Adaptations of entomopathogenic nematodes to plant-derived benzoxazinoids

Inaugural dissertation
of the Faculty of Science,
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presented by

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Supervisors of the doctoral thesis:

Prof. Dr. Christelle A. M. Robert, Institute of Plant Sciences, University of Bern

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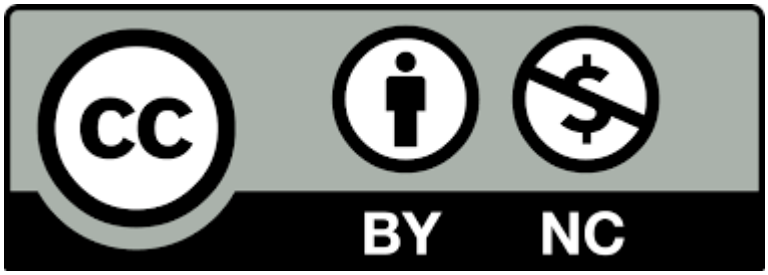

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General Abstract

Benzoxazinoids (BXs) are plant specialized metabolites involved in herbivore-defenses of cereals such as maize and wheat. The Western Corn Rootworm (WCR, Diabrotica virgifera virgifera), a specialist root herbivore, stabilizes, sequesters and accumulate them into their bodies. The interactions of these WCR accumulated toxins with the WCR natural enemies, entomopathogenic nematodes (EPNs) are not fully understood. This thesis aimed to investigate EPN benzoxazinoid tolerance and resistance mechanisms and or strategies.

Chapter 2 demonstrated using an evolutionary experiment that EPNs evolved BX resistance within three host cycles. Additionally, we also found that before adaptation, BX repulsive effect can explain the initial differences between BX- sensitive and Bx resistance isolates. However, this phenomenon (repulsion) cannot explain difference between BX-adapted and non-adapted isolates. These findings highlight the capacity of EPNs to rapidly adapt to plant chemical defenses, providing new insights into enhancing the efficacy of biocontrol strategies against BX-sequestering pests like WCR.

Chapter 3, revealed that EPNs can fight back benzoxazinoid toxin effects by biodegrading them to their breakdown derivates, thus, showing EPNs ability to respond in stressful situations. This work findings will be helpful in elucidation mechanistic bases of inter-strain benzoxazinoid tolerance variation. Additionally, this will make prediction of benzoxazinoid effect on various strains possible. Furthermore, this paves way for nematode breeders for development of genetic markers linked benzoxazinoid tolerance.

Chapter 4, indicated cooperative acts between EPN and some of its associates in times of trouble which may in turn benefit the EPN to grow, develop and survive in stressful situations. These findings will motivate nematologists to study and understand other symbiosis relationships between EPNs and their associates. Understanding the influences of such EPN associations in EPN biological, physiological and biochemical process may give crucial information for EPN-contents manipulations programs, which may be used in maximizing EPN- control potential and protection.

General introduction

The domestication of plants is believed to have started about 12000 years ago when farmers decided to switch from nomadism to permanent settlement (Hillel, 2010; Sushma Naithani, 2021). This was later threatened by impending famine due to populations increase (Wu & William, 2004). This led to introduction of the "green revolution", which refer to the large scale transfer and adaptation of new technologies in agriculture that boosted crop yield since the mid-20th century (Khush, 1999). This resulted in a remarkable yield increase, which, for example, in countries like China, a five -fold increase in food productivity was reported ((FAO, 2010; Horlings & Marsden, 2011). However, today, food insecurity is still being reported as this increase in food production cannot meet the present challenge of human population growth pressure which is estimated to be causing about a billion additional mouths every 12-14 years ((Rajendra Prasad, 2013) with a census prediction of (~10 billion people) globally by 2050 (Fess *et al.*, 2011; UN, 2022).

Continuing to expand harvests to meet this population growth pressure is constrained by the availability of arable land (Ehrlich *et al.*, 1993; Fess *et al.*, 2011). The demand for arable land will increase as the population grows and the demand for food increases. Any attempts to expand arable will be at the expense of natural forests, which are habitats for wildlife, wild crops as well as crop pests natural enemies (Popp *et al.*, 2013). Thus, there is urgent need to develop sustainable and productive agroecosystems measures. Such measures can target nutrient cycling, as well as ecological management of crop weeds, crop pathogens as well as crop animal pests (Reddy, 2017; Ahmad *et al.*, 2020). Pests greatly threaten food security (Deguine *et al.*, 2023). Global studies on crop losses due to pests and disease are estimated to be approximately 40% every year (Oerke, 2006; FAO, 2025). This clearly indicates that minimizing crop yield and quality losses which are mostly lost to crop herbivore will significantly boost production.

Plant defenses

Plants have evolved some defense strategies against herbivory ranging from specialized morphological structures, secondary metabolites and proteins that have toxic, repellent,

and/or antinutritional effects on the herbivores ((Kogan & Paxton; War *et al.*, 2011; War *et al.*, 2012). One of the defense mechanisms in maize plants is the production of benzoxazinoids

Benzoxazinoids

Benzoxazinoids (BXs) are specialized indole-derived metabolites that have been studied primarily in Poaceae. In this plant family, BXs were most isolated from wild grasses and major agricultural crops like maize, wheat, and rye. Besides Poaceae, BXs have also been isolated from dicotyledonous medicinal plant families Acanthaceae, Calceolariaceae, Lamiaceae, Plantaginaceae, and Ranunculaceae. (Baumeler *et al.*, 2000 ; Sicker *et al.*, 2000; Bruijn *et al.*, 2018; Machado *et al.*, 2020). Due to the agricultural importance and well-developed genetic resources of maize, many of the recent advances in benzoxazinoid research have been made in this species. (Zhou *et al.*, 2018).

Structure and classification of benzoxazinoids

Chemically, they are double ringed aromatic compounds which can be classified into two main classes. Benzoxazinoids are normally stored as stable benzoxazinones glucosides, which upon tissue maceration by herbivores, will be converted to their corresponding unstable benzoxazinones aglucones (Wouters *et al.*, 2016). The unstable aglucones are very reactive and can pose allelopathic, toxicity, repulsion and anti- herbivory effects against herbivores (Sicker *et al.*, 2000; Sicker & Schulz, 2002; Bruijn *et al.*, 2018, 2018). The aglucones can later be converted by microbes to either aminophenoxazinones, malonamic acids, or acetamides, some of which are also bioactive (Zikmundová *et al.*, 2002; Krogh *et al.*, 2006; Schulz *et al.*, 2013; Robert & Mateo, 2022). Efforts have been made to use BXs for pest control (Zhou *et al.*, 2018).). Their interaction with various organisms is clearly shown with their tritrophic interaction with the western corn rootworm and its natural enemies the entomopathogenic nematodes sparked a lot of research interest (Erb & Robert, 2016 ; Robert *et al.*, 2017; Zhang *et al.*, 2019; Bruno *et al.*, 2020; Machado *et al.*, 2020).

Below ground herbivory

Subterranean pests pose a significant economic threat due to their substantial impact on food security. These pests have been observed to cause substantial damage to forests and agricultural crops, resulting in considerable yield losses. The damage of these underground pests may begin soon after seed planting when they feed on seed and continue till harvesting when they mostly feed on stalk just below soil level as well as plant roots and root hairs (Khudoykulov *et al.*, 2021). Plant roots are mostly important for acquisition of water and nutrients, anchoring of the plant as well as the production of herbivory defense compounds (van Dam, 2009). Any disturbance of plant roots due to root feeding pests can impact plant fitness and yield (Blossey & Hunt-Joshi, 2003). It has been reported that, if root herbivores are not detected and treated in time, their damage, as well as the effects of related secondary stresses such as drought, can cause dramatic declines in plant populations (Blossey & Hunt-Joshi, 2003).

Their impact on crop production is worsened by the fact that belowground-feeding herbivores are difficult to see and manipulate (Andersen, 1987; Brown & Gange, 1990; Blossey & Hunt-Joshi, 2003). To farmers, this also pose a serious challenge as their treatment and management normally occur late, mostly after severe symptoms appear. Below ground herbivory is mainly from rodents, nematodes, molluscs, and insect (Brown & Gange, 1990). Some examples of belowground insect herbivores include seed corn maggots, wireworms, white grubs, cutworms as well as northern and western corn rootworms (Heather & Sebe, 2017; Khudoykulov *et al.*, 2021)

Western corn rootworm (WCR)

The western corn rootworm (WCR), or *Diabrotica virgifera virgifera* LeConte is a beetle belonging to the family Chrysomelidae. The beetles are specialised maize root herbivores that were introduced to Europe in the early 1990s from North America (Miller *et al.*, 2005; Bažok *et al.*, 2021). The pest is believed to have originated from the north America and has been introduced to Europe without its natural enemies through air travel and or shipment of goods (Hummel, 2003). The beetles are good flyers as they can cover more than 20 km in a single flight and can also be carried by weather such as storms and cold fronts (Grant & Seevers, 1989). Together with its high fecundity, the pest, rapidly spreads

and infests vast maize fields in Europe. According to (Lemic *et al.*, 2015) , the pest had, by 2011, spread to most maize growing areas in Europe where it is causing maize crop yield and quality losses.

The female WCR mostly lay eggs in autumn, and the eggs can overwinter underground and will hatch in spring. The hatched WCR larvae has three instars which are all specialized maize root feeders (St Clair & Gassmann, 2021).They larvae feed on seed kernels, root hairs and burrow tunnels in maize roots as they feed. The damages caused by WCR to maize growth and yield were elaborated by (Hoffmann *et al.*, 2000).The authors stated that larval feeding on roots result in plant lodging, stunting and root injuries are pathogen entry points. They also stated that adult WCR can feed on corn silk, pollen leaves and immature kernels, all of which can cause yield losses. Finally, the authors also stated that the pest is also a virus and fungus vector to plants. The adults also can feed and oviposit on alternative hosts like Soybeans, Cucurbitaceous, and Lucerne (Gray *et al.*, 2009). Thus, the pest has a potential to cause other crop losses to farmers. In the United States, an annual estimated loss exceeding USB~1-2 billion (Darlington *et al.*, 2022) through pest management and crop damage losses has been reported ((Miller & Krysan, 1986; St Clair & Gassmann, 2021). Other past studies in Europe, reported that the annual economic benefits of controlling WCR in best case scenario and worst case scenario range between 143 million Euro and 1739 million Euro respectively (Wesseler & Fall, 2010). With all these impacts, WCR poses serious threats to farmers worldwide , who have implemented various ways in affected areas against WCR to try to reduce losses (Vidal *et al.*, 2005; Meissle *et al.*, 2010; Meissle *et al.*, 2011).

Crop rotation

Rotation of maize with WCR non hosts has been effective, as the pest preferably lay eggs on maize and the devastating larval instars do not survive well on other crop roots (Boriani *et al.*, 2006). In North America, maize rotation with soy beans has been effective till the mid-1990s when the cultural practice failed as the pest had developed behavioural adaptations (Knolhoff *et al.*, 2006; Gray *et al.*, 2009). Similar behavioural adaptations were also reported in Europe where the beetle were discovered in Slovenia in pumpkin oil plants (Hummel, 2007). The time when the beetles were seen in the pumpkin oil plants coincides with the time when the females are looking for oviposition sites. Thus, longer

crop rotations are needed to suppress WCR populations, which come with their own costs in terms of complexity and crop choice.

Chemical control

The most common pest control strategy of reducing the WCR density, root lodging, and yield loss in Europe is the application of soil insecticides at planting. This application can be performed in seed furrow during planting operation or through seed treatment, using pyrethroids or neonicotinoids (Sutter *et al.*, 1990; Blandino *et al.*, 2017; Souza *et al.*, 2020; Ferracini *et al.*, 2021). However, WCR has evolved resistance against several of these insecticides (Sutter *et al.*, 1989; Meinke *et al.*, 1998; Souza *et al.*, 2020; St Clair *et al.*, 2020; Meinke *et al.*, 2021). Lifetime WCR females lay 500 to 1000 eggs, which hatch across several weeks, thus resulting in variable insecticide exposure and control success (Branson & Johnson, 1973; Hill, 1975; Rondon & Gray, 2004). Furthermore, neonicotids have major adverse impacts on non-target organisms and are therefore being banned in the EU (Kluser *et al.*, 2010; Laurino *et al.*, 2011; Alemanno, 2013; European Commission, 2013; Blake, 2018; Kathage *et al.*, 2018). Alternative control strategies for WCR and other soil-dwelling pests are thus needed.

Transgenic maize

Crops engineered to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt) provide an effective management tool against WCR(Gassmann, 2012). These maize lines express crystalline (Cry) proteins whose mode of action involves insect intoxication by disrupting midgut epithelial tissues upon ingestion (Vachon *et al.*, 2012). However, in 2009, fields were discovered in Iowa, USA, with populations of western corn rootworm that had evolved resistance to maize producing the Bt toxin Cry3Bb1. (Andow *et al.*, 2016). Laboratory selection experiments confirmed that evolution of Bt resistance is possible in insects (Tabashnik, 1994). To make matters worse, a single insect can develop resistance to many Bt strains and/or toxins, even when many toxins are used simultaneously. (Tabashnik *et al.*, 1993a; Tabashnik *et al.*, 1993b).

The other avenue that has been explored to control WCR has been the use of RNA *interference (RNAi*), which refers to a set of processes that involve introduction of double-stranded RNAs of the gene of interest to silence gene of interest expression. (Yang

et al., 2011). Oral admission of the dsRNA into WCR using artificial diets bioassays has been successful targeting a putative endoglucanase (*DvvENGase*I) gene that is involved in cellulose digestion (Valencia *et al.*, 2013), however, this oral feeding does not apply to field situation. Thus, the challenge was to have transgenic plants expressing dsRNA for the target gene to allow continuous oral delivery for effective silencing hindered progress (Narva et al., 2013). Research in this area is ongoing.

Biological control with natural enemies

Several natural enemy species or groups appeared to be promising candidates for WCR control strategies, including parasitoid, fungi, viruses, and nematodes. (Kuhlmann *et al;* Kuhlmann & van der Burgt, 1998; Pilz *et al.*, 2009). One of the biological control agents being investigated is Celatoria *compressa* (Wulp) (Diptera: Tachinidae), a parasitoid of adult chrysomelid beetles of the subtribe Diabrotica in North America, which has been selected as a candidate for classical biological control of the European invasive bark beetle WCR. In the study, *C. compressa* parasitized about 45 % of the tested WCR larvae (Toepfer *et al.*, 2009). *Gaeolaelaps aculeifer*, a soil-dwelling predatory mite that inhabits the first few centimeters of the soil, was also assessed for its predatory potential against WCR larvae. The results of the study showed that *G. aculeifer* has the potential to control WCR at densities starting from 100 mites/plant (Pasquier *et al.*, 2021). The infection rate of these two agents is too low to be used as reliable biological control agents.

Entomopathogenic fungi were another biocontrol agent tested against the WCR. In one study, twenty strains of *Metarhizium anisopliae, Beauveria bassiana* and *Beauveria brongniartii* were used in bioassays in the laboratory. The results of the study showed that adults were significantly more susceptible to entomopathogenic fungi than larvae (Pilz *et al.*, 2007) and the strain *Metarhizium anisopliae* showed the greatest WCR larvae infectivity. (Pilz *et al.*, 2007; Rudeen *et al.*, 2013). Viral research has demonstrated that the presence of viral particles in the male reproductive organs of *D. virgifera virgifera* is associated with a high incidence of abnormal sperm. (Degrugillier *et al.*, 1991), However, these viruses had no effect on insects in the family Chrysomelidae, which includes WCR. The viruses did not affect the insects' lifespan, mobility, or mating behavior. After several unsatisfactory attempts with most of the above WCR natural enemies, hope was raised when scientists tested the efficacy of entomopathogenic nematodes (EPNs) against

WCR, which became one of the successful candidates. (Journey & Ostlie, 2000; Kurtz *et al.*, 2007).

Entomopathogenic nematodes (EPNs)

EPNs are non-segmented, soil-dwelling roundworms and are a discovered biological control agent that have been successful in controlling insect pests such as white grubs, black vine weevil, turf grass pests, fungus gnats, mole crickets, weevils, and cutworms, etc.(Singh *et al.*, 2022). Species in two families (Heterorhabditidae and Steinernematidae) have been effectively used as biological insecticides in pest management programs (Grewal & Georgis, 1999; Grewal, 2012). The high virulence, broad host range, ease of mass production, and host-seeking ability of these worms are desirable traits. (Timper & Kaya, 1989).Both groups of EPNs have similar life-cycles where the infective juvenile (IJ), a modified third stage juveniles (dauer juveniles) (Ciche *et al.*, 2006), which are the only free living and infective stage of EPNs. The only infective stage (the third-stage (L3) duer juveniles) can survive outside the host. They are environmentally stress resistant, nonfeeding and ensheathed with a second cuticle (Poinar & Leutenegger, 1968; Timper & Kaya, 1989; Donald L. Lee, 2002). The cuticular sheath uncovering the L3 is as a result of incomplete molt of the second stage juveniles which may seem to play a role in environmental stress tolerance (Timper & Kaya, 1989; Campbell & Gaugler, 1992; Donald L. Lee, 2002)). Exsheathment of the L3 second cuticle, which is usually stimulated by the host, marks the transition of these L3s from free -living to parasitic stage (Campbell & Gaugler, 1992; Donald L. Lee, 2002)). EPNs live in association with other microbes and even cooperate with some during host infection.

EPN-associated microorganisms

The two EPN Genera (Heterorhabditis and Steinernema) have evolved mutualisms with entomopathogenic bacteria spp (*Photorhabdus and Xenorhabdus*), which they carry in their intestines (Boemare *et al.*, 1993; Kaya & Gaugler, 1993; Forst *et al.*, 1997; Grewal & Georgis, 1999; Tomar *et al.*, 2022). Studies on EPN microbiota revealed that this association is not monoxenic, but occurs within more complex bacterial communities, which also seem to aid in nematode entomopathenogenity (Jiménez-Cortés *et al.*, 2016; Goda *et al.*, 2020; Ogier *et al.*, 2020; Ogier *et al.*, 2023). The microbiota of studied Steinernema spp confirmed the presence of dozens of Proteobacteria (Pseudomonas,

Stenotrophomonas, Alcaligenes, Achromobacter, Pseudochrobactrum, Ochrobactrum, Brevundimonas, Deftia) among others (Ogier *et al.*, 2020). Studies with Heterorhabditis also revealed a bacterial community consisting of different bacteria, including *Pseudochrobactrum sp., Comamonas sp., Alcaligenes sp., Klebsiella sp., Acinetobacter sp., and Leucobacter spp* (Jiménez-Cortés *et al.*, 2016). Whether these microbes are from the inside or the surface of nematodes still needs more research, although some studies with different nematodes including the entomopathogenic nematode strain EN01 have managed to target only potentially endogenous microbes as recovered microbes were obtained after fsurface sterilizing nematodes with sodium hypo chloride (Loulou *et al.*, 2023).

Efficacy of entomopathogenic nematodes against WCR

A lot of research on the efficacy of EPNs against WCR has been reported. One of the research projects involved laboratory screening of eight EPN spp (7 *Steinernema spp* and *H. bacteriophora*) against larvae and adults of WCR. All tested species were able to infect WCR larvae, but not adults. *Heterorhabditis bacteriophora* was among the spp that caused high mortality rate by reducing populations of WCR by 81% (Toepfer *et al.*, 2005). *Heterorhabditis bacteriophora* was later mostly used in other EPN efficacy studies. It was proven that nematodes have a great potential to reduce WCR populations in all types of soil, with the highest efficacy in clay maize fields (Toepfer *et al.*, 2010). Some studies showed that the mortality of WCR larvae increases with the increase in nematode initial populations (Hoffmann *et al.*, 2014) showing that nematodes work in a concentration dependent manner.

The WCR has a number of resistance mechanisms that reduce EPN infectivity. One of these are maize defense metabolites (benzoxazinoids, BXs), which it can ingest and accumulate (sequester) (Erb & Robert, 2016; Robert *et al.*, 2017). No negative effects of BXs on WCR growth and development have been found (Alouw & Miller, 2015). The accumulated benzoxazinoids include the glucosides HDMBOA-Glc and MBOA-Glc. In addition, WCR larvae can hydrolyze HDMBOA-Glc, to produce another toxic BX: MBOA. Both HDMBOA-Glc and MBOA have a toxic effect on EPN, while MBOA-Glc acts as a repellent (Robert *et al.*, 2017). Benzoxazinoids can interact with both EPNs and their associated microbes. However, many questions regarding this interaction remain open.

Aim and scope of the thesis

This thesis explores the multitrophic interactions involving plant defense metabolites (benzoxazinoids), herbivores (western corn rootworm), their natural enemies (entomopathogenic nematodes), and their associated microbes.

In Chapter 1, EPN response strategies to various biotic (viruses, bacteria, fungi, and predatory insects) as well as abiotic (starvation, low/elevated temperatures, desiccation, osmotic stress, hypoxia, and ultra-violet light) stresses are reviewed. The reports provide new avenues and targets to select and engineer nematodes for better adaptations to field conditions.

Chapter 2 assesses the benzoxazinoid tolerance of various EPNs by comparing their infectivity success towards benzoxazinoid-fed WCR. It furthermore investigates the possibility to enhance infectivity of benzoxazinoid-susceptible EPN strains through forward evolution. Based on earlier experiments, we hypothesized that exposure to BX-containing WCR should result in the rapid evolution of BX-dependent infectivity.

Chapter 3 investigates the capacity of EPNs to metabolize BXs . We hypothesized that BX resistant strains may have a higher ability to metabolize and/or degrade BXs than non-resistant strains.

Chapter 4 aimed at understanding the role of microbes in benzoxazinoid metabolization by EPNs. We hypothesized that surface microbes may mediate the rapid metabolization of BXs in the EPN environment.

Together, these chapters provide novel insights into EPN-environment interactions and reveal the dynamic nature of BX metabolization and BX-dependent infectivity.

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Chapter 1: Stress tolerance in entomopathogenic nematodes: Engineering superior nematodes for precision agriculture.

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Abstract

Entomopathogenic nematodes (EPNs) are soil-dwelling parasitic roundworms commonly used as biocontrol agents of insect pests in agriculture. EPN dauer juveniles locate and infect a host in which they will grow and multiply until resource depletion. During their free-living stage, EPNs face a series of internal and environmental stresses. Their ability to overcome these challenges is crucial to determine their infection success and survival. In this review, we provide a comprehensive overview of EPN response to stresses associated with starvation, low/elevated temperatures, desiccation, hypoxia, and ultra-violet light. We further report EPN defense strategies to cope with biotic stressors such as viruses, bacteria, fungi, and predatory insects. By comparing the genetic and biochemical basis of these strategies to the nematode model *Caenorhabditis elegans*, we provide new avenues and targets to select and engineer precision nematodes adapted to specific field conditions.

Keywords: Entomopathogenic nematodes, biological control, stress tolerance, soil environment, *Caenorhabditis elegans*

1. Introduction

Sustainable strategies to increase crop productivity are urgently required to ensure food safety and food security worldwide (Vågsholm et al., 2020). Biological control is a promising and environmentally friendly avenue to increase food production by decreasing herbivore-associated crop losses.

Animal pests are responsible for 8-16% crop losses annually (Birch et al., 2011; Oerke, 2006). Among these pests, root feeding insects are particularly damaging (Agrawal, 2011; Anbesse et al., 2013; Hunter, 2001; Johnson and Murray, 2008; Kergunteuil et al., 2016). For example, the western corn rootworm, *Diabrotica virgifera virgifera* Le Conte, causes damage and control costs exceeding two billion USD annually in the USA alone (Gray et al., 2009; Wechsler and Smith, 2018). Wireworms, larvae of click beetles (Coleoptera: Elateridae), inflict potato yield losses and control costs of about six million USD (Vernon et al., 2009). The greyback grub, *Dermolepida albohirtum*, causes about 28 million USD losses annually in Australian sugar industry (Chandler, 2002). While soil-dwelling herbivores are out-of-sight, their populations can be controlled by biocontrol agents such as entomopathogenic nematodes (EPNs) (Grewal et al., 2005; Koppenhöfer et al., 2020).

Entomopathogenic nematodes (EPNs) are obligate pathogenic roundworms that can infect and kill soil-living arthropods (Poinar, 2018; Shapiro-Ilan et al., 2014; Weischer and Brown, 2000). They belong to the Heterorhabditidae and Steinernematidae families. EPNs shelter a community of endosymbiotic bacteria, referred to as the EPN pathobiome (Machado et al., 2018; Ogier et al., 2020; Pervez et al., 2020; Poinar and Grewal, 2012; Sajnaga and Kazimierczak, 2020). Infective juvenile (IJ) nematodes penetrate their host through natural apertures or by puncturing the cuticle (Bedding and Molyneux, 1982; Castelletto et al., 2014; Ciche and Ensign, 2003; Dowds and Peters, 2002). Once in the body, EPNs release their bacterial symbionts by regurgitation or defecation (Ciche et al., 2006; Ciche and Ensign, 2003; Martens et al., 2004; Poinar, 1966). The bacteria produce digestive enzymes, immunosuppressors, and toxins, which ultimately kill the host within a few days (Brivio and Mastore, 2018; Ciche and Ensign, 2003; Clarke, 2020; Duchaud et

334 al., 2003; Gaugler and Kaya, 1990; Kaya and Gaugler, 1993). EPNs then feed on the bac-
335 teria and reproduce in their insect host until resource depletion (Ciche and Ensign, 2003).
336 At this stage, the nematodes produce new IJs that leave the body and seek for a new host
337 (Gaugler and Kaya, 1990; Grewal and Georgis, 1999; Zhang et al., 2021). EPNs have
338 evolved several strategies, ranging from ambush to cruising, to locate and/or to increase
339 probabilities of encountering preys (for review see Zhang et al., 2021). Their high viru-
340 lence, together with the ease of mass production (Ehlers, 2001), suggest them as a bio-
341 logical agent of high potential for controlling soil-dwelling pests (Gaugler and Kaya, 1990;
342 Kaya and Gaugler, 1993). To make use of this potential, however, it is crucial to attain
343 high consistency of EPN's impact on herbivore populations that is independent of envi-
344 ronmental conditions and pest characteristics, and robust to changes in application
345 strategies (Gaugler, 1988; Griffin, 2012; Khatri-Chhetri et al., 2011; Koppenhöfer et al.,
346 2020; Shapiro-Ilan et al., 2006b). This lack of understanding brings an urgent need to
347 identify and characterize nematode traits that enhance their success in different condi-
348 tions to generate superior EPNs.. To make use of this potential, however, it is crucial to
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352 2020; Shapiro-Ilan et al., 2006b). This lack of understanding brings an urgent need to
353 identify and characterize nematode traits that enhance their success in different condi-
354 tions to generate superior EPNs

355 Traits conferring superior efficacy include attributes increasing EPN persistence in soil,
356 host encountering probability, infectivity success, and multiplication potential (Shapiro-
357 Ilan et al., 2012; Shapiro-Ilan et al., 2006a). Traits involved in EPN commercial suitability,
358 such as EPN production, formulation, and shelf-life, should also be considered for ap-
359 plied perspectives (Georgis and Gaugler, 2002; Glazer, 2015; Vernon and van Herk,
360 2022), but are not under the scope of this review. Although the identification of relevant
361 traits by comparing EPN populations of different geographical origins or through artificial
362 selection, hybridization or genomics-assisted breeding offers valuable insights, the func-
363 tional characterization of genes encoding for specific traits remains a milestone to be

achieved in developing genetic improvement programs (GIPs), and ultimately superior nematode strains adapted to specific field conditions and pest targets.

Multiple genomes of EPNs and their endosymbionts are available to characterize the genetic pathways and gene families underlying specific traits (Table 1). Initial resources, such as the *H. bacteriophora* and *H. indica* draft genomes that are 77 Mb (~16'000 annotated protein coding genes) and 91 Mb (~10'000 annotated protein coding genes) long respectively, are available for comparative genomics (Bai et al., 2013; Bhat et al., 2021; McLean et al., 2018). *Steinernema* genomes have been assembled for multiples species including *S. carpocapsae*, *S. diaprepesi* and *S. feltiae* (Table 1). While the dissection of the genetic pathways involved in EPN biology and stress tolerance are still at an early stage, the knowledge gained from the model nematode *Caenorhabditis elegans* offers a unique opportunity to accelerate EPN research and genetic improvement in stress tolerance.

In this review, we provide an overview of EPN traits that modulate nematode tolerance to endogenous and exogenous stressors and thus their persistence in soil as IJs. We highlight the current knowledge about the involved genetic pathways underlying stress resistance in EPNs and in *C. elegans*. Finally, we emphasize some of the limitations associated with the breeding of superior EPNs and advocate mitigation plans for effective biocontrol strategies.

2. Endogenous stresses

2.1. Nutritional stress tolerance

IJs are nonfeeding organisms that rely on internal lipids (mainly triacylglycerols) and glycogen reserves for survival (Grewal and Georgis, 1999; The C. elegans Research Community). Neutral lipids represent between 24 and 31% of the nematode dry weight (Patel et al., 1997b). In IJs of *H. bacteriophora* and *H. megidis*, unsaturated fatty acids represent 57% and 62% of the detected fatty acids, respectively . Interestingly, steinernematid species contain relatively more saturated fatty acids, as the latest can reach up to 70% of the lipid pool (Patel and Wright, 1997a; Selvan et al., 1993b; Selvan et al., 1993a). Among

the 18 fatty acids detected in *Steinernema* species, oleic acid (C18:1n-9), palmitic acid (C16:0), and stearic acid (C18:0) decline over storage time (100 days), suggesting that these fatty acids are preferentially used as energy suppliers (Patel and Wright, 1997a). On the other hand, glycogen reserves in steinernematids ranges from 8% to 18% dry weight (Patel and Wright, 1997b) and their level seem to decline more slowly than lipids in young IJs, but tend to decline faster after lipid depletion. Thus, it is hypothesized that glycogen might be the alternative source of energy after lipids reserves depletion in aged IJs. This was experimentally shown in a study of (Wright et al., 1997) where *S. carpocapsae* maintained its infectivity potential at low lipid levels when glycogen levels were high. Infectivity later markedly decreased with decline in glycogen reserves. IJ reserves are critical factors shaping the nematode survival, stress tolerance, motility, behaviour, and ultimately infectivity (Abu Hatab and Gaugler, 1997; Fitters and Griffin, 2006; Grewal and Georgis, 1999; Griffin and Fitters, 2004; Hass et al., 2002; Jagdale and Gordon, 1997; Patel et al., 1997b; Qiu and Bedding, 2000). Although the role of neutral lipids and glycogen as energy storage for IJs seems established, manipulative studies using the available information from *C. elegans* (e.g., 471 putative genes involved in the lipid metabolism, fatty acid pathway (Rappleye et al., 2003; Watts and Ristow, 2017), should be undertaken to disentangle their specific roles in IJ nutritional stress tolerance.

2.2. Oxidative stress tolerance

Oxidative stress is caused by an imbalance between reactive species (RS) production and antioxidant defenses (Sies, 2018; Sies, 1985a). The production of RS, including reactive oxygen species (ROS) and reactive carbonyl species (RCS), results from the reduction-oxidation reactions associated with the use of oxygen (Halliwell and Gutteridge, 2015; Pamplona and Costantini, 2011). For instance, ROS are mostly produced by the electron transport system of mitochondria during ATP production (Zorov et al., 2014). As highly reactive molecules, RS can disrupt the cellular metabolism and their overproduction results in cell death (Frijhoff et al., 2015; Nanette and Tim, 2013; Sies, 2018; Sies, 1985b; Zorov et al., 2014). RS are detoxified through several mechanisms, such as conjugation (e.g., to glutathione), oxidation (e.g., by aldehyde dehydrogenases), or reduction (e.g., by aldoketoreductases) (Detienne et al., 2018; Hulbert et al., 2007; Pamplona and

Costantini, 2011; West and Marnett, 2006). In *H. bacteriophora*, IJ tolerance to H_2O_2 exposure correlates with their lifespan in sand (Sumaya et al., 2017). The crossing of two strains and mutagenesis further revealed that tolerance to oxidative stress can be enhanced (Sumaya et al., 2018). These results are consistent with studies conducted in *C. elegans* demonstrating the role of the oxidation level on nematode lifespan. For instance, silencing antioxidant enzymes such as catalases (CTL-2), thioredoxins (TRX-1), peroxidases (PRDX-2 and PRDX-3), or glutathione-S-transferases (GST-5, GST-10) accelerates aging and decreases lifespan (Ayyadevara et al., 2007; Ha et al., 2006; Jee et al., 2005; Miranda-Vizuete et al., 2006; Oláhová et al., 2008; Petriv and Rachubinski, 2004). Noteworthy, not all RS detoxification enzymes are associated with nematode lifespan and some of them have tissue-specific functions (for a review in *C. elegans* see Shields et al. 2021). The impact of RS and their detoxification machinery on *C. elegans* is well advanced, however, how oxidative stress shapes EPN IJs persistence in soil remains elusive and should be further investigated.

3. Abiotic stresses

3.1. Low temperatures

EPN juveniles can persist in the soil for over a year in continental climates (Kurtz et al., 2007) and can cope with cold temperatures (Ali and Wharton, 2013). Yet, EPN tolerance to low temperature and the underlying mechanisms are species- and likely even strain-specific (Godina et al., 2022; Grewal et al., 1994; Jagdale and Grewal, 2003).

IJs can withstand and acclimate to falling temperatures by lowering their metabolism activity (Ali and Wharton, 2013; Brown and Gaugler, 1996). While IJs/dauer nematodes already have a hypometabolism, IJs subjected to cold conditions further slacken their metabolism, as reflected by the slower use rate of their lipid reserves (Andaló et al., 2011). In another example, *H. megidis* IJs decrease the production of proteins involved in metabolism and protein synthesis (e.g., ribosomal proteins), and *S. carpocapsae* decreases the production of proteins involved in intermediary metabolism and oxidative phosphorylation (Jagdale and Grewal, 2003). Reducing the metabolism and protein synthesis, as

in *H. megidis*, may be an effective strategy to prevent proteotoxic stress and to spare energy for the cruiser nematodes (Lillis et al., 2022). Decreasing intermediary metabolism and oxidative phosphorylation may limit energy loss and reactive oxygen species (ROS) production (Lillis et al., 2022).

Additionally, EPNs can enhance their tolerance to low temperatures by producing trehalose, a natural disaccharide (α-d-glucopyranosyl-1,1-α-d-glucopyranoside) involved in resistance to freezing in numerous organisms (Ali and Wharton, 2015; Grewal and Jagdale, 2002; Jagdale and Grewal, 2003; Kandror et al., 2002; Liu et al., 2019; NDong et al., 2002; Virgilio et al., 1994). For instance, cold shock induces the transient activity of the trehalose-6-phosphate synthase (T6PS), trehalose accumulation, and cold tolerance in *S. carpocapsae* and *H. bacteriophora* (Jagdale et al., 2005; Pellerone et al., 2003). Trehalose protects the cells from thermal injury by stabilizing proteins in their native state and preventing denaturation or aggregation (Erkut et al., 2011; Hottiger et al., 1994; Jain and Roy, 2009; Singer and Lindquist, 1998), decreasing damage by oxygen radicals (Benaroudj et al., 2001), and stabilizing lipid membranes (Crowe et al., 1998a; Crowe et al., 1984; Leslie et al., 1994). In EPNs and in *C. elegans*, two trehalose phosphate synthases, Tps-1 and Tps-2, were reported (Depuydt et al., 2014; Murphy et al., 2003; Sonoda et al., 2016). In *C. elegans*, the two isoforms are under the control of one transcription factor (DAF-16) (Depuydt et al., 2014; Murphy et al., 2003; Sonoda et al., 2016). Trehalose is likely released in the intestines and transported via the glucose transporter FGT-1 over the plasma membrane into the pseudocoel (Feng, 2010; Kitaoka et al., 2013). Finally, trehalose and late embryogenesis proteins can promote bioglass formation and enhance EPN tolerance to freezing temperatures (Qiu and Bedding, 2002). Understanding the regulation of trehalose production may offer promising targets to enhance cold tolerance in EPNs.

Some EPN species further respond to cold stress by producing stress and detoxification proteins (Lillis et al., 2022). Incubating EPNs at 9°C promotes the production of dehydrogenases, thioredoxins, glutathione-S-transferases (GSTs), catalase, and oxidoreductases in *S. carpocapsae* (Lillis et al., 2022). In nematodes, the antioxidant-, detoxification-, and unfolding protein response-pathways are regulated by the transcription factor

SKN-1, a critical player for nematode longevity (Choe et al., 2012; Ewald et al., 2015; Gillet et al., 2017).

In *C. elegans*, low temperatures induce epidermal-specific autophagy (Chen et al., 2019). Autophagy is a process that maintains intracellular homeostasis by degrading and recycling cytoplasmic components through a lysosomal pathway (Deter et al., 1967; Levine et al., 2011; Megalou and Tavernarakis, 2009; Mizushima, 2007; Palmisano and Meléndez, 2019). The genetic and molecular pathways involved in autophagy are well characterized in the model nematode species and are likely to be conserved across the different nematode clades (Fu et al., 2022; Palmisano and Meléndez, 2019). The product of PAQR-2 detects low temperatures and upregulates poly-unsaturated fatty acids, gamma-linolenic acid and arachidonic acid, which in turn increases the ratio between unsaturated and saturated fatty acids and increases epidermal autophagy (Chen et al., 2019; Svensk et al., 2013). Silencing bec-1, let-512, or epg-1 in *C. elegans* shortens the nematode lifespan when exposed to low, but not to ambient, temperatures (Chen et al., 2019; Liang et al., 2012; Takacs-Vellai et al., 2005). Interestingly, in their study, Lillis et al (2022) noted that an autophagy-related protein accumulated over storage time in *H. megidis*, and that the protein levels increased faster at 9°C than at 20°C, suggesting a possible role of autophagy in cold tolerance in EPNs.

Additional cold-tolerance mechanisms were described in *C. elegans*, including sensing by transient receptor potential (TRP) channels, activation of a PKC-2/$Ca2^{+}$-sensitive protein kinase C, activation of DAF-16/FOXO, or phosphorylation (Okahata et al., 2022). Whether similar mechanisms also exist in EPNs remains to be investigated.

3.2. Elevated temperatures

Elevated temperatures (>32 °C) have detrimental effect on EPN reproduction, growth, survival and pathogenicity (Grewal et al., 1994; Kung et al., 1991; Zervos et al., 1991). However some species have been isolated from hot deserts (Edgington et al., 2011; Glazer et al., 1991; Kusakabe et al., 2019). This shows that some EPNs have evolved adaptive mechanisms to tolerate elevated temperatures. Up to date, whether the variation in tolerance is genetically determined, or plastic remains unclear.

509 Efforts have been made to elucidate genetic information linked to high temperature tol-
510 erance. For example, *H. bacteriophora* isolates with contrasting heat tolerance were
511 compared based on their specific expression patterns from RNA-seq and highlighted sig-
512 nificant downregulation of transcript levels in the heat tolerant (EN01) than in the heat
513 sensitive (Grofit) nematodes (Levy et al., 2020). In the study, highly tolerant line (EN01)
514 transcripts specific include glycerol kinase (GK), fatty acid desaturase (FAD), and a zinc
515 finger protein (ZFP). Down regulation of TRE (trehalose)-stress responding in low tolerant
516 lines (Grofit) while the heat shock proteins (HSPs) and related pathways were up regu-
517 lated in of both natural variants. HSPs catalyze the unfolding of either native or denatured
518 proteins enabling the cell to eliminate or renature proteins damaged by high temperature
519 (Candido et al., 1989). High genetic variation in one of the family protein, HSP70, has long
520 been reported using the Restriction Fragment Length Polymorphism (RFLP) among 6 dif-
521 ferent high temperature surviving EPN species from different geographical locations
522 (Hashmi et al., 1997). However the involvement of these HSPs in heat tolerance of stud-
523 ied EPN isolates is not known (Glazer, 1996). In other studies, heat stress causes accu-
524 mulation of trehalose as well as a shift in enzyme activities in the trehalose metabolism
525 (T6PS and trehalase). For example the activity of T6PS and trehalase increase and de-
526 crease, respectively, during the heat shock (Jagdale et al., 2005; Jagdale and Grewal,
527 2003). The trehalose accumulation might be an adaptive way to cope with thermal stress
528 or preparation for desiccation stress that will subsequently follow due to evaporation. In
529 other studies, heat stress causes accumulation of trehalose as well as a shift in enzyme
530 activities in the trehalose metabolism (T6PS and trehalase). The genetic manipulation
531 and or modification to attain heat tolerance in EPNs is possible through continuous ex-
532 posure of EPNs to heat stress. For instance, in one study, hybrids of *H. bacteriophora*
533 (PS7 and H06 crosses), have increased their mean temperature tolerance from 38.5 to
534 39.2°C after four selection steps (Ehlers et al., 2005).

535 Although the genetic mechanisms of heat tolerance in EPNs are not clear yet, studies in
536 *C. elegans* have shown that the model organism is equipped with systems helping them
537 in tolerating the adverse effects of high temperatures stress. The Heat shock transcrip-
538 tion factor-1 (HSF-1) and the forkhead box O (FOXO) transcription factor DAF-16 upreg-
539 ulate chaperone expression after acute heat exposure (Park et al., 2017). According to

previous studies, HSF1 targets genes encoding molecular chaperones, that are essential for protein folding, preventing misfolding, and restoring the native conformation of misfolded proteins. Thus HSF1 promotes stability by preventing protein aggregation and subsequent proteome imbalance (Li et al., 2017; Servello and Apfeld, 2020).

3.3. Desiccation

EPNs are soil organisms living in large soil pores on surface films of water or at relative humidity > 97% (Jung et al., 2012; Navaneethan et al., 2010; Neher, 2010). They possess a hydrostatic skeleton, inferring that the nematode body is supported by fluid pressure (Neher, 2010; Riddle et al., 1997; Wallace, 1971). Low water content may thus drastically limit their motility and survival. (Glazer, 2002; Grant and Villani, 2003; Kaya, 2018).

