Little and Kraaijeveld, 2004; Vigneron *et al.*, 2019). Several authors have suggested making use of immune priming in the commercial production of insects (Grau *et al.*, 2017b; Maciel-Vergara and Ros, 2017). The application of immune priming has been shown to be successful in another invertebrate system, the production of giant tiger prawns (*Penaeus monodon*), providing protection from infections caused by white spot syndrome virus (Witteveldt *et al.*, 2004). In *T. molitor,* immune priming has been shown to have both intra- and transgenerational effects (Dhinaut *et al.*, 2018). Immune priming of *T. molitor* using Gram-positive bacteria conferred protection from infections with pathogenic Gram-positive and -negative bacteria within generation and the next generation (Dhinaut *et al.*, 2018). To reduce the risk of insects becoming infected during the immune priming treatment, heat inactivated microorganisms could be used, as it has been successfully demonstrated in *T. molitor* larvae (González-Acosta *et al.*, 2022). This could be useful in the future as a preventative treatment for parasites known to be problematic in insect facilities.

Modification of diet components might be another useful tool to prevent or treat parasites. For example, beneficial compounds found in diets, like flavonoids, could confer protection from parasites. In a study on amoeba in locusts, hosts collected in the field had lower infection rates by the amoeba *Malamoeba locustae* (Abdel Rahman *et al.*, 2015), compared to reared hosts (King and Taylor, 1936; Kleespies *et al.*, 2010). Abdel Rahman *et al.*, (2015) hypothesised that orthopterans living in natural conditions acquired immunity associated with feeding on the plant *Portulaca oleracea*, which contains flavonoids with potential anti-protist properties. Moreover, prevention of harmful symbionts could also be achieved by providing probiotics, as discussed in section 3.

Temperature treatments could prevent and treat disease outbreaks in *T. molitor* populations, in particular heat shock. Curative heat treatments can, for example, reduce the effects of viral pathogens in insects (Cevallos and Sarnow, 2010; Inoue and Tanada, 1977). Another interesting finding in this regard is that temperature stress can pre-emptively increase the immune responses of insect hosts and thereby decrease the susceptibility to pathogen infection (Browne *et* *al.*, 2014; Wojda and Taszłow, 2013). These findings have, however, not been tested in *T. molitor* thus far.

Previous work in selective breeding has shown it is possible to fix particular traits and produce lines of *T. molitor* with altered phenotypes (Song *et al.*, 2022). In the future, selective breeding of *Tenebrio* for the purposes of withstanding certain conditions or diseases might be of interest to insect producers. The knowledge necessary for producing resistant lines of insects will be developed from our understanding of different aspects that contribute to disease resistance, like insect behaviour, ecology, and evolution. For example, it might be possible to promote grooming behaviours, which is important in high-density conditions, based on our understanding of insect grooming.

5. Concluding remarks

The mass-production of *T. molitor* is a relatively young industry, and information on symbionts of this insect species is therefore still limited. However, *T. molitor* has been used as a model organism to study host-parasite interactions for several decades, providing valuable insights into its life history and ecology (Barnes and Siva-Jothy, 2000; Dhinaut *et al.*, 2018). In the future, it will be important to consider how diseases are classified in terms of host range, as insect rearing facilities are neither natural conditions nor optimised laboratory conditions. Under unnatural, high-density breeding conditions, it is possible that new and emerging parasites may adapt to infect insects that were formerly only capable of colonisation under experimental circumstances. It is largely unknown what effects altered environments like mass rearing facilities will have on host-parasite interactions and disease outcomes, which could be positive or negative for insect production. For this reason, it is important to understand a parasite's physiological as well as ecological host range, and the environmental and evolutionary forces driving adaptation and host-shifts. Interactive effects arising from co-infection must also be considered. Different parasite species or strains might infect simultaneously, resulting in unpredictable outcomes that are impossible to determine when studying parasites individually (Cory and Deschodt, 2018).

As the mass production of *T. molitor* grows alongside global demand for insect protein, it will be important to maintain awareness of the type and severity of organisms affecting insect stocks. This is especially true for disease-causing agents, given that mass-rearing is practised at high insect densities that are conducive to outbreaks. Likewise, continued research into the possible benefits of mutualistic organisms will also help to ensure the health and well-being of farmed insects. These areas of research could largely benefit from partnerships between academic institutions, government programs, and industry in order to identify and address emerging parasites of particular concern and ensure the best practices for maintaining insect health are known and implemented.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2022.0171.

Table S1. Overview of *Tenebrio molitor* parasites, symptoms, detection methods, and prevention or treatment.

Authors contributions

A.R.S., P.H., E.B., F.S.L., L.H.-P., C.S. conceptualisation, investigation, writing – original draft preparation. A.R.S. and P.H. writing – reviewing and editing.

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Conflict of interest

The authors declare no conflict of interest.

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