Nematodes have evolved behavioural and biochemical strategies to cope with unfavourable soil moisture levels. The model worm *C. elegans* can navigate towards more favourable environment following a gradient in soil moisture, a process referred to as hygrotaxis (El-Borai et al., 2016). The nematode perceives and responds to changes in water gradients as shallow as 0.03% (Russell et al., 2014). Hygrotaxis requires the activation of both mechanosensitive- and thermosensitive pathways (Hibshman et al., 2020; Russell et al., 2014). The mechanosensitive pathway relies on a conserved DEG/ENaC/ASIC mechanoreceptor complex in the FLP neuron pair in the nematode cuticle (Russell et al., 2014). The FLP neurons are located below the epidermis and are likely sensitive to changes in cuticle stretch by hydration (Russell et al., 2014). The thermosensitive pathway is activated through cGMP-gated channels in the AFD neuron pair (Russell et al., 2014). The relevance of thermosensing during the detection and response to dry environment was suggested to reflect the drop of temperatures due to water evaporation in moister soil layers (Russell et al., 2014). Signalling pathways involving a patched related protein (DAF-6), a notch ligand (OSM-11), and mitogen-activated protein kinases (MAPK) were further suggested to mediate the nematode behaviour (Banton and Tunnacliffe, 2012). Interestingly, hygrotaxis depends on the nutritional status of the nematode, as dry conditions only trigger *C. elegans* directional movements in starved conditions (Russell et al., 2014). Although yet untested, similar mechanisms likely drive moisture perception

569 and response in EPNs. Nematodes have evolved behavioural and biochemical strategies
570 to cope with unfavourable soil moisture levels.

571 Under severe dehydration, EPNs enter in anhydrobiosis (Gal et al., 2005a). Anhydrobiosis
572 is a reversible physiological process by which an organism loses up to 98% of its body
573 water up to metabolism arrest, a state called cryptobiosis (Cooper and van Gundy, 1971;
574 Womersley, 1981). EPNs can reach partial anhydrobiosis (decreasing oxygen consump-
575 tion by ~80%, (Grewal, 2000a)), but not cryptobiosis, and are thus considered as quies-
576 cent anhydrobiotes (Gaugler and Kaya, 1990; Simons and Poinar, 1973; Womersley,
577 1990). Desiccation elicits a transient increase in EPN metabolism, prior to slowing it
578 down to levels below the normal metabolic rate (Grewal, 2000b). The glycogen produc-
579 tion decreases, as reflected by the down-regulation of the glycogen synthase (*gsy-1* while
580 trehalose and glycerol synthesis from existing glycogen and neutral lipid reserves in-
581 creases (Behm, 1997; Crowe and Crowe, 1992; Gal et al., 2001; Qiu et al., 2000; Wom-
582 ersley, 1990). Trehalose progressively replaces water in membranes and contributes to
583 maintaining the structures and stabilizing proteins (Behm, 1997; Crowe et al., 1998b;
584 Crowe and Crowe, 1992; Elbein et al., 2003). In *S. feltiae*, desiccation triggers a two-fold
585 increase in trehalose contents (Solomon and Glazer, 1999). Additionally, the casein ki-
586 nase (CK2) is induced and elicits the transcriptional activation of a nucleosome-assem-
587 bly protein (NAP-1) through physical interaction (Gal et al., 2005b; Gal et al., 2003;
588 Somvanshi et al., 2008). Osmoregulant molecules (e.g. produced by ALDH), antioxidants
589 (e.g., Gg., DESC47, HSP40) are further synthesized and may further protect the cells from
590 desiccation-induced damage (Gal et al., 2003; Solomon et al., 2000; Somvanshi et al.,
591 2008).

592 While *C. elegans* dauer nematodes are true anhydrobiotes which are able to lose more
593 than 98% of their water content (Erkut et al., 2011; Madin and Crowe, 1975), some of the
594 genetic and molecular mechanisms underlying desiccation responses are likely to be
595 similar to those of EPNs' (for review see Hibshman et al., 2020). These mechanisms in-
596 clude the production of trehalose (by trehalose 6-phosphate synthases TPS-1 and TPS-
597 2), an upregulation of the glyoxylate shunt to synthesize succinate and malate (by the
598 isocitrate lyase and malate synthase enzyme ICL-1), the synthesis of late embryogenesis

599 abundant proteins (LEA proteins), heat shock proteins (F08H9.3 and F08H9.4), and anti-
600 oxidant enzymes (SOD-1, GPX-2, GPX-6, GPX-7, CTL-1) and the desaturation of fatty ac-
601 ids by fatty acid desaturases (FAT-3 through FAT-7) (Abusharkh et al., 2014; Braeckman
602 et al., 2009; Erkut et al., 2016; Erkut et al., 2013; Gal et al., 2004). The accumulation of
603 trehalose, heat-shock proteins, and LEA proteins is further associated with the formation
604 of glasses in a dry state, a process referred to as vitrification, which stabilize biomaterials
605 and avoid free-radical oxidation, lipid phase transition and protein crystallization
606 (Crowe, 2002; Crowe et al., 1998a; Sun and Leopold, 1997). Finally, LEA proteins act as
607 molecular shields around proteins and prevent them from aggregating (Goyal et al.,
608 2005; Wise and Tunnacliffe, 2004). EPN ability for water replacement, vitrification, and
609 molecular shielding under desiccation may be targeted as promising trait for EPN selec-
610 tion (Hibshman et al., 2020).

611 Desiccation-induced quiescence can increase (e.g., *S. carpocapsae*) or decrease (e.g.,
612 in *S. feltiae*) EPN lifespan, but has no or limited effect on their virulence when stored at
613 room temperature (25°C) (Grewal, 2000a; Grewal, 2000b, 1998; Matadamas-Ortiz et al.,
614 2014). The differential effect of quiescence on EPN lifespan has been attributed to their
615 foraging lifestyles. Ambushers (e.g., *S. carpocapsae*) may be more exposed to desicca-
616 tion during nictation than mobile cruisers (e.g., *S. feltiae*) and may thus have been under
617 higher selection pressure to tolerate desiccation stresses (Grewal, 2000a; Patel et al.,
618 1997a). However, a concomitant storage of desiccated EPNs at lower temperatures re-
619 duce EPN lifespan (Grewal, 2000a). This sharp effect may be explained by the costs as-
620 sociated with desiccation and low temperature tolerance costs (Grewal, 2000a).

621 Desiccation tolerance is increased in EPNs being exposed to dehydration gradually ra-
622 ther than rapidly (Simons and Poinar, 1973; Womersley, 1990). Preconditioning EPNs to
623 sub-lethal dehydration levels elicit an adaptive response that enhances their tolerance
624 to subsequent, stronger, desiccating conditions (Kung et al., 1991; Nimkingrat et al.,
625 2013; Patel et al., 1997a; Strauch et al., 2004) . The potential of the adaptive response is
626 species and strain specific (Grewal, 2000a; Nimkingrat et al., 2013; Somvanshi et al.,
627 2008). The selection of desiccation tolerant strains was successful in several studies
628 (Mukuka et al., 2010b; Mukuka et al., 2010a), although this trait is rapidly lost during *in*

vivo mass production when the selection pressure is relieved (Anbesse et al., 2013; Mukuka et al., 2010c).

3.4. Hypoxia

Nematodes are aerobic organisms. They use oxygen to fuel mitochondrial energy production and to ensure a proper metabolism. In soil, oxygen levels are naturally lower than ambient levels (21%) due to root- and microbial respiration and/or soil water contents, and ranges between 0.5 and 21% (Félix and Braendle, 2010; Grant and Villani, 2003). Hypoxia (<1% oxygen for worms like *C. elegans*) limits the cell mitochondrial respiration and energy production, disrupts proteostasis (Fawcett et al., 2015). EPN tolerance to hypoxia is highly variable among species and strains (Burman and Pye, 1980; Grewal et al., 2002; Kour et al., 2021; Morton and García-del-Pino, 2009). For instance, the survival rate of different *H. bacteriophora* strains ranges from 10 to 90% after four day exposure to hypoxia (Grewal et al., 2002). Nematodes have evolved behavioural and physiological strategies to prevent hypoxia-induced detrimental effects on their virulence, longevity, and survival.

Nematodes can perceive environmental oxygen levels and migrate up or down its concentration gradients. While this behaviour remains elusive in EPNs, it has been investigated in *C. elegans* (Choudhry and Harris, 2018; Gray et al., 2004; Kitazume et al., 2018; Kumar, 2016). Interestingly, the oxygen sensing neurons (AQR, PQR, URX, BAG) involved in *C. elegans* repellence from elevated (21%) levels of oxygen do not mediate the nematode response to hypoxia (Zhao et al., 2022). Instead, the sensory neurons ADL and ASH seem to interact with signals from neurons producing 3',5'-cyclic guanosine monophosphate (cGMP), a major second messenger, to modulate the nematode response (Zhao et al., 2022). Additionally, mitochondria are critical in sensing and responding to hypoxic conditions. Hypoxia triggers a reversible oxidation of a cysteine residue (Cys366) in the mitochondrial complex I subunit NDUF2.1, causing a decrease in the complex activity (Onukwufor et al., 2022). This decrease in turn modulates the production of ROS in a site-specific manner, a process sufficient to elicit a behavioural response to hypoxia (Kaelin and Ratcliffe, 2008; Onukwufor et al., 2022). In parallel, the conserved oxygen-activated

prolyl hydroxylases (PHDs) can also act as oxygen sensors and activate the hypoxia adaptation program (Bruick and McKnight, 2001; Darby et al., 1999; Epstein et al., 2001; Ivan et al., 2002; Kaelin and Ratcliffe, 2008; Trent et al., 1983). In *C. elegans*, a single family member of PHD is known (EGL-9) (Epstein et al., 2001). Under hypoxic conditions, the lower PHD activity leads to the inhibition of the glutamate receptor (GLR-1) recycling, itself inducing a roaming behaviour, and to the upregulation of the hypoxia inducible factor (HIF-1) and its target genes, including genes involved in the energy metabolism, pathophysiological processes, cell differentiation, and apoptosis (Dengler et al., 2014; Kumar, 2016; Liu et al., 2012; Ma et al., 2012; Park and Rongo, 2016; Powell-Coffman, 2010; Shen et al., 2006; Vora et al., 2022). Finally, the nuclear hormone receptor NHR-49 is required to orchestrate a *hif-1* independent response to hypoxia, including detoxification, and autophagy genes (Doering et al., 2022). The orchestrated response to low oxygen levels enables the nematode to switch from mitochondrial respiration to anaerobic glycolysis while maintaining its homeostasis (physiological functions), and ultimately support the nematode survival (Doering et al., 2022; Shih et al., 1996).

3.5. Ultra-violet (UV) tolerance

UV light exposure elevates the mutation rates in the genome. EPN IJs can suffer from UV irradiation, although an important variability between strains and between studies should be noted. For example, (Fujiie and Yokoyama, 1998; Gaugler and Boush, 1978; Jagdale and Grewal, 2007) showed that UV radiations drastically decreased IJ virulence and survival in *S. carpocapsae*, but (Shapiro-Ilan et al., 2015) found that the effect was neglectable. While UV exposure was similar in both studies in terms of wavelength (254 nm) and exposure duration (7-10 min), several hypotheses can explain the apparent discrepancy. First, the UV irradiation intensity is a crucial parameter to consider. Studies in *C. elegans* demonstrate that UV irradiations at 20 J/m^2/min triggers severe stresses, while irradiations at 10 J/m^2/min elicit limited toxicity (Wang et al., 2010). Second, pre-exposure to a mild, sub-lethal, stress can elicit an adaptive response of the organism, leading to increased tolerance to higher stress levels (Yanase et al., 1999). Finally, different exogenous (e.g., temperature) and endogenous (e.g., nutritious status) conditions may modulate the ability of EPNs to cope with specific stresses. When compared within

688 the same study, steinernematids have a higher tolerance to UV exposure than het-
689 erorhabditids (Shapiro-Ilan et al., 2015). Yet, the tolerance spectrum of different *Het-*
690 *erorhabditis* strain remains large, suggesting possible genetic differences in UV toler-
691 ance between strains (Shapiro-Ilan et al., 2015).

692 Several UV tolerance mechanisms were elucidated in *C. elegans*. The model nema-
693 tode can perceive and avoid UV light through (at least) four sensory neurons (ASJ, ASK,
694 AWB, ASH) (Bargmann, 2006; Ward et al., 2008). ASH and ASK neurons express a seven-
695 transmembrane protein (LITE-1) UV light receptor (Gong et al., 2016) whose activation
696 triggers the release of a neurotransmitter, glutamate (Ozawa et al., 2022). Glutamate re-
697 ceptors (*glc-3*, *mgl-1*, *mgl-2*) are expressed only in ASH and ASK (glutaminergic neurons)
698 (Ozawa et al., 2022). The mechanisms involved in ASJ and AWB activation in response to
699 UV light remain yet more elusive. Using similar technologies than in *C. elegans* (e.g, neu-
700 ron ablation, genetically inserted synapses, RNAi) will contribute to better understanding
701 whether the same cellular and molecular mechanisms are involved in EPNs perception
702 and behavioral responses to UV irradiations. Upon exposure to UV, animal cells activate
703 surveillance mechanisms responsible for cell cycle arrest, DNA repair, and apoptosis in
704 case of elevated DNA damage (Bailly and Gartner, 2013; Fortunato et al., 2021). These
705 cellular mechanisms have been extensively studied in *C. elegans* and their underlying
706 pathways have been mostly elucidated (for review see (Canchaya et al., 2003; Elsakrmy
707 et al., 2020)). Because DNA damage response pathways are highly conserved in animals
708 (Clancy, 2008), it is likely that EPNs undergo similar processes upon UV exposure. Un-
709 derstanding the impact of UV light on EPN biology is critical when using inundative or
710 foliar pest control strategies.

711 # 4. Biotic stresses

712 ## 4.1. Viruses

713 To date, no viral pathogen is known to infect EPNs in soil, although the presence of
714 bacteriophages has been described in the nematode endosymbiont genomes (Can-
715 chaya et al., 2003; Kaya et al., 1998). Accumulating genomic resources such as RNAseq

716 data will likely identify a diversity of viruses interacting with EPNs. Several viruses are in-
717 deed known to infect *C. elegans* (Félix et al., 2011). The nematode anti-viral response
718 includes RNA interference (RNAi), RNA uridylation, and intracellular pathogen response
719 (IPR) (for review see Martineau et al., 2021). The natural variation in *C. elegans* resistance
720 to viral infections revealed the crucial role of the dicer related helicase DHR-1 protein, a
721 pattern recognition receptor (PRR) that mediates the intracellular pathogen response
722 (IPR) program (van Sluijs et al., 2021). Whether and which viruses can infect EPNs, and
723 which markers are critical for EPN immune response remains to be elucidated.

724 4.2. Bacteria

725 Soil bacteria can attach to nematodes' cuticle (Adam et al., 2014; Topalović et al.,
726 2019) Pathogenic bacteria have been isolated from free-living nematodes, (e.g., *C. ele-*
727 *gans*, (Schulenburg and Félix, 2017)), plant pathogenic nematodes, (e.g., *Meloidogyne*
728 *hapla,* (Adam et al., 2014; Elhady et al., 2017), and EPNs (Bajaj and Walia, 2005; Enright
729 et al., 2003). For instance, the screening of EPN soil isolates identified *Pasteuria* sp. on
730 the cuticle and inside *S. pakistense* IJs (Bajaj and Walia, 2005). In addition to that, re-
731 cently, several other bacteria were washed-off from soil borne nematodes´ cuticle and
732 most of them proved to be pathogenetic against *Galleria mellonella* larvae (Loulou et al.,
733 2023). The entomopathogenic bacterial strains isolated by the authors include *Lysini-*
734 *bacillus fusiformis, Kaistia sp., Alcaligenes sp., Enterobacter sp., Klebsiella quasipneu-*
735 *moniae* subsp. *quasipneumoniae, Bacillus cereus, Acinetobacter sp., Pseudomonas ae-*
736 *ruginosa, and Morganella morganii* subsp. *morganii* (Loulou et al., 2023). The presence
737 of the endospore-forming bacteria is associated with the abolition of virulence in EPNs
738 (Bajaj and Walia, 2005). The EPN immune response to bacterial agents remains unclear.
739 In *C. elegans*, a pathogen attack is detected through PRRs that detect pathogen-associ-
740 ated molecular patterns and/or disturbances in cellular homeostasis and triggers cell
741 autonomous and non-autonomous responses (Martineau et al., 2021). Interestingly, *C.*
742 *elegans* is able to detect and evade pathogenic bacteria (Bai et al., 2013; Kim and Flavell,
743 2020; Schulenburg and Müller, 2004; Tran et al., 2017). For example, the worm can detect

and avoid bacterial products, such as the exolipid serrawettin W2 from *Serratia marcescens* (Pradel et al., 2007). Understanding how EPNs detect and respond to specific pathogens may provide target markers that enhance EPN survival in soil.

4.3. Fungi

Nematophagous fungi are common nematode enemies and can be classified as nematode-trapping (predators), endoparasitic, or egg- and cyst- parasites (Lopez-Llorca and Jansson, 2007; Pathak et al., 2017; Soares et al., 2018). The presence of nematophagous fungi in the soil can decrease EPN populations and their biocontrol efficacy (Kaya and Koppenhöfer, 1996; Soares et al., 2018). EPNs can protect themselves from the fungal predators/parasites through several mechanisms. First, cruising nematodes with high mobility can move away from a fungal trap (Bright et al., 2009; Karthik Raja et al., 2021). Second, Heterorhabdits species conserve their second-stage cuticle after moulting for some time, often until invasion into the insect, which provides them an enhanced protection from fungal infection (Timper and Kaya, 1989). Third, EPNs can detect and avoid fungal chemical cues, although several fungal species have evolved to produce nematode attractants (El-Borai et al., 2011). Finally, EPN can learn cues associated with the presence of fungi and avoid them in subsequent exposure (Willett et al., 2017). In *C. elegans*, the detection of nematophagus fungi is achieved through mechanical and chemical perception, via the tyramine signalling (Maguire et al., 2011; O'Donnell et al., 2020). Fungus-induced cuticular damage and fungal polysaccharides further activate the worm immune system (e.g., production of antimicrobial peptides) via pattern recognition receptors (PRRs) (Martineau et al., 2021). EPNs with enhanced physical or immune defenses, as well as “educated” EPNs, may provide future avenues to treat fields with high nematophagous fungi density and ensure EPN efficacy.

4.4. Insects

Predatory collembolas, such as *Folsomia candida, Sinella curviseta*, or *Hypogasturas cotti*, prey on EPNs, reducing their biocontrol efficacy in natural conditions (Epsky et al., 1988; Gilmore and Potter, 1993; Hodson et al., 2012; Ishibashi et al., 1987; Read et al., 2006; Ulug et al., 2014). Although very few studies investigated the EPN defenses against

insects, it can be hypothesized that nematodes and/or their endosymbionts, are producing and exuding a range of chemicals that repels their enemies.

5. Engineering superior nematodes

5.1. Artificial selection and genetic engineering methods

Understanding mechanisms that modulate EPN IJ persistence in soil will be key to develop “superior” (more resistant or tolerant) nematodes and thus enhance pest biocontrol. Engineering superior EPNs can be achieved through artificial selection or genetic engineering (Lu et al., 2016). The short life cycle of EPNs and their ease of mass cultivation render them ideal to select traits of interest.

Artificial selection can be conducted by exposing EPNs to a chosen selection pressure (e.g., desiccation, low temperature) for several generations. Genetic traits that promote EPN fitness under the imposed stress will be selected. Many studies successfully improved EPN tolerance to diverse stressors, such as cold (Koppenhöfer et al., 2000), plant secondary metabolite (Zhang et al., 2019), plant volatiles (Hiltpold et al., 2010), etc. Sequencing the genome of the first and last, selected, EPN generations may further allow to elucidate the genetic pathways/markers involved in tolerance. However, as several pathways can be concomitantly involved in tolerance to one stress, it is judicious to split the initial EPN population in several sub-populations each ongoing artificial selection in parallel to compare the obtained results. Additionally, selecting several superior strains in variable genetic backgrounds is recommended to prevent trait deterioration through inter-crossing (Bilgrami et al., 2006; Chaston et al., 2011). Not only have EPNs been selected under stressful conditions, but also their endosymbiontic bacteria alone. The best example was shown in an experimental evolution study where five *Photorhabdus* symbionts from different nematodes were selected in benzoxazinoids. The selected endosymbionts were later reintroduced to their host strain and one of the selected bacteria significantly improved the infectivity of the host strain against benzoxazinoid-sequestering western corn rootworm (Machado et al., 2020).

Genetic engineering tools include mutagenesis, transgene insertion, and genome editing tools. Mutagenesis can be performed by exposing nematodes to chemical agents such as ethyl methane sulfonate (EMS) (Liu et al., 2012; Sumaya et al., 2018). The induced DNA mutations may (or may not) lead to the appearance of a desired phenotype that can then be stabilized. Transgenes can be inserted into the gonads of adult hermaphrodites, resulting in extrachromosomal arrays that can be further incorporated into the genome (Mello et al., 1991). However, the insertion site of the sequences is not controlled in these assays (Praitis et al., 2001; Wilm et al., 1999). To palliate these limitations, several genome editing technologies were developed, such as zinc-finger nucleases (ZFNs), transcriptional activator-like nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) (Sugi, 2016). ZFNs and TALENs are engineered site specific nucleases that induce double strand breaks at desired loci (Wood et al., 2011). The breaks are then repaired by non-homologous end-joining, resulting in insertions and deletions at the site of interest (Wei et al., 2014; Wood et al., 2011). CRIPSR/Cas9 allows targeted genome editing by incorporating foreign nucleotides into the CRISPR locus of the host genome, resulting in the production of CRISPR RNAs (crRNAs) and to the sequence-specific cleavage of homologous target dsDNA by Cas endonucleases (Jinek et al., 2012). CRISPR/Cas9 can induce heritable changes in the nematodes' genome (Bortesi and Fischer, 2015; Chiu et al., 2013; Frøkjær-Jensen, 2013; Lo et al., 2013; Tzur et al., 2013).

5.2. Selection limitations

Engineering EPNs that are superior in tolerating some stresses can be associated with some limitations. First, the success of selection depends on the trait heritability (h^2) (Hartl and Clark, 1997). For instance, the heritability of oxidative stress tolerance is of h^2>0.9 in *H. bacteriophora*, implying a high probability for the tolerance trait to be transmitted to the next generation (Sumaya et al., 2018). Engineering EPNs that are superior in tolerating some stresses can be associated with some limitations. Firstly, the selected traits may deteriorate in absence of the selective pressure (Anbesse et al., 2013; Chaston et al., 2011). Second, selecting specific traits may come at the expense of other traits relevant for EPN efficacy (persistence in soil, but also infectivity, reproduction, or storage

ability). Neutral (e.g., between desiccation and freezing tolerance) (Sumaya et al., 2017), negative (e.g., between desiccation tolerance and EPN fitness) (Gaugler et al., 1990), and positive (e.g., between heat-tolerance and longevity) (Grewal et al., 2002) correlations were reported. A better understanding of the genetic mechanisms underlying the different responses will be crucial in predicting the occurrence of possible cross-talks. Genetic improvement should always be associated with a thorough characterization of the EPN virulence, longevity, and reproduction abilities, ideally under field conditions (Bilgrami et al., 2006; Gaugler et al., 1989).

6. Conclusion

EPNs are promising biological control agents of numerous agricultural pests. Yet, their variable outcome in controlling pest populations in the field has hindered their use. Engineering superior EPNs with enhanced tolerance to endogenous and exogenous stresses encountered at the free-living stage (IJs) will likely promote their efficacy. Importantly, the selection of traits of interest should be performed according to the specific characteristics of the field and target pest. Using such a precision approach may reinforce the use of biological control in sustaining food production and food security.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

Abu Hatab, M.A., Gaugler, R., 1997. Influence of growth temperature on fatty acids and phospholipids of *Steinernema riobravis* infective juveniles. J. Therm. Biol. 22, 237–244. https://doi.org/10.1016/S0306-4565(97)00017-X.

Abusharkh, S.E., Erkut, C., Oertel, J., Kurzchalia, T.V., Fahmy, K., 2014. The role of phospholipid headgroup composition and trehalose in the desiccation tolerance of *Caenorhabditis elegans*. Langmuir 30, 12897–12906. https://doi.org/10.1021/la502654j.

Adam, M., Westphal, A., Hallmann, J., Heuer, H., 2014. Specific microbial attachment to root knot nematodes in suppressive soil. Appl. Environ. Microbiol. 80, 2679–2686. https://doi.org/10.1128/AEM.03905-13.

Agrawal, A.A., 2011. Current trends in the evolutionary ecology of plant defence. Funct. Ecol. 25, 420–432. https://doi.org/10.1111/j.1365-2435.2010.01796.x.

Ali, F., Wharton, D.A., 2013. Cold tolerance abilities of two entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora*. Cryobiology 66, 24–29. https://doi.org/10.1016/j.cryobiol.2012.10.004.

Ali, F., Wharton, D.A., 2015. Infective juveniles of the entomopathogenic nematode, *Steinernema feltiae* produce cryoprotectants in response to freezing and cold acclimation. PLOS ONE 10, e0141810. https://doi.org/10.1371/journal.pone.0141810.

Anbesse, S., Sumaya, N.H., Dörfler, A.V., Strauch, O., Ehlers, R.-U., 2013. Selective breeding for desiccation tolerance in liquid culture provides genetically stable inbred lines of the entomopathogenic nematode *Heterorhabditis bacteriophora*. Appl. Microbiol. Biotechnol. 97, 731–739. https://doi.org/10.1007/s00253-012-4227-5.

Andaló, V., Moino, A., Jr., Maximiniano, C., Campos, V.P., Mendonça, L.A., 2011. Influence of temperature and duration of storage on the lipid reserves of entomopathogenic nematodes. Rev. Colomb. Entomol. 37, 203–209.

Ayyadevara, S., Dandapat, A., Singh, S.P., Siegel, E.R., Shmookler Reis, R.J., Zimniak, L., Zimniak, P., 2007. Life span and stress resistance of *Caenorhabditis elegans* are

differentially affected by glutathione transferases metabolizing 4-hydroxynon-2-enal. Mech. Ageing Dev. 128, 196–205. https://doi.org/10.1016/j.mad.2006.11.025.

Bai, X., Adams, B.J., Ciche, T.A., Clifton, S., Gaugler, R., Kim, K.-S., Spieth, J., Sternberg, P.W., Wilson, R.K., Grewal, P.S., 2013. A lover and a fighter: the genome sequence of an entomopathogenic nematode *Heterorhabditis bacteriophora*. PLOS ONE 8, e69618. https://doi.org/10.1371/journal.pone.0069618.

Bailly, A., Gartner, A., 2013. Germ cell apoptosis and DNA damage responses, in: Tim Schedl (Ed.), Germ cell development in *C. elegans*. Springer, New York, NY, pp. 249–276.

Bajaj, H., Walia, K.K., 2005. Studies on a *Pasteuria* isolate from an entomopathogenic nematode, *Steinernema pakistanense* (Nematoda: Steinernematidae). Nematol. 7, 637–640. https://doi.org/10.1163/156854105774384813.

Baniya, A., DiGennaro, P., 2021. Genome announcement of *Steinernema khuongi* and its associated symbiont from Florida. G3-Genes. Genom. Genet. 11, jkab053. https://doi.org/10.1093/g3journal/jkab053.

Baniya, A., Huguet-Tapia, J.C., DiGennaro, P., 2020. A draft genome of *Steinernema diaprepesi*. J. Nematol. 52, 1–4. https://doi.org/10.21307/jofnem-2020-069.

Banton, M.C., Tunnacliffe, A., 2012. MAPK phosphorylation is implicated in the adaptation to desiccation stress in nematodes. J. Exp. Biol. 215, 4288–4298. https://doi.org/10.1242/jeb.074799.

Bargmann, C.I., 2006. Chemosensation in *C. elegans*. WormBook, 1–29. https://doi.org/10.1895/wormbook.1.123.1.

Bedding, R.A., Molyneux, A.S., 1982. Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). Nematol. 28, 354–359. https://doi.org/10.1163/187529282X00402.

Behm, C.A., 1997. The role of trehalose in the physiology of nematodes. Int. J. Parasitol. 27, 215–229. https://doi.org/10.1016/S0020-7519(96)00151-8.

Benaroudj, N., Lee, D.H., Goldberg, A.L., 2001. Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals. J. Biol. Chem. 276, 24261–24267. https://doi.org/10.1074/jbc.M101487200.

Bhat, C.G., Somvanshi, V.S., Budhwar, R., Godwin, J., Rao, U., 2021. Nematode genome announcement: The draft genome sequence of entomopathogenic nematode

Heterorhabditis indica. J. Nematol. 53, e2021-101. https://doi.org/10.21307/jofnem-2021-101.

Bilgrami, A.L., Gaugler, R., Shapiro-Ilan, D.I., Adams, B.J., 2006. Source of trait deterioration in entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* during in vivo culture. Nematol. 8, 397–409. https://doi.org/10.1163/156854106778493394.

Birch, E., Nicholas, A., Begg, G.S., Squire, G.R., 2011. How agro-ecological research helps to address food security issues under new IPM and pesticide reduction policies for global crop production systems. J. Exp. Bot. 62, 3251–3261. https://doi.org/10.1093/jxb/err064.

Bortesi, L., Fischer, R., 2015. The CRISPR/Cas9 system for plant genome editing and beyond. Biotechnol. Adv. 33, 41–52. https://doi.org/10.1016/j.biotechadv.2014.12.006.

Braeckman, B.P., Houthoofd, K., Vanfleteren, J.R., 2009. Intermediary metabolism. https://doi.org/10.1895/wormbook.1.146.1.

Bright, D., El-Borai, F., Stuart, R., Graham, J., Cubero, J., Duncan, L., 2009. Differential susceptibility of entomopathogenic nematodes to nematophagous fungi from Florida citrus orchards. Nematol. 11, 231–241. https://doi.org/10.1163/156854109X429565.

Brivio, M.F., Mastore, M., 2018. Nematobacterial complexes and insect hosts: Different weapons for the same war. Insects 9, 117. https://doi.org/10.3390/insects9030117.

Brown, I.M., Gaugler, R., 1996. Cold tolerance of steinernematid and heterorhabditid nematodes. J. Therm. Biol. 21, 115–121. https://doi.org/10.1016/0306-4565(95)00033-X.

Bruick, R.K., McKnight, S.L., 2001. A conserved family of prolyl-4-hydroxylases that modify HIF. Science 294, 1337–1340. https://doi.org/10.1126/science.1066373.

Burman, M., Pye, A.E., 1980. *Neoaplectana carpocapsae*: Respiration of infective juveniles. Nematol. 26, 214–219. https://doi.org/10.1163/187529280X00107.

Canchaya, C., Proux, C., Fournous, G., Bruttin, A., Brüssow, H., 2003. Prophage genomics. Microbiol. Mol. Biol. Rev. 67, 238-276. https://doi.org/10.1128/MMBR.67.2.238-276.2003.

951 Candido, E.P., Jones, D., Dixon, D.K., Graham, R.W., Russnak, R.H., Kay, R.J., 1989.
952 Structure, organization, and expression of the 16-kDa heat shock gene family of
953 *Caenorhabditis elegans*. Genome 31, 690–697. https://doi.org/10.1139/g89-126.
954 Castelletto, M.L., Gang, S.S., Okubo, R.P., Tselikova, A.A., Nolan, T.J., Platzer, E.G., Lok,
955 J.B., Hallem, E.A., 2014. Diverse host-seeking behaviors of skin-penetrating
956 nematodes. PLOS Pathog. 10, e1004305.
957 https://doi.org/10.1371/journal.ppat.1004305.
958 Chandler, K.J., 2002. Strategies to control greyback canegrub in early harvested ratoon
959 crops: SRDC final report IPB001. BSES.
960 Chaston, J.M., Dillman, A.R., Shapiro-Ilan, D.I., Bilgrami, A.L., Gaugler, R., Hopper, K.R.,
961 Adams, B.J., 2011. Outcrossing and crossbreeding recovers deteriorated traits in
962 laboratory cultured *Steinernema carpocapsae* nematodes. Int. J. Parasitol. 41, 801–
963 809. https://doi.org/10.1016/j.ijpara.2011.02.005.
964 Chen, Y.-L., Tao, J., Zhao, P.-J., Tang, W., Xu, J.-P., Zhang, K.-Q., Zou, C.-G., 2019.
965 Adiponectin receptor PAQR-2 signaling senses low temperature to promote *C.*
966 *elegans* longevity by regulating autophagy. Nat. Commun. 10, 2602.
967 https://doi.org/10.1038/s41467-019-10475-8.
968 Chiu, H., Schwartz, H.T., Antoshechkin, I., Sternberg, P.W., 2013. Transgene-free
969 genome editing in *Caenorhabditis elegans* using CRISPR-Cas. Genetics 195, 1167–
970 1171. https://doi.org/10.1534/genetics.113.155879.
971 Choe, K.P., Leung, C.K., Miyamoto, M.M., 2012. Unique structure and regulation of the
972 nematode detoxification gene regulator, SKN-1: Implications to understanding and
973 controlling drug resistance. Drug Metab. Rev. 44, 209–223.
974 https://doi.org/10.3109/03602532.2012.684799.
975 Choudhry, H., Harris, A.L., 2018. Advances in hypoxia-inducible factor biology. Cell
976 Metab. 27, 281–298. https://doi.org/10.1016/j.cmet.2017.10.005.
977 Ciche, T.A., Darby, C., Ehlers, R.-U., Forst, S., Goodrich-Blair, H., 2006. Dangerous
978 liaisons: The symbiosis of entomopathogenic nematodes and bacteria. Biol. Control
979 38, 22–46. https://doi.org/10.1016/j.biocontrol.2005.11.016.
980 Ciche, T.A., Ensign, J.C., 2003. For the insect pathogen *Photorhabdus luminescens*,
981 which end of a nematode is out? Appl. Environ. Microbiol. 69, 1890–1897.
982 https://doi.org/10.1128/AEM.69.4.1890-1897.2003.

983 Clancy, S., 2008. DNA damage & repair: Mechanisms for maintaining DNA integrity.
984 Nature Education.
985 Clarke, D.J., 2020. *Photorhabdus*: A tale of contrasting interactions. Microbiology 166,
986 335–348. https://doi.org/10.1099/mic.0.000907.
987 Cooper, A.F., van Gundy, S.D., 1971. Senescence, quiescence, and cryptobiosis, in:
988 Zuckerman, B.M., Mai, W.F., Rhode R.A. (Eds.), Plant parasitic nematodes. Academic
989 Press, New York, USA, pp. 297–318.
990 Crowe, J.H., Carpenter, J.F., Crowe, L.M., 1998a. The role of vitrification in
991 anhydrobiosis. Annu. Rev. Physiol. 60, 73–103.
992 https://doi.org/10.1146/annurev.physiol.60.1.73.
993 Crowe, J.H., Clegg, J.S., Crowe, L.M., 1998b. Anhydrobiosis: The water replacement
994 hypothesis 6, 440–455. https://doi.org/10.1007/978-1-4613-0311-4_20.
995 Crowe, J.H., Crowe, L.M., 1992. Membrane integrity in anhydrobiotic organisms: Toward
996 a mechanism for stabilizing dry cells, in: Somero, G.N., Osmond, C.B., Bolis, C.L.
997 (Eds.), Water and life: Comparative analysis of water relationships at the organismic,
998 cellular, and molecular levels. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 87–
999 103.
1000 Crowe, J.H., Crowe, L.M., Chapman D., 1984. Preservation of membranes in
1001 anhydrobiotic organisms: the role of trehalose. Science 223, 701–703.
1002 https://doi.org/10.1126/science.223.4637.701.
1003 Crowe, L.M., 2002. Lessons from nature: The role of sugars in anhydrobiosis. Comp.
1004 Biochem. Physiol 131, 505–513. https://doi.org/10.1016/s1095-6433(01)00503-7.
1005 Darby, C., Cosma, C.L., Thomas, J.H., Manoil, C., 1999. Lethal paralysis of
1006 *Caenorhabditis elegans* by *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. U.S.A.
1007 96, 15202–15207. https://doi.org/10.1073/pnas.96.26.15202.
1008 Dengler, V.L., Galbraith, M., Espinosa, J.M., 2014. Transcriptional regulation by hypoxia
1009 inducible factors. Crit. Rev. Biochem. Mol. Biol. 49, 1–15.
1010 https://doi.org/10.3109/10409238.2013.838205.
1011 Depuydt, G., Xie, F., Petyuk, V.A., Smolders, A., Brewer, H.M., Camp, D.G., Smith, R.D.,
1012 Braeckman, B.P., 2014. LC-MS proteomics analysis of the insulin/IGF-1-deficient
1013 *Caenorhabditis elegans* daf-2(e1370) mutant reveals extensive restructuring of

1014 intermediary metabolism. J. Proteome Res. 13, 1938–1956.
1015 https://doi.org/10.1021/pr401081b.
1016 Deter, R.L., Baudhuin, P., Duve, C. de, 1967. Participation of lysosomes in cellular
1017 autophagy induced in rat liver by glucagon. J. Cell Biol. 35, C11–C16.
1018 https://doi.org/10.1083/jcb.35.2.c11.
1019 Detienne, G., Haes, W. de, Mergan, L., Edwards, S.L., Temmerman, L., van Bael, S.,
1020 2018. Beyond ROS clearance: Peroxiredoxins in stress signaling and aging. Ageing
1021 Res. Rev. 44, 33–48. https://doi.org/10.1016/j.arr.2018.03.005.
1022 Dillman, A.R., Chaston, J.M., Adams, B.J., Ciche, T.A., Goodrich-Blair, H., Stock, S.P.,
1023 Sternberg, P.W., 2012. An entomopathogenic nematode by any other name. PLOS
1024 Pathog. 8, e1002527. https://doi.org/10.1371/journal.ppat.1002527.
1025 Doering, K.R., Cheng, X., Milburn, L., Ratnappan, R., Ghazi, A., Miller, D.L., Taubert, S.,
1026 2022. Nuclear hormone receptor NHR-49 acts in parallel with HIF-1 to promote
1027 hypoxia adaptation in *Caenorhabditis elegans*. eLife.
1028 Dowds, B.C.A., Peters, A., 2002. Virulence mechanisms, in: Gaugler, R. (Ed.),
1029 Entomopathogenic nematology. CABI Publishing, UK, pp. 79–98.
1030 Duchaud, E., Rusniok, C., Frangeul, L., Buchrieser, C., Givaudan, A., Taourit, S., Bocs,
1031 S., Boursaux-Eude, C., Chandler, M., Charles, J.-F., Dassa, E., Derose, R., Derzelle,
1032 S., Freyssinet, G., Gaudriault, S., Médigue, C., Lanois, A., Powell, K., Siguier, P.,
1033 Vincent, R., Wingate, V., Zouine, M., Glaser, P., Boemare, N., Danchin, A., Kunst, F.,
1034 2003. The genome sequence of the entomopathogenic bacterium *Photorhabdus*
1035 *luminescens*. Nat. Biotechnol. 21, 1307–1313. https://doi.org/10.1038/nbt886.
1036 Edgington, S., Buddie, A.G., Moore, D., France, A., Merino, L., Hunt, D.J., 2011.
1037 *Heterorhabditis atacamensis* n. sp. (Nematoda: Heterorhabditidae), a new
1038 entomopathogenic nematode from the Atacama Desert, Chile. J. Helminthol. 85,
1039 381–394. https://doi.org/10.1017/S0022149X10000702.
1040 Ehlers, R.-U., 2001. Mass production of entomopathogenic nematodes for plant
1041 protection. Appl. Microbiol. Biotechnol. 56, 623–633.
1042 https://doi.org/10.1007/s002530100711.
1043 Ehlers, R.-U., Oestergaard, J., Hollmer, S., Wingen, M., Strauch, O., 2005. Genetic
1044 selection for heat tolerance and low temperature activity of the entomopathogenic

1045 nematode–bacterium complex *Heterorhabditis bacteriophora–Photorhabdus*
1046 *luminescens*. Biocontrol 50, 699–716. https://doi.org/10.1007/s10526-005-5079-z.
1047 Elbein, A.D., Pan, Y.T., Pastuszak, I., Carroll, D., 2003. New insights on trehalose: A
1048 multifunctional molecule. Glycobiology 13, 17R-27R.
1049 https://doi.org/10.1093/glycob/cwg047.
1050 El-Borai, F., Killiny, N., Duncan, L.W., 2016. Concilience in entomopathogenic nematode
1051 responses to water potential and their geospatial patterns in Florida. Front.
1052 Microbiol. 7, 356. https://doi.org/10.3389/fmicb.2016.00356.
1053 El-Borai, F.E., Campos-Herrera, R., Stuart, R.J., Duncan, L.W., 2011. Substrate
1054 modulation, group effects and the behavioral responses of entomopathogenic
1055 nematodes to nematophagous fungi. J. Invertebr. Pathol. 106, 347–356.
1056 https://doi.org/10.1016/j.jip.2010.12.001.
1057 Elhady, A., Giné, A., Topalovic, O., Jacquiod, S., Sørensen, S.J., Sorribas, F.J., Heuer, H.,
1058 2017. Microbiomes associated with infective stages of root-knot and lesion
1059 nematodes in soil. PLOS ONE 12, e0177145.
1060 https://doi.org/10.1371/journal.pone.0177145.
1061 Elsakrmy, N., Zhang-Akiyama, Q.-M., Ramotar, D., 2020. The base excision repair
1062 pathway in the nematode *Caenorhabditis elegans*. Front. Cell Dev. Biol. 8, 598860.
1063 https://doi.org/10.3389/fcell.2020.598860.
1064 Enright, M.R., McInerney, J.O., Griffin, C.T., 2003. Characterization of endospore-forming
1065 bacteria associated with entomopathogenic nematodes, *Heterorhabditis* spp., and
1066 description of *Paenibacillus nematophilus* sp. nov. Int. J. Syst. Evol. Microbiol. 53,
1067 435–441. https://doi.org/10.1099/ijs.0.02344-0.
1068 Epsky, N.D., Walter, D.E., Capinera, J.L., 1988. Potential role of nematophagous
1069 microarthropods as biotic mortality factors of entomogenous nematodes
1070 (Rhabditida: *Steinernematidae*, *Heterorhabditidae*). J. Econ. Entomol. 81, 821–825.
1071 https://doi.org/10.1093/jee/81.3.821.
1072 Epstein, A.C., Gleadle, J.M., McNeill, L.A., Hewitson, K.S., O'Rourke, J., Mole, D.R.,
1073 Mukherji, M., Metzen, E., Wilson, M.I., Dhanda, A., Tian, Y.-M., Masson, N., Hamilton,
1074 D.L., Jaakkola, P., Barstead, R., Hodgkin, J., Maxwell, P.H., Pugh, C.W., Schofield,
1075 C.J., Ratcliffe, P.J., 2001. *C. elegans* EGL-9 and mammalian homologs define a family

of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 107, 43–54. https://doi.org/10.1016/s0092-8674(01)00507-4.

Erkut, C., Gade, V.R., Laxman, S., Kurzchalia, T.V., 2016. The glyoxylate shunt is essential for desiccation tolerance in *C. elegans* and budding yeast. eLife. https://doi.org/10.7554/eLife.13614.

Erkut, C., Penkov, S., Khesbak, H., Vorkel, D., Verbavatz, J.-M., Fahmy, K., Kurzchalia, T.V., 2011. Trehalose renders the dauer larva of *Caenorhabditis elegans* resistant to extreme desiccation. Curr. Biol. 21, 1331–1336. https://doi.org/10.1016/j.cub.2011.06.064.

Erkut, C., Vasilj, A., Boland, S., Habermann, B., Shevchenko, A., Kurzchalia, T.V., 2013. Molecular strategies of the *Caenorhabditis elegans* dauer larva to survive extreme desiccation. PLOS ONE 8, e82473. https://doi.org/10.1371/journal.pone.0082473.

Ewald, C.Y., Landis, J.N., Porter Abate, J., Murphy, C.T., Blackwell, T.K., 2015. Dauer-independent insulin/IGF-1-signalling implicates collagen remodelling in longevity. Nature 519, 97–101. https://doi.org/10.1038/nature14021.

Fawcett, E.M., Hoyt, J.M., Johnson, J.K., Miller, D.L., 2015. Hypoxia disrupts proteostasis in *Caenorhabditis elegans*. Aging Cell 14, 92–101. https://doi.org/10.1111/acel.12301.

Félix, M.-A., Ashe, A., Piffaretti, J., Wu, G., Nuez, I., Bélicard, T., Jiang, Y., Zhao, G., Franz, C.J., Goldstein, L.D., Sanroman, M., Miska, E.A., Wang, D., 2011. Natural and experimental infection of *Caenorhabditis* nematodes by novel viruses related to nodaviruses. PLOS Biol. 9, e1000586. https://doi.org/10.1371/journal.pbio.1000586.

Félix, M.-A., Braendle, C., 2010. The natural history of *Caenorhabditis elegans*. Curr. Biol. 20, R965-9. https://doi.org/10.1016/j.cub.2010.09.050.

Feng, Y., 2010. Study of glucose transporters in *C. elegans*. PhD. UK.

Fitters, P.F., Griffin, C.T., 2006. Survival, starvation, and activity in *Heterorhabditis megidis* (Nematoda: Heterorhabditidae). Biol. Control 37, 82–88. https://doi.org/10.1016/j.biocontrol.2005.08.005.

Fortunato, A., Fleming, A., Aktipis, A., Maley, C.C., 2021. Upregulation of DNA repair genes and cell extrusion underpin the remarkable radiation resistance of *Trichoplax adhaerens*. PLOS Biol. 19, e3001471. https://doi.org/10.1371/journal.pbio.3001471.

1107 Frijhoff, J., Winyard, P.G., Zarkovic, N., Davies, S.S., Stocker, R., Cheng, D., Knight, A.R.,
1108 Taylor, E.L., Oettrich, J., Ruskovska, T., Gasparovic, A.C., Cuadrado, A., Weber, D.,
1109 Poulsen, H.E., Grune, T., Schmidt, H.H.H.W., Ghezzi, P., 2015. Clinical relevance of
1110 biomarkers of oxidative stress. Antioxid. Redox Signal. 23, 1144–1170.
1111 https://doi.org/10.1089/ars.2015.6317.
1112 Frøkjær-Jensen, C., 2013. Exciting prospects for precise engineering of *Caenorhabditis*
1113 *elegans* genomes with CRISPR/Cas9. Genetics 195, 635–642.
1114 https://doi.org/10.1534/genetics.113.156521.
1115 Fu, R., Jiang, X., Yang, Y., Wang, C., Zhang, Y., Zhu, Y., Zhang, H., 2022. Bidirectional
1116 regulation of structural damage on autophagy in the *C. elegans* epidermis.
1117 Autophagy, 1–15. https://doi.org/10.1080/15548627.2022.2047345.
1118 Fu, Z., Li, Y., Elling, A.A., Snyder, W.E., 2020. A draft genome of a field-collected
1119 *Steinernema feltiae* strain NW. J. Nematol. 52, 1–7. https://doi.org/10.21307/jofnem-
1120 2020-003.
1121 Fujiie, A., Yokoyama, T., 1998. Effects of ultraviolet light on the entomopathogenic
1122 nematode, *Steinernema kushidai* and its symbiotic bacterium, *Xenorhabdus*
1123 *japonicus*. Appl. Entomol. Zool. 33, 263–269. https://doi.org/10.1303/aez.33.263.
1124 Gal, T.Z., Glazer, I., Koltai, H., 2003. Differential gene expression during desiccation
1125 stress in the insect-killing nematode *Steinernema feltiae* IS-6. J. Parasitol. 89, 761–
1126 766. https://doi.org/10.1645/GE-3105.
1127 Gal, T.Z., Glazer, I., Koltai, H., 2004. An LEA group 3 family member is involved in survival
1128 of *C. elegans* during exposure to stress. FEBS Lett. 577, 21–26.
1129 https://doi.org/10.1016/j.febslet.2004.09.049.
1130 Gal, T.Z., Glazer, I., Koltai, H., 2005a. Stressed worms: responding to the post-genomics
1131 era. Mol. Biochem. Parasitol. 143, 1–5.
1132 https://doi.org/10.1016/j.molbiopara.2005.04.011.
1133 Gal, T.Z., Glazer, I., Sherman, A., Koltai, H., 2005b. Protein interaction of nucleosome
1134 assembly protein 1 and casein kinase 2 during desiccation response in the insect-
1135 killing nematode *Steinernema feltiae* IS-6. J. Parasitol. 91, 691–693.
1136 https://doi.org/10.1645/GE-402R.

1137 Gaugler, R., 1988. Ecological considerations in the biological control of soil-inhabiting
1138 insects with entomopathogenic nematodes. Agric. Ecosyst. Environ. 24, 351–360.
1139 https://doi.org/10.1016/0167-8809(88)90078-3.
1140 Gaugler, R., Boush, G., 1978. Effects of ultraviolet radiation and sunlight on the
1141 entomogenous nematode, *Neoaplectana carpocapsae*. J. Invertebr. Pathol. 32, 291–
1142 296. https://doi.org/10.1016/0022-2011(78)90191-X.
1143 Gaugler, R., Campbell, J.F., McGuire, T.R., 1990. Fitness of a genetically improved
1144 entomopathogenic nematode. J. Invertebr. Pathol. 56, 106–116.
1145 https://doi.org/10.1016/0022-2011(90)90151-U.
1146 Gaugler, R., Kaya, H.K., 1990. Entomopathogenic nematodes in biological control, 1st
1147 ed. CRC Press, 381 pp.
1148 Gaugler, R., McGuire, T., Campbell, J., 1989. Genetic variability among strains of the
1149 entomopathogenic nematode *Steinernema feltiae*. J. Nematol. 21, 247–253.
1150 Georgis, R., Gaugler, R., 2002. The Biosys experiment: an insider's perspective.
1151 https://doi.org/10.1079/9780851995670.0357.
1152 Gillet, F.-X., Bournaud, C., Antonino de Souza Júnior, J.D., Grossi-de-Sa, M.F., 2017.
1153 Plant-parasitic nematodes: Towards understanding molecular players in stress
1154 responses. Ann. Bot. 119, 775–789. https://doi.org/10.1093/aob/mcw260.
1155 Gilmore, S.K., Potter, D.A., 1993. Potential role of Collembola as biotic mortality agents
1156 for entomopathogenic Nematodes. Pedobiologia (Germany).
1157 Glazer, I., 1996. Survival mechanisms of entomopathogenic nematodes. Biocontrol Sci.
1158 Technol. 6, 373–378. https://doi.org/10.1080/09583159631343.
1159 Glazer, I., 2002. Survival biology, in: Gaugler, R. (Ed.), Entomopathogenic nematology,
1160 pp. 169–188.
1161 Glazer, I., 2015. Improvement of entomopathogenic nematodes: A genetic approach, in:
1162 Raquel Campos Herrera (Ed.), Nematode pathogenesis of insects and other pests,
1163 pp. 29–55.
1164 Glazer, I., Liran, N., Steinberger, Y., 1991. A survey of entomopathogenic nematodes
1165 (Rhabditida) in the Negev desert. Phytoparasitica 19, 291–300.
1166 https://doi.org/10.1007/BF02980963.
1167 Godina, G., Kirsch, C., Dörfler, V., Barg, M., Singh, R., Vandenbossche, B., Strauch, O.,
1168 Ehlers, R.-U., Molina, C., 2022. Single nucleotide polymorphism markers in

Heterorhabditis bacteriophora associated with virulence at low temperature. Nematol. 24, 1–14. https://doi.org/10.1163/15685411-bja10181.

Gong, J., Yuan, Y., Ward, A., Kang, L., Zhang, B., Wu, Z., Peng, J., Feng, Z., Liu, J., Xu, X.Z.S., 2016. The *C. elegans* taste receptor homolog LITE-1 is a photoreceptor. Cell 167, 1252-1263.e10. https://doi.org/10.1016/j.cell.2016.10.053.

Goyal, K., Walton, L.J., Tunnacliffe, A., 2005. LEA proteins prevent protein aggregation due to water stress. Biochem. 388, 151–157. https://doi.org/10.1042/BJ20041931.

Grant, J.A., Villani, M.G., 2003. Soil moisture effects on entomopathogenic nematodes. Environ. Entomol. 32, 80–87. https://doi.org/10.1603/0046-225X-32.1.80.

Gray, J.M., Karow, D.S., Lu, H., Chang, A.J., Chang, J.S., Ellis, R.E., Marletta, M.A., Bargmann, C.I., 2004. Oxygen sensation and social feeding mediated by a *C. elegans* guanylate cyclase homologue. Nature 430, 317–322. https://doi.org/10.1038/nature02714.

Gray, M.E., Sappington, T.W., Miller, N.J., Moeser, J., Bohn, M.O., 2009. Adaptation and invasiveness of western corn rootworm: Intensifying research on a worsening pest. Annu. Rev. Entomol. 54, 303–321.

Grewal, P., Georgis, R., 1999. Entomopathogenic nematodes, in: Julius J. M.,Franklin R. H. (Ed.), Biopesticides: Use and delivery. Humana Press, pp. 271–299.

Grewal, P.S., 1998. Formulations of entomopathogenic nematodes for storage and application. Jpn. J. Nematol. 28, 68–74. https://doi.org/10.3725/jjn1993.28.supplement_68.

Grewal, P.S., 2000a. Anhydrobiotic potential and long-term storage of entomopathogenic nematodes (Rhabditida: Steinernematidae). Int. J. Parasitol. 30, 995–1000. https://doi.org/10.1016/S0020-7519(00)00080-1.

Grewal, P.S., 2000b. Enhanced ambient storage stability of an entomopathogenic nematode through anhydrobiosis. Pest Manag. Sci. 56, 401–406. https://doi.org/10.1002/(SICI)1526-4998(200005)56:5<401:AID-PS137>3.0.CO;2-4.

Grewal, P.S., Ehlers, R.-U., Shapiro-Ilan, D.I., 2005. Nematodes as biological control agents. CAB International, Wallingford, 523 pp.

Grewal, P.S., Jagdale, G.B., 2002. Enhanced trehalose accumulation and desiccation survival of entomopathogenic nematodes through cold preacclimation. Biocontrol Sci. Technol. 12, 533–545. https://doi.org/10.1080/0958315021000016207.

1201 Grewal, P.S., Selvan, S., Gaugler, R., 1994. Thermal adaptation of entomopathogenic
1202 nematodes: Niche breadth for infection, establishment, and reproduction. J. Therm.
1203 Biol. 19, 245–253. https://doi.org/10.1016/0306-4565(94)90047-7.
1204 Grewal, P.S., Wang, X., Taylor, R., 2002. Dauer juvenile longevity and stress tolerance in
1205 natural populations of entomopathogenic nematodes: Is there a relationship? Int. J.
1206 Parasitol. 32, 717–725. https://doi.org/10.1016/S0020-7519(02)00029-2.
1207 Griffin, C.T., 2012. Perspectives on the behavior of entomopathogenic nematodes from
1208 dispersal to reproduction: traits contributing to nematode fitness and biocontrol
1209 efficacy. J. Nematol. 44, 177–184.
1210 Griffin, C.T., Fitters, P., 2004. Spontaneous and induced activity of *Heterorhabditis*
1211 *megidis* infective juveniles during storage. Nematol. 6, 911–917.
1212 https://doi.org/10.1163/1568541044038597.
1213 Ha, M.K., Soo Cho, J., Baik, O.-R., Lee, K.H., Koo, H.-S., Chung, K.Y., 2006.
1214 *Caenorhabditis elegans* as a screening tool for the endothelial cell-derived putative
1215 aging-related proteins detected by proteomic analysis. Proteomics 6, 3339–3351.
1216 https://doi.org/10.1002/pmic.200500395.
1217 Halliwell, B., Gutteridge, J.M.C., 2015. Free radicals in biology and medicine. Oxford
1218 University Press, Oxford.
1219 Hartl, D.L., Clark, A.G., 1997. Principle of population genetics. Sinauer Associates, Inc
1220 Publishers.
1221 Hashmi, G., Hashmi, S., Selvan, S., Grewal, P., Gaugler, R., 1997. Polymorphism in heat
1222 shock protein gene (hsp70) in entomopathogenic nematodes (Rhabditida). J. Therm.
1223 Biol. 22, 143–149. https://doi.org/10.1016/S0306-4565(97)00005-3.
1224 Hass, B., Downes, M.J., Griffin, C.T., 2002. Persistence of four *Heterorhabditis* spp.
1225 isolates in soil: Role of lipid reserves. J. Nematol. 34, 151–158.
1226 Hibshman, J.D., Clegg, J.S., Goldstein, B., 2020. Mechanisms of desiccation tolerance:
1227 Themes and variations in brine shrimp, roundworms, and tardigrades. Front. Physiol.
1228 11, 592016. https://doi.org/10.3389/fphys.2020.592016.
1229 Hiltpold, I., Baroni, M., Toepfer, S., Kuhlmann, U., Turlings, T.C.J., 2010. Selection of
1230 entomopathogenic nematodes for enhanced responsiveness to a volatile root signal
1231 helps to control a major root pest. The Journal of experimental biology 213, 2417–
1232 2423. https://doi.org/10.1242/jeb.041301.

1233 Hodson, A.K., Siegel, J.P., Lewis, E.E., 2012. Ecological influence of the
1234 entomopathogenic nematode, *Steinernema carpocapsae*, on pistachio orchard soil
1235 arthropods. Pedobiologia 55, 51–58. https://doi.org/10.1016/j.pedobi.2011.10.005.
1236 Hottiger, T., Virgilio, C. de, Hall, M.N., Boller, T., Wiemken, A., 1994. The role of trehalose
1237 synthesis for the acquisition of thermotolerance in yeast. II. Physiological
1238 concentrations of trehalose increase the thermal stability of proteins in vitro. Eur. J.
1239 Biochem. 219, 187–193. https://doi.org/10.1111/j.1432-1033.1994.tb19929.x.
1240 Hulbert, A.J., Pamplona, R., Buffenstein, R., Buttemer, W.A., 2007. Life and death:
1241 Metabolic rate, membrane composition, and life span of animals. Physiol. Rev. 87,
1242 1175–1213. https://doi.org/10.1152/physrev.00047.2006.
1243 Hunter, M.D., 2001. Out of sight, out of mind: The impacts of root-feeding insects in
1244 natural and managed systems. Agric. For. Entomol. 3, 3–9.
1245 https://doi.org/10.1046/j.1461-9563.2001.00083.x.
1246 Ishibashi, N., Young, F.Z., Nakashima, M., Agabeyru, C., Haraguchi, N., 1987. Effects of
1247 application of DD-136 on silkworm, *Bombyx mori*, predatory insect, *Agriosphodorus*
1248 *dohrni*, parasitoid, *Trichomalusa panteloctenus*, soil mites, and other non-target soil
1249 arthropods, with brief notes on feeding behavior and predatory pressure of soil
1250 mites, tardigrates, and predatory nematodes on DD-136 nematodes, in: Ishibashi, N.
1251 (Ed.), Recent Advances in biological control of insect pests by entomogenous
1252 nematodes in Japan. Ministry of Education, Culture and Science, Japan, pp. 158–
1253 164.
1254 Ivan, M., Haberberger, T., Gervasi, D.C., Michelson, K.S., Günzler, V., Kondo, K., Yang, H.,
1255 Sorokina, I., Conaway, R.C., Conaway, J.W., Kaelin, W.G., 2002. Biochemical
1256 purification and pharmacological inhibition of a mammalian prolyl hydroxylase
1257 acting on hypoxia-inducible factor. Proc. Natl. Acad. Sci. U.S.A. 99, 13459–13464.
1258 https://doi.org/10.1073/pnas.192342099.
1259 Jagdale, G., Grewal, P., 2003. Acclimation of entomopathogenic nematodes to novel
1260 temperatures: trehalose accumulation and the acquisition of thermotolerance. Int.
1261 J. Parasitol. 33, 145–152. https://doi.org/10.1016/S0020-7519(02)00257-6.
1262 Jagdale, G.B., Gordon, R., 1997. Effect of temperature on the composition of fatty acids
1263 in total lipids and phospholipids of entomopathogenic nematodes. J. Therm. Biol.
1264 22, 245–251. https://doi.org/10.1016/S0306-4565(97)00019-3.

1265 Jagdale, G.B., Grewal, P.S., 2007. Storage temperature influences desiccation and ultra
1266 violet radiation tolerance of entomopathogenic nematodes. J. Therm. Biol. 32, 20–
1267 27. https://doi.org/10.1016/j.jtherbio.2006.07.004.
1268 Jagdale, G.B., Grewal, P.S., Salminen, S.O., 2005. Both heat-shock and cold-shock
1269 influence trehalose metabolism in an entomopathogenic nematode. J. Parasitol. 91,
1270 988–994. https://doi.org/10.1645/GE-504R.1.
1271 Jain, N.K., Roy, I., 2009. Effect of trehalose on protein structure. Protein Sci. 18, 24–36.
1272 https://doi.org/10.1002/pro.3.
1273 Jee, C., Vanoaica, L., Lee, J., Park, B.J., Ahnn, J., 2005. Thioredoxin is related to life span
1274 regulation and oxidative stress response in *Caenorhabditis elegans*. Genes Cells 10,
1275 1203–1210. https://doi.org/10.1111/j.1365-2443.2005.00913.x.
1276 Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., Charpentier, E., 2012. A
1277 programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity.
1278 Science 337, 816–821. https://doi.org/10.1126/science.1225829.
1279 Johnson, S.N., Murray, P.J., 2008. Root feeders: An ecosystem approach. CABI,
1280 Wallingford, 213 pp.
1281 Jung, J., Nakajima, M., Kojima, M., Ooe, K., Fukuda, T., 2012. Microchip device for
1282 measurement of body volume of *C. elegans* as bioindicator application. J. Micro-
1283 Nano Mech. 7, 3–11. https://doi.org/10.1007/s12213-011-0036-7.
1284 Kaelin, W.G., Ratcliffe, P.J., 2008. Oxygen sensing by metazoans: the central role of the
1285 HIF hydroxylase pathway. Mol. Cell 30, 393–402.
1286 https://doi.org/10.1016/j.molcel.2008.04.009.
1287 Kandror, O., DeLeon, A., Goldberg, A.L., 2002. Trehalose synthesis is induced upon
1288 exposure of *Escherichia coli* to cold and is essential for viability at low temperatures.
1289 Proc. Natl. Acad. Sci. U.S.A. 99, 9727–9732.
1290 https://doi.org/10.1073/pnas.142314099.
1291 Karthik Raja, R., Arun, A., Touray, M., Hazal Gulsen, S., Cimen, H., Gulcu, B., Hazir, C.,
1292 Aiswarya, D., Ulug, D., Cakmak, I., Kaya, H.K., Hazir, S., 2021. Antagonists and
1293 defense mechanisms of entomopathogenic nematodes and their mutualistic
1294 bacteria. Biol. Control 152, 104452.
1295 https://doi.org/10.1016/j.biocontrol.2020.104452.

1296 Kaya, H.K., 2018. Soil ecology, in: Gaugler, R., Kaya, H.K. (Eds.), Entomopathogenic
1297 nematodes in biological control. CRC Press, Taylor & Francis Group, Boca Raton.
1298 Kaya, H.K., Gaugler, R., 1993. Entomopathogenic Nematodes. Annu. Rev. Entomol. 38,
1299 181–206. https://doi.org/10.1146/annurev.en.38.010193.001145.
1300 Kaya, H.K., Koppenhöfer, A.M., 1996. Effects of microbial and other antagonistic
1301 organism and competition on entomopathogenic nematodes. Biocontrol Sci.
1302 Technol. 6, 357–372. https://doi.org/10.1080/09583159631334.
1303 Kaya, H.K., Koppenhöfer, A.M., Johnson, M., 1998. Natural enemies of
1304 entomopathogenic nematodes. Jpn. J. Nematol. 28, 13–21.
1305 https://doi.org/10.3725/jjn1993.28.supplement_13.
1306 Kergunteuil, A., Bakhtiari, M., Formenti, L., Xiao, Z., Defossez, E., Rasmann, S., 2016.
1307 Biological control beneath the feet: A review of crop protection against insect root
1308 herbivores. Insects 7. https://doi.org/10.3390/insects7040070.
1309 Khatri-Chhetri, H.B., Timsina, G.P., Manandhar, H.K., Moens, M., 2011. Potential of
1310 Nepalese entomopathogenic nematodes as biocontrol agents against *Holotrichia*
1311 *longipennis* Blanch. (Coleoptera: Scarabaeidae). J. Pest Sci. 84, 457–469.
1312 https://doi.org/10.1007/s10340-011-0370-5.
1313 Kim, D.H., Flavell, S.W., 2020. Host-microbe interactions and the behavior of
1314 *Caenorhabditis elegans*. J. Neurogenet. 34, 500–509.
1315 https://doi.org/10.1080/01677063.2020.1802724.
1316 Kitaoka, S., Morielli, A.D., Zhao, F.-Q., 2013. FGT-1 is a mammalian GLUT2-like
1317 facilitative glucose transporter in *Caenorhabditis elegans* whose malfunction
1318 induces fat accumulation in intestinal cells. PLOS ONE 8, e68475.
1319 https://doi.org/10.1371/journal.pone.0068475.
1320 Kitazume, H., Dayi, M., Tanaka, R., Kikuchi, T., 2018. Assessment of the behaviour and
1321 survival of nematodes under low oxygen concentrations. PLOS ONE 13, e0197122.
1322 https://doi.org/10.1371/journal.pone.0197122.
1323 Koppenhöfer, A., Ganguly, S., Kaya, H., 2000. Ecological characterisation of
1324 *Steinernema monticolum*, a cold-adapted entomopathogenic nematode from
1325 Korea. Nematol. 2, 407–416. https://doi.org/10.1163/156854100509268.

1326 Koppenhöfer, A.M., Shapiro-Ilan, D.I., Hiltpold, I., 2020. Entomopathogenic nematodes
1327 in sustainable food production. Front. Sustain. Food Syst. 4, 125, 125.
1328 https://doi.org/10.3389/fsufs.2020.00125.
1329 Kour, S., Khurma, U., Brodie, G., 2021. Ecological characterisation of native isolates of
1330 *Heterorhabditis indica* from Viti Levu, Fiji Islands. J. Nematol. 53.
1331 https://doi.org/10.21307/jofnem-2021-085.
1332 Kumar, G.K., 2016. Hypoxia: Adapt or avoid. eLife 5, e14345.
1333 https://doi.org/10.7554/eLife.14345.
1334 Kung, S.-P., Gaugler, R., Kaya, H.K., 1991. Effects of soil temperature, moisture, and
1335 relative humidity on entomopathogenic nematode persistence. J. Invertebr. Pathol.
1336 57, 242–249. https://doi.org/10.1016/0022-2011(91)90123-8.
1337 Kurtz, B., Toepfer, S., Ehlers, R.-U., Kuhlmann, U., 2007. Assessment of establishment
1338 and persistence of entomopathogenic nematodes for biological control of western
1339 corn rootworm. J. Appl. Entomology 131, 420–425. https://doi.org/10.1111/j.1439-
1340 0418.2007.01202.x.
1341 Kusakabe, A., Peterson, B.F., Rivera Orduño, B., Stock, S.P., 2019. Ecological
1342 characterization of *Heterorhabditis sonorensis* (Caborca strain) (Nematoda:
1343 Heterorhabditidae), an entomopathogenic nematode from the Sonoran Desert.
1344 Zoology (Jena) 135, 125689. https://doi.org/10.1016/j.zool.2019.05.001.
1345 Leslie, S.B., Teter, S.A., Crowe, L.M., Crowe, J.H., 1994. Trehalose lowers membrane
1346 phase transitions in dry yeast cells. Biochim. Biophys. Acta 1192, 7–13.
1347 https://doi.org/10.1016/0005-2736(94)90136-8.
1348 Levine, B., Mizushima, N., Virgin, H.W., 2011. Autophagy in immunity and inflammation.
1349 Nature 469, 323–335. https://doi.org/10.1038/nature09782.
1350 Levy, N., Faigenboim, A., Salame, L., Molina, C., Ehlers, R.-U., Glazer, I., Ment, D., 2020.
1351 Characterization of the phenotypic and genotypic tolerance to abiotic stresses of
1352 natural populations of *Heterorhabditis bacteriophora*. Sci Rep 10, 10500.
1353 https://doi.org/10.1038/s41598-020-67097-0.
1354 Li, J., Labbadia, J., Morimoto, R.I., 2017. Rethinking HSF1 in stress, development, and
1355 organismal health. Trends Cell Biol. 27, 895–905.
1356 https://doi.org/10.1016/j.tcb.2017.08.002.

1357 Liang, Q., Yang, P., Tian, E., Han, J., Zhang, H., 2012. The *C. elegans* ATG101 homolog
1358 EPG-9 directly interacts with EPG-1/Atg13 and is essential for autophagy. Autophagy
1359 8, 1426–1433. https://doi.org/10.4161/auto.21163.
1360 Lillis, P.E., Griffin, C.T., Carolan, J.C., 2022. The effect of temperature conditioning (9°C
1361 and 20°C) on the proteome of entomopathogenic nematode infective juveniles.
1362 PLOS ONE 17, e0266164. https://doi.org/10.1371/journal.pone.0266164.
1363 Liu, W., Shen, S.-M., Zhao, X.-Y., Chen, G.-Q., 2012. Targeted genes and interacting
1364 proteins of hypoxia inducible factor-1. Int. J. Biochem. Mol. Biol. 3, 165–178.
1365 Liu, Z., Li, Y., Pan, L., Meng, F., Zhang, X., 2019. Cold adaptive potential of pine wood
1366 nematodes overwintering in plant hosts. Biol. Open 8.
1367 https://doi.org/10.1242/bio.041616.
1368 Lo, T.-W., Pickle, C.S., Lin, S., Ralston, E.J., Gurling, M., Schartner, C.M., Bian, Q.,
1369 Doudna, J.A., Meyer, B.J., 2013. Precise and heritable genome editing in
1370 evolutionarily diverse nematodes using TALENs and CRISPR/Cas9 to engineer
1371 insertions and deletions. Genetics 195, 331–348.
1372 https://doi.org/10.1534/genetics.113.155382.
1373 Lopez-Llorca, L.V., Jansson, H.B., 2007. Fungal parasites in invertebrates: Multimodal
1374 biocontrol agents?, in: Robson, G.D., Van Vest, P., Gadd, G.M. (Eds.), Exploitation of
1375 Fungi. Cambridge Univ. Press, Cambridge, pp. 310–335.
1376 Loulou, A., Mastore, M., Caramella, S., Bhat, A.H., Brivio, M.F., Machado, R.A.R., Kallel,
1377 S., 2023. Entomopathogenic potential of bacteria associated with soil-borne
1378 nematodes and insect immune responses to their infection. PLOS ONE 18,
1379 e0280675. https://doi.org/10.1371/journal.pone.0280675.
1380 Lu, D., Baiocchi, T., Dillman, A.R., 2016. Genomics of entomopathogenic nematodes
1381 and implications for pest control. Trends Parasitol. 32, 588–598.
1382 https://doi.org/10.1016/j.pt.2016.04.008.
1383 Ma, D.K., Vozdek, R., Bhatla, N., Horvitz, H.R., 2012. CYSL-1 interacts with the O2-
1384 sensing hydroxylase EGL-9 to promote H2S-modulated hypoxia-induced behavioral
1385 plasticity in *C. elegans*. Neuron 73, 925–940.
1386 https://doi.org/10.1016/j.neuron.2011.12.037.
1387 Machado, R.A.R., Thönen, L., Arce, C.C.M., Theepan, V., Prada, F., Wüthrich, D., Robert,
1388 C.A.M., Vogiatzaki, E., Shi, Y.-M., Schaeren, O.P., Notter, M., Bruggmann, R.,

1389 Hapfelmeier, S., Bode, H.B., Erb, M., 2020. Engineering bacterial symbionts of
1390 nematodes improves their biocontrol potential to counter the western corn
1391 rootworm. Nat. Biotechnol. 38, 600–608. https://doi.org/10.1038/s41587-020-0419-
1392 1.
1393 Machado, R.A.R., Wüthrich, D., Kuhnert, P., Arce, C.C.M., Thönen, L., Ruiz, C., Zhang, X.,
1394 Robert, C.A.M., Karimi, J., Kamali, S., Ma, J., Bruggmann, R., Erb, M., 2018. Whole-
1395 genome-based revisit of *Photorhabdus* phylogeny: proposal for the elevation of most
1396 *Photorhabdus* subspecies to the species level and description of one novel species
1397 *Photorhabdus bodei* sp. nov., and one novel subspecies *Photorhabdus laumondii*
1398 subsp. *clarkei* subsp. nov. Int. J. Syst. Evol. Microbiol. 68, 2664–2681.
1399 https://doi.org/10.1099/ijsem.0.002820.
1400 Madin, K.A.C., Crowe, J.H., 1975. Anhydrobiosis in nematodes: Carbohydrate and lipid
1401 metabolism during dehydration. J. Exp. Zool. 193, 335–342.
1402 https://doi.org/10.1002/jez.1401930309.
1403 Maguire, S.M., Clark, C.M., Nunnari, J., Pirri, J.K., Alkema, M.J., 2011. The *C. elegans*
1404 touch response facilitates escape from predacious fungi. Curr. Biol. 21, 1326–1330.
1405 https://doi.org/10.1016/j.cub.2011.06.063.
1406 Martens, E.C., Vivas, E.I., Heungens, K., Cowles, C.E., Goodrich-Blair, H., 2004.
1407 Investigating mutualism between entomopathogenic bacteria and nematodes, in:
1408 Cook, R., Hunt, D. (Eds.), Proceedings of the Fourth International Congress of
1409 Nematology, 8-13 June 2002, Tenerife, Spain. Brill, pp. 447–462.
1410 Martineau, C.N., Kirienko, N.V., Pujol, N., 2021. Chapter Ten - Innate immunity in *C.*
1411 *elegans*, in: Jarriault, S., Podbilewicz, B. (Eds.), Current Topics in Developmental
1412 Biology : Nematode Models of Development and Disease, vol. 144. Academic Press,
1413 pp. 309–351.
1414 Matadamas-Ortiz, P.T., Ruiz-Vega, J., Vazquez-Feijoo, J.A., Cruz-Martínez, H., Cortés-
1415 Martínez, C.I., 2014. Mechanical production of pellets for the application of
1416 entomopathogenic nematodes: Factors that determine survival time of *Steinernema*
1417 *glaseri*. Biocontrol Sci. Technol. 24, 145–157.
1418 https://doi.org/10.1080/09583157.2013.852161.

1419 McLean, F., Berger, D., Laetsch, D.R., Schwartz, H.T., Blaxter, M., 2018. Improving the
1420 annotation of the *Heterorhabditis bacteriophora* genome. Gigascience 7.
1421 https://doi.org/10.1093/gigascience/giy034.
1422 Megalou, E.V., Tavernarakis, N., 2009. Autophagy in *Caenorhabditis elegans*. Biochim.
1423 Biophys Acta 1793, 1444–1451. https://doi.org/10.1016/j.bbamcr.2008.12.010.
1424 Mello, C.C., Kramer, J.M., Stinchcomb, D., Ambros, V., 1991. Efficient gene transfer in *C.*
1425 *elegans*: Extrachromosomal maintenance and integration of transforming
1426 sequences. EMBO J. 10, 3959–3970. https://doi.org/10.1002/j.1460-
1427 2075.1991.tb04966.x.
1428 Miranda-Vizuete, A., Fierro González, J.C., Gahmon, G., Burghoorn, J., Navas, P.,
1429 Swoboda, P., 2006. Lifespan decrease in a *Caenorhabditis elegans* mutant lacking
1430 TRX-1, a thioredoxin expressed in ASJ sensory neurons. FEBS Lett. 580, 484–490.
1431 https://doi.org/10.1016/j.febslet.2005.12.046.
1432 Mizushima, N., 2007. Autophagy: process and function. Genes Dev. 21, 2861–2873.
1433 https://doi.org/10.1101/gad.1599207.
1434 Morton, A., García-del-Pino, F., 2009. Ecological characterization of entomopathogenic
1435 nematodes isolated in stone fruit orchard soils of Mediterranean areas. J. Invertebr.
1436 Pathol. 102, 203–213. https://doi.org/10.1016/j.jip.2009.08.002.
1437 Mukuka, J., Strauch, O., Ehlers, R.-U., 2010a. Variability in desiccation tolerance among
1438 different strains of the entomopathogenic nematode *Heterorhabditis bacteriophora*.
1439 Nematol. 12, 711–720. https://doi.org/10.1163/138855409X12607871174454.
1440 Mukuka, J., Strauch, O., Hoppe, C., Ehlers, R.-U., 2010b. Improvement of heat and
1441 desiccation tolerance in *Heterorhabditis bacteriophora* through cross-breeding of
1442 tolerant strains and successive genetic selection. Biocontrol 55, 511–521.
1443 https://doi.org/10.1007/s10526-010-9271-4.
1444 Mukuka, J., Strauch, O., Waeyenberge, L., Viaene, N., Moens, M., Ehlers, R.-U., 2010c.
1445 Heat tolerance among different strains of the entomopathogenic nematode
1446 *Heterorhabditis bacteriophora*. Biocontrol 55, 423–434.
1447 https://doi.org/10.1007/s10526-009-9255-4.
1448 Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li,
1449 H., Kenyon, C., 2003. Genes that act downstream of DAF-16 to influence the lifespan

of *Caenorhabditis elegans*. Nature 424, 277–283. https://doi.org/10.1038/nature01789.

Nanette, P., Tim, S., 2013. Germ cell development in *C. elegans*. Springer, New York.

Navaneethan, T., Strauch, O., Besse, S., Bonhomme, A., Ehlers, R.-U., 2010. Influence of humidity and a surfactant-polymer-formulation on the control potential of the entomopathogenic nematode Steinernema feltiae against diapausing codling moth larvae (Cydia pomonella L.) (Lepidoptera: Tortricidae). Biocontrol 55, 777–788. https://doi.org/10.1007/s10526-010-9299-5.

NDong, C., Danyluk, J., Wilson, K.E., Pocock, T., Huner, N.P.A., Sarhan, F., 2002. Cold-regulated cereal chloroplast late embryogenesis abundant-like proteins. Molecular characterization and functional analyses. Plant Physiol. 129, 1368–1381. https://doi.org/10.1104/pp.001925.

Neher, D.A., 2010. Ecology of plant and free-living nematodes in natural and agricultural soil. Annu. Rev. Phytopathol. 48, 371–394. https://doi.org/10.1146/annurev-phyto-073009-114439.

Nimkingrat, P., Uhlmann, F., Strauch, O., Ehlers, R.-U., 2013. Desiccation tolerance of dauers of entomopathogenic nematodes of the genus *Steinernema*. Nematol. 15, 451–458. https://doi.org/10.1163/15685411-00002692.

O'Donnell, M.P., Fox, B.W., Chao, P.-H., Schroeder, F.C., Sengupta, P., 2020. A neurotransmitter produced by gut bacteria modulates host sensory behaviour. Nature 583, 415–420. https://doi.org/10.1038/s41586-020-2395-5.

Oerke, E.-C., 2006. Crop losses to pests. J. Agric. Sci. 144, 31–43. https://doi.org/10.1017/s0021859605005708.

Ogier, J.-C., Pagès, S., Frayssinet, M., Gaudriault, S., 2020. Entomopathogenic nematode-associated microbiota: From monoxenic paradigm to pathobiome. Microbiome 8, 25. https://doi.org/10.1186/s40168-020-00800-5.

Okahata, M., Motomura, H., Ohta, A., Kuhara, A., 2022. Molecular physiology regulating cold tolerance and acclimation of *Caenorhabditis elegans*. Proc Jpn Acad Ser B Phys Biol Sci 98, 126–139. https://doi.org/10.2183/pjab.98.009.

Oláhová, M., Taylor, S.R., Khazaipoul, S., Wang, J., Morgan, B.A., Matsumoto, K., Blackwell, T.K., Veal, E.A., 2008. A redox-sensitive peroxiredoxin that is important for

longevity has tissue- and stress-specific roles in stress resistance. Proc. Natl. Acad. Sci. U.S.A. 105, 19839–19844. https://doi.org/10.1073/pnas.0805507105.

Onukwufor, J.O., Farooqi, M.A., Vodičková, A., Koren, S.A., Baldzizhar, A., Berry, B.J., Beutner, G., Porter, G.A., Belousov, V., Grossfield, A., Wojtovich, A.P., 2022. A reversible mitochondrial complex I thiol switch mediates hypoxic avoidance behavior in *C. elegans*. Nat. Commun. 13, 2403. https://doi.org/10.1038/s41467-022-30169-y.

Ozawa, K., Shinkai, Y., Kako, K., Fukamizu, A., Doi, M., 2022. The molecular and neural regulation of ultraviolet light phototaxis and its food-associated learning behavioral plasticity in *C. elegans*. Neurosci. Lett. 770, 136384. https://doi.org/10.1016/j.neulet.2021.136384.

Palmisano, N.J., Meléndez, A., 2019. Autophagy in *C. elegans* development. Dev. Biol. 447, 103–125. https://doi.org/10.1016/j.ydbio.2018.04.009.

Pamplona, R., Costantini, D., 2011. Molecular and structural antioxidant defenses against oxidative stress in animals. Am. J. Physiol. Regul. Integr. Comp. Physiol. 301, R843-63. https://doi.org/10.1152/ajpregu.00034.2011.

Park, E.C., Rongo, C., 2016. The p38 MAP kinase pathway modulates the hypoxia response and glutamate receptor trafficking in aging neurons. eLife 5. https://doi.org/10.7554/eLife.12010.

Park, H.-E.H., Jung, Y., Lee, S.-J.V., 2017. Survival assays using *Caenorhabditis elegans*. Mol. Cells 40, 90–99. https://doi.org/10.14348/molcells.2017.0017.

Patel, M.N., Perry, R.N., Wright, D.J., 1997a. Desiccation survival and water contents of entomopathogenic nematodes, *Steinernema* spp. (Rhabditida: Steinernematidae). Int. J. Parasitol. 27, 61–70. https://doi.org/10.1016/s0020-7519(96)00154-3.

Patel, M.N., Stolinski, M., Wright, D.J., 1997b. Neutral lipids and the assessment of infectivity in entomopathogenic nematodes: Observations on four *Steinernema* species. Parasitology 114 (Pt 5), 489–496. https://doi.org/10.1017/S0031182096008748.

Patel, M.N., Wright, D.J., 1997a. Fatty acid composition of neutral lipid energy reserves in infective juveniles of entomopathogenic nematodes. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 118, 341–348. https://doi.org/10.1016/S0305-0491(97)00057-6.

1512 Patel, M.N., Wright, D.J., 1997b. Glycogen: its importance in the infectivity of aged
1513 juveniles of *Steinernema carpocapsae*. Parasitology 114, 591–596.
1514 https://doi.org/10.1017/S0031182097008780.
1515 Pathak, E., Campos-Herrera, R., El-Borai, F.E., Duncan, L.W., 2017. Spatial relationships
1516 between entomopathogenic nematodes and nematophagous fungi in Florida citrus
1517 orchards. J. Invertebr. Pathol. 144, 37–46. https://doi.org/10.1016/j.jip.2017.01.005.
1518 Pellerone, F., Archer, S., Behm, C., Grant, W., Lacey, M., Somerville, A., 2003. Trehalose
1519 metabolism genes in *Caenorhabditis elegans* and filarial nematodes. Int. J. Parasitol.
1520 33, 1195–1206. https://doi.org/10.1016/s0020-7519(03)00173-5.
1521 Pervez, R., Lone, S.A., Pattnaik, S., 2020. Characterization of symbiotic and associated
1522 bacteria from entomopathogenic nematode *Heterorhabditis* sp. (nematode:
1523 Heterorhabditidae) isolated from India. Egypt. J. Biol. Pest Control 30.
1524 https://doi.org/10.1186/s41938-020-00343-9.
1525 Petriv, O.I., Rachubinski, R.A., 2004. Lack of peroxisomal catalase causes a progeric
1526 phenotype in *Caenorhabditis elegans*. J. Biol. Chem. 279, 19996–20001.
1527 https://doi.org/10.1074/jbc.M400207200.
1528 Poinar, G.O., 1966. The presence of *Achromobacter nematophilus* in the infective stage
1529 of a *Neoaplectana* sp. (Steinernematidae: Nematoda). Nematol. 12, 105–108.
1530 https://doi.org/10.1163/187529266X00068.
1531 Poinar, G.O., 2018. Nematodes for biological control of insects, 1st ed. CRC Press, Boca
1532 Raton.
1533 Poinar, G.O., Grewal, P.S., 2012. History of entomopathogenic nematology. J. Nematol.
1534 44, 153–161.
1535 Powell-Coffman, J.A., 2010. Hypoxia signaling and resistance in *C. elegans*. Trends
1536 Endocrinol. Metab. 21, 435–440. https://doi.org/10.1016/j.tem.2010.02.006.
1537 Pradel, E., Zhang, Y., Pujol, N., Matsuyama, T., Bargmann, C.I., Ewbank, J.J., 2007.
1538 Detection and avoidance of a natural product from the pathogenic bacterium
1539 *Serratia marcescens* by *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U.S.A. 104,
1540 2295–2300. https://doi.org/10.1073/pnas.0610281104.
1541 Praitis, V., Casey, E., Collar, D., Austin, J., 2001. Creation of low-copy integrated
1542 transgenic lines in *Caenorhabditis elegans*. Genetics 157, 1217–1226.
1543 https://doi.org/10.1093/genetics/157.3.1217.

1544 Qiu, L., Bedding, R., 2000. Energy metabolism and its relation to survival and infectivity
1545 of infective juveniles of *Steinernema carpocapsae* under aerobic conditions.
1546 Nematol. 2, 551–559. https://doi.org/10.1163/156854100509330.
1547 Qiu, L., Bedding, R.A., 2002. Characteristics of protectant synthesis of infective
1548 juveniles of *Steinernema carpocapsae* and importance of glycerol as a protectant
1549 for survival of the nematodes during osmotic dehydration. Comp. Biochem. Physiol.
1550 B. Biochem. Mol. Biol. 131, 757–765. https://doi.org/10.1016/S1096-4959(02)00019-
1551 2.
1552 Rappleye, C.A., Tagawa, A., Le Bot, N., Ahringer, J., Aroian, R.V., 2003. Involvement of
1553 fatty acid pathways and cortical interaction of the pronuclear complex in
1554 *Caenorhabditis elegans* embryonic polarity. BMC Dev. Biol. 3, 8.
1555 https://doi.org/10.1186/1471-213X-3-8.
1556 Read, D.S., Sheppard, S.K., Bruford, M.W., Glen, D.M., Symondson, W.O.C., 2006.
1557 Molecular detection of predation by soil micro-arthropods on nematodes. Mol. Ecol.
1558 15, 1963–1972. https://doi.org/10.1111/j.1365-294X.2006.02901.x.
1559 Riddle, D.L., Blumenthal, T., Meyer, B.J., Priess, J.R. (Eds.), 1997. *C. elegans* II. 2nd
1560 edition. Cold Spring Harbor Laboratory Press.
1561 Rougon-Cardoso, A., Flores-Ponce, M., Ramos-Aboites, H.E., Martínez-Guerrero, C.E.,
1562 Hao, Y.-J., Cunha, L., Rodríguez-Martínez, J.A., Ovando-Vázquez, C., Bermúdez-
1563 Barrientos, J.R., Abreu-Goodger, C., Chavarría-Hernández, N., Simões, N., Montiel,
1564 R., 2016. The genome, transcriptome, and proteome of the nematode *Steinernema*
1565 *carpocapsae*: Evolutionary signatures of a pathogenic lifestyle. Sci. Rep. 6, 37536.
1566 https://doi.org/10.1038/srep37536.
1567 Russell, J., Vidal-Gadea, A.G., Makay, A., Lanam, C., Pierce-Shimomura, J.T., 2014.
1568 Humidity sensation requires both mechanosensory and thermosensory pathways in
1569 *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U.S.A. 111, 8269–8274.
1570 https://doi.org/10.1073/pnas.1322512111.
1571 Sajnaga, E., Kazimierczak, W., 2020. Evolution and taxonomy of nematode-associated
1572 entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus*: An
1573 overview. Symbiosis 80, 1–13. https://doi.org/10.1007/s13199-019-00660-0.
1574 Schulenburg, H., Félix, M.-A., 2017. The natural biotic environment of *Caenorhabditis*
1575 *elegans*. Genetics 206, 55–86. https://doi.org/10.1534/genetics.116.195511.

1576 Schulenburg, H., Müller, S., 2004. Natural variation in the response of *Caenorhabditis*
1577 *elegans* towards *Bacillus thuringiensis*. Parasitology 128, 433–443.
1578 https://doi.org/10.1017/S003118200300461X.
1579 Selvan, S., Gaugler, R., Grewal, P.S., 1993a. Water content and fatty acid composition of
1580 infective juvenile entomopathogenic nematodes during storage. J. Parasitol. 79, 510.
1581 https://doi.org/10.2307/3283375.
1582 Selvan, S., Gaugler, R., Lewis, E.E., 1993b. Biochemical energy reserves of
1583 entomopathogenic nematodes. J. Parasitol. 79, 167.
1584 https://doi.org/10.2307/3283503.
1585 Serra, L., Macchietto, M., Macias-Muñoz, A., McGill, C.J., Rodriguez, I.M., Rodriguez, B.,
1586 Murad, R., Mortazavi, A., 2019. Hybrid assembly of the genome of the
1587 entomopathogenic nematode *Steinernema carpocapsae* identifies the X-
1588 chromosome. G3-Genes Genom Genet. 9, 2687–2697.
1589 https://doi.org/10.1534/g3.119.400180.
1590 Servello, F.A., Apfeld, J., 2020. The heat shock transcription factor HSF-1 protects
1591 *Caenorhabditis elegans* from peroxide stress. Transl. Med. Aging 4, 88–92.
1592 https://doi.org/10.1016/j.tma.2020.07.002.
1593 Shapiro-Ilan, D.I., Gouge, D.H., Piggott, S.J., Fife, J.P., 2006a. Application technology and
1594 environmental considerations for use of entomopathogenic nematodes in biological
1595 control. Biol. Control. 38, 124–133.
1596 https://doi.org/10.1016/j.biocontrol.2005.09.005.
1597 Shapiro-Ilan, D.I., Han, R., Dolinksi, C., 2012. Entomopathogenic nematode production
1598 and application technology. J. Nematol. 44, 206–217.
1599 Shapiro-Ilan, D.I., Han, R., Qiu, X., 2014. Production of Entomopathogenic Nematodes,
1600 in: Mass Production of Beneficial Organisms. Elsevier, pp. 321–355.
1601 Shapiro-Ilan, D.I., Hazir, S., Lete, L., 2015. Viability and virulence of entomopathogenic
1602 nematodes exposed to ultraviolet radiation. J. Nematol. 47, 184–189.
1603 Shapiro-Ilan, D.I., Stuart, R.J., McCoy, C.W., 2006b. A comparison of entomopathogenic
1604 nematode longevity in soil under laboratory conditions. J. Nematol. 38, 119–129.
1605 Shen, C., Shao, Z., Powell-Coffman, J.A., 2006. The *Caenorhabditis elegans* rhy-1 gene
1606 inhibits HIF-1 hypoxia-inducible factor activity in a negative feedback loop that does

not include vhl-1. Genetics 174, 1205–1214. https://doi.org/10.1534/genetics.106.063594.

Shields, H.J., Traa, A., van Raamsdonk, J.M., 2021. Beneficial and detrimental effects of reactive oxygen species on lifespan: A comprehensive review of comparative and experimental studies. Front. Cell Dev. Biol. 9, 628157. https://doi.org/10.3389/fcell.2021.628157.

Shih, J.M., Platzer, E.G., Thompson, S.N., Carroll, E.J., 1996. Characterization of key glycolytic and oxidative enzymes in *Steinernema carpocapsae*. J. Nematol. 28, 431–441.

Sies, H., 1985a. Introductory Remarks, in: Sies, H. (Ed.), Oxidative Stress. Academic Press, London, pp. 1–8.

Sies, H. (Ed.), 1985b. Oxidative Stress. Academic Press, London.

Sies, H., 2018. On the history of oxidative stress: Concept and some aspects of current development. Curr. Opin. Toxicol. 7, 122–126. https://doi.org/10.1016/j.cotox.2018.01.002.

Simons, W.R., Poinar, G.O., 1973. The ability of *Neoaplectana carpocapsae* (Steinernematidae: Nematodea) to survive extended periods of desiccation. J. Invertebr. Pathol. 22, 228–230. https://doi.org/10.1016/0022-2011(73)90138-9.

Singer, M.A., Lindquist, S., 1998. Multiple effects of trehalose on protein folding in vitro and in vivo. Mol. Cell. 1, 639–648. https://doi.org/10.1016/s1097-2765(00)80064-7.

Soares, F.E.d.F., Sufiate, B.L., Queiroz, J.H. de, 2018. Nematophagous fungi: Far beyond the endoparasite, predator and ovicidal groups. Agric. Nat. Resour. (Agriculture and Natural Resources) 52, 1–8. https://doi.org/10.1016/j.anres.2018.05.010.

Solomon, A., Glazer, I., 1999. Desiccation survival of the entomopathogenic nematode *Steinernema feltiae*: induction of anhydrobiosis. Nematol. 1, 61–68. https://doi.org/10.1163/156854199507983.

Solomon, A., Solomon, R., Paperna, I., Glazer, I., 2000. Desiccation stress of entomopathogenic nematodes induces the accumulation of a novel heat-stable protein. Parasitology 121, 409–416. https://doi.org/10.1017/s0031182099006563.

Somvanshi, V.S., Koltai, H., Glazer, I., 2008. Expression of different desiccation-tolerance related genes in various species of entomopathogenic nematodes. Mol. Biochem. Parasitol. 158, 65–71. https://doi.org/10.1016/j.molbiopara.2007.11.012.

1639 Sonoda, S., Ohta, A., Maruo, A., Ujisawa, T., Kuhara, A., 2016. Sperm affects head
1640 sensory neuron in temperature tolerance of *Caenorhabditis elegans*. Cell Rep. 16,
1641 56–65. https://doi.org/10.1016/j.celrep.2016.05.078.
1642 Strauch, O., Oestergaard, J., Hollmer, S., Ehlers, R.-U., 2004. Genetic improvement of
1643 the desiccation tolerance of the entomopathogenic nematode *Heterorhabditis*
1644 *bacteriophora* through selective breeding. Biol. Control 31, 218–226.
1645 https://doi.org/10.1016/j.biocontrol.2004.03.009.
1646 Sugi, T., 2016. Genome editing in *C. elegans* and other nematode species. Int. J. Mol.
1647 Sci. 17. https://doi.org/10.3390/ijms17030295.
1648 Sumaya, N.H., Aryal, S., Vandenbossche, B., Barg, M., Doerfler, V., Strauch, O., Molina,
1649 C., Ehlers, R.-U., 2017. Phenotyping dauer juvenile oxidative stress tolerance,
1650 longevity and persistence within wild type and inbred lines of the entomopathogenic
1651 nematode *Heterorhabditis bacteriophora*. Nematol. 19, 971–986.
1652 https://doi.org/10.1163/15685411-00003100.
1653 Sumaya, N.H., Gohil, R., Okolo, C., Addis, T., Doerfler, V., Ehlers, R.-U., Molina, C., 2018.
1654 Applying inbreeding, hybridization and mutagenesis to improve oxidative stress
1655 tolerance and longevity of the entomopathogenic nematode *Heterorhabditis*
1656 *bacteriophora*. J. Invertebr. Pathol. 151, 50–58.
1657 https://doi.org/10.1016/j.jip.2017.11.001.
1658 Sun, W.Q., Leopold, A., 1997. Cytoplasmic vitrification and survival of anhydrobiotic
1659 organisms. Comp. Biochem. Physiol. 117, 327–333. https://doi.org/10.1016/S0300-
1660 9629(96)00271-X.
1661 Svensk, E., Ståhlman, M., Andersson, C.-H., Johansson, M., Borén, J., Pilon, M., 2013.
1662 PAQR-2 regulates fatty acid desaturation during cold adaptation in *C. elegans*. PLoS
1663 Genet. 9, e1003801. https://doi.org/10.1371/journal.pgen.1003801.
1664 Takacs-Vellai, K., Vellai, T., Puoti, A., Passannante, M., Wicky, C., Streit, A., Kovacs, A.L.,
1665 Müller, F., 2005. Inactivation of the autophagy gene bec-1 triggers apoptotic cell
1666 death in *C. elegans*. Curr. Biol. 15, 1513–1517.
1667 https://doi.org/10.1016/j.cub.2005.07.035.
1668 The C. elegans Research Community (Ed.). WormBook.

1669 Timper, P., Kaya, H.K., 1989. Role of the second-stage cuticle of entomogenous
1670 nematodes in preventing infection by nematophagous fungi 54, 314–321.
1671 https://doi.org/10.1016/0022-2011(89)90115-8.
1672 Topalović, O., Elhady, A., Hallmann, J., Richert-Pöggeler, K.R., Heuer, H., 2019. Bacteria
1673 isolated from the cuticle of plant-parasitic nematodes attached to and antagonized
1674 the root-knot nematode *Meloidogyne hapla*. Sci. Rep. 9, 11477.
1675 https://doi.org/10.1038/s41598-019-47942-7.
1676 Tran, A., Tang, A., O'Loughlin, C.T., Balistreri, A., Chang, E., Coto Villa, D., Li, J.,
1677 Varshney, A., Jimenez, V., Pyle, J., Tsujimoto, B., Wellbrook, C., Vargas, C., Duong, A.,
1678 Ali, N., Matthews, S.Y., Levinson, S., Woldemariam, S., Khuri, S., Bremer, M., Eggers,
1679 D.K., L'Etoile, N., Miller Conrad, L.C., VanHoven, M.K., 2017. *C. elegans* avoids toxin-
1680 producing *Streptomyces* using a seven transmembrane domain chemosensory
1681 receptor. eLife 6. https://doi.org/10.7554/eLife.23770.
1682 Trent, C., Tsuing, N., Horvitz, H.R., 1983. Egg-laying defective mutants of the nematode
1683 *Caenorhabditis elegans*. Genetics 104, 619–647.
1684 https://doi.org/10.1093/genetics/104.4.619.
1685 Tzur, Y.B., Friedland, A.E., Nadarajan, S., Church, G.M., Calarco, J.A., Colaiácovo, M.P.,
1686 2013. Heritable custom genomic modifications in *Caenorhabditis elegans* via a
1687 CRISPR-Cas9 system. Genetics 195, 1181–1185.
1688 https://doi.org/10.1534/genetics.113.156075.
1689 Ulug, D., Hazir, S., Kaya, H.K., Lewis, E., 2014. Natural enemies of natural enemies: The
1690 potential top-down impact of predators on entomopathogenic nematode
1691 populations. Ecol. Entomol. 39, 462–469. https://doi.org/10.1111/een.12121.
1692 Vågsholm, I., Arzoomand, N.S., Boqvist, S., 2020. Food security, safety, and
1693 sustainability—Getting the trade-offs right. Front. Sustain. Food Syst. 4, 16, 16.
1694 https://doi.org/10.3389/fsufs.2020.00016.
1695 van Sluijs, L., Bosman, K.J., Pankok, F., Blokhina, T., Wilten, J.I.H.A., Te Molder, D.M.,
1696 Riksen, J.A.G., Snoek, B.L., Pijlman, G.P., Kammenga, J.E., Sterken, M.G., 2021.
1697 Balancing selection of the intracellular pathogen response in natural *Caenorhabditis*
1698 *elegans* populations. Front. Cell. Infect. Microbiol. 11, 758331.
1699 https://doi.org/10.3389/fcimb.2021.758331.

1700 Vernon, B., van Herk, W., 2022. Chapter7-Wireworms as pests of potato. Academic
1701 Press.
1702 Vernon, R.S., van Herk, W.G., Clodius, M., Harding, C., 2009. Wireworm management I:
1703 Stand protection versus wireworm mortality with wheat seed treatments. J. Econ.
1704 Entomol. 102, 2126–2136. https://doi.org/10.1603/029.102.0616.
1705 Virgilio, C. de, Hottiger, T., Dominguez, J., Boller, T., Wiemken, A., 1994. The role of
1706 trehalose synthesis for the acquisition of thermotolerance in yeast. I. Genetic
1707 evidence that trehalose is a thermoprotectant. Eur. J. Biochem. 219, 179–186.
1708 https://doi.org/10.1111/j.1432-1033.1994.tb19928.x.
1709 Vora, M., Pyonteck, S.M., Popovitchenko, T., Matlack, T.L., Prashar, A., Kane, N.S.,
1710 Favate, J., Shah, P., Rongo, C., 2022. The hypoxia response pathway promotes PEP
1711 carboxykinase and gluconeogenesis in *C. elegans*. Nat. Commun. 13, 6168.
1712 https://doi.org/10.1038/s41467-022-33849-x.
1713 Wallace, H.R., 1971. Abiotic influences in the soil environment., in: Zuckerman, B.M.,
1714 Mai, W.F., Rhode R.A. (Eds.), Plant parasitic nematodes, vol. 1. Academic Press, New
1715 York, USA, pp. 257–280.
1716 Wang, D., Liu, P., Xing, X., 2010. Pre-treatment with mild UV irradiation increases the
1717 resistance of nematode *Caenorhabditis elegans* to toxicity on locomotion behaviors
1718 from metal exposure. Environ. Toxicol. Pharmacol. 29, 213–222.
1719 https://doi.org/10.1016/j.etap.2010.01.002.
1720 Ward, A., Liu, J., Feng, Z., Xu, X.Z.S., 2008. Light-sensitive neurons and channels
1721 mediate phototaxis in *C. elegans*. Nat. Neurosci. 11, 916–922.
1722 https://doi.org/10.1038/nn.2155.
1723 Watts, J.L., Ristow, M., 2017. Lipid and carbohydrate metabolism in *Caenorhabditis*
1724 *elegans*. Genetics 207, 413–446. https://doi.org/10.1534/genetics.117.300106.
1725 Wechsler, S., Smith, D., 2018. Has resistance taken root in U.S. corn fields? Demand for
1726 insect control. Am. J. Agric. Econ. 100, 1136–1150.
1727 https://doi.org/10.1093/ajae/aay016.
1728 Wei, Q., Shen, Y., Chen, X., Shifman, Y., Ellis, R.E., 2014. Rapid creation of forward-
1729 genetics tools for *C. briggsae* using TALENs: lessons for nonmodel organisms. Mol.
1730 Biol. Evol. 31, 468–473. https://doi.org/10.1093/molbev/mst213.

1731 Weischer, B., Brown, D.J.F., 2000. An introduction to nematodes: General nematology;
1732 Student's textbook. Pensoft; Eurospan, Sofia, London, 187 pp.
1733 West, J.D., Marnett, L.J., 2006. Endogenous reactive intermediates as modulators of cell
1734 signaling and cell death. Chem. Res. Toxicol. 19, 173–194.
1735 https://doi.org/10.1021/tx050321u.
1736 Willett, D.S., Alborn, H.T., Stelinski, L.L., 2017. Multitrophic effects of belowground
1737 parasitoid learning. Sci. Rep. 7. https://doi.org/10.1038/s41598-017-02193-2.
1738 Wilm, T., Demel, P., Koop, H.-U., Schnabel, H., Schnabel, R., 1999. Ballistic
1739 transformation of *Caenorhabditis elegans*. Gene 229, 31–35.
1740 https://doi.org/10.1016/s0378-1119(99)00043-8.
1741 Wise, M.J., Tunnacliffe, A., 2004. POPP the question: what do LEA proteins do? Trends
1742 Plant Sci. 9, 13–17. https://doi.org/10.1016/j.tplants.2003.10.012.
1743 Womersley, C., 1981. Biochemical and physiological aspects of anhydrobiosis. Comp.
1744 Biochem. Physio.l B Biochem. Mol. Biol. 70, 669–678. https://doi.org/10.1016/0305-
1745 0491(81)90001-8.
1746 Womersley, C.Z., 1990. Entomopathogenic nematodes in biological control. CRC Press,
1747 Boca Raton, Fla.
1748 Wood, A.J., Lo, T.-W., Zeitler, B., Pickle, C.S., Ralston, E.J., Lee, A.H., Amora, R., Miller,
1749 J.C., Leung, E., Meng, X., Zhang, L., Rebar, E.J., Gregory, P.D., Urnov, F.D., Meyer, B.J.,
1750 2011. Targeted genome editing across species using ZFNs and TALENs. Science 333,
1751 307. https://doi.org/10.1126/science.1207773.
1752 Wright, D.J., Grewal, P.S., Stolinski, M., 1997. Relative importance of neutral lipids and
1753 glycogen as energy stores in dauer larvae of two entomopathogenic nematodes,
1754 *Steinernema carpocapsae* and *Steinernema feltiae*. Comp. Biochem. Physiol. B.
1755 Biochem. Mol. Biol. 118, 269–273. https://doi.org/10.1016/S0305-0491(97)00165-X.
1756 Yanase, S., Hartman, P.S., Ito, A., Ishii, N., 1999. Oxidative stress pretreatment
1757 increases the X-radiation resistance of the nematode *Caenorhabditis elegans*.
1758 Mutat. Res. 426, 31–39. https://doi.org/10.1016/s0027-5107(99)00079-2.
1759 Zervos, S., Johnson, S.C., Webster, J.M., 1991. Effect of temperature and inoculum size
1760 on reproduction and development of *Heterorhabditis heliothidis* and *Steinernema*
1761 *glaseri* (Nematoda: Rhabditoidea) in *Galleria mellonella*. Can. J. Zool. 69, 1261–1264.
1762 https://doi.org/10.1139/z91-177.

1763 Zhang, X., Li, L., Kesner, L., Robert, C.A.M., 2021. Chemical host-seeking cues of
1764 entomopathogenic nematodes. Curr. Opin. Insect. Sci. 44, 72–81.
1765 https://doi.org/10.1016/j.cois.2021.03.011.
1766 Zhang, X., Machado, R.A., van Doan, C., Arce, C.C., Hu, L., Robert, C. am, 2019.
1767 Entomopathogenic nematodes increase predation success by inducing cadaver
1768 volatiles that attract healthy herbivores. eLife 8, e46668.
1769 https://doi.org/10.7554/eLife.46668.
1770 Zhao, L., Fenk, L.A., Nilsson, L., Amin-Wetzel, N.P., Ramirez-Suarez, N.J., Bono, M. de,
1771 Chen, C., 2022. ROS and cGMP signaling modulate persistent escape from hypoxia
1772 in *Caenorhabditis elegans*. PLOS Biol. 20, e3001684.
1773 https://doi.org/10.1371/journal.pbio.3001684.
1774 Zorov, D.B., Juhaszova, M., Sollott, S.J., 2014. Mitochondrial reactive oxygen species
1775 (ROS) and ROS-induced ROS release. Physiol. Rev. 94, 909–950.
1776 https://doi.org/10.1152/physrev.00026.2013.

Chapter 2: Experimental adaptation of *Heterorhabditis bacteriophora* strains to plant specialized metabolites.

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Abstract

Benzoxazinoids are plant herbivory defense special metabolites produced by cereal crops like maize and wheat. Western corn rootworm (WCR, *Diabrotica virgifera virgifera)* larvae, a serious specialised maize root pest, has evolved some benzoxazinoid adaptation mechanisms. The pest has the capacity to stabilise and sequester (accumulate) some of these benzoxazinoids into its own body. Suppressive impacts of these plant metabolites on WCR natural enemies [entomopathogenic nematode, (EPN)], which prey on WCR, has been observed. Very few studies focused looking for possible ways of enhancing EPN performance and fitness under such benzoxazinoids stressful environment like the haemolymph of the WCR. In this study, we first assessed the benzoxazinoid tolerance of various EPNs by comparing their infectivity success towards benzoxazinoid-fed WCR to determine their BX tolerance level. We, furthermore, investigated the possibility of enhancing infectivity of benzoxazinoid-susceptible EPN strains through a forward evolutionary experiment. Our results identified six *Heterorhabditis bacteriophora* strains with benzoxazinoid susceptibility with regards to infectivity success. These susceptible EPN strains later evolved benzoxazinoid resistance within three host cycles of continuous exposure to benzoxazinoids in WCR, achieving infectivity rates comparable to those reared initially resistant ones. With these results we managed to show that EPN benzoxazinoid tolerance is strain specific. Furthermore, our findings from the evolution experiment highlighted the capacity of EPNs to rapidly adapt to plant chemical defenses, providing new insights into enhancing biocontrol strategies against BX-sequestering pests like WCR.

Keywords: Entomopathogenic nematodes, benzoxazinoids, adaptation, western corn rootworm

Introduction

Insect pests are regulated by both bottom-up forces, such as plant defenses that reduce herbivore survival and reproduction, as well as top-down forces, including natural enemies like predators, parasitoids, and pathogens, which suppress herbivore abundance (Gripenberg & Roslin, 2007). These forces are critical determinants of ecosystem functioning and biodiversity, as they influence species interactions, population dynamics, and the balance between herbivores and their natural enemies (Price *et al.*, 1980). Yet, some specialized herbivores like the WCR have evolved the ability to hijack plant defenses by sequestering toxic compounds, which they can repurpose for their own defense against their natural enemies, entomopathogenic nematodes (EPNs) (Erb & Robert, 2016). This strategy, while beneficial WCR, can turn WCR into significantly resistant agricultural pests. However EPNs can still succeed in controlling WCR and mitigating their negative impacts on plant health and yield (Hajek & Eilenberg, 2018). Understanding how pest enemies have evolved to counter plant defenses sequestered or utilized by insects could open new avenues for developing sustainable biocontrol strategies in pest management and to ensure food security.

Predators and parasitoids have evolved different strategies to resist or to tolerate plant defense metabolites. For example, it has been demonstrated that birds in Mexico that prey on monarch butterflies have become insensitive to the bitter-taste-plant cardenolides that the butterflies sequester from plant (Fink & Brower, 1981). The same scenario was also shown by the predators, big-eyed bugs which prey on nicotine sequestering -*Manduca sexta* larvae equally as those fed on nicotine-free plants (Kumar *et al.*, 2014). Furthermore, development of the specialist parasitoid *Cotesia melitaearum* was unaffected by plant- sequestered iridoid glycosides sequestered by its host *Melitaea cinxia* (Reudler *et al.*, 2011). Additionally , upto date, the underlying mechanisms of such adaptations remain unclear (Ode, 2006).

A notable example of a specialized herbivore that can tolerate, sequester, and use plant specialized metabolites is the western corn rootworm (*Diabrotica virgifera virgifera* LeConte, WCR) (Robert *et al.*, 2012; Robert *et al.*, 2017), which is one of the most economically significant agricultural pests worldwide, responsible for over $2 billion in annual

losses due to yield reduction and pest management costs in the United States alone (Gray *et al.*, 2009). WCR larvae tolerate the benzoxazinoid compounds by sequestering HDMBOA-Glc, and glucosylating MBOA to MBOA-Glc and again sequestering the resultant MBOA-Glc (Robert *et al.*, 2017). Entomopathogenic nematodes (EPNs) infectivity towards BX-sequestering WCR larvae can be drastically reduced (Robert *et al.*, 2017; Zhang *et al.*, 2019). First, WCR larvae release MBOA-Glc through their skin, acting as a repellent to EPNs (Robert *et al.*, 2017). Second, EPNs that enter the insect host encounter elevated level of HDMBOA-Glc, which is also toxic to EPNs (Robert *et al.*, 2017). Finally, HDMBOA-Glc is locally deglucosylated, leading to the rapid formation of MBOA, which is again toxic for both EPNs and their endosymbiotic bacteria (Robert *et al.*, 2017). It very clear that EPN effectiveness is greatly compromised against these WCR and any possible ways of improving EPN performances in benzoxazinoid stressful situation will likely boost crop (maize) productivity.

In this study, we first assessed the benzoxazinoid tolerance of various EPNs by comparing their infectivity success towards benzoxazinoid-fed larvae to that of benzoxazinoid-free larvae. We later adapted initially susceptible to benzoxazinoids by continually exposing them to benzoxazinoid in WCR larvae for 5 successive generation. We hypothesised that benzoxazinoid tolerance may be explained by metabolic and behavioural tolerance of EPNs.

Material and Methods

Biological resources

Maize seeds of the wild type inbred line W22 and benzoxazinoid-deficient mutant bx1::W22 (gene identifier GRMZM2G085381; Ds, B.W06.0775 for *bx1* and Ds) on the *bx1* gene (Tzin *et al.*, 2015) were kindly provided by Georg Jander (Boyce Thompson Institute, Ithaca, NY; USA). Western corn rootworm (WCR, *Diabrotica virgifera virgifera*) eggs were obtained from USDA-ARS-NCARL (North Central Agricultural Research Laboratory, US Department of Agriculture, Agriculture Research Service, Brookings, SD, USA). WCR eggs were incubated at room temperature and hatching larvae were raised on either bx1::W22 ("bx^- mutant") or W22 (Bx+) seedlings. WCR larvae fed on bx1::W22 are hereafter referred to as bx^--fed WCR. WCR larvae fed on W22 are hereafter referred to as BX^+-fed WCR. Second and third instar larvae were used for infectivity assays. Entomopathogenic nematodes (EPNs) were originally provided by Prof. Raquel Campos Herrera (Institute of Grapevine and Wine Sciences, Spain) and Prof. Ralf Udo Ehlers (e-nema GmbH, Germany) and maintained in the Biotic Interactions and Chemical Ecology collection. The detailed description of 8 individual EPN strains used in this study can be found in Table S1, and whole genome sequencing data for all of the strains (Ogi et al., under review.). EPNs were amplified in *Galleria mellonella* larvae bought from a local fish store (Fischereibedarf N. Wenger AG, Kasernenstrasse, Bern). Emerging EPNs were collected using white traps (White, 1927), filtered through 25 µm sieves, and kept in tap water in cell culture flasks (Thermo Fisher scientific, Switzerland). All EPNs were stored in 8°C fridges until use. Infective juveniles (IJs) were used in all experiments unless specified otherwise.

Phenotyping benzoxazinoid tolerance of different entomopathogenic nematodes strains.

For this, 26 nematodes strains in our laboratory had their susceptibility against BX-sequestering WCR larvae assessed. Briefly, W22 and bx1::W22 fed larvae collected from rearing pots were separately infected with each of the nematode strains. For this, 5 WCR larvae fed on either W22 (BX^+) or bx1::W22 (bx^-) were placed into individual solo cups (30

mL; Frontier Scientific Services, Inc.) containing 3.5g of moist, autoclaved sand (Selmaterra, Bigler Samen AG). Each treatment was replicated 5 times. An approximate of about 100 nematodes suspended in 700 µL tap water were applied in each solo cups. Incubating these treatments at 25 ± 0.5 °C promote nematodes infectivity against WCR larvae. The percentage of nematode-infected larvae (mostly brick reddish, pinked or yellowish) in each solo cup was determined. For each nematode strain, BX tolerance phenotype (resistant or susceptible) was determined by statistical comparison of infected BX containing- larvae (W22-fed) to infected BX-free- larvae (bx1::W22-fed).

Experimental evolution experiment

Four suceptible strain (09_43, EN01, MG618b and DE6) and 4 resistant strains (HU2, VM1, IT6, and DE2) from the infectivity phenotyping experiment results above (Table 1) were selected and later divided into four subpopulations named A-D. The individual EPN subpopulations were then selected in either W22-fed or bx1::W22 -fed WCR larvae, resulting in 64 replicates within subpopulations (8 strains x 4 subpopulations x 2 host diet). EPNs were multiplied by adding 50 EPNs to solo cups (30 mL; Frontier Scientific Services, Inc.) containing 3.5g of moist, autoclaved sand (Selmaterra, Bigler Samen AG) and 5 WCR larvae over five host cycles (called F_0 to F_5, n= 5 per subpopulation). Each host cycle represents approximately 2-3 EPN generations depending on host size (Dillman & Sternberg, 2012; Trejo-Meléndez *et al.*, 2024) . EPN infectivity success in BXs, BXs preference, and BX survival rate were tested at F0, F3, and F5 generations.

Entomopathogenic nematodes benzoxazinoids survival rate

To test EPN susceptibility to BXs, 2’000 living IJs were placed in microtubes (1.5 mL, Nolato Treff AG, Degersheim, Switzerland) containing either 25 µg/mL MBOA or 150 µg/mL HDMBOA-Glc suspended in 1 mL tap water. These doses represent physiological WCR concentrations (Robert *et al.*, 2017) Control EPNs were placed in water also included. The tubes were left at ambient conditions (25 +/-0.5°C) at ambient light levels. After 48 h, all samples were homogenised, and 3 aliquots of 20 µL were used to record the number of live and or dead EPNs under a binocular microscope (10x16). The average percentage of living nematodes per sample for EPNs of the F0, and F5 generation (n=5 per strain (F0)/per subpopulation (F5)) was determined.

Host preference of entomopathogenic nematodes

EPN preference assays were conducted as previously described (Robert *et al.*, 2017). Briefly, a 5 mm layer of 0.5 % agarose (Sigma Aldrich CHEMIE GmbH, USA) was poured into 94 mm diameter petri dishes (Huberlab). Three wells were made along the dish diameter. The side wells (5 mm diameter) were filled with 50 uL exudates of bx-fed and BX-fed WCR larvae respectively. WCR exudates were collected by rinsing WCR larvae with tap water. The central well (1 cm diameter) was used to add 100 EPNs in 100 µL tap water. The preference assays were conducted for EPNs of the F0, and F5 generation (n=10 per strain (F0)/per subpopulation (F5)). After 24 h, the number of EPNs in each of the small wells (left or right side) and their surrounding three marked ring sectors was recorded under the microscope.

Statistical analyses

For the first BX-tolerance phenotyping test, infectivity of each strain to BX-fed larvae was compared to its infectivity to BX-free larvae using t-test (parametric test requirements met) or Wilcox test as an alternative non-parametric test in R. (https://www.r-project.org/ , version 4.4.2). For the evolution experiment, infectivity assays statistical comparisons between diets (BX-fed larvae vs BX-free larvae) or between EPN phenotypes (resistant vs susceptible strains) per each day of the, we performed a Two-Way Repeated Measures ANOVA (One Factor Repetition), after data passed both Normality Test (Shapiro-Wilk) and Equal Variance Test (Brown-Forsythe) in SigmaPlot. For significant different groups, all pairwise comparisons were done using Bonferroni t-test. Differences in EPN preferences were assessed by One sample t tests on the difference of EPN numbers on BX (MBOA-Glc) treated well side to control side containing just water, using GraphPad (https://www.graphpad.com/quickcalcs/oneSampleT1/). For the mortality assays, mortality effect of the compounds (HDMBOA-GLc or MBOA) were tested using a t-test which compared mortality of nematodes in BXs and control treatments of just nematodes in water in R and since we didnt observe the toxicity effect, no interaction tests were done.

Results

Benzoxazionoids supressed infectivity success of some EPN isolates

A total of Six (DE6, 09_43,EN01,MG618b,TT01 and RW14) out of 26 worldwide screened EPN strains suffered infectivtiy suppressions against BX-containing (W22-fed) larvae (Table 1). Infectivity phenotype was determined after comparing infectivity success perecentage towards BX-containing larvae (W22-fed) to that of against BX-free larvae(bx1::W22-fed) for each strain 6 days post infection (T-tests or Wilcox tests, $P<0.05$). All strains with a significantly infectivity supresssion againsts W22-fed larvae [Figure S1. (red bars)] as compare to that of bx1::W22 [Figure S1. (orange bars)] were later termed susceptible isolates (S) while the unsupressed ones were termed the resistant isolates (R) throughout this research work. After statistical tests (4 out of 6) showed much significant differences 6 days post infection (Figure S1.A.). Remaining two, MG618b and and TT01 showed susceptibilty 3 (Figure S1.B.) and 4 (Figure S1.C) days post infection respectively to finally make a total of all 6 out of 26 screened strains (Table 1).

Table 1. **Summary table showing benzoxazinoids resistance phenotype of 26 screened IPS nematode. In the table, the first column iis the name of the tested isolate followed by its country of origini in the seconf colum**. The third column is the statistical test comaprison of BX-fed-d to BX-free larave infectivity the correspondin strain, were, **(*)** denotes that the coresponding strain showed significant BX-fed-larvae to BX- free-larvae infectivity diffrences. Thus these strains all these strain with an asterik were termed susceptible isolates. The sympol (**ns)** denotes no infectictivity differences and thus these strains were termed resistant isolates.

Strain	Origin	bx1::W22 vs W22 fed infectivity	Phenotype
Hb17	Turkey	ns	resistant
Hbbio	USA	ns	resistant
S5P8	United States	ns	resistant
S12	United States	ns	resistant
S14	United States	ns	resistant
S15	United States	ns	resistant
MEX23	Mexico	ns	resistant
MEX32	Mexico	ns	resistant
MEX37	Mexico	ns	resistant
TT01	Trinidad and Tobago	*	Susceptible
MG618b	Switzerland	*	Susceptible
RM102	Spain	ns	resistant
VM1	Spain	ns	resistant
EN01	Germany	*	susceptible
IT6	Italy	ns	resistant
IR2	Iran	ns	resistant
DE2	Germany	ns	resistant
PT1	Portugal	ns	resistant
HU2	Hungary	ns	resistant
IL9	Australia	ns	resistant
DE6	Germany	*	susceptible
09-43	Turkey	*	susceptible
Boj	Iran	ns	resistant
RW14	Rwanda	*	resistant

Susceptible EPN isolates evolved resistance to benzoxazinoids within three growth cycles in BX-fed WCR larvae

In average, F0 resistant isolates showed a similar infectivity rate of BX^+-fed and bx^--fed WCR larvae, even though individual differences were noted during the assay.In particular, HU2 was more successful in infecting BX^+-fed WCR larvae, DE2 and VM1 in infecting bx^--fed larvae, and IT6 showed no difference (Figure S2). However, no difference could be noted between infectivity rates of BX^+ and bx^--fed WCR larvae within any of the recording days. As expected, F0 susceptible isolates displayed a higher infectivity in bx^--fed WCR larvae than in BX^+-fed ones at all timepoints, a pattern that was consistently observed for each of the four isolates (Figure S2). These observations confirmed the categorization of HU2, IT6, VM1, and DE2 as resistant isolates and of DE6, 0943,MG618b, and EN01 as BX susceptible isolates (Table1).

After three host cycles (F_3) in BX^+-fed WCR larvae, BX-resistant and BX-susceptible isolates did show non-significant differences in infecting BX^+ or bx^--fed WCR larvae (Figure 1), a behaviour that was consistently observed through all individual isolates except for 09_43 and EN01 (Figure S3) suggesting the adaptation has already been at least partly successful. As expected, after five host cycles (F_5), BX-resistant and BX-susceptible isolates similarly infected BX^+ or bx^--fed WCR larvae (Figure 1), a behaviour that was consistently observed through all individual isolates (Figure S5). Overall, three host cycles in BX^+-fed WCR larvae were sufficient to clear the initial differences observed between BX-resistant and BX-susceptible isolates (F_0) (Figure 1).

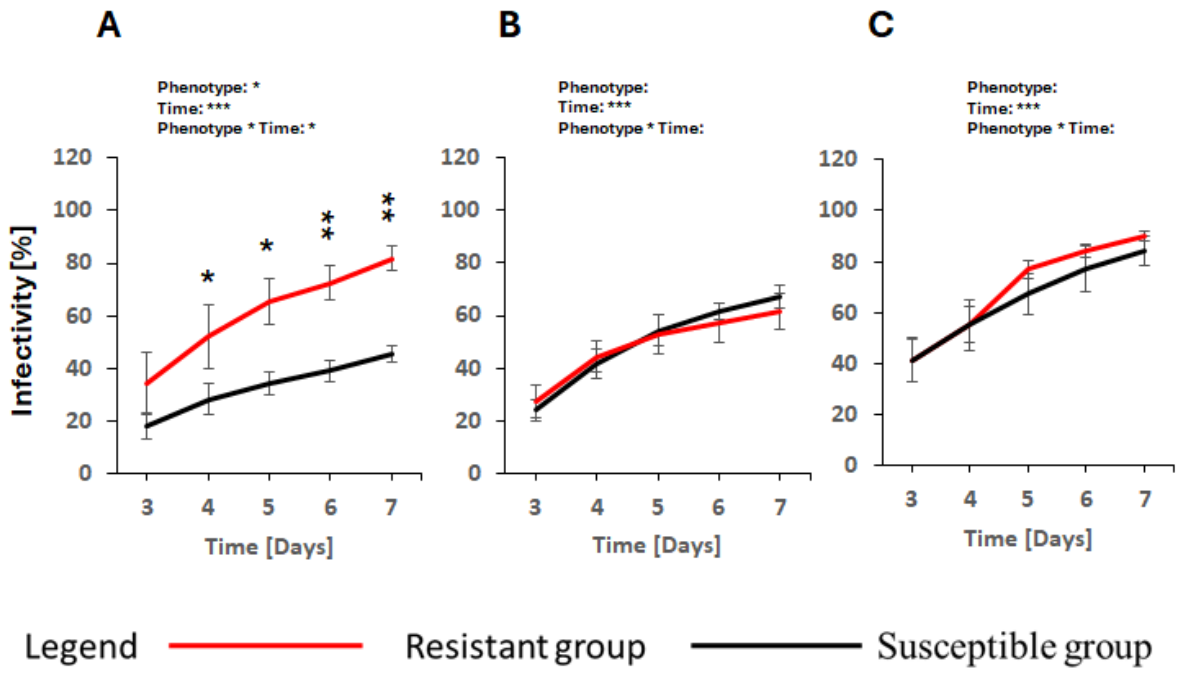

Figure 1. Comparing the infectivity of susceptible and resistant isolates in benzoxazinoid-containing larvae (BX^+-fed) over several generations (F_0s (A), F_3s(B) and F_5s (C)) Asterisk (*) indicate significant difference in infectivity between resistant and susceptible nematode isolates at a time point between day 3 and 7 post exposure ($P<0.05$). The legend on the right of each Figure shows the significance of the contribution the factors phenotype and time have in determining these differences.,**:$0.001<p<0.01$,*: $0.01<p<0.05$, Error Bars: MSe.

The results showed that generation F_0 started with significant infectivity differences ($P<0.05$) between susceptible (black line) and resistant isolates (red line, Figure 1A). However, at F_3 as well as F_5 (Figures 1B and 1C), infectivity differences between resistant and susceptible isolates disappeared for all data points ($P>0.05$).Thus, the difference between susceptible and resistant isolates in benzoxazinoid tolerance disappeared after continuous exposure to benzoxazinoids, suggesting that adaptation to BXs has taken place within these few generations

Entomopathogenic nematodes benzoxazinoid infectivity success linked to their behavioral resistance but not on their metabolic resistance

Our results revealed that, given a choice, non-adapted (F0s), susceptible isolates significantly moved away from MBOA-Glc treated well side and preferred water treated side more (P>0.05), while resistant isolates move randomly to either side (Figure 2A). Individual isolates preferences statistical tests revealed that 3 out 4 susceptible isolates, strongly moved away from MBOA-glc treated wells (Figure S4A.), meaning MBOA-Glc may exert some repelling effect mostly on non-adapted susceptible isolates than resistant ones. Thus, the infectivity differences of the F0s resistant and susceptible strain may be linked to this phenomenon. The MBOA-Glc effect on these susceptible isolates, however, get shifted after benzoxazinoid adaptation (F_5) as the susceptible isolates reared in benzoxazinoids (W222-fed larvae) show significant preference to the MBOA-Glc treated wells [Figure 2B, (F5)], which may also mean that due to continuous exposure, sensitive isolates may have evolved adaptation to this compound. This shift of MBOA-Glc effect was not observed in strains selected in benzoxazinoid-free larvae (Figure 2B). Surprisingly, no benzoxazinoid toxicity effects were observed from both compound (MBOA and HDMBOA-Glc) tested in this experiment as all t-test comparison show no difference in mortality between treatments (BXs) and control (water) (P>0.05) for all strains Figure S5B-C, and within each phenotype (Figure 2C-F).

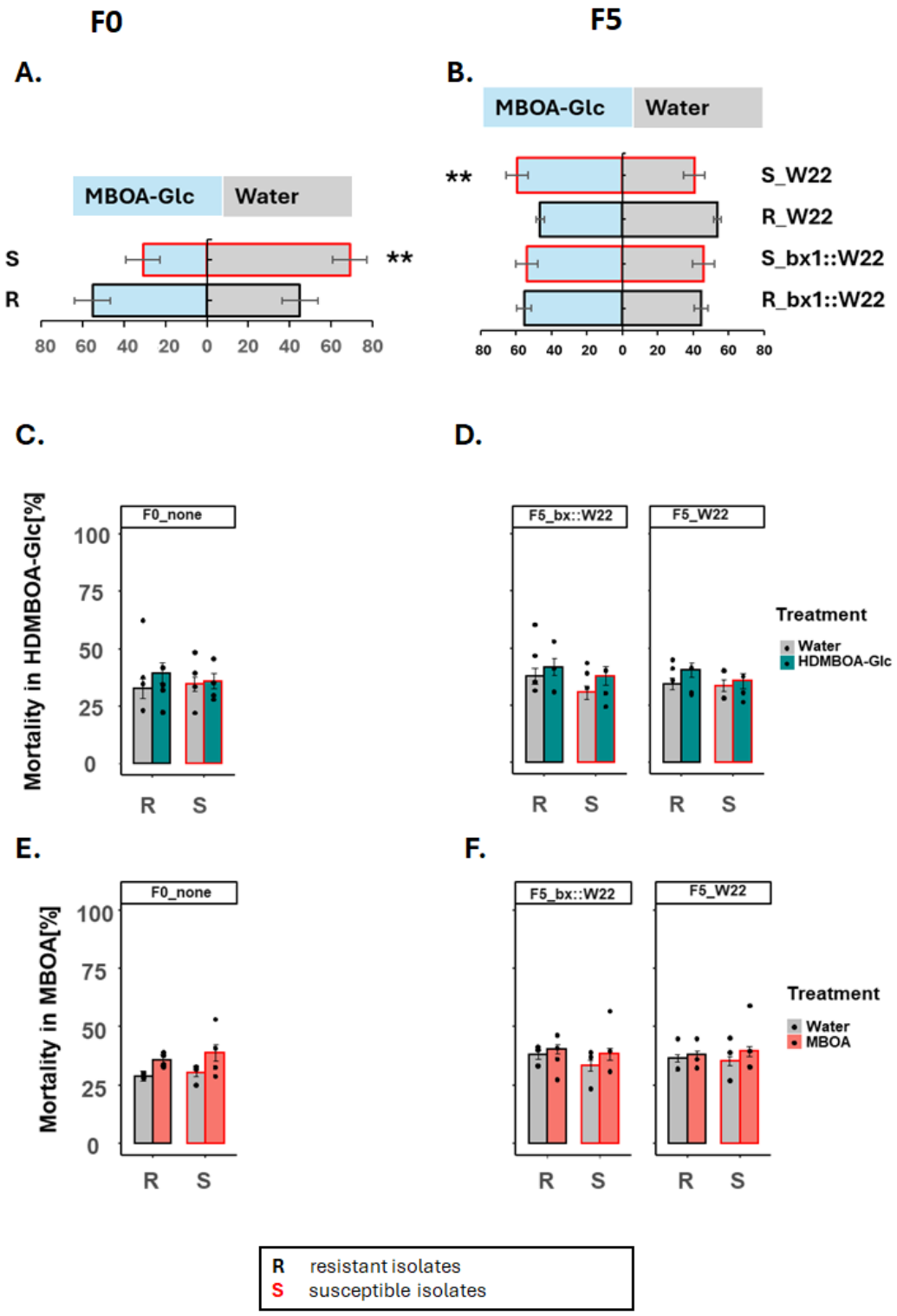

Figure 2. The relationship between EPN infectivity success with EPN behavioural and metabolic resistance. Comparison of the choice of isolated groups between BX (MBOA-Glc) or and control (Water) treated wells of the resistant and susceptible isolates was compared before [F0(A.)] and after [F5(B.)] adaptation. Nematode isolates were adapted in either benzoxazinoid- fed (W22) or benzoxazinoid -free larvae (bx1::W22-fed). An asterisk **(*)** and positioning (left or right) indicates significant preference of the compound contained in that well side ($P<0.05$). Survival potential of isolated EPN groups after 48 hrs exposure to before [F0(C)] or [F0(E)] and after [F5(D)] or [F0(F)] benzoxazinoid (HDMBOA-Glc or MBOA) adaptation respectively. Error Bars: Mse. For all graphs, red border lines indicate susceptible isolates group (S) while black indicate resistant isolates group (R) to match the selection lines in Figure 1.

Discussion

In this work, we discovered 6 *Heterorhabditis bacteriophora* strains which are susceptible (infectivity suppressed) to WCR larvae sequestered BXs. This shows that, these strains effectiveness is compromised against WCR and or in soils with benzoxazinoids as some benzoxazinoids are actively released to the environment in relatively large quantities through root exudation (Belz & Hurle, 2005; Wouters *et al.*, 2016b; Wouters *et al.*, 2016a; Robert & Mateo, 2022). EPN infectivity suppression against WCR been reported before (Zhang *et al.*, 2019; Bruno *et al.*, 2020).This infectivity suppression towards WCR may be due to sequestered benzoxazinoids in WCR (Robert *et al.*, 2017). Also, in general most benzoxazinoids are reported have allelopathic and biocidal effects on microorganisms (Gerber & Lechevalier, 1964; Gagliardo & Chilton, 1992; Kumar *et al.*, 1993; Neal *et al.*, 2012). However, some other defense maize metabolite may also be ingested from maize by larvae which may also be in different concentrations between the two maize lines we used as maize also produce chlorogenic acid (Cortés-Cruz *et al.*, 2003), maysin (Rector *et al.*, 2003), protease inhibitors (Tamayo *et al.*, 2000), cysteine protease (Pechan *et al.*, 2000), ribosome-inactivating proteins (Chuang *et al.*, 2014) as well as non-protein amino acid; 5-hydroxynorvaline (Yan *et al.*, 2015) in response to herbivory. Another interesting observation was EN01 which is a commercial strain was also among these 6 identified susceptible strains. Thus, there is need to think of all possible ways of maintaining infectivity in benzoxazinoid environments.

Our adaptation experiment results revealed a rapid adaptation of these susceptible EPNs strains to benzoxazinoid environments. The significant differences in infection rates between susceptible and resistant EPN isolates, which were evident at the start of the experiment (F_0), disappeared after only three generations of exposure to BX-sequestering WCR larvae. The rapid adaptation to BXs highlights the remarkable plasticity and adaptability of EPNs. Thus, artificial selection is one useful tool in superior EPN engineering. In the past, experimental attempts to improve EPNs stress tolerance like benzoxazinoids (Zhang *et al.*, 2019), desiccation (Strauch *et al.*, 2004), temperature (Griffin & Downes, 1994; Grewal *et al.*, 1996; Ehlers *et al.*, 2005) through continuous stress exposure has been successful. This has been previously hypothesized to be due to EPNs have short generation time, small genome size and are also ease of culture (Hiltpold *et*

al., 2010) and also showing that EPNs, can quickly adjust diverse situations. Interestingly, EPN endosymbionts bacteria alone can also be selected under stressful conditions to be become more infectious (Machado *et al.*, 2020). Proving again, that still artificial selection is a useful tool for engineering superior EPNs.

We later observed that before adaptation (F_0s), susceptible isolates infectivity suppression was linked to their behaviour in BXs. Our results show significant repulsion effect of MBOA-Glc towards the susceptible strains group but not the resistant one. Meaning that maybe infectivity suppression of susceptible strains may be driven by repellent forces of BXs against EPNs. Repulsion effect of this WCR larvae sequestered plant metabolite (MBOA-Glc) we used in these assays against EPNs has been reported (Robert *et al.*, 2017; Zhang *et al.*, 2019). Not only BXs impacts EPNs, but also other plant metabolites too with effects ranging from attractants, repellents, hatching stimulants or inhibitors have been reported (Sikder & Vestergård, 2019; Mathesius & Costa, 2021; Sikder *et al.*, 202). For example of such plant compound is the plant signal, (E)-beta-caryophyllene attracts EPNs (Rasmann *et al.*, 2005) and exudates from green pea induced reversible quiescence in EPN (Hiltpold *et al.*, 2015). Thus, plant metabolites are of economic importance as the compromise EPN potential.

In conclusion, in this study, we observed the supressive effect of WCR benzoxazinoid againsts some susceptible *Heterorhabditis bacteriophora* strains. We later demonstrated a remarkable ability of these suceptible strains to rapidly adapt to the host-sequestered plant defense compounds benzoxazinoids within as few as three generations of selection. These findings highlight potential targets for enhancing the efficacy of EPNs in biocontrol strategies and provide insights into mechanisms by which organisms adapt benzoxazinoid toxins. This work provides material which can used to make before(F_0) and after(F_5) adaptation genomic comparison useful in development of genetic markers that may explain benzoxazinoid tolerance in EPNs.

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Author contributions

ME, CR, and CP designed and supervised the research. VO and DM conducted the assays, DM, CR analysed and interpreted the data. DM wrote the work.

Conflicts of interest

The authors declare no conflicts of interest.

Figures and tables information

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Table 1. Summary table showing benzoxazinoids resistance phenotype of 26 screened IPS nematode. In the table, the first column iis the name of the tested isolate followed by its country of origini in the seconf colum. The third column is the statistical test comaprison of BX-fed-d to BX-free larave infectivity the correspondin strain, were, **(*)** denotes that the coresponding strain showed significant BX-fed-larvae to BX- free-larvae infectivity diffrences. Thus these strains all these strain with an asterik were termed susceptible isolates. The sympol (**ns)** denotes no infectictivity differences and thus these strains were termed resistant isolates

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Figure 1: Comparing the infectivity of susceptible and resistant isolates in benzoxazinoid-containing larvae (BX^+-fed) over several generations (F_0s (A), F_3s(B) and F_5s (C)) Asterisk (*) indicate significant difference in infectivity between resistant and susceptible nematode isolates at a time point between day 3 and 7 post exposure ($P<0.05$). The legend on the right of each figure shows the significance of the contribution the factors phenotype and time have in determining these differences. (**:$0.001<p<0.01$, *: $0.01<p<0.05$), Error Bars: MSe.

Figure 2. The relationship between EPN infectivity success with EPN behavioural and metabolic resistance. Comparison of the choice of isolated groups between BX (MBOA-Glc) or and control (Water) treated wells of the resistant and susceptible isolates was compared before [F0(A.)] and after [F5(B.)] adaptation. Nematode isolates were adapted in either benzoxazinoid- fed (W22) or benzoxazinoid -free larvae (bx1::W22-fed). An asterisk **(*)** and positioning (left or right) indicates significant preference of the compound contained in that well side ($P<0.05$). Survival potential of isolated EPN groups after 48 hrs exposure to before [F0(C)] or [F0(E)] and after [F5(D)] or [F0(F)] benzoxazinoid (HDMBOA-Glc or MBOA) adaptation respectively. Error Bars: Mse. For all graphs, red border lines indicate susceptible isolates group (S) while black indicate resistant isolates group (R) to match the selection lines in Figure 1.

Supplementary information

Supplementary Tables

Table S1: List with nematode strain names, origin and suppliers of nematode used in this study.

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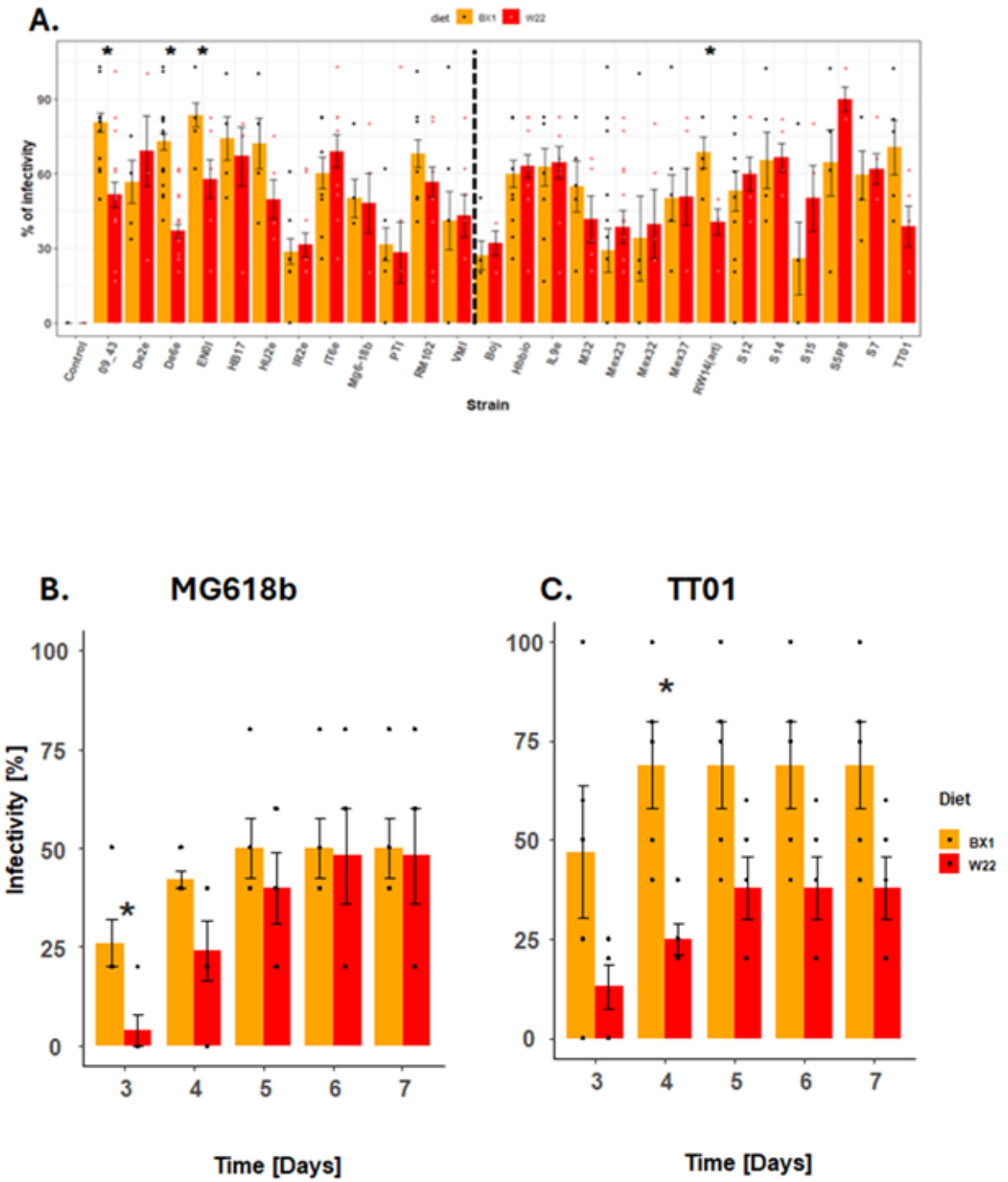
Figure S4: F_5 infectivity rates of individual entomopathogenic nematode (EPN) isolates (F_0) in Western Corn Rootworm larvae (WCR) fed on wild type plants containing benzoxazinoids (BX^+) and bx mutant plants (bx^-)

Figure S5. Comparison of characteristics of benzoxazinoid adapted and non-adapted nematode isolates in benzoxazinoid environments

2127 **Table S1: List with nematode strain names, origin and suppliers of nematode used in this study.**

Strain	Origin	Donator
Hb17	Turkey	Ismael Susurluk
Hbbio	USA	Bruce Hibbard
S5-P8	United States	BIN-CE collection
S12	United States	BIN-CE collection
S14	United States	BIN-CE collection
S15	United States	BIN-CE collection
MEX23	Mexico	Ted Turlings (FARCE)
MEX32	Mexico	Ted Turlings (FARCE)
MEX37	Mexico	Ted Turlings (FARCE)
TT01	Trinidad and Tobago	David Clarke
MG618b	Switzerland	Raquel Campos Herrera
RM102	Spain	Raquel Campos Herrera
VM1	Spain	Raquel Campos Herrera
EN01	Germany (Commercial)	Ralf Udo Ehlers (e-nema)
IT6	Italy	Ralf Udo Ehlers (e-nema)
IR2	Iran	Ralf Udo Ehlers (e-nema)
DE2	Germany	Ralf Udo Ehlers (e-nema)
PT1	Portugal	Ralf Udo Ehlers (e-nema)
HU2	Hungary	Ralf Udo Ehlers (e-nema)
IL9	Australia	Ralf Udo Ehlers (e-nema)
DE6	Germany	Ralf Udo Ehlers (e-nema)
09-43	Turkey	Ralf Udo Ehlers (e-nema)
Boj	Iran	Ralf Udo Ehlers (e-nema)
RW14	Rwanda	Stefan Toepfer



2128

2129 **Fig S1. Infectivity of 21 Heterorhabditis bacteriophora isolates on WCR larvae fed on BX$^+$ or bx$^-$ maize.** Figure S1A.
2130 Comparison of Infectivity success towards WCR larvae fed on WT (red bar) to that of bx::W22 mutant maize line (orange
2131 bar) by each strain after 6 days of infection. A. comparison of Infectivity success towards WCR larvae fed on WT (red
2132 bar) to that of bx::W22 mutant maize line (orange bar) by nematode strain MG618b (Figure S1.B) and strain TT01 (Figure
2133 S1.C) from 3 to 7 days after infection. An asterisk (*) indicate significant differences between infections of a bx::W22
2134 and W22 fed larvae ($P < 0.05$) by same strain. Error Bars: MSe.

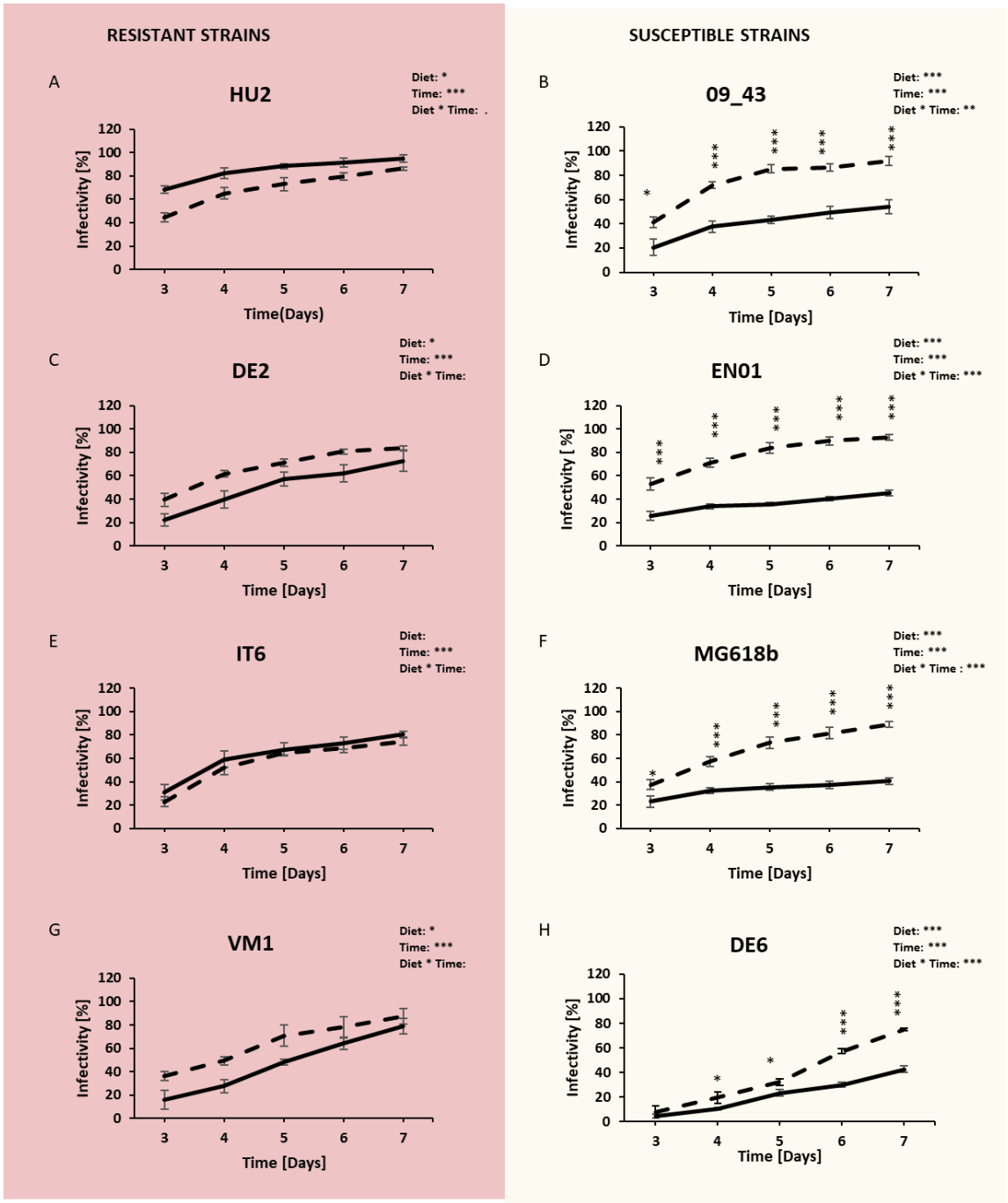


Figure S1: F_0 infectivity rates of individual entomopathogenic nematode (EPN) isolates (F_0) in western corn rootworm larvae (WCR) fed on wild type plants containing benzoxazinoids (BX^+) and bx mutant plants (bx^-). EPN infectivity rate (Mean ± SEM) was recorded by adding 50 EPNs to 5 WCR larvae and visually inspecting the larvae for colour change daily between day 3 and day 7 after EPN addition (n=5). Red panel (left): BX-resistant EPN isolates. Yellow panel (right): BX susceptible EPN isolates. Solid line: EPN infectivity in BX^+-fed WCR. Dotted line: EPN infectivity in bx^--fed WCR. Diet: Diet of the WCR larvae used for infectivity. Two-Way ANOVA on repeated measured were conducted. Stars indicate significant differences between EPN infectivity on BX^+ and bx^--fed WCR larvae within the day of observation. *: p<0.05; **: p<0.01; ***: p<0.001.

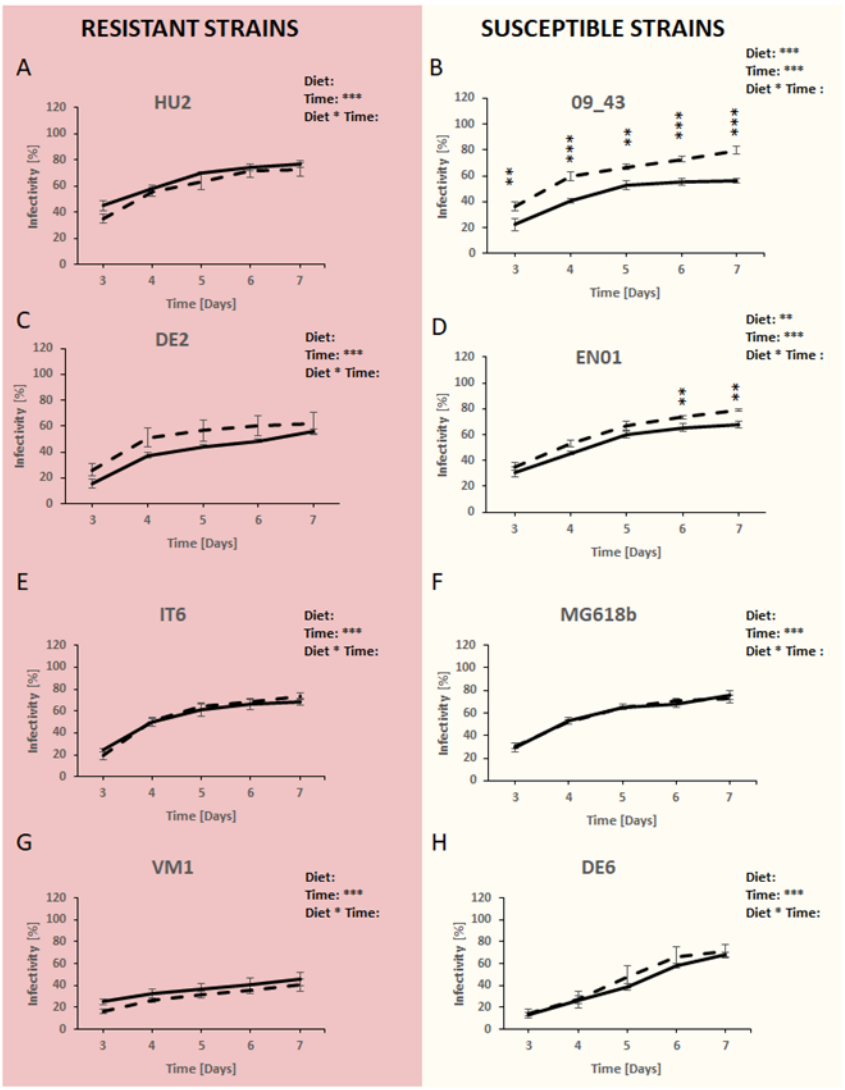
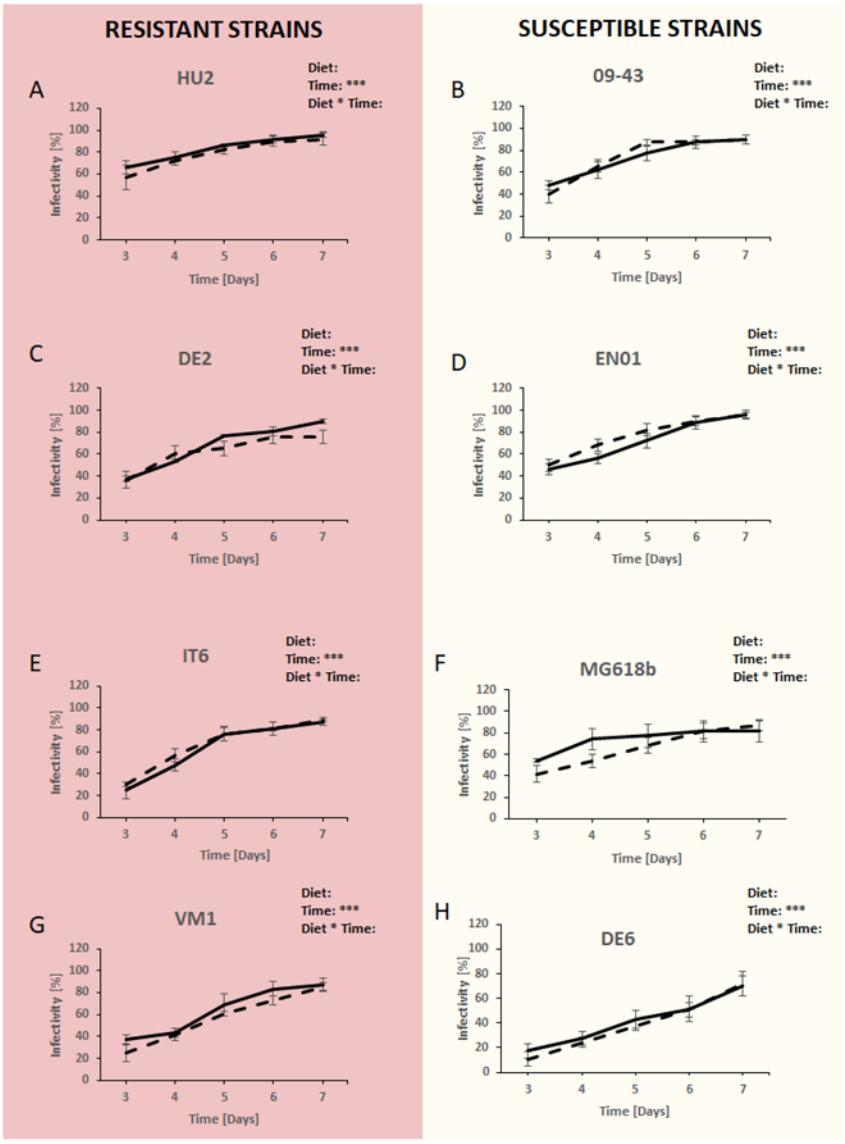


Figure S3: F_3 infectivity rates of individual entomopathogenic nematode (EPN) isolates (F_0) in Western Corn Rootworm larvae (WCR) fed on wild type plants containing benzoxazinoids (BX^+) and bx mutant plants (bx^-). EPN infectivity rate (Mean ± SEM) was recorded by adding 50 EPNs to 5 WCR larvae and visually inspecting the larvae for colour change daily between day 3 and day 7 after EPN addition (n=5). Red panel (left): BX-resistant EPN isolates. Yellow panel (right): BX susceptible EPN isolates. Solid line: EPN infectivity in BX^+-fed WCR. Dotted line: EPN infectivity in bx^--fed WCR. Diet: Diet of the WCR larvae used for infectivity. Two-Way ANOVA on repeated measured were conducted. Stars indicate significant differences between EPN infectivity on BX^+ and bx^--fed WCR larvae within the day of observation. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.



2152

2153 **Figure S4: F_5 infectivity rates of individual entomopathogenic nematode (EPN) isolates (F_0) in Western Corn Root-**
2154 **worm larvae (WCR) fed on wild type plants containing benzoxazinoids (BX^+) and bx^- mutant plants (bx^-)**. EPN in-
2155 fectivity rate (Mean ± SEM) was recorded by adding 50 EPNs to 5 WCR larvae and visually inspecting the larvae for
2156 colour change daily between day 3 and day 7 after EPN addition (n=5). Red panel (left): BX-resistant EPN isolates. Yel-
2157 low panel (right): BX susceptible EPN isolates. Solid line: EPN infectivity in BX^+-fed WCR. Dotted line: EPN infectivity in
2158 bx^--fed WCR. Diet: Diet of the WCR larvae used for infectivity. Two-Way ANOVA on repeated measured were con-
2159 ducted. Stars indicate significant differences between EPN infectivity on BX^+ and bx^--fed WCR larvae within the day of
2160 observation. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

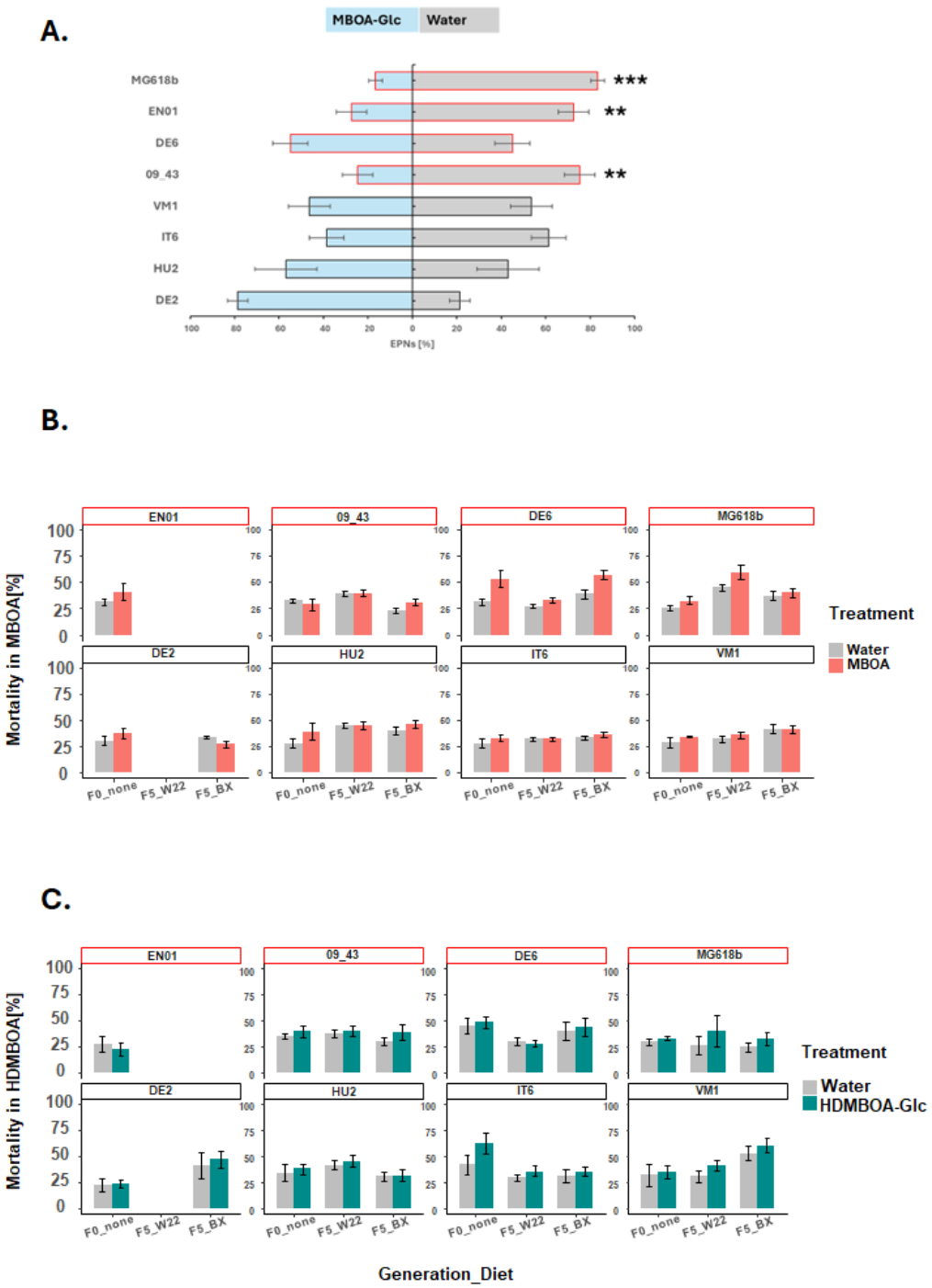


Figure S5. Comparison of characteristics of benzoxazinoid adapted and non-adapted nematode isolates in benzoxazinoid environments. (Figure S4A.) show the non- adapted (F_0S) individual isolate preference difference between well treated with BX(MBOA-Glc) and control well with water. Asterisk (*) and the side where its placed indicate significant preference of the compound contained in that well side compared to the other side ($P<0.05$). The individual test on BX survival potential of each individual isolate before (F_0s) and after adaptation in either BX-fed (W22) or BX-free larvae for 5 generation (F_5s) (Figure S4B-C). red border lines, indicate susceptible isolates while black indicate resistant isolates

References

Bruno, P., Machado, R.A.R., Glauser, G., Köhler, A., Campos-Herrera, R., Bernal, J., Toepfer, S., Erb, M., Robert, C.A.M., Arce, C.C.M. *et al.* (2020). Entomopathogenic nematodes from Mexico that can overcome the resistance mechanisms of the western corn rootworm. *Scientific reports* 10, 8257. DOI: 10.1038/s41598-020-64945-x

Campos, F., Atkinson, J., Arnason, J.T., Philogéne, B.J., Morand, P., Werstiuk, N.H., Timmins, G. (1988). Toxicity and toxicokinetics of 6-methoxybenzoxazolinone (MBOA) in the european corn borer,*Ostrinia nubilalis* (Hübner). *Journal of Chemical Ecology* 14, 989–1002. DOI: 10.1007/BF01018788

Chang, D.Z., Serra, L., Lu, D., Mortazavi, A., Dillman, A.R. (2019). A core set of venom proteins is released by entomopathogenic nematodes in the genus Steinernema. *PLOS Pathogens* 15, e1007626. DOI: 10.1371/journal.ppat.1007626

Chuang, W.-P., Herde, M., Ray, S., Castano-Duque, L., Howe, G.A., Luthe, D.S. (2014). Caterpillar attack triggers accumulation of the toxic maize protein RIP2. *The New phytologist* 201, 928–939. DOI: 10.1111/nph.12581

Cortés-Cruz, M., Snook, M., McMullen, M.D. (2003). The genetic basis of C-glycosyl flavone B-ring modification in maize *(Zea mays* L.) silks. *Genome* 46, 182–194. DOI: 10.1139/g02-119

Dillman, A.R. & Sternberg, P.W. (2012). Entomopathogenic nematodes. *Current biology CB* 22, R430-1. DOI: 10.1016/j.cub.2012.03.047

Ehlers, R.-U., Oestergaard, J., Hollmer, S., Wingen, M., Strauch, O. (2005). Genetic selection for heat tolerance and low temperature activity of the entomopathogenic nematode–bacterium complex *Heterorhabditis Bacteriophora–Photorhabdus Luminescens*. *Biocontrol* 50, 699–716. DOI: 10.1007/s10526-005-5079-z

Erb, M. & Robert, C. am (2016). Sequestration of plant secondary metabolites by insect herbivores: Molecular mechanisms and ecological consequences. *Current Opinion in Insect Science* 14, 8–11. DOI: 10.1016/j.cois.2015.11.005

Erenstein, O., Jaleta, M., Sonder, K., Mottaleb, K., Prasanna, B.M. (2022). Global maize production, consumption and trade: trends and R&D implications. *Food Security* 14, 1295–1319. DOI: 10.1007/s12571-022-01288-7

Fink, L.S. & Brower, L.P. (1981). Birds can overcome the cardenolide defence of monarch butterflies in Mexico. *Nature* 291, 67–70. DOI: 10.1038/291067a0

Glauser, G., Marti, G., Villard, N., Doyen, G.A., Wolfender, J.-L., Turlings, T.C.J., Erb, M. (2011). Induction and detoxification of maize 1,4-benzoxazin-3-ones by insect herbivores. *The Plant journal for cell and molecular biology* 68, 901–911. DOI: 10.1111/j.1365-313X.2011.04740.x

Gray, M.E., Sappington, T.W., Miller, N.J., Moeser, J., Bohn, M.O. (2009). Adaptation and invasiveness of western corn rootworm: Intensifying research on a worsening pest. *Annual Reviews on Entomology* 54, 303–321.

Grewal, P.S., Gaugler, R., Shupe, C. (1996). Rapid changes in thermal sensitivity of entomopathogenic nematodes in response to selection at temperature extremes. *Journal of Invertebrate Pathology* 68, 65–73. DOI: 10.1006/jipa.1996.0059

Griffin, C.T. & Downes, M.J. (1994). Selection of *Heterorhabditis* sp. (Nematoda: Heterorhabditidae) for improved infectivity at low temperature. *Nematologica*, *37*(1-4), 83-91.

Gripenberg, S. & Roslin, T. (2007). Up or down in space? Uniting the bottom-up versus top-down paradigm and spatial ecology. *Oikos* 116, 181–188. DOI: 10.1111/j.0030-1299.2007.15266.x

Hajek, A.E. & Eilenberg, J. (2018). Natural enemies: An introduction to biological control. Cambridge University Press.

Hiltpold, I., Baroni, M., Toepfer, S., Kuhlmann, U., Turlings, T.C.J. (2010). Selection of entomopathogenic nematodes for enhanced responsiveness to a volatile root signal helps to control a major root pest. *The Journal of experimental biology* 213, 2417–2423. DOI: 10.1242/jeb.041301

Hiltpold, I., Jaffuel, G., Turlings, T.C.J. (2015). The dual effects of root-cap exudates on nematodes: from quiescence in plant-parasitic nematodes to frenzy in entomopathogenic nematodes. *Journal of experimental botany* 66, 603–611. DOI: 10.1093/jxb/eru345

Kaya, H.K. & Gaugler, R. (1993). Entomopathogenic Nematodes. *Annual review of entomology* 38, 181–206. DOI: 10.1146/annurev.en.38.010193.001145

Kumar, P., Pandit, S.S., Steppuhn, A., Baldwin, I.T. (2014). Natural history-driven, plant-mediated RNAi-based study reveals CYP6B46's role in a nicotine-mediated anti-predator herbivore defense. *Proceedings of the National Academy of Sciences* 111, 1245–1252. DOI: 10.1073/pnas.1314848111

Lu, D., Macchietto, M., Chang, D., Barros, M.M., Baldwin, J., Mortazavi, A., Dillman, A.R. (2017). Activated entomopathogenic nematode infective juveniles release lethal venom proteins. *PLOS Pathogens* 13, e1006302. DOI: 10.1371/journal.ppat.1006302

Maag, D., Kohler, A., Robert, C.A.M., Frey, M., Wolfender, J.-L., Turlings, T.C.J., Glauser, G., Erb, M. (2016). Highly localized and persistent induction of Bx1-dependent herbivore resistance factors in maize. *The Plant journal for cell and molecular biology* 88, 976–991. DOI: 10.1111/tpj.13308

Machado, R.A.R., Thönen, L., Arce, C.C.M., Theepan, V., Prada, F., Wüthrich, D., Robert, C.A.M., Vogiatzaki, E., Shi, Y.-M., Schaeren, O.P. *et al.* (2020). Engineering bacterial symbionts of nematodes improves their biocontrol potential to counter the western corn rootworm. *Nature biotechnology* 38, 600–608. DOI: 10.1038/s41587-020-0419-1

Mathesius, U. & Costa, S.R. (2021). Plant signals differentially affect rhizosphere nematode populations. *Journal of experimental botany* 72, 3496–3499. DOI: 10.1093/jxb/erab149

Meihls, L.N., Handrick, V., Glauser, G., Barbier, H., Kaur, H., Haribal, M.M., Lipka, A.E., Gershenzon, J., Buckler, E.S., Erb, M. *et al.* (2013). Natural variation in maize aphid resistance is associated with 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside methyltransferase activity. *The Plant Cell* 25, 2341–2355. DOI: 10.1105/tpc.113.112409

Ode, P.J. (2006). Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. *Annual review of entomology* 51, 163–185. DOI: 10.1146/annurev.ento.51.110104.151110

Pechan, T., Ye, L., Chang, Y., Mitra, A., Lin, L., Davis, F.M., Williams, W.P., Luthe, D.S. (2000). A unique 33-kD cysteine proteinase accumulates in response to larval feeding in maize genotypes resistant to fall armyworm and other Lepidoptera. *The Plant Cell* 12, 1031–1040. DOI: 10.1105/tpc.12.7.1031

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N., Weis, A.E. (1980). Interactions Among Three Trophic Levels: Influence of Plants on Interactions Between Insect Herbivores and Natural Enemies. *Annual Review of Ecology and Systematics* 11, 41–65.

Ranum, P., Peña-Rosas, J.P., Garcia-Casal, M.N. (2014). Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences* 1312, 105–112. DOI: 10.1111/nyas.12396

Rasmann, S., Köllner, T.G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J., Turlings, T.C.J. (2005). Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* 434, 732–737. DOI: 10.1038/nature03451

Rector, B.G., Liang, G., Guo, Y. (2003). Effect of Maysin on Wild-Type, Deltamethrin-Resistant, and Bt-Resistant Helicoverpa armigera (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 96, 909–913. DOI: 10.1093/jee/96.3.909

Reudler, J.H., Biere, A., Harvey, J.A., van Nouhuys, S. (2011). Differential performance of a specialist and two generalist herbivores and their parasitoids on *Plantago lanceolata*. *Journal of Chemical Ecology* 37, 765–778. DOI: 10.1007/s10886-011-9983-7

Robert, C.A., Zhang, X., Machado, R.A., Schirmer, S., Lori, M., Mateo, P., Erb, M., Gershenzon, J. (2017). Sequestration and activation of plant toxins protect the western corn rootworm from enemies at multiple trophic levels. *eLife* 6. DOI: 10.7554/eLife.29307

Robert, C.A.M. & Mateo, P. (2022). The Chemical Ecology of Benzoxazinoids. *CHIMIA* 76, 928. DOI: 10.2533/chimia.2022.928

Robert, C.A.M., Veyrat, N., Glauser, G., Marti, G., Doyen, G.R., Villard, N., Gaillard, M.D.P., Köllner, T.G., Giron, D., Body, M. *et al.* (2012). A specialist root herbivore exploits defensive metabolites to locate nutritious tissues. *Ecology Letters* 15, 55–64. DOI: 10.1111/j.1461-0248.2011.01708.x

Sikder, M.M. & Vestergård, M. (2019). Impacts of Root Metabolites on Soil Nematodes. *Frontiers in plant science* 10, 1792. DOI: 10.3389/fpls.2019.01792

Sikder, M.M., Vestergård, M., Kyndt, T., Fomsgaard, I.S., Kudjordjie, E.N., Nicolaisen, M. (2021). Benzoxazinoids selectively affect maize root-associated nematode taxa. *Journal of experimental botany* 72, 3835–3845. DOI: 10.1093/jxb/erab104

Strauch, O., Oestergaard, J., Hollmer, S., Ehlers, R.-U. (2004). Genetic improvement of the desiccation tolerance of the entomopathogenic nematode *Heterorhabditis bacteriophora* through selective breeding. *Biological Control* 31, 218–226. DOI: 10.1016/j.biocontrol.2004.03.009

Tamayo, M.C., Rufat, M., Bravo, J.M., San Segundo, B. (2000). Accumulation of a maize proteinase inhibitor in response to wounding and insect feeding, and characterization of its activity toward digestive proteinases of *Spodoptera littoralis* larvae. *Planta* 211, 62–71. DOI: 10.1007/s004250000258

Trejo-Meléndez, V.J., Ibarra-Rendón, J., Contreras-Garduño, J. (2024). The evolution of entomopathogeny in nematodes. *Ecol. Evol. Ecology and evolution* 14, e10966. DOI: 10.1002/ece3.10966

Tzin, V., Fernandez-Pozo, N., Richter, A., Schmelz, E.A., Schoettner, M., Schäfer, M., Ahern, K.R., Meihls, L.N., Kaur, H., Huffaker, A. *et al.* (2015). Dynamic Maize Responses to Aphid Feeding Are Revealed by a Time Series of Transcriptomic and Metabolomic Assays. *Plant Physiology* 169, 1727–1743. DOI: 10.1104/pp.15.01039

White, G.F. (1927). A Method for obtaining infective nematode larvae from cultures. *Science* 66, 302–303. DOI: 10.1126/science.66.1709.302.b

Yan, J., Lipka, A.E., Schmelz, E.A., Buckler, E.S., Jander, G. (2015). Accumulation of 5-hydroxynorvaline in maize (Zea mays) leaves is induced by insect feeding and abiotic stress. *Journal of experimental botany* 66, 593–602. DOI: 10.1093/jxb/eru385

Zhang, X., Li, L., Kesner, L., Robert, C.A.M. (2021). Chemical host-seeking cues of entomopathogenic nematodes. *Current Opinion in Insect Science* 44, 72–81. DOI: 10.1016/j.cois.2021.03.011

Zhang, X., van Doan, C., Arce, C.C.M., Hu, L., Gruenig, S., Parisod, C., Hibbard, B.E., Hervé, M.R., Nielson, C., Robert, C.A.M. *et al.* (2019). Plant defense resistance in natural enemies of a specialist insect herbivore. *Proceedings of the National Academy of Sciences of the United States of America* 116, 23174–23181. DOI: 10.1073/pnas.1912599116

Chapter 3: Benzoxazinoid (BXs) metabolization by entomopathogenic nematodes (EPNs)

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Abstract

One of the endogenous stressful factors of entomopathogenic nematodes (EPNs) inside the western corn rootworm (WCR) host are the benzoxazinoids toxins accumulated in the body of the host. Our previous Chapter 2 of this work, have revealed that these can significantly suppress infectivity of some EPN strain including the commercial strains EN01. This gives researchers homework on how to maintain and or enhance infectivity even in fields with plants which produce these toxic defense compounds. One stepping-stone in tackling such challenges is assessing EPNs response in stressful situation and link responses to some biological processes then later use the information in EPN breeding and manipulation programs. Here, we assessed the response of EPN strains to all three benzoxazinoids (HDMBOA-Glc, MBOA-Glc and MBOA) accumulated in WCR body. To do this, we incubated nematodes in benzoxazinoids for some specified time and the monitor the stability of the initially added compound over time. Our results revealed that, EPNs can biodegrade all three WCR accumulated benzoxazinoids to their breakdown derivates in a strain specific manner. These results revealed that EPNs can fight back benzoxazinoid toxin effects by biodegrading them to their breakdown derivates, thus, showing EPNs ability to respond in stressful situations. This work findings will be helpful in elucidation mechanistic bases of inter-strain benzoxazinoid tolerance variation. Additionally, this will make predictions of benzoxazinoid effect on various other strains possible. Furthermore, this paves way for nematode breeders for development of genetic markers linked to benzoxazinoid tolerance.

Keywords: Benzoxazinoids, entomopathogenic nematodes, biodegradation or biotransformation

Introduction

Organisms encounter biotic as well as abiotic stressful environments, which in turn, compromise their lifestyle and or lifespan. In response to stress, organisms employ various resistance and tolerance strategies. Entomopathogenic nematodes (EPNs) face numerous stresses, which compromise their efficacy as pest biological control agents (Kaya, 2002; Maushe *et al.*, 2023). A clear understanding of stress mitigation strategies of EPNs, may help to breed EPNs that perform better as biological control agents under variable biotic and abiotic conditions.

EPNs encounter biotic stressors such as viruses and phages, bacteria, nematophagus fungi, insects, mites (Kaya, 2002; Maushe *et al.*, 2023) and abiotic factors such as heat, cold, starvation, desiccation, hypoxic conditions, oxidative stress, and ultraviolet radiation (Robert *et al.*, 2017; Sharmila *et al.*, 2018; Maushe *et al.*, 2023). Previous research showed that in response to stress, EPNs have adopted unique structural, biochemical, and behavioral stress adaptations (Glazer, 2002; Perry & Wharton, 2011; Perry *et al.*, 2012). Natural variation in stress resistance can be leveraged through selection or genetic engineering of EPNs and or their endosymbiotic bacteria to improve their performance (Lu *et al.*, 2016; Machado *et al.*, 2020; Maushe *et al.*, 2023)

When they invade their host, EPNs can be exposed to toxic plant secondary metabolites such as benzoxazinoids (BXs). Benzoxazinoids are indole- derived plants herbivory defence metabolites, store as stable glucosides conjugates (Frey *et al.*, 2003; Jonczyk *et al.*, 2008; Wouters *et al.*, 2016a). Upon tissue maceration these stable BX glucosides can be hydrolysed by endogenous β-glucosidases enzymes, resulting in the liberation of unstable and more toxic to plant herbivore aglucones(Czjzek *et al.*, 2000; Oikawa *et al.*, 2004; Glauser *et al.*, 2011; Marti *et al.*, 2013; Handrick *et al.*, 2016; Wouters *et al.*, 2016a). BXs are reported to have allelopathic and anti-herbivory effects against plant herbivores and pathogenic fungi and bacteria in cereal plants (Sicker *et al.*, 2000; Sicker & Schulz, 2002; Bruijn *et al.*, 2018).

The western corn rootworm (WCR), or *Diabrotica virgifera virgifera* LeConte, a specialised maize root feeder and target of EPN biological control, can tolerate and sequester

benzoxazinoids. In studies of BX effects on WCR, no negative effect on growth and development has been reported of these ingested BX to WCR (Alouw & Miller, 2015). This observation was later explained by the revelation that WCR has evolved resistance to BXs. WCR larvae accumulates two BXs: HDMBOA- Glc and MBOA- Glc. In addition, WCR larvae can hydrolyze HDMBOA-Glc, to produce MBOA. HDMBOA- Glc and MBOA have a toxic effect on EPNs, while MBOA- Glc, repels EPNs (Robert *et al.*, 2017).

Interestingly, EPNs from the natural range of WCR are resistant to BXs (Zhang *et al.*, 2019). However, the mechanism underlying this phenomenon are unclear. EPNs may resist BXs either by resistance or tolerance. Tolerance encompasses processes such as avoidance,exclusion, compensation, insensitivity as well as enzyme inhibition (Jeckel *et al.*, 2022) and resistance includes processes like metabolization, excretion and sequestration (Jeckel *et al.*, 2022).

To gain deeper insights into potential resistance strategies of EPNs to BXs, we investigated their potential to metabolize BXs. We hypothesized that BX resistant strains may have a higher ability to metabolize and/or degrade BXs than non-resistant strains. To assess BX metabolization by EPNs, we incubated them with purified BXs and then measured the accumulation of breakdown products. We then compared metabolization between BX resistant and tolerant EPN strains.

Material and Methods

Biological resources

Maize seeds of the wild type inbred line W22 and benzoxazinoid-deficient mutant bx1::W22 (gene identifier GRMZM2G085381; Ds, B.W06.0775 for *bx1* and Ds) on the *bx1* gene (Tzin *et al.*, 2015) were kindly provided by Georg Jander (Boyce Thompson Institute, Ithaca, NY; USA). Western corn rootworm (WCR, *Diabrotica virgifera virgifera*) eggs were obtained from USDA-ARS-NCARL (North Central Agricultural Research Laboratory, US Department of Agriculture, Agriculture Research Service, Brookings, SD, USA). WCR eggs were incubated at room temperature and hatching larvae were raised on either bx1::W22 or W22 seedlings. WCR larvae fed on bx1::W22 are hereafter referred to as bx^--fed WCR. WCR larvae fed on W22 are hereafter referred to as BX^+-fed WCR. Second and third instar larvae were used for infectivity assays.

A total of 21 isolates of the EPN *Heterorhabditis bacteriophora* were obtained from collaborators and commercial providers as described in Supplementary Table 1 (Zhang *et al.*, 2019) and maintained in the Biotic Interactions and Chemical Ecology groups (University of Bern, Switzerland). The EPN isolates (09_43, DE2, DE6, EN01, Hb 17, Hb bio, HU2, IL9, IR2, IT6, MEX-17, MEX-21, MEX-33, MG618b, PT1, S12, S14, S15, S5-P8, S7 and VM1) were chosen according to the following criteria: (i) known genomes (Ogi et al., under review), (ii) know susceptibility level to BXs (Ogi *et al.*, 2024), and (iii) diverse geographic origins including regions where EPNs have co-evolved with western corn rootworms (WCR), and regions where WCRs are absent or introduced less than 50 years ago (Miller *et al.*, 2005; Zhang *et al.*, 2019). All isolates were multiplied in greater wax moth (*Galleria mellonella*) larvae, bought from local fish store (Fischereibedarf Wenger AG, Bern, Switzerland) and kept at 8 °C until use. EPN amplification was performed following a previous protocol by (White 1927) with some modifications. Briefly 400 µl of nematodes were added to about five *G. mellonella* larvae place in a 5 cm diameter petri dish (Greiner Bio-One, Frickenhausen, Germany) on filter paper (55 mm diameter, Whatman, China). The infected larvae were then incubated at 24 ± 2°C in an incubator. After seven days infected *G. mellonella* larvae were transferred to white traps and incubated in darkness at 24 ± 2°C. Hatching EPNs (infective juveniles, IJs) were collected and concentrated using a 25 µm pore diameter sieve (Rentsch GmbH, Germany) and poured with tap water

into cell culture flasks (160 mL, Thermo Fisher scientific, Switzerland). All collected EPNs were stored at 8°C in a fridge until use.

Benzoxazinoids metabolization assays

Benzoxazinoid metabolization by different EPN isolates was tested by adding 2-O-β-D-glucopyranosyl-2-hydroxy-4,7-dimethoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (HDMBOA-Glc), 6-methoxy-benzoxazolin-2(3*H*)-one (MBOA), or 3-β-D-glucopyranosyl-6-methoxy-benzoxazolin-2(3*H*)-one (MBOA- Glc) to an EPN-containing solution in 6 independent experiments. HDMBOA-Glc was isolated from maize plants in our laboratory and resulted in a >99% purity (Mateo et al.; Unpublished). MBOA was bought MBOA were purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). MBOA-Glc were synthesized in our laboratory directly from or adapting published protocols, (Sicker *et al.*, 2001; Macías *et al.*, 2006; Li *et al.*, 2013). Preliminary assays demonstrated that (i) these BXs are stable in water solution under ambiant conditions for 10 days (Supplementary Figure S1), (ii) none of the 21 H. *bacteriophora* isolates produce these BXs (Supplementary Figure S2), and (iii) EPNs can transform some of these after one day (Supplementary Figure S3), suggesting that longer periods of incubation might highlight conversion products.

In a first series of three experiments, BX metabolization was tested in the commercial *H. bacteriophora* strain EN01 after 1, 2, 3, and 7 days (HDMBOA- Glc assay) or 1, 3, 7, and 10 days (MBOA- Glc assays) and 1, 3 and 10 days (MBOA assays). In a second series of two experiments, HDMBOA- Glc and MBOA- Glc metabolization was tested in 21 *H. bacteriophora* strains after 3 days. In all assays, approximately 10'000 EPNs were placed in 1 mL tap water. HDMBOA- Glc, MBOA, or MBOA- Glc, were added to half of the tubes to reach a final concentration of 150 µg/mL, 100 µg/mL, 50 µg/mL respectively (n=5). These concentrations correspond to BXs concentrations reported in WCR (Robert *et al.*, 2017). All samples were incubated at 25 ± 2 ℃. After incubation, 500 µL aliquots were collected from the samples and mixed with 500 µL methanol (MeOH, Fisher Chemica). The samples were then centrifuged at 10 °C at 13'500 RPM for 10 min. The supernatant were collected into 1.5 mL HPLC-glass vials (VWR International, UK)). The pellets, containing EPN bodies, were rinsed three times adding distilled water and centrifuging. The final pellet mass was measured, and 10 µL extraction buffer (50% MeOH with 0.1% formic acid (FA,

Fisher Chemical, Waltham, MA, USA) was added per 1 mg pellet. 1.4 mm zirconium oxide beads (Precellys, Bertin Technologies, France) were added to the tubes for grinding in a bead-beater at 30 X speed for 3 minutes before centrifugation at 13'500 RPM at 10 °C for 15 min. The supernatant was collected in 1.5mL glass vials (VWR International, UK) for benzoxazinoid analyses.

Benzoxazinoid analyses

All the collected analytic glass vials were later sent for accurate quantitation of most known benzoxazinoids and their breakdown products and conjugates. This was done using an ultra-high performance liquid chromatography-mass spectrometry system equipped with an electrospray source (UHPLC-QDA) to detect, quantify, and identify known new structures through exact mass and fragmentation analysis by MSE as analytic methods. Briefly, for detection and identification of recovered benzoxazinoid recovered breakdown derivatives after their incubation with EPNs, using UHPLC-MS system equipped with an electrospray source (Waters i-Class UHPLC-QDA, USA). Recovered benzoxazinoids and their breakdown derivates were separated on a BEH C18 column (2.1 × 100 mm i.d., 1.7 µm particle size). Mobile phase A and B of Water (0.1% FA) and acetonitrile (0.1% FA) respectively were set with respective elution profiles of: 0–9.65 min, 97–83.6% A in B; 9.65–13 min, 100% B; 13.1–15 min 97% A in B which equivalate to 0.4 mL/min. A stable column temperature of 40°C was maintained, as well as an injection volume of 5µL. The MS was set to operate in a negative reverse mode, and data acquisition scan range of (m/z 150–650) using a cone voltage of 10V. default setting were maintained for all other MS parameters as suggested by the manufacturer. Absolute BX concentrations were determined using standard curves obtained from purified benzoxazinoid compounds. Briefly for the synthesis of the standards: - BOA (benzoxazolin-2(3H)-one) and MBOA (6-methoxy-benzoxazolin-2(3H)-one) were purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). HMPMA (N-(3-methoxy-2-hydroxyphenyl) malonamic acid) was received as a gift from Prof. Dr. Francisco A. Macías (University of Cádiz, Spain). DIMBOA-Glc (2-O-β-D-glucopyranosyl-2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one) and HDMBOA-Glc (2-O-β-D-glucopyranosyl-2-hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3(4H)-one) were isolated from maize plants in our laboratory. DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one), MBOA-Glc (3-β-

D-glucopyranosyl-6-methoxy-benzoxazolin-2(3H)-one), HMBOA (2-hydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one), APO (2-amino-3H-phenoxazin-3-one), AMPO (9-methoxy-2-amino-3H-phenoxazin-3-one), AAMPO (9-methoxy-2-acetylamino-3H-phenoxazin-3-one), were synthesized in our laboratory directly from or adapting published protocols.

Infectivity assays

The infectivity of the selected subpopulations was tested in WCR larvae fed on either W22 (maize plants which produces benzoxazinoids) or bx::W22 mutant plants (benzoxazinoids -free plants). Briefly, 5 WCR larvae were placed into solo cups (30 mL; Frontier Scientific Services, Newark, USA) containing 3.5 g of moist, autoclaved, sand (Selmaterra, Bigler Samen AG, Steffisburg, Switzerland). Approximately 100 EPNs suspended in 700 µL tap water were added into the solo cups and incubated at 25 ± 0.5 °C for 7 days. As infected larvae with a reddish or yellowish colour phenotype were considered infected by nematodes and the infectivity rate was recorded visually 3-7 days post exposure (n=5 per each of the strain).

Screening for homologous enzyme corresponding to benzoxazinoid biodegradation in EPNs and their endosymbiont bacteria.

The benzoxazinoid pathway is not yet fully known but some of the enzymes responsible for benzoxazinoids degradation have been reported in past studies. Deglucosylating of benzoxazinone glucosides like HDMBOA-Glc and MBOA-Glc to benzoxazinone aglucone like MBOA and HMBOA by β-glucosidases enzyme like maize reported *ZmGLU1* and *ZmGLU2* (Sue *et al.*, 2011; Schulz *et al.*, 2016) while further breaking down of aglucones has been also reported to involves microbe enzymes like BxdA (Thoenen *et al.*, 2024) as well as CbaA and Mbl1 (Saunders & Kohn, 2008). Using this information from past reports, we were curious to explore presence of these five enzymes (Table 1, 1st column) in either or both, the EPNs or their endosymbiont bacteria genomes. For this, the potential presence of reported sequence of genes known to be involved in the degradation of benzoxazinoids has been used in a blast search both against a blast database built on a reference genome for *Heterorhabditis bacteriophora* ((Ogi *et al.*, 2024) as well as a blast database built on the genome of *Photorhabdus luminescens* ((Thoenen *et al.*, 2023).The

blast sear was run as a blastn, with a maximum E-value of 1e-1 and a gap cost of 5 2. The resulting hits were evaluated based on the length of the match, the percentage of the query sequence that showed a match to the reference and the percentage of the match between the sequences.

Statistical analyses

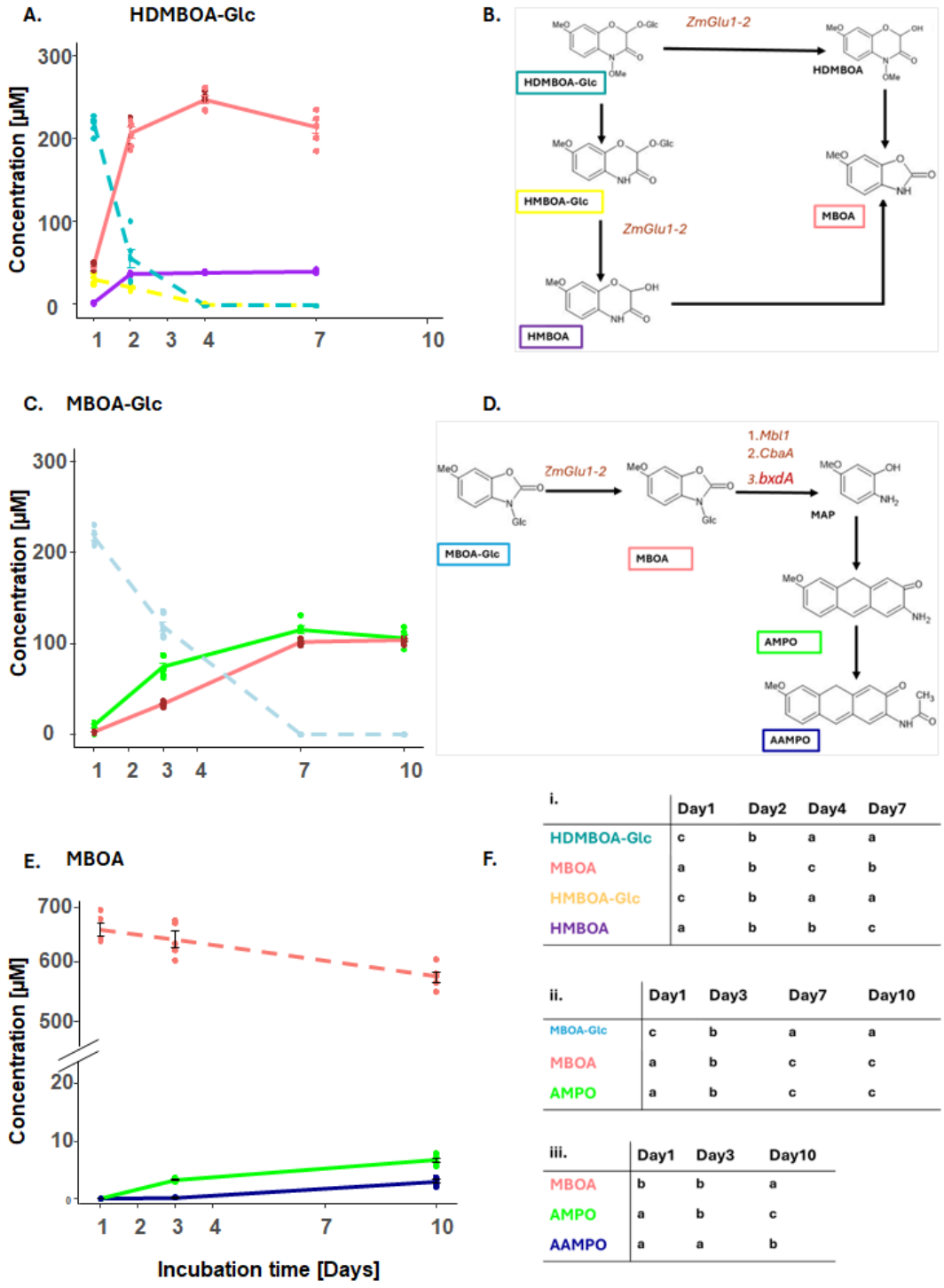
All statistical analyses were conducted in R (https://www.r-project.org/, version 4.4.2)). Effect of different isolates as well as differently treated nematode on benzoxazinoids recovery after degaradation was tested using One-way ANOVA if all parametric test requirements were meet. For non parametric data, its alternative Kruskal-Wallis was used. Whenever there was a significant effect ($P<0.05$), post hoc pairwise comparisons between treatments or isolates was carried out by extracting "estimated marginal means" using “emmeans multicomp” package in R (https://doi.org/10.32614/CRAN.package.emmeans).

Results

Entomopathogenic nematode (isolate EN01) metabolizes benzoxazinoids

HDMBOA-Glc, MBOA-Glc and MBOA were stable over the course of the experiments, and were not endogenously produced by EPNs. (Supplementary Figure S1) (Supplementary. Figure S2). However, the presence of EPNs resulted in their rapid transformation. A slightly lower recovery of HDMBOA-Glc, MBOA-Glc and MBOA at the beginning was observed in presence of EPNs as compared to the benzoxazinoid only control, suggesting potential matrix effects in EPN samples (Supplementary Figure S3C. and Supplementary Figure S4A.). At day 2, a significant reduction in all three BXs was observed in the supernatant (Figure 1A-C). No BXs were detected in the EPN bodies, suggesting rapid metabolization. (Supplementary Figure S4). Incubating EPNs in HDMBOA-Glc resulted in a complete disappearance of the compound after four days of incubation (Figure 1A). Similarly, the traces of HMBOA-Glc present in the original HDMBOA-Glc standard decreased over time (Figure 1A). The decline was accompanied by an increase in MBOA and HMBOA (Figure1A). A similar profile was observed after 10 days, where MBOA was dominant in the matrix (Supplementary Figure S5).

Whether the formation of HMBOA arose from a direct deglucosylation of HMBOA-Glc and/or from a demethylation of HDMBOA-Glc to HMBOA-Glc prior deglucosylation could not be established at this stage. However, the mass balances between HDMBOA- Glc and MBOA, as well as between HMBOA-Glc and HMBOA corresponded to each other and would suggest that these two pathways were independent, with HDMBOA- Glc being first deglucosylated to HDMBOA, a rapid and highly unstable compound (Czjzek *et al.*, 2000)), itself spontaneously hydrolyzed to MBOA (Figure 1B); and HMBOA- Glc being deglucosylated to HMBOA. Adding MBOA-Glc to EPNs showed a steep decrease in initial MBOA- Glc concentrations with total disappearance after 7 days (Figure1C). The disappearance of MBOA-Glc was accompanied by a sharp increase in MBOA and AMPO concentrations (Figure1C, D). Thus, in the presence of EPNs, MBOA was slowly converted to AMPO and AAMPO (Figure1D,E).



i.

	Day1	Day2	Day4	Day7
HDMBOA-Glc	c	b	a	a
MBOA	a	b	c	b
HMBOA-Glc	c	b	a	a
HMBOA	a	b	b	c

ii.

	Day1	Day3	Day7	Day10
MBOA-Glc	c	b	a	a
MBOA	a	b	c	c
AMPO	a	b	c	c

iii.

	Day1	Day3	Day10
MBOA	b	b	a
AMPO	a	b	c
AAMPO	a	a	b

Figure.1 **Metabolisation pattern of benzoxazinoids (BXs) by entomopathogenic nematodes (EPNs).** Biotransformation of HDMBOA-Glc (A), MBOA-Glc (C) and MBOA (E) over time (Mean ±SEM). Recovered BXs compounds and their derivates were measured from a matrix of about 10 000 nematodes of starin EN01 incubated in BXs suspended in 1mL water over a period of time ranging from 1-10 days. Broken lines are the initially added compound while solid lines represent derivative compounds from the intialy added compound measure after the stipulated time. Benzoxazinoid full names and structures can be found in Supplementary Table SX. The proposed breakdown pathways and proposed responsible enzymes (using past reports) in HDMBOA-Glc (Figure 1B.), MBOA-Glc (Figure 1D) biotransformation. The statistical comparison of the benzoxazionoids and the dreakdown derivates of HDMBOA-Glc (Figure Fi.), MBOA-Glc (Figure Fii.),) as well as MBOA (Figure Fiii.),) at each point of data collection and different letters (a,b etc) indicates statistical concentration differences of the measured compound for that specific day.

Benzoxazinoid metabolization is widespread among EPN (*Heterhabdtis bacteriophora*) isolates

The conversion of HDMBOA-Glc and of MBOA-Glc to MBOA after 3 days was observed in all 21 tested isolates (Figure 2). The metabolization of HDMBOA-Glc seemed to be faster than of MBOA-Glc (Figure 2). The rate of metabolization of each compound was different between isolates (Figure 2A and Figure 2B). In particular, AMPO was only detected in 9 out of 21 isolates after the addition of MBOA- Glc, suggesting either different metabolization rates or capabilities among strains (Figure 2B). Additionally, for some isolates, such as MG618b, during HDMBOA-Glc metabolization experiment and 09_43 in the MBOA-glc experiment, the recovered compounds were far much less that the initial control concentration which may be due to undetected compound production or isolate-specific matrix effects. Interestingly, the metabolization of HDMBOA-Glc after those 3 days was possible for all strains while only 11 isolates had significant degradation of MBOA-Glc.

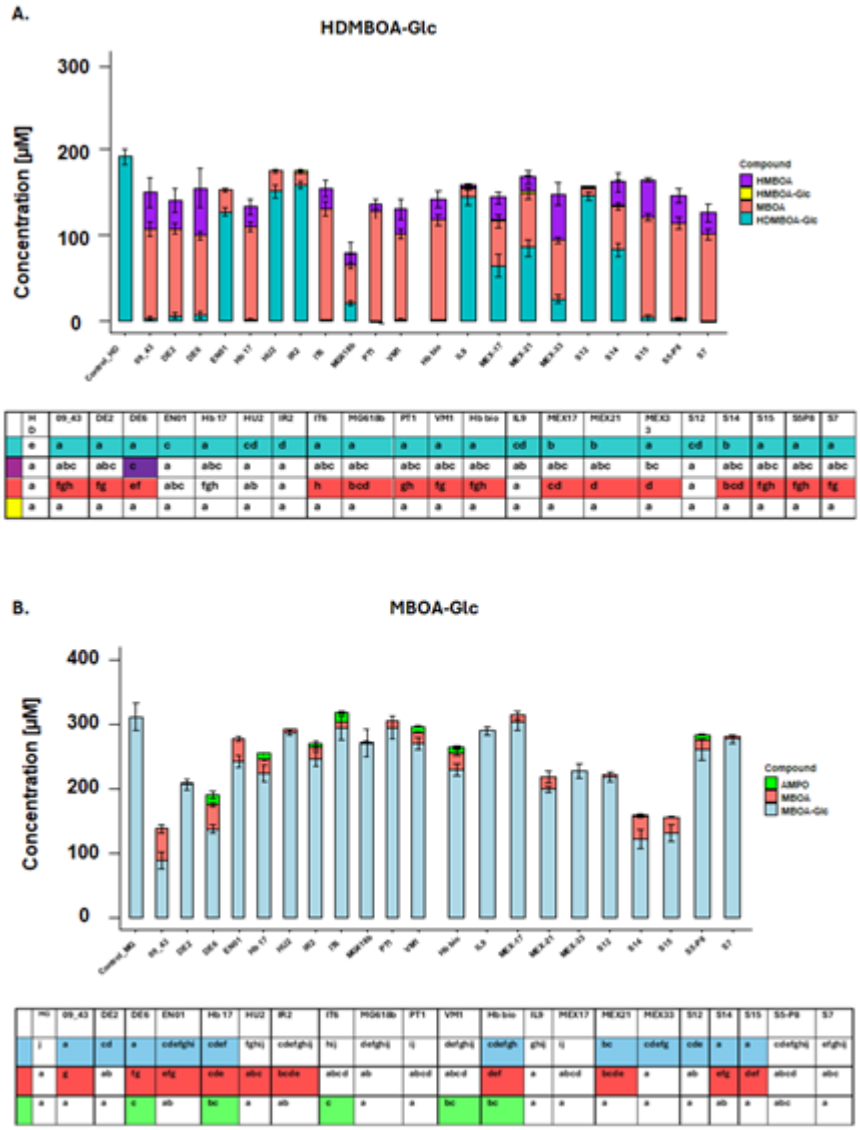


Figure.2. **Benzoxazinoid metabolization is widespread among Heterorhabditis bacteriophora isolates. Assessing the potential of different EPN isolate to metabolise HDMBOA-Glc (Figure 2A.) and MBOA-Glc (Figure 2B.) after 3 days of incubations.** Each bar represent a unique EPN isolate and the colour and height of each bar rer represent the different kinds of recovered benzoxazinoids derivates and their concentrations in the matrix respectively. The legend table below show the statistical comparison for each recovered derivative after transformation and are represented by their respective colour on the graphs. Different lettters in the table (a,b,c,etc) denotes significant concentration diffrence among strains for the respective compound and columns with compond color in a raw show strains signnificantly different from controls treatment of just compound [HD= HDMBOA-Glc and MG= MGBOA-Glc] without nematodes. The first coloured column represent colour of all recovered derivatives as in the transformation graphs shown on top of each table. acronomy HD and MG respectively.Error Bars: MSe

Benzoxazinoid metabolization is independent from WCR co-evolution and benzoxazinoid susceptibility

To test whether EPNs share an evolutionary history with WCR show differential metabolization, we used the data we compiled on origin of these 21 tested strains (supplementary table S2., second column). From the table nine out of the 21 tested strains shared a common origin with the WCR (USA and Mexico) while the rest do not. We compared the average percentage of remaining initial compound (HDMBOA-Glc or MBOA-Glc) between these two groups of strains. We did not see any statistically significant differences ($P>0.05$) between the two groups of WCR origin in both cases (Figure 3.A and Figure 3B.)

In the previous chapter 2, infectivity assays demonstrated that 6 of the 21 isolates were susceptible to benzoxazinoids sequestered by WCR (Supplementary Figure S6 and Supplementary Table S2.). As previously stated, these 6 isolates showed significant infectivity suppression towards BX-containing (W22 maize line fed) WCR larvae as compared to their benzoxazinoid -free (bx1::W22 maize line- fed) WCR larvae. Two of these 6 BXs susceptible strain (TT01 and RW14) could not be recovered during the time of the metabolization experiment, thus the average of the benzoxazinoid degradation rate of remaining 4 isolates was compared to that of the remaining isolates. No significant relationships between EPN BX resistanceand BX metabolization rate were found (Figure 3C. and Figure 3D.).

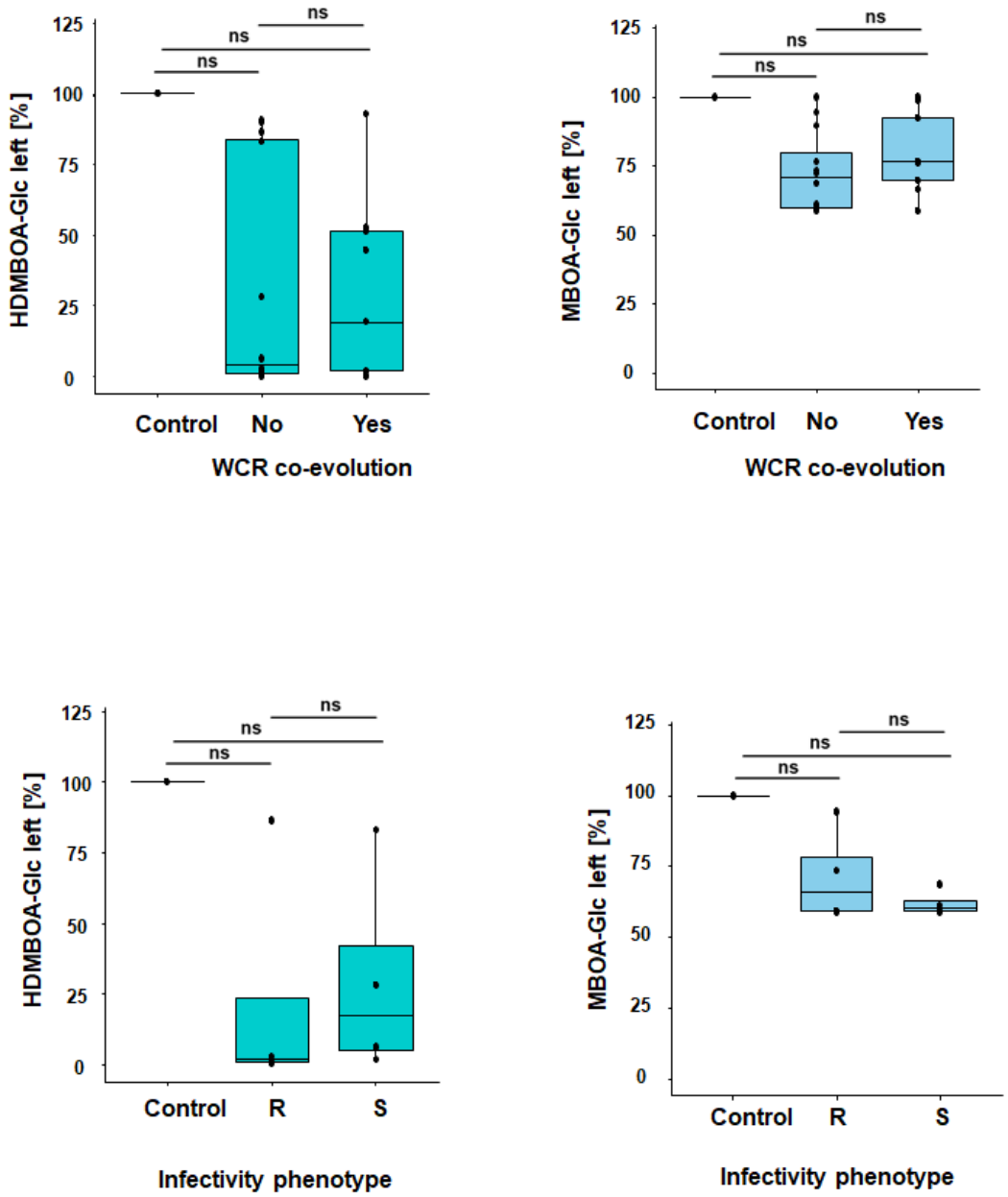


Figure 3. **The relationship between EPN geographical distance of where the host (WCR) originated as well as infectivity success towards BXs- accumulating host (WCR) to EPN response in benzoxazinoid environments.** Comparison of degradation rate of HDMBOA-Glc and MBOA-Glc between isolates which share evolutionary history (Yes group) with the WCR pest to that of those isolates which do not share evolutionary history (No group) with the WCR pest Figure 3A. and Figure 3B.)**.** Comparison of degradation rate of HDMBOA-Glc and MBOA-Glc between isolates with infectivity resistant (R group) to that of those isolates with infectivity suppression [susceptible isolates, (S group)] against benzoxazinoid-Fed WCR (Figure 3C. and Figure 3D). In both case the 2 resultant groups of relationship with WCR and benzoxazinoid infectivity success were also compared to "control group" of treatments with no nematodes but just benzoxazinoid (HDMBOA-Glc or MBOA-Glc) which obviously had 100% of initial compound remained as their was no biotransformation of compound there. The letters **("ns")** of each of the 2 bars denotes not statistical diffrefences in comapraison of any of the indicated groups as demarcated by the start and end point of each small bar on top. (P>0.05) after some pairwise t.test. All cyan colloured bars represent group comparison of HDMBOA-Glc degradation while all sky blue bars represent MBOA-Glc degradation comparisons.

A benzoxazinoid biodegrading enzyme is present in EPN endosymbiont bacteria

To gain first insights into the mechanisms of benzoxazinoid metabolization in EPNs, we screened for homologous sequences of reported responsible benzoxazinoid degrading enzymes in the genome of (*Heterorhabditis bacteriophora*) and their endosymbiont (*Photorhabdus luminescens)*. We discovered two of the bacteria sequences shared more than 70 % homology with the *BdxA*: - an enzyme recently discovered soil bacteria to be responsible for the conversion of MBOA to AMPO (Table 1). No strong candidates were found for the other candidate enzymes. This may mean there are other enzymes, not yet reported in literature, are responsible for benzoxazinoid biodegradation in EPNs.

Table1. **Output of sequence holomology search of reported benzoxazinoid degrading enzymes to nematode (*Heterorhabidtis bacteriophora*) and endosymbiont bacteria (*Photorhabdus luminescens)* genomes.** The first column is the name of the reported protein followed by the organism where the protein homology was searched, third from last column reports length of match between the sequences, second from last column report how perfect the match within those bits of sequence and last column report how much of the sequence we wanted blast against the genome is actually represented in the match.

Protein	Organism	scaffold	length of match	%match within residual sequence	query coverage
BdxA	***Photorhabdus***	**NA**	**586**	**63.70%**	**72.85%**
BxdA	***Photorhabdus***	**NA**	**710**	**63.00%**	**87.88%**
Mbl1	*Photorhabdus*	NA	NA	NA	NA
PhsA	*Photorhabdus*	NA	NA	NA	NA
CbaA	*Photorhabdus*	NA	24	NA	NA
ZmGlu1	*H. bacteriophora*	HiC_scaffold_3	26	96.20%	1.31%
ZmGlu2	*H. bacteriophora*	HiC_scaffold_3	26	100%	1.33%

Discussion

This study, revealed that, EPNs, when exposed to BXs in WCR such as HDMBOA- Glc. MBOA- Glc and MBOA (Robert *et al.*, 2017), they will metabolize them. However, as we did not observe a direct correlation between BX metabolization and infectivity success of a BX-sequestering insect herbivore, in which context BX metabolization may be adaptive is unclear.

BX glucosides were deglycosylated by EPNs. Although HDMBOA- Glc is known to be toxic to EPNs and MBOA- Glc is also known to repel EPNs (Robert *et al.*, 2017), degrading them to unstable benzoxazinone aglucones and benzoxazolinone might not be a smart move, as past observations have reported that benzoxazinone aglucones and their degradation products are more reactive and may cause enzyme or protein activity modification (Niemeyer *et al.*, 1982; Atkinson *et al.*, 1991; Niemeyer, 2009). BX activation upon disturbances work in a similar pattern like compounds such as glucosinolates and cyanogenic and iridoid glycosides ((Morant *et al.*, 2008; Pentzold *et al.*, 2014). An example being the observed increased activity of oat cell wall peroxidase enzyme which resulted in enhanced rate of H_2O_2 production from the oxidation of NADH (González & Rojas, 1999; Niemeyer, 2009) due to presence of BX aglucones (Wouters *et al.*, 2016a). DIMBOA also causes enzymes such as papain (Pérez & Niemeyer, 1989; Niemeyer, 2009) and aphid cholinesterase's (Cuevas & Niemeyer, 1993; Niemeyer, 2009) to be inactivated. This may be due the reported reaction of benzoxazinone aglucone with thiols and with amines (Atkinson *et al.*, 1991; Wouters *et al.*, 2016a). This may also be the reason behind the discovered toxicity effect of MBOA to EPNs (Robert *et al.*, 2017). Past studies reported impaired growth of *Arabidopsis thaliana* through inhibiting histone deacetylase (HDAC) activity (Venturelli *et al.*, 2015) due to AMPO presence. However, further tests need to be done on the specific effects of HMBOA and AMPO and other derivatives on EPNs to understand whether metabolization is beneficial or harmful to EPNs.

EPNs slowly degrade MBOA to the benzoxazolinones AMPO and AAMPO. This conversion also happens in soil environments (Macías *et al.*, 2004, 2005; Hussain *et al.*, 2022). Past reports stated that the instability of aglucones will lead to spontaneously further break down to benzoxazolinones which are even more toxic to herbivores (Oikawa *et al.*,

2004; Glauser *et al.*, 2011; Marti *et al.*, 2013; Handrick *et al.*, 2016; Wouters *et al.*, 2016b). However, we did not observe the spontaneous part in our work as benzoxazinoid biodegradation was only observed were we added nematodes and not in the control treatment with just benzoxazinoids. This is in line with work showing that benzoxazolinone production is facilitated by specific enzymes.

Some of the reported enzymes for this step of MBOA to AMPO conversion include *CbaA* and *Mbl1* from *Pigmentiphaga* spp and *Fusarium Verticillioides* (Saunders & Kohn, 2008) respectively. As well as the recently reported enzyme namely *BxdA* enzymes in *Microbacterium* spp. (Thoenen *et al.*, 2024), which we also discovered to be present in EPN endosymbionts bacteria in our work. Our search for other benzoxazinoid degrading enzymes like endogenous β-glucosidases (*ZmGLU1* and *ZmGLU2*)previously reported (Sue *et al.*, 2011; Schulz *et al.*, 2016) to hydrolyse the glucose moiety from benzoxazinone glucosides liberating resulting in the liberation of aglucones (Czjzek *et al.*, 2000) did not result in any hits. How EPNs deglycosylate BXs, thus, remains unclear. One possibility is that surface microbes may contribute to this reaction as EPNs have them (Jiménez-Cortés *et al.*, 2016; Goda *et al.*, 2020; Ogier *et al.*, 2020; Ogier *et al.*, 2023). This hypothesis requires further testing.

Our hypothesis that EPN resistance to WCR sequestered BXs may be related to their ability to metabolize BXs could not be confirmed, as we did not observe any associations between these two traits across different EPN isolates. In accordance with earlier work (Zhang *et al.*, 2019), EPN resistance may be more strongly related to behavior, and possibly BX uptake. Further work is required to assess these mechanisms.

In conclusion, this work shows that EPNs can metabolize BXs in their environment in a strain-specific manner. This may influence BX toxicity and microbial as well as plant interactions. To what extent the ability of EPNs to metabolize BXs is currently unclear, but it seems unlikely that metabolization is a major factor that contributes to EPN BX resistance and biocontrol success against BX sequestering insects such as the western corn rootworm.

Acknowledgements

We are very grateful to Dr. Vera Ogi for all her efforts in searching for homologous enzymes sequences in nematode and bacteria genome

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Author contributions

ME and CR designed and supervised the research. VO and DM conducted the assays, DM, CR and ME analyzed and interpreted the data. DM wrote the first draft. CR and ME contributed to writing the final version.

Conflicts of interest

The authors declare no conflicts of interest.

Figures and tables information

List of Tables

Table1. **Output of sequence holomology search of reported benzoxazinoid degrading enzymes to nematode (*Heterorhabidtis bacteriophora*) and endosymbiont bacteria (*Photorhabdus laumondii)* genomes.** The first column is the name of the reported protein followe by the organism where the protein homology was searched, third from last column reports length of match between the sequences, second from last column report how perfect the match within those bits of sequence and last column report how much of the sequence we wanted blast against the genome is actually represented in the match

List of figures

Figure.1 **Metabolisation pattern of benzoxazinoids (BXs) by entomopathogenic nematodes (EPNs).** Biotransformation of HDMBOA-Glc (A), MBOA-Glc (C) and MBOA (E) over time (Mean ±SEM). Recovered BXs compounds and their derivates were measured from a matrix of about 10 000 nematodes of starin EN01 incubated in BXs suspended in 1mL water over a period of time ranging from 1-10 days. Broken lines are the initially added compound while solid lines represent derivative compounds from the intialy added compound measure after the stipulated time. Benzoxazinoid full names and structures can be found in Supplementary Table SX. The proposed breakdown pathways and proposed responsible enzymes (using past reports) in HDMBOA-Glc (Figure 1B.), MBOA-Glc (Figure 1D) biotransformation. The statistical comparison of the benzoxazionoids and the dreakdown derivates of HDMBOA-Glc (Figure Fi.), MBOA-Glc (Figure Fii.),) as well as MBOA (Figure Fiii.),) at each point of data collection and different letters (a,b etc) indicates statistical concentration differences of the measured compound for that specific day.

Figure.2. **Benzoxazinoid metabolization is widespread among Heterorhabditis bacteriophora isolates. Assessing the potential of different EPN isolate to metabolise HDMBOA-Glc (Figure.2.A.) and MBOA-Glc (Figure.2.B.) after 3 days of incubations.** Each bar represent a unique EPN isolate and the colour and height of each bar rer represent the different kinds of recovered benzoxazinoids derivates and their concentrations in the matrix respectively. The legend table below show the statistical comparison for each recovered derivative after transformation and are represented by their respective colour on the graphs. Different lettters in the table (a,b,c,etc) denotes significant concentration diffrence among strains for the respective compound and columns with compond color in a raw show strains signnificantly different from controls treatment of just compound [HD= HDMBOA-Glc and MG= MGBOA-Glc] without nematodes. The first coloured column represent colour of all recovered derivatives as in the transformation graphs shown on top of each table. acronomy HD and MG respectively.Error Bars: Mse

Figure 3. **The relationship between EPN geographical distance of where the host (WCR) originated as well as infectivity success towards BXs- accumulating host (WCR) to EPN response in benzoxazinoid environments.** Comparison of degradation rate of HDMBOA-Glc and MBOA-Glc between isolates which share evolutionary history (Yes group) with the WCR pest to that of those isolates which do not share evolutionary history (No group) with the WCR pest Figure 3.A. and Figure 3.B.). Comparison of degradation rate of HDMBOA-Glc and MBOA-Glc between isolates with infectivity resistant (R group) to that of those isolates with infectivity suppression [susceptible isolates, (S group)] against benzoxazinoid-Fed WCR (Figure 3.C. and Figure 3.D). In both case the 2 resultant groups of relationship with WCR and benzoxazinoid infectivity success were also compared to “control group” of treatments with no nematodes but just benzoxazinoid (HDMBOA-Glc or MBOA-Glc) which obviously had 100% of initial compound remained as their was no biotransformation of compound there. The letters **(“ns”)** of each of the 2 bars denotes not statistical diffrefences in comapraison of any of the indicated groups as demarcated by the start and end point of each small bar on top. (P>0.05) after some pairwise t.test. All cyan colloured bars represent group comparison of HDMBOA-Glc degradation while all sky blue bars represent MBOA-Glc degradation comparisons.

Supplementary information

Supplementary Tables

Supplementary Table S2. Summary table showing benzoxazinoids resistance phenotype of 26 screened IPS nematode. In the table, the first column iis the name of the tested isolate followed by its country of origini in the seconf colum

Supplementary Table S2. Abrrevation (1st column), Full names (2nd column), benzoxazinoid class (3rd column),moplecular mass (4th column) and chemical formular(5th column) for all the benzoxazinoid reported in **this work**

Supplementary Figures

Supplementary Figure S1. BXs are stable in water solution under ambient conditions for 10 days

Supplementary Figure S2. EPNs do not produce BXs.

Supplementary Figure S3. EPNs can transform some BX after 3 days.

Supplementary Figure S4. BXs do not penetrate EPN bodies and are metabolized on the EPN surface.

Supplementary Figure S5. HDMBOA- Glc metabolization by EN01 after 10 days.

Supplementary Figure S6. Infectivity of 21 *Heterorhabditis bacteriphora* isolates on WCR larvae fed on BX+ or bx- maize.

Table S1. **Abrrevation (1st column), Full names (2nd column), benzoxazinoid class (3rd column),moplecular mass (4th column) and chemical formular(5th column) for all the benzoxazinoid reported in this work**. The colour in the 1st column represent the the colour used to represent the repect compound throughout the whole of this in graphing

Abbreviation	Full name	Class	Mass [g/mol]	Formula
AAMPO	2-acetylamino-7-methoxy-phenoxazin-3-one	Amino phenoxazine	284.27	C15H12N2O4
AMPO	2-amino-7-methoxy-phenoxazin-3-one	Amino phenoxazine	242.23	C13H10N2O3
HMBOA	2-Hydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one	Benzoxazinone	195.17	C9H9NO4
HDMBOA-Glc	2-O-Glucosyl-7-methoxy-1,4(2H) benzoxazin-3-one	Benzoxazinone glucoside	357.31	C15H19NO9
MBOA	6-methoxybenzoxazolin-2(3H)-one	Benzoxazolinone	165.15	C8H7NO3
HMBOA-Glc	4,7-dimethoxy-2-{[3,4,5-trihydroxy-6 (hydroxymethyl) oxan-2-yl]oxy}-3,4-dihydro-2H-1,4-benzoxazin-3-one	Benzoxazinone glucoside	387.34	C16H21NO10
MBOA-Glc	3-β-D-Glucopyranosyl-6-methoxy-2	Benzoxazolinone glucoside	195.17	C14H18NO8

Table S2. Summary table showing benzoxazinoids resistance phenotype of 26 screened IPS nematode. In the table, the first column iis the name of the tested isolate followed by its country of origini in the seconf colum. The third column is the statistical test comparison of BX-fed-d to BX-free larave infectivity the correspondin strain, were, **(*)** denotes that the coresponding strain showed significant BX-fed-larvae to BX- free-larvae infectivity diffrences. Thus these strains all these strain with an asterik were termed susceptible isolates. The sympol (**ns)** denotes no infectictivity differences and thus these strains were termed resistant isolates.

Strain	Origin	bx1::W22 VS W22 fed comparisons (t-tests)	Phenotype
Hb17	Turkey	ns	resistant
Hbbio	USA	ns	resistant
S5P8	United States	ns	resistant
S12	United States	ns	resistant
S14	United States	ns	resistant
S15	United States	ns	resistant
MEX23	Mexico	ns	resistant
MEX32	Mexico	ns	resistant
MEX37	Mexico	ns	resistant
TT01	Trinidad and Tobago	*	Susceptible
MG618b	Switzerland	*	Susceptible
RM102	Spain	ns	resistant
VM1	Spain	ns	resistant
EN01	Germany	*	susceptible
IT6	Italy	ns	resistant
IR2	Iran	ns	resistant
DE2	Germany	ns	resistant
PT1	Portugal	ns	resistant
HU2	Hungary	ns	resistant
IL9	Australia	ns	resistant
DE6	Germany	*	susceptible
09-43	Turkey	*	susceptible
Boj	Iran	ns	resistant
RW14	Rwanda	*	resistant

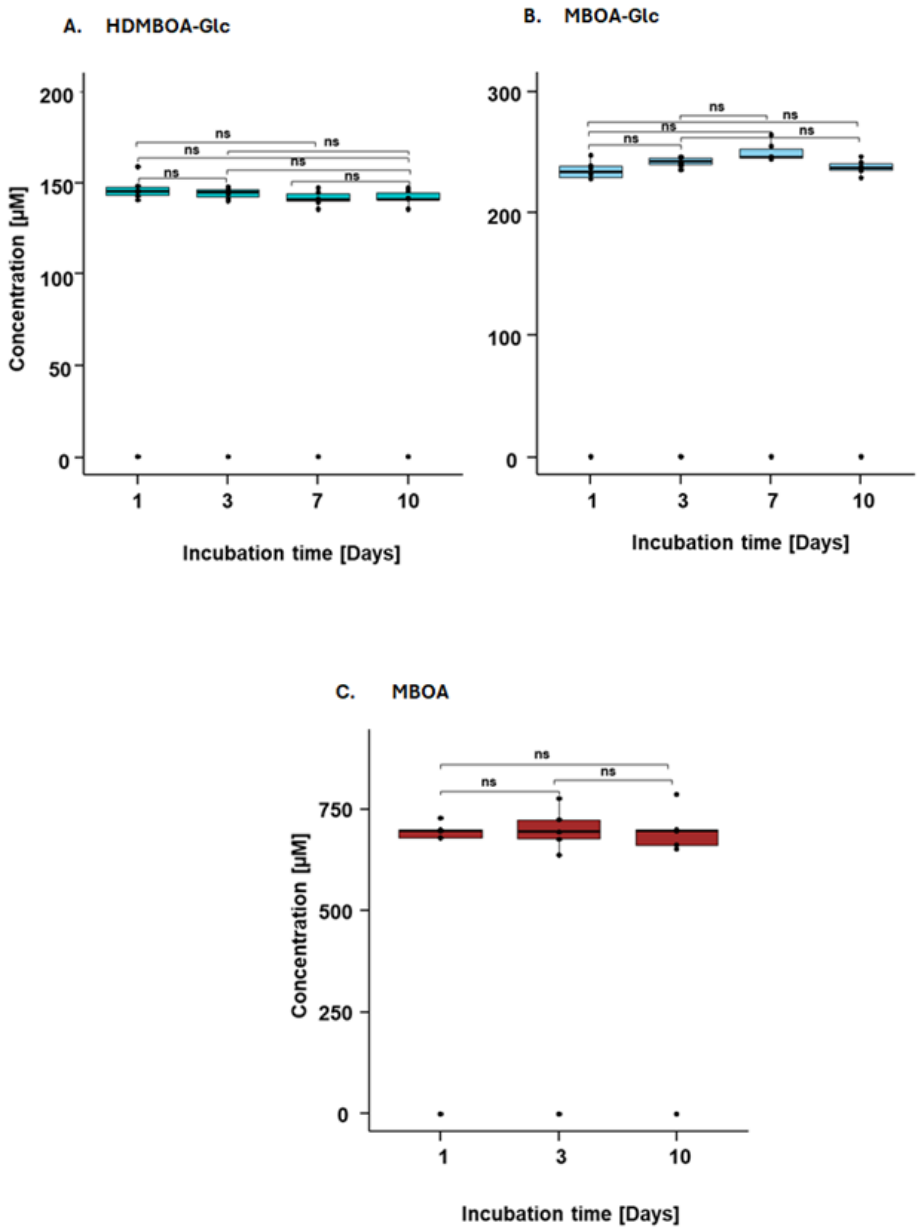


Figure S1. BXs are stable in water solution under ambient conditions for 10 days. Comparison of quantified BXs concentarations in the control tretaments. Figure S1.A. Comparisons of HDMBOA-Glc concentrations in control treatments with just HDMBOA-Glc from the start (day1) to the end (day 10) of the experiment. Figure S1.B. Comparisons of MBOA-Glc concentrations in control treatments with just MBOA-Glc from the start (day1) to the end (day 10) of the experiment. Figure S1.C. Comparisons of MBOA concentrations in control treatments with just MBOA from the start (day1) to the end (day 10) of the experiment. The bars of top any 2 points show the points or day being compared and the sympol on to reperent the statistical results after a two sample t-test analysis. Sympol "**ns"**denotes a non sugnificant difference in benzoxazinoid concentration between the two compared time points. Error Bars: MSe

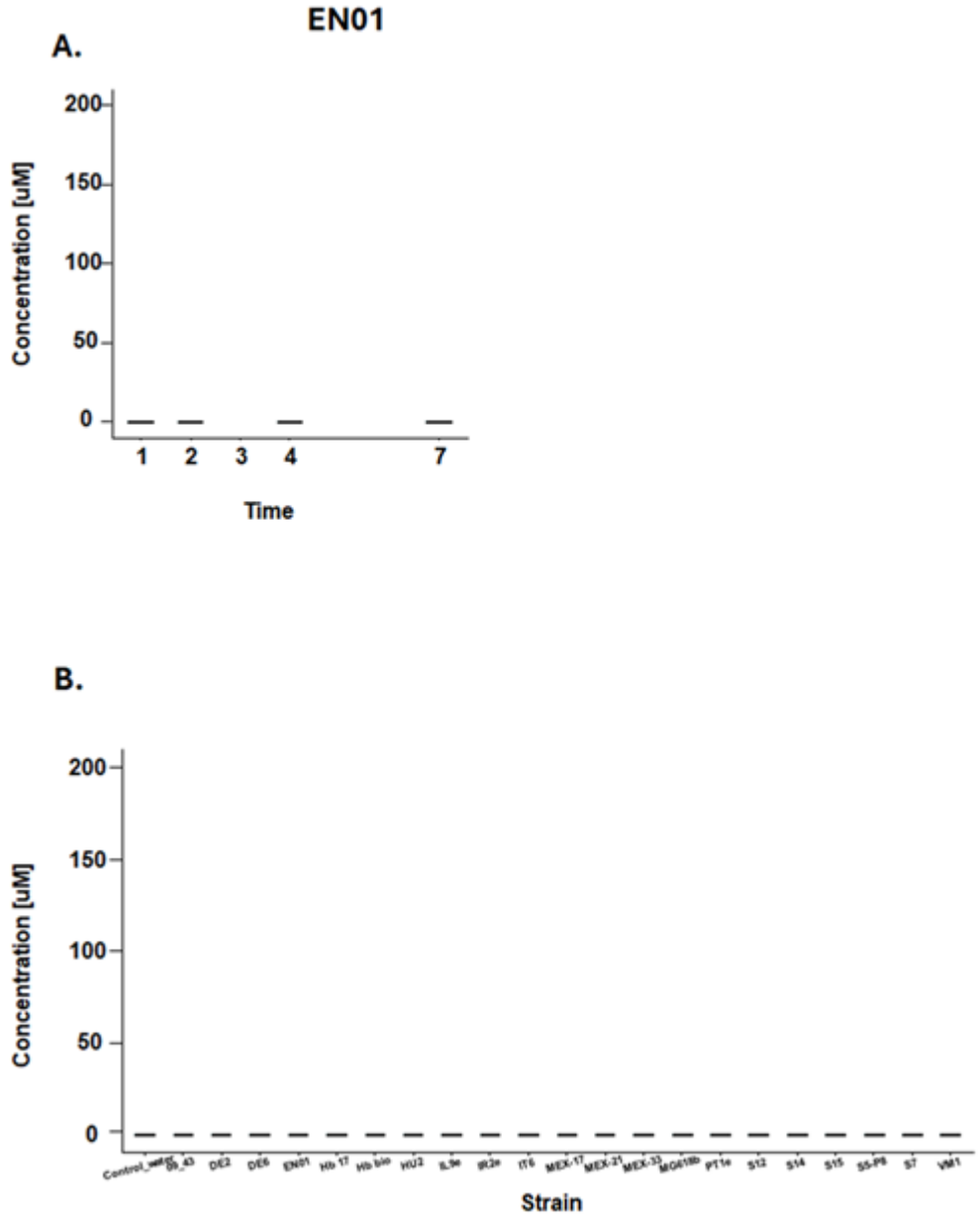


Figure S2. EPNs do not produce BXs. Figure S2.A. **T**he concentration of benzoxazinoids recovered inside EPN bodies strain EN01 over a period of 7 days. Figure S2.B. **T**he concentration of benzoxazinoids recovered inside EPN bodies of 21 different strains after 3 days of incubation.

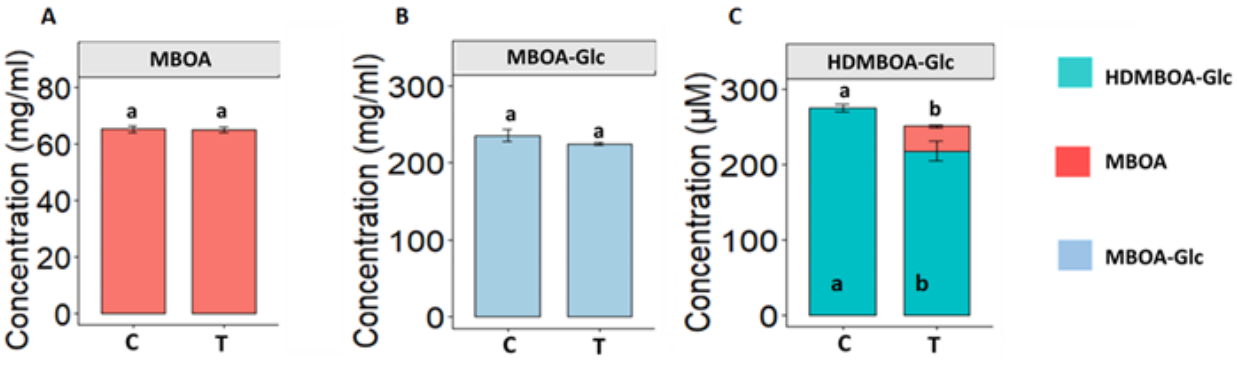
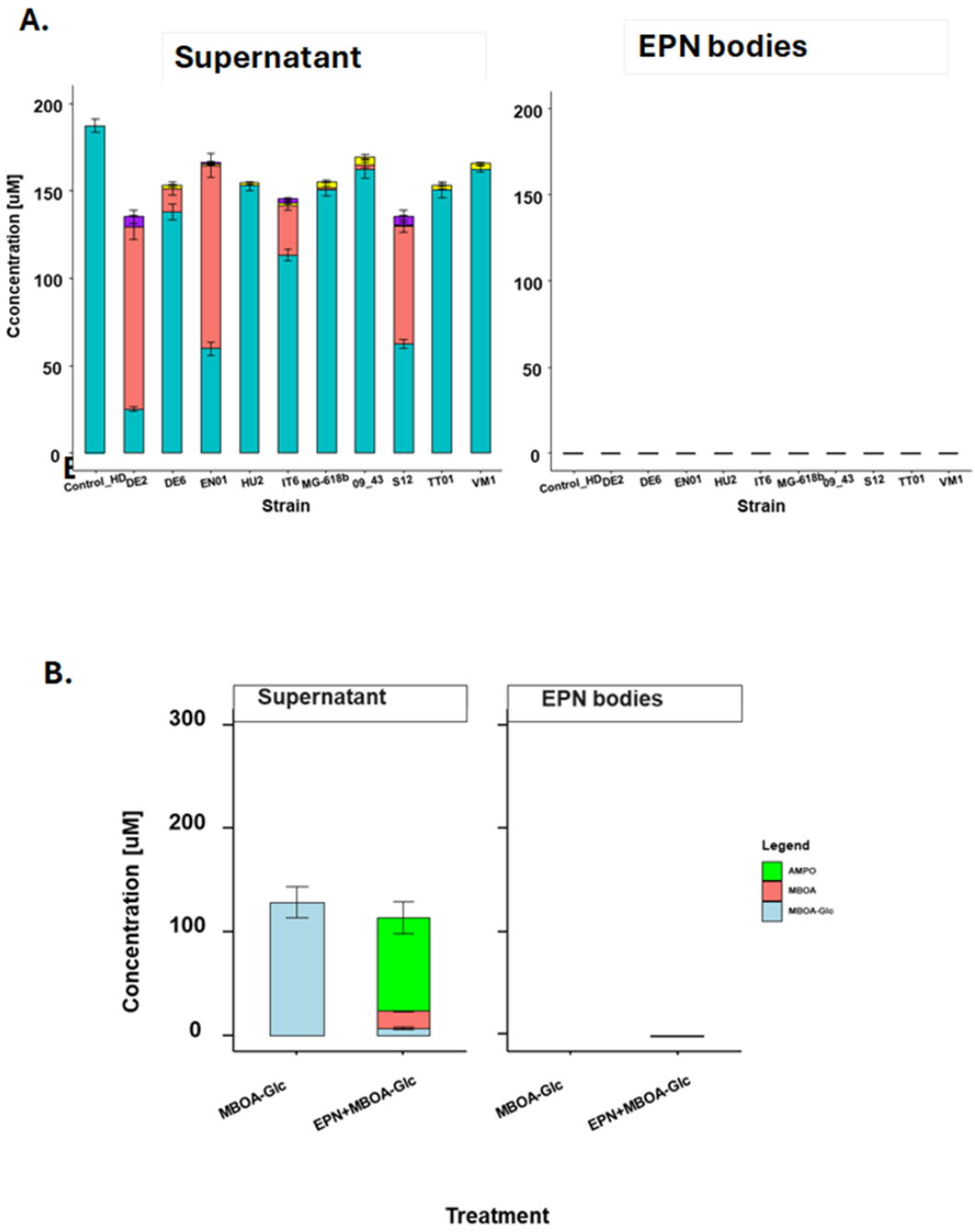
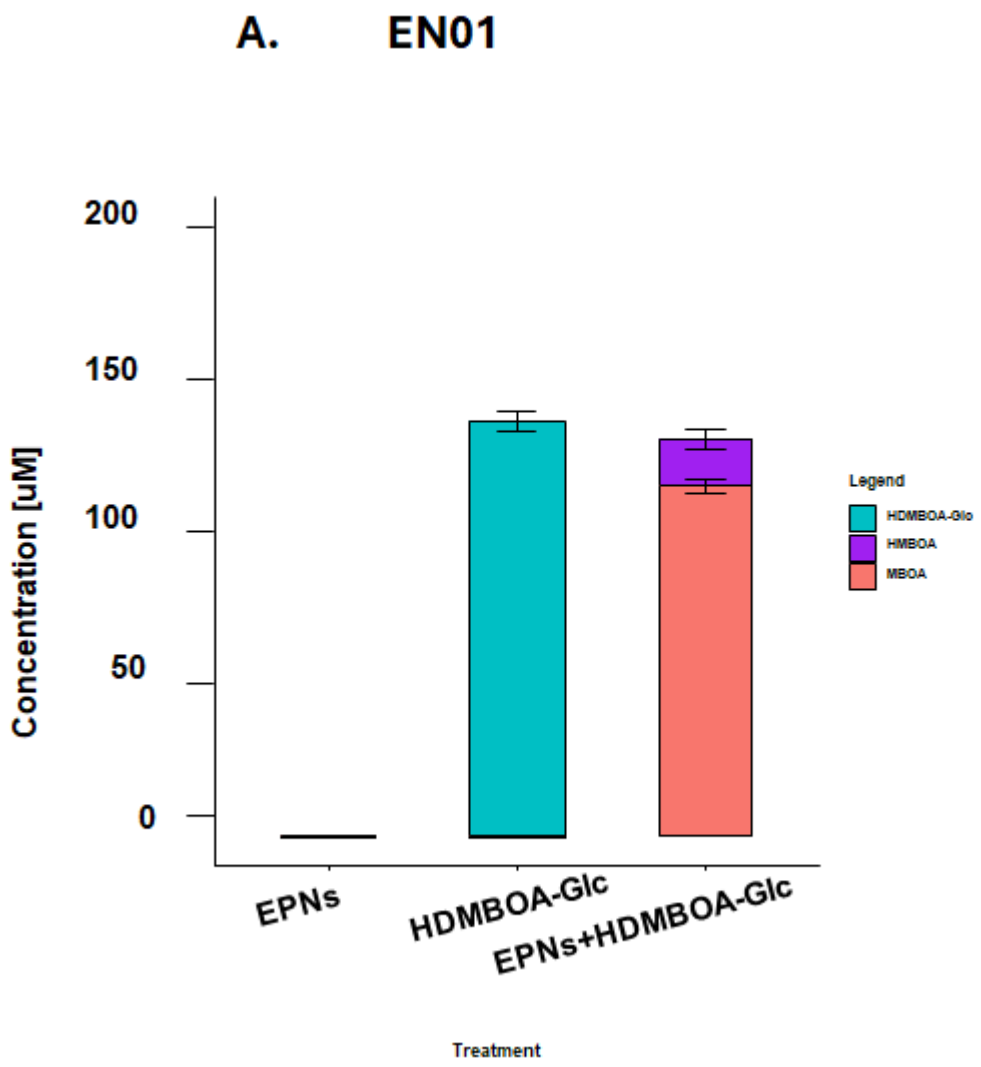


Figure S3. EPNs can transform some BX after 3 days. The comparison of recovered BXs concentration of and their breakdown derivates in samples with EN01 and BXs to their control treatments with just the BXs. Figure S3.A. was treatments with MBOA (orange bars) while Figure S3.B. were treatments with MBOA-Glc (skyblue bars) while Figure S3.C. were treatments with HDMBOA-Glc (cyan bars). Letters **"a" a**nd or **"b"** denotes significant benzoxazinoid concentrations difference between treatment of EPN+ BXs ((T) and control of BXs only (C) for that respective BX. Different bar colors represent different BX compound recovered after 24 hrs incubation time. Error Bars: MSe.



2804

2805 **Figure S4. BXs do not penetrate EPN bodies and are metabolized on the EPN surface. Figure S4.A. T**he concentra-
2806 tion of HDMBOA-glc (light green), and its derivatives: MBOA (orange) HMBOA-Glc (yellow) and HMBOA (purple) recov-
2807 ered on the supernatant as well as inside EPN bodies of 10 EPN strain after 3 incubation time. Figure S4.B. **T**he con-
2808 centration of MBOA-glc (lskyblue), and its derivatives: MBOA (orange) AMPO (green) recovered on the supernatant as
2809 well as inside EPN bodies of EPN strain EN01 after 3 incubation time. Error Bars: MSe



2810

2811 **Figure S5. HDMBOA- Glc metabolization by EN01 after 10 days. T**he concentration of HDMBOA-glc (light green), and
2812 its derivatives: MBOA (orange), and HMBOA (purple) recovered IN three different treatments of just EN01 in water,
2813 HDMBOA-Glc in water and EN01 with HDMBOA-Glc also suspended in water after 3 incubation time. Error Bars: MSe

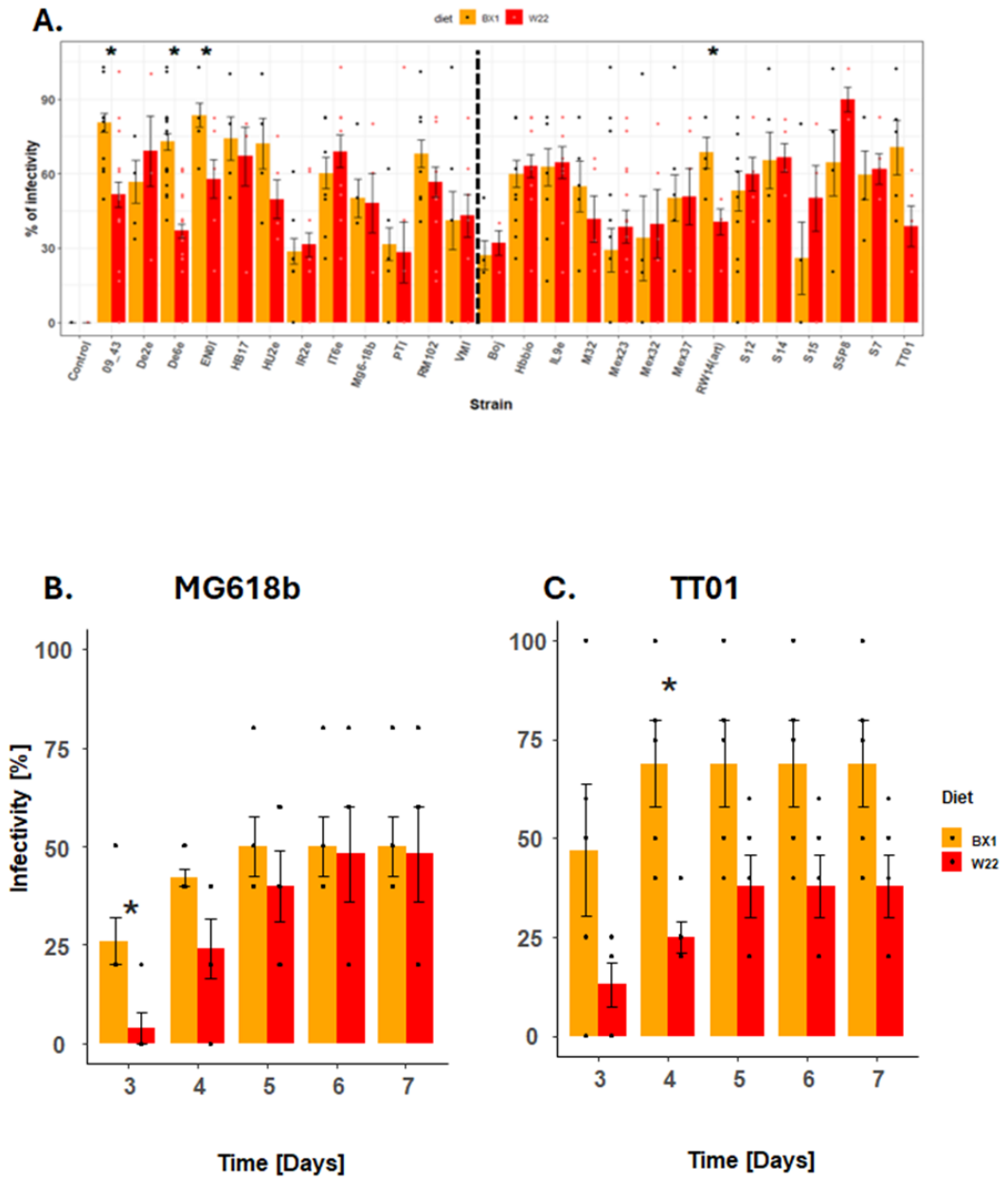


Figure S6. Infectivity of 21 *Heterorhabditis bacteriphora* isolates on WCR larvae fed on BX+ or bx- maize. Figure S6.A. Comparison of Infectivity success towards WCR larvae fed on WT (red bar) to that of bx:W22 mutant maize line (orange bar) by each strain after 6 days of infection. A. comparison of Infectivity success towards WCR larvae fed on WT (red bar) to that of bx:W22 mutant maize line (orange bar) by nematode strain MG618b (Figure S6.B) and strain TT01 (Figure S6.C) from 3 to 7 days after infection. An asterisk (*) indicate significant differences between infections of a bx::W22 and W22 fed larvae ($P < 0.05$) by same strain. Error Bars: MSe.

References

Alouw, J.C. & Miller, N.J. (2015). Effects of benzoxazinoids on specialist and generalist *Diabrotica* species. *Journal of Applied Entomology* 139, 424–431. DOI: 10.1111/jen.12194

Atkinson, J., Morand, P., Arnason, J.T. (1991). Analogs of the cyclic hydroxamic acid 2, 4-dihydroxy-7-methoxy-2H-1, 4-benzoxazin-3-one (DIMBOA): Decomposition to benzoxazolinones and reaction with .beta-mercaptoethanol. T*he Journal of Organic Chemistry* 56, 1788–1800. DOI: 10.1021/jo00005a025

Bruijn, W.J.C. de, Gruppen, H., Vincken, J.-P. (2018). Structure and biosynthesis of benzoxazinoids: Plant defence metabolites with potential as antimicrobial scaffolds. *Phytochemistry* 155, 233–243. DOI: 10.1016/j.phytochem.2018.07.005

Cuevas, L. & Niemeyer, H.M. (1993). Effect of hydroxamic acids from cereals on aphid cholinesterases. *Phytochemistry* 34, 983–985. DOI: 10.1016/S0031-9422(00)90698-8

Czjzek, M., Cicek, M., Zamboni, V., Bevan, D.R., Henrissat, B., Esen, A. (2000). The mechanism of substrate (aglycone) specificity in beta-glucosidases is revealed by crystal structures of mutant maize beta-glucosidase-DIMBOA, -DIMBOAGlc, and dhurrin complexes. *Proceedings of the National Academy of Sciences of the United States of America* 97, 13555–13560. DOI: 10.1073/pnas.97.25.13555

Frey, M., Huber, K., Park, W.J., Sicker, D., Lindberg, P., Meeley, R.B., Simmons, C.R., Yalpani, N., Gierl, A. (2003). A 2-oxoglutarate-dependent dioxygenase is integrated in DIMBOA-biosynthesis. *Phytochemistry* 62, 371–376. DOI: 10.1016/S0031-9422(02)00556-3

Glauser, G., Marti, G., Villard, N., Doyen, G.A., Wolfender, J.-L., Turlings, T.C.J., Erb, M. (2011). Induction and detoxification of maize 1,4-benzoxazin-3-ones by insect herbivores. *The Plant journal for cell and molecular biology* 68, 901–911. DOI: 10.1111/j.1365-313X.2011.04740.x

Glazer, I. (2002). Survival biology. In: Gaugler, R. (Ed). *Entomopathogenic nematology*. UK, CABI Publishing, pp. 169–187. DOI: 10.1079/9780851995670.0000

Goda, N., Mirzae, M., Brunetti, M. (2020). Potentially entomopathogenic nematode isolated from *Popillia japonica*: bioassay, molecular characterization and the associated microbiota. *Bulletin of Insectology*, *73*(2), 295-301

González, L.F. & Rojas, M. (1999). Role of wall peroxidases in oat growth inhibition by DIMBOA. *Phytochemistry* 50, 931–937. DOI: 10.1016/S0031-9422(98)00635-9

Handrick, V., Robert, C.A.M., Ahern, K.R., Zhou, S., Machado, R.A.R., Maag, D., Glauser, G., Fernandez-Penny, F.E., Chandran, J.N., Rodgers-Melnik, E. *et al.* (2016). Biosynthesis of 8-O-Methylated benzoxazinoid defense compounds in maize. *The Plant Cell* 28, 1682–1700. DOI: 10.1105/tpc.16.00065

Hussain, M.I., Araniti, F., Schulz, M., Baerson, S., Vieites-Álvarez, Y., Rempelos, L., Bilsborrow, P., Chinchilla, N., Macías, F.A., Weston, L.A. *et al.* (2022). Benzoxazinoids in

wheat allelopathy – From discovery to application for sustainable weed management. *Environmental and Experimental Botany* 202, 104997. DOI: 10.1016/j.envexpbot.2022.104997

Jeckel, A.M., Beran, F., Züst, T., Younkin, G., Petschenka, G., Pokharel, P., Dreisbach, D., Ganal-Vonarburg, S.C., Robert, C.A.M. (2022). Metabolization and sequestration of plant specialized metabolites in insect herbivores: Current and emerging approaches. *Frontiers in physiology* 13, 1001032. DOI: 10.3389/fphys.2022.1001032

Jiménez-Cortés, J.G., Canales-Lazcano, J., Lara-Reyes, N., Rosenblueth, M., Martínez-Romero, E., Contreras-Garduño, J. (2016). Microbiota from Rhabditis regina may alter nematode entomopathogenicity. *Parasitology research* 115, 4153–4165. DOI: 10.1007/s00436-016-5190-3

Jonczyk, R., Schmidt, H., Osterrieder, A., Fiesselmann, A., Schullehner, K., Haslbeck, M., Sicker, D., Hofmann, D., Yalpani, N., Simmons, C. *et al.* (2008). Elucidation of the final reactions of DIMBOA-glucoside biosynthesis in maize: Characterization of Bx6 and Bx7. *Plant Physiology* 146, 1053–1063. DOI: 10.1104/pp.107.111237

Kaya, H.K. (2002). Natural enemies and other antagonists. In: Gaugler, R. (Ed). *Entomopathogenic nematology*. UK, CABI Publishing, pp. 189–203. DOI: 10.1079/9780851995670.0000

Li, J., Liu, X., Don, F., Xu, J., GUO, L., Kong, Z., Tian, Y., Wu, Y., Zheng, Y. (2013). A Simple Method for the Isolation and Purification of 2,4-Dihydroxy-7-Methoxy-2H-1,4-Benzoxazin-3(4H)-One (DIMBOA) from Maize (*Zea mays* L.) Seedlings. *Journal of Integrative Agriculture* 12, 95–102. DOI: 10.1016/S2095-3119(13)60209-9

Lu, D., Baiocchi, T., Dillman, A.R. (2016). Genomics of Entomopathogenic Nematodes and Implications for Pest Control. *Trends in parasitology* 32, 588–598. DOI: 10.1016/j.pt.2016.04.008

Machado, R.A.R., Thönen, L., Arce, C.C.M., Theepan, V., Prada, F., Wüthrich, D., Robert, C.A.M., Vogiatzaki, E., Shi, Y.-M., Schaeren, O.P. *et al.* (2020). Engineering bacterial symbionts of nematodes improves their biocontrol potential to counter the western corn rootworm. *Nature biotechnology* 38, 600–608. DOI: 10.1038/s41587-020-0419-1

Macías, F.A., Marín, D., Oliveros-Bastidas, A., Chinchilla, D., Simonet, A.M., Molinillo, J.M.G. (2006). Isolation and synthesis of allelochemicals from gramineae: benzoxazinones and related compounds. *Journal of agricultural and food chemistry* 54, 991–1000. DOI: 10.1021/jf050896x

Macías, F.A., Oliveros-Bastidas, A., Marín, D., Castellano, D., Simonet, A.M., Molinillo, J.M.G. (2004). Degradation studies on benzoxazinoids. Soil degradation dynamics of 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA) and its degradation products, phytotoxic allelochemicals from gramineae. *Journal of agricultural and food chemistry* 52, 6402–6413. DOI: 10.1021/jf0488514

Macías, F.A., Oliveros-Bastidas, A., Marín, D., Castellano, D., Simonet, A.M., Molinillo, J.M.G. (2005). Degradation studies on benzoxazinoids. Soil degradation dynamics of

(2R)-2-O-beta-D-glucopyranosyl-4-hydroxy-(2H)- 1,4-benzoxazin-3(4H)-one (DIBOA-Glc) and its degradation products, phytotoxic allelochemicals from Gramineae. *Journal of agricultural and food chemistry* 53, 554–561. DOI: 10.1021/jf048702l

Marti, G., Erb, M., Boccard, J., Glauser, G., Doyen, G.R., Villard, N., Robert, C.A.M., Turlings, T.C.J., Rudaz, S., Wolfender, J.-L. (2013). Metabolomics reveals herbivore-induced metabolites of resistance and susceptibility in maize leaves and roots. *Plant, Cell & Environment* 36, 621–639. DOI: 10.1111/pce.12002

Maushe, D., Ogi, V., Divakaran, K., Verdecia Mogena, A.M., Himmighofen, P.A., Machado, R.A.R., Towbin, B.D., Ehlers, R.-U., Molina, C., Parisod, C. *et al.* (2023). Stress tolerance in entomopathogenic nematodes: Engineering superior nematodes for precision agriculture. *Journal of invertebrate pathology* 199, 107953. DOI: 10.1016/j.jip.2023.107953

Miller, N., Estoup, A., Toepfer, S., Bourguet, D., Lapchin, L., Derridj, S., Kim, K.S., Reynaud, P., Furlan, L., Guillemaud, T. (2005). Multiple transatlantic introductions of the western corn rootworm. *Science* 310, 992. DOI: 10.1126/science.1115871

Morant, A.V., Jørgensen, K., Jørgensen, C., Paquette, S.M., Sánchez-Pérez, R., Møller, B.L., Bak, S. (2008). beta-Glucosidases as detonators of plant chemical defense. *Phytochemistry* 69, 1795–1813. DOI: 10.1016/j.phytochem.2008.03.006

Niemeyer, H.M. (2009). Hydroxamic acids derived from 2-hydroxy-2H-1,4-benzoxazin-3(4H)-one: Key defense chemicals of cereals. *Journal of agricultural and food chemistry* 57, 1677–1696. DOI: 10.1021/jf8034034

Niemeyer, H.M., Corcuera, L.J., Pérez, F.J. (1982). Reaction of a cyclic hydroxamic acid from gramineae with thiols. *Phytochemistry* 21, 2287–2289. DOI: 10.1016/0031-9422(82)85192-3

Ogi, V., Grob, S., Schmid, M., Poretti, M., Machado, R., Maushe, D., Erb, M., Robert, C., Parisod, C. (2024). *Chromosome-scale genome of Heterorhabditis bacteriophora reveals genetic basis of parasitism and plant toxin resistance*. DOI: 10.22541/au.172892720.02570799/v1

Ogier, J.-C., Akhurst, R., Boemare, N., Gaudriault, S. (2023). The endosymbiont and the second bacterial circle of entomopathogenic nematodes. *Trends in microbiology* 31, 629–643. DOI: 10.1016/j.tim.2023.01.004

Ogier, J.-C., Pagès, S., Frayssinet, M., Gaudriault, S. (2020). Entomopathogenic nematode-associated microbiota: from monoxenic paradigm to pathobiome. *Microbiome* 8, 25. DOI: 10.1186/s40168-020-00800-5

Oikawa, A., Ishihara, A., Tanaka, C., Mori, N., Tsuda, M., Iwamura, H. (2004). Accumulation of HDMBOA-Glc is induced by biotic stresses prior to the release of MBOA in maize leaves. *Phytochemistry* 65, 2995–3001. DOI: 10.1016/j.phytochem.2004.09.006

Pentzold, S., Zagrobelny, M., Rook, F., Bak, S. (2014). How insects overcome two-component plant chemical defence: plant β-glucosidases as the main target for herbivore adaptation. *Biological reviews of the Cambridge Philosophical Society* 89, 531–551. DOI: 10.1111/brv.12066

Pérez, F.J. & Niemeyer, H.M. (1989). Reaction of dimboa, a resistance factor from cereals, with papain. *Phytochemistry* 28, 1597–1600. DOI: 10.1016/S0031-9422(00)97806-3

Perry, R.N., Ehlers, R.-U., Glazer, I. (2012). A Realistic Appraisal of Methods to Enhance Desiccation Tolerance of Entomopathogenic Nematodes. *Journal of Nematology* 44, 185–190.

Perry, R.N. & Wharton, D.A. (2011). *Molecular and physiological basis of nematode survival*. UK, CAB International. DOI: 10.1079/9781845936877.0000

Robert, C.A., Zhang, X., Machado, R.A., Schirmer, S., Lori, M., Mateo, P., Erb, M., Gershenzon, J. (2017). Sequestration and activation of plant toxins protect the western corn rootworm from enemies at multiple trophic levels. *eLife* 6. DOI: 10.7554/eLife.29307

Saunders, M. & Kohn, L.M. (2008). Host-synthesized secondary compounds influence the in vitro interactions between fungal endophytes of maize. *Applied and environmental microbiology* 74, 136–142. DOI: 10.1128/AEM.01538-07

Schulz, M., Filary, B., Kühn, S., Colby, T., Harzen, A., Schmidt, J., Sicker, D., Hennig, L., Hofmann, D., Disko, U. *et al.* (2016). Benzoxazolinone detoxification by N-Glucosylation: The multi-compartment-network of Zea mays L. *Plant signaling & behavior* 11, e1119962. DOI: 10.1080/15592324.2015.1119962

Sharmila, R., Subramanian, S., Shanmuga, P., Poornima, K., Pandiyan, M. (2018). *Review on ecology of entomopathogenic nematodes*. *J. Entomol. Zool. Stud*, *6*(4), 1086-1093.

Sicker, D., Frey, M., Schulz, M., Gierl, A. (2000). Role of natural benzoxazinones in the survival strategy of plants. In: *International review of cytology*, Academic Press, pp. 319–346.

Sicker, D., Schneider, B., Hennig, L., Knop, M., Schulz, M. (2001). Glycoside carbamates from benzoxazolin-2(3H)-one detoxification in extracts and exudates of corn roots. *Phytochemistry* 58, 819–825. DOI: 10.1016/S0031-9422(01)00299-0

Sicker, D. & Schulz, M. (2002). Benzoxazinones in plants: Occurrence, synthetic access, and biological activity. In: *Bioactive natural products (Part H)*, Elsevier, pp. 185–232.

Sue, M., Nakamura, C., Nomura, T. (2011). Dispersed benzoxazinone gene cluster: molecular characterization and chromosomal localization of glucosyltransferase and glucosidase genes in wheat and rye. *Plant Physiology* 157, 985–997. DOI: 10.1104/pp.111.182378

Thoenen, L., Giroud, C., Kreuzer, M., Waelchli, J., Gfeller, V., Deslandes-Hérold, G., Mateo, P., Robert, C.A.M., Ahrens, C.H., Rubio-Somoza, I. *et al.* (2023). Bacterial tolerance to host-exuded specialized metabolites structures the maize root microbiome. *Proceedings of the National Academy of Sciences of the United States of America* 120, e2310134120. DOI: 10.1073/pnas.2310134120

Thoenen, L., Kreuzer, M., Pestalozzi, C., Florean, M., Mateo, P., Züst, T., Wei, A., Giroud, C., Rouyer, L., Gfeller, V. *et al.* (2024). The lactonase BxdA mediates metabolic specialisation of maize root bacteria to benzoxazinoids. *Nature communications* 15, 6535. DOI: 10.1038/s41467-024-49643-w

Tzin, V., Fernandez-Pozo, N., Richter, A., Schmelz, E.A., Schoettner, M., Schäfer, M., Ahern, K.R., Meihls, L.N., Kaur, H., Huffaker, A. *et al.* (2015). Dynamic Maize Responses to Aphid Feeding Are Revealed by a Time Series of Transcriptomic and Metabolomic Assays. *Plant Physiology* 169, 1727–1743. DOI: 10.1104/pp.15.01039

Venturelli, S., Belz, R.G., Kämper, A., Berger, A., Horn, K. von, Wegner, A., Böcker, A., Zabulon, G., Langenecker, T., Kohlbacher, O. *et al.* (2015). Plants Release Precursors of Histone Deacetylase Inhibitors to Suppress Growth of Competitors. *The Plant Cell* 27, 3175–3189. DOI: 10.1105/tpc.15.00585

Wouters, F.C., Blanchette, B., Gershenzon, J., Vassão, D.G. (2016a). Plant defense and herbivore counter-defense: Benzoxazinoids and insect herbivores. *Phytochemistry reviews proceedings of the Phytochemical Society of Europe* 15, 1127–1151. DOI: 10.1007/s11101-016-9481-1

Wouters, F.C., Gershenzon, J., Vassão, D.G. (2016b). Benzoxazinoids: Reactivity and Modes of Action of a Versatile Class of Plant Chemical Defenses. *Journal of the Brazilian Chemical Society*. DOI: 10.5935/0103-5053.20160177

Zhang, X., van Doan, C., Arce, C.C.M., Hu, L., Gruenig, S., Parisod, C., Hibbard, B.E., Hervé, M.R., Nielson, C., Robert, C.A.M. *et al.* (2019). Plant defense resistance in natural enemies of a specialist insect herbivore. *Proceedings of the National Academy of Sciences of the United States of America* 116, 23174–23181. DOI: 10.1073/pnas.1912599116

Chapter 4: Role of EPN associated micro-organisms in benzoxazinoid metabolization

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Abstract

Entomopathogenic nematodes and microorganisms have evolved some association relationships ranging from the well-known mutual symbiosis relationship with their endosymbiotic bacteria to some unknown but constant association with other microbes both inside their bodies and on their surface cuticle. While their mutualistic relationship with their endosymbionts bacteria during host infectivity has been well documented, the impacts of other endosymbionts as well as surface-associated microbiome on other nematode biological, physiological and biochemical processes is an area that still needs scientist attention and resource investment. In our previous chapter we observed benzoxazinoid biotransformation to their breakdown derivates by incubated nematodes. Since nematodes live in association with other living microbes both outside and inside its body, we were curious to know which among the three factors (the EPNs, endogenous microbes or surface cuticle associates) carry enzymes responsible for this EPN-benzoxazinoid biotransformation phenomenon. Laboratory incubation of nematodes with manipulated microbe content, in benzoxazinoids revealed that nematode-surface associated microbes play a very significant role in benzoxazinoid biotransformation by EPNs. These findings indicate cooperative acts between EPN and some of its associates in times of trouble which may in turn benefit EPN to grow, develop and survive in stressful situations. These findings will motivate nematologists to study and understand other symbiosis relationships between EPNs from their associates. Understanding the influences of such EPN associations in EPN biological, physiological and biochemical process may give crucial information for EPN-contents manipulations programs, which may be used in maximizing EPN-control potential and protection. Additionally, the occurrence of commonly used biocontrol organisms (microbes and EPNs) in a single entity gives hope for future integrated/combined biocontrol agents programs for additive/synergistic entomopathogenecity.

Keywords: Benzoxazinoids, entomopathogenic nematodes (EPNs), EPN-associated microbes, biotransformation

Introduction

Interactions between organisms range from predation, competition, commensalism, parasitism to mutualism and symbiosis (Sheehy *et al.*, 2022). The type of relation between these organisms may differ according to environmental situation, age of and developmental stage of an organism, among other factors. Filarial nematodes (helminth parasites) for instance dependent on their symbiotic intracellular bacterium, *Wolbachia* for their fertility and development (Taylor *et al.*, 2005); Stilbonematinae and Desmodoridae marine nematode families depend on cuticle associated sulphur-oxidizing bacteria (*Robbea spp*.) for food (Bayer *et al.*, 2009); *Phasmarhabditis hermaphrodita nematode spp* use *Moraxella osloensis* bacteria to achieve their malacopathogenic (kills slugs and snails) behaviour (Tan & Grewal, 2001; Sheehy *et al.*, 2022).

Entomopathogenic nematodes (EPNs), which are successful crop insect biological agents live in symbiosis with entomopathogenic bacteria, which they carry in their intestines (Boemare *et al.*, 1993; Kaya & Gaugler, 1993; Forst *et al.*, 1997; Grewal & Georgis, 1999; Tomar *et al.*, 2022). In addition, EPNs harbour more complex bacterial communities, whose role is less clear(Jiménez-Cortés *et al.*, 2016; Goda *et al.*, 2020; Ogier *et al.*, 2020; Ogier *et al.*, 2023).

Most EPN-associated microbes are found on their outer surface. In third stage infective juveniles (J3s), the surface includes a second cuticular sheath as a result of incomplete molt of the second stage juveniles (J2s). This sheath may play a role in environmental stress tolerance (Timper & Kaya, 1989; Campbell & Gaugler, 1992; Donald L. Lee, 2002). Exsheathment of the J3 second cuticle, which is usually stimulated by the host, marks the transition of these J3s from free -living to parasitic stage (J4s) (Campbell & Gaugler, 1992; Donald L. Lee, 2002). Thus, it will be interesting to assess changes in EPN biological process in the presence or absence of this surface cuticle as this affect EPN-associated surface microbe abundance.

In our previous work, we observed metabolization of benzoxazinoids (BXs) by EPNs only occur on their surface cuticle. Microbes are also well known to be able to metabolize benzoxazinoids, similar to what we observed with EPNs (Friebe *et al.*, 1998; Fomsgaard *et al.*, 2004; Kettle *et al.*, 2015; Glenn *et al.*, 2016; Schütz *et al.*, 2019; Thoenen *et al.*,

2024). Thus, it is possible that microbes on the surface of EPNs contribute to benzoxazinoid metabolization.

Here, we aimed at understanding the role of microbes in benzoxazinoid metabolization by EPNs. We used different approaches to manipulate the presence and activity of endogenous and surface microbes. We then incubated the manipulated EPNs with benzoxazinoids and measured metabolization. We found that surface microbes contribute to benzoxazinoid metabolization, and that the second cuticular sheath acts as a physical barrier that prevents the entry of benzoxazinoids into the nematode bodies.

Material and methods

Biological Resources

All benzoxazinoid metabolization experiments of this work were conducted with the commercial *Heterorhabditis bacteriophora* strain EN01 (e-nema GmbH, Germany). Each experiments was done using nematode concentartion of about 10.000 nematodes per mL per replicate. Nematodes were multiplied in greater wax moth (*Galleria mellonella*) larvae (Fischereibedarf Wenger AG, Bern, Switzerland) and kept at 8 °C until use. EPN amplification was performed following a previous protocol by (White 1927) with some modifications. Briefly, 400 µl of nematodes were added to about five *G. mellonella* larvae place in a 5 cm diameter petri dish (Greiner Bio-One, Frickenhausen, Germany) on filter paper (55 mm diameter, Whatman, China). The infected larvae were then incubated at 24 ± 2℃ in an incubator. After seven days infected *G. mellonella* larvae were transferred to white traps and incubated in darkness at 24 ± 2°C. Hatching EPNs (infective juveniles, IJs) were collected and concentrated using a 25 µm pore diameter sieve (Rentsch GmbH, Germany) and poured with tap water into cell culture flasks (160 mL, Thermo Fisher scientific, Switzerland). All collected EPNs were stored at 8°C in a fridge until use.

To study benzoxazinoid degradation by nematodes, endosymbiont bacteria and surface associated microbes, different approaches were used.

i. ***EPN_wash treated and washed-EPNs***: Briefly, for these two linked treatments, about 10000 nematodes were incubated 1mL of added fresh tap water for 24hrs at room temperature. The next day, the liquid part of the mixture (“EPN-wash”) was separated from the nematodes (“washed-EPNs”).

ii. ***Bleached_EPNs***:- Nematodes were treated with 0.05% of bleach (Migros, Bern). The mixture was left to incubate for 15 mins at room temperature. The bleach was washed away by pouring the mixture with nematode-bleach mixture in a nematode collection (retaining) sieve and running tap water over the sieve for 3 min.

iii. ***Streptomycin + EPNs***:- The antibiotic streptomycin, was added to 50ml falcon tube containing concentrated nematodes at a concentration of 200 mg/ml. The antibiotic

was left to act for 4 hrs and after that, the nematodes were washed under running water through a nematode collecting sieve for 2 min.

iv. ***Filtered wash***:- For this, the EPN wash (see point **(i)** above) was filtered with 0.2 µm filters to filter out microbes and the collected filtrate was taken for further tests.

v. ***Boiled_EPNs***:- About 10 000 nematodes in in 1mL of water were heated for 5 minutes in a heating block at 100°C.

The different extracts above where then tested separately for their capacity to metabolize benzoxazinoids. The extracts were incubated with 2-O-β-D-glucopyranosyl-2-hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3(4H)-one) (HDMBOA-Glc) and 3-β-D-glucopyranosyl-6-methoxy-benzoxazolin-2(3H)-one (MBOA-Glc) in separate experiments. HDMBOA-Glc was isolated from maize plants in our laboratory and resulted in a >99% purity (Mateo et al.; Unpublished). MBOA-Glc was synthesized in our laboratory directly from or adapting published protocols, (Sicker *et al.*, 2001; Macías *et al.*, 2006; Li *et al.*, 2013). Benzoxazinoid metabolization was assessed in the incubation water (EPN supernatant) or inside the EPN bodies. In all assays, approximately 10'000 EPNs were placed in 1 mL tap water. HDMBOA-Glc or MBOA-Glc, were added to reach a final concentration of 150 µg/mL, 50 µg/mL respectively (n=5). These concentrations correspond to BXs concentrations reported in WCR (Robert *et al.*, 2017a). Control solutions with benzoxazinoids or nematodes only in water were also included. All samples were incubated at 25 ±2°C. After incubation, 500 µL aliquots were collected from the samples and mixed with 500 µL methanol (MeOH, Fisher Chemica). The samples were then centrifuged at 10 °C at 13'500 RPM for 10 min. The supernatant was collected into 1.5 mL HPLC-glass vials (VWR International, UK)). The pellets, containing EPN bodies, were rinsed three times adding distilled water and centrifuging. The final pellet mass was measured, and 10 µL extraction buffer (50% MeOH with 0.1% formic acid (FA, Fisher Chemical, Waltham, MA, USA) was added per 1 mg pellet. 1.4 mm zirconium oxide beads (Precellys, Bertin Technologies, France) were added to the tubes for grinding in a bead-beater at 30 X speed for 3 minutes before centrifugation at 13'500 RPM at 10 °C for 15 min. The supernatant was collected in 1.5mL glass vials (VWR International, UK) for benzoxazinoid analyses.

Benzoxazinoid analyses

All the vials were later sent for accurate quantitation of most known benzoxazinoids and their recovered breakdown products and conjugates. This was done using an ultra-high performance liquid chromatography-mass spectrometry system equipped with an electrospray source (UHPLC-QDA) to detect, quantify, and identify known new structures through exact mass and fragmentation analysis by MSE as analytic methods. Briefly, for detection and identification of recovered benzoxazinoid recovered breakdown derivatives after their incubation with EPNs isolates EN01 and or its associated microbes, we used an UHPLC-MS system equipped with an electrospray source (Waters i-Class UHPLC-QDA, USA). Recovered benzoxazinoids and their breakdown derivates were separated on a BEH C18 column (2.1 × 100 mm i.d., 1.7 µm particle size). Mobile phase A and B of Water (0.1% FA) and acetonitrile (0.1% FA) respectively were set with respective elution profiles of : 0–9.65 min, 97–83.6% A in B; 9.65–13 min, 100% B; 13.1–15 min 97% A in B which equivalate to 0.4 mL/min. A stable column temperature of 40°C was maintained, as well as an injection volume of 5 µL. The MS was set to operate in a negative reverse mode, and data acquisition scan range of (m/z 150–650) using a cone voltage of 10V. default setting were maintained for all other MS parameters as suggested by the manufacturer. Absolute BX concentrations were determined using standard curves obtained from purified benzoxazinoid compounds. Briefly for the synthesis of the standards: - BOA (benzoxazolin-2(3H)-one) and MBOA (6-methoxy-benzoxazolin-2(3H)-one) were purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). HMPMA (N-(3-methoxy-2-hydroxyphenyl) malonamic acid) was received as a gift from Prof. Dr. Francisco A. Macías (University of Cádiz, Spain). DIMBOA-Glc (2-O-β-D-glucopyranosyl-2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one) and HDMBOA-Glc (2-O-β-D-glucopyranosyl-2-hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3(4H)-one) were isolated from maize plants in our laboratory. DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one), MBOA-Glc (3-β-D-glucopyranosyl-6-methoxy-benzoxazolin-2(3H)-one), HMBOA (2-hydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one), APO (2-amino-3H-phenoxazin-3-one), AMPO (9-methoxy-2-amino-3H-phenoxazin-3-one), AAMPO (9-methoxy-2-acetylamino-3H-phenoxazin-3-one), were synthesized in our laboratory directly from or adapting published protocols.

Statistical analyses

Influences and comparisons of different nematode treatments on biotransformation potential and rate in all this work experiments, were tested using One-way ANOVA. Whenever there was a significant effect ($P<0.05$), post hoc pairwise comparisons between treatments was carried out by extracting "estimated marginal means" using “emmeans multicomp” package in R (https://doi.org/10.32614/CRAN.package.emmeans). And for data that was not normal, Comparison of concentration of different recovered compounds between different nematodes treatments was done using Kruskal-Wallis test computed with R software again**.** Later if significant difference ($p<0.05$) between samples was observed, a post hoc analysis was done using Dunn test again in R software.

Results

Bleach treatment enhances permeability, but abolishes benzoxazinoid metabolization

We previously observed that benzoxazinoid metabolization is restricted to the supernatant of EPN solutions and does not occur in EPN bodies. Our experiments here confirmed this observation (Figure 1A. and Figure 1C, left panels) and (Figure 1A. and Figure 1S right panels) as of EPN + benzoxazinoid (HD=HDMBOA-Glc or MG= MBOA-Glc). We hypothesized that this may be due to the fact that EPN third stage infective juveniles are protected through the second outer cuticle (Timper & Kaya, 1989; Campbell & Gaugler, 1992; Donald L. Lee, 2002). To test this hypothesis,, we removed this cuticle by a short bleach treatment.

Removal of this outer second cuticle by bleaching resulted in the loss of benzoxazinoid transformation in the supernatant. [Figure 1B. and Figure 1 D right panels, (bleached_ EPN treatment)]. At the same time, the EPNs became permeable to HDMBOA-Glc [Figure 1B. left panel). Although, HDMBOA-Glc could now enter the nematodes body, we didn't observe any metabolization (only HDMBOA-Glc but not breakdown derivates). The entrance of benzoxazinoid into nematode bodies after removal of the outer cuticle was not observed with MBOA-Glc [Figure 1D. left panel). Past studies with the western corn rootworm has also reported that MBOA-Glc is mostly concentrated on the larvae cuticle (Robert *et al.*, 2017b). This work shows that bleach-susceptible factors determine benzoxazinoid metabolization and nematode permeability.

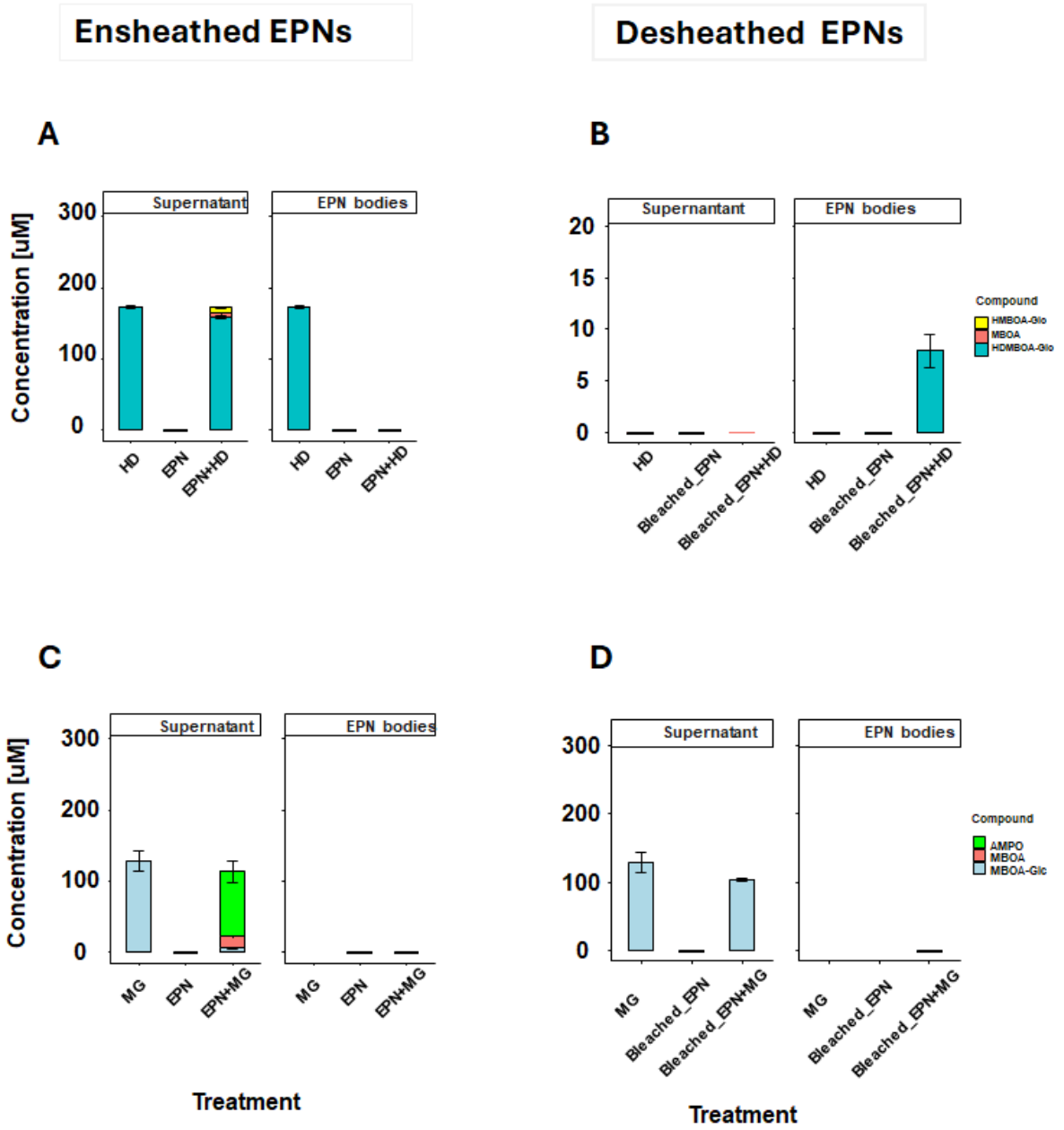
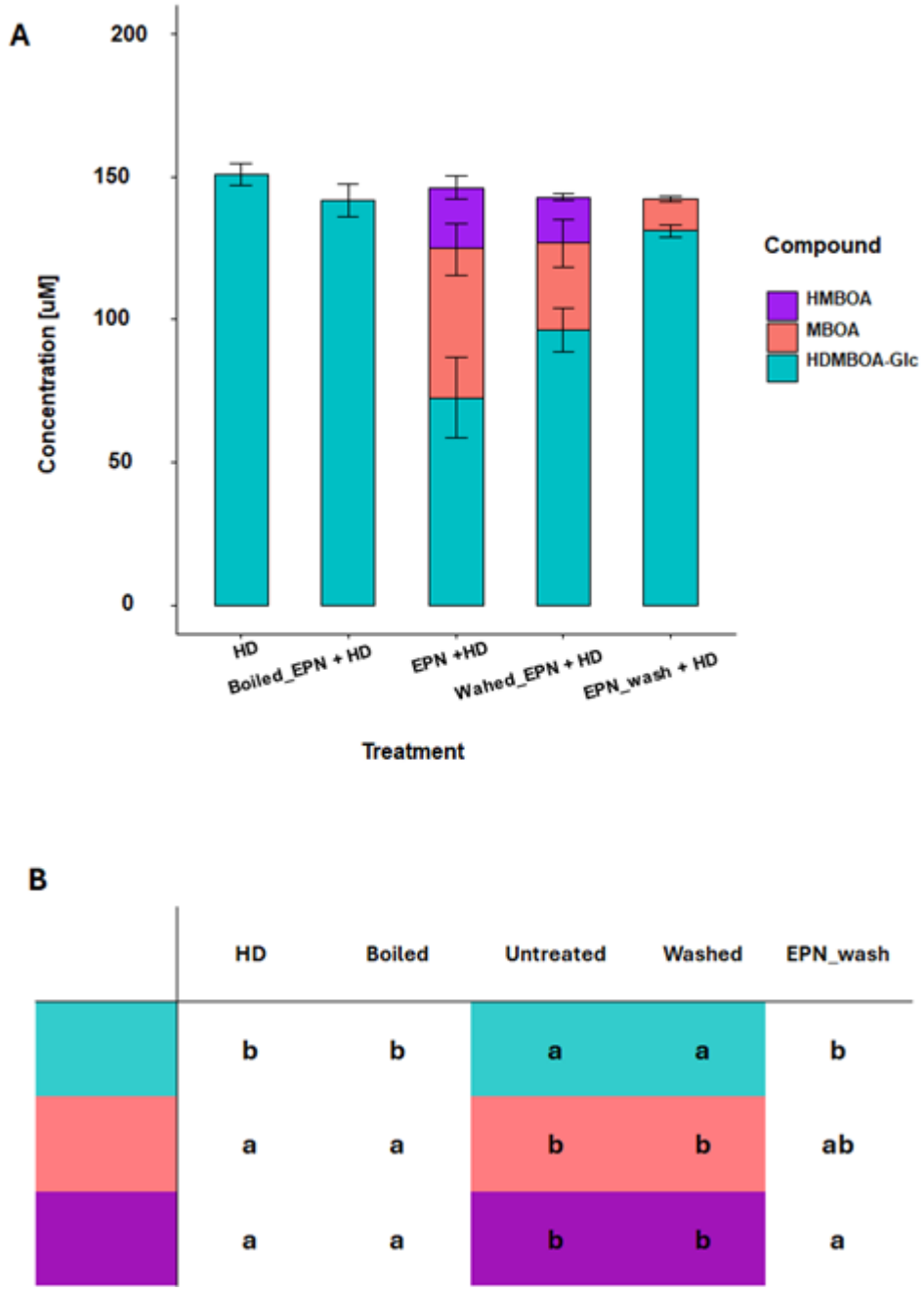


Figure1. Metabolization of benzoxazinoids by nematode (EN01) from both inside its body and on its surface. Biotransformation of HDMBOA-Glc (cyan-colored bar portion) to its breakdown derivatives as analyzed on nematode surface cuticle (Supernatant) as well as from inside nematode bodies of nematode with (Figure 1A.) and without (Figure 1B.) second cuticle. Biotransformation of MBOA-Glc (skyblue-coloured bar portion) as analyzed on nematode surface cuticle(Supernatant) as well as from inside nematode bodies of nematode with (Figure 1C.) and without (Figure 1D.) second cuticle. For surface metabolization analyses were made on only the supernatant obtained after incubation of nematodes in benzoxazinoids for a stipulated time. And for assessment of benzoxazinoids inside the nematodes, the benzoxazinoid incubated nematodes were first crushed and analyzed done from nematode contents. Removal of second cuticle was done by soaking nematodes in 5% for about 15 minutes. Each bar represents uniquely treated EPNs (EN01), and the colors and height of each bar represent the different kinds of recovered benzoxazinoids derivates and their concentrations in the matrix respectively. Acronomy HD and MG respectively.Error Bars: Mse. The red line on Figure 1B. represent an omitted bar for that treated for a control treatment so as to put emphasis on the treatments of interest as they had so much differences in concentration.

EPN surface content manipulations influence benzoxazinoid metabolization

Past studies reported presence of other microbes on the nematode surface (Jiménez-Cortés *et al.*, 2016; Goda *et al.*, 2020; Ogier *et al.*, 2020; Ogier *et al.*, 2023). Both nematode-derived factors as well as surface microbes may mediate these effects. We thus investigated the effects of modulating the composition and condition of the EPN surface. The first manipulation was to heat the nematodes to kill surface microbes and degrade heat-labile factors. Boiled nematodes no longer metabolized HDMBOA-Glc (Figure 2A). We then assessed washed EPNs and the EPN-wash separately. We observed that washed EPN can still metabolise HDMBOA-Glc (Figure 2A), albeit with slightly lower efficiency (Figure 2B.). The EPN-wash was also able to metabolize HDMBOA-Glc to MBOA (Figure 2A., last bar in the graph, though, at a significantly lower rate (Figure 2B.).



3219

3220 **Figure 2. Metabolization of benzoxazinoids by nematode (EN01) from differently manipulated EPN surfaces**.
3221 Graphical presentation of the biotransformation of HDMBOA-Glc from nematodes with double- cuticle (third bars),,
3222 nematode-cuticle wash (l5th bars) water washed cuticle nematodes (4th bars), nematodes with boiled cuticle (2nd bars)
3223 as well as in control treatments (HD) with just HDMBOA-Glc in tap water. Each bar represents uniquely treated EPNs
3224 (EN01), and the colors and height of each bar represent the different kinds of recovered benzoxazinoids derivates and
3225 their concentrations in the matrix respectively. Error Bars: Mse. A legnd table at the bottomof the graph, represent a
3226 statistical comparison of recovered HDMBOA-Glc and its breakdown derivates among differently treated nematodes.
3227 And treatments with different letters (a, b, c etc) had different concentration for the respective compound with the one
3228 shaded with the color that represents that respective compound in this work being compound concentration differ-
3229 ence from the control treatments of just HDMBOA-Glc in water without nematodes

EPN surface microbes métabolise HDMBOA-Glc

To further investigate the potential contribution of microbes to benzoxazinoid metabolization, we filtered them (microbes) from the wash. The HDMBOA-Glc breakdown product MBOA was no longer present in filtered extracts (Figure 3B-C.). We then surface-sterilized nematodes. This resulted in a significant reduction of HDMBOA-Glc metabolization, although residual activity was still observed (Figure 3A.). Thus, surface microbes are likely to play a significant role in benzoxazinoid metabolization by EPNs.

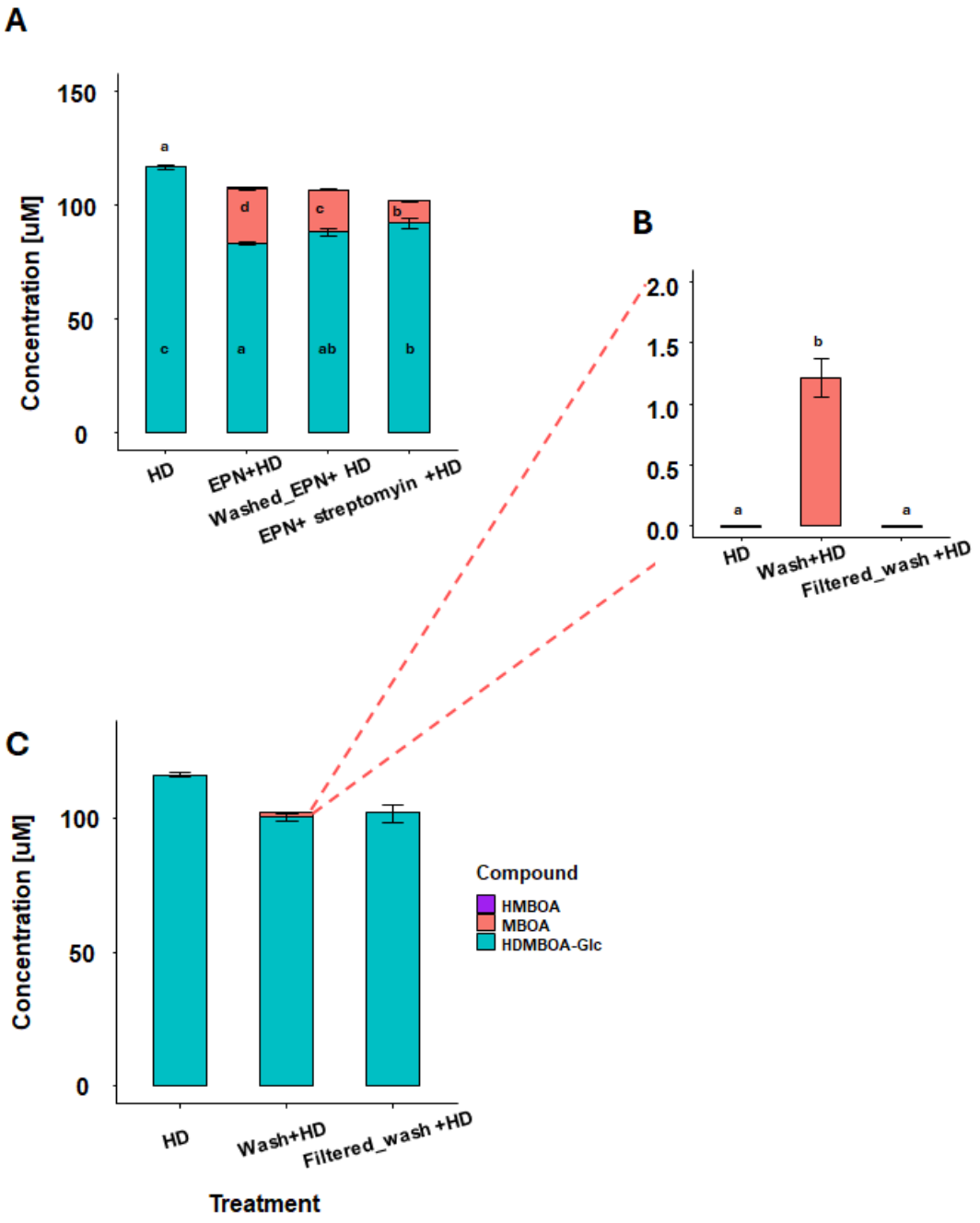


Figure 3. Metabolization of HDMBOA-Glc from nematodes (EN01) with different quantities surfaces associated microbes. Assessing and comparing biotransformation of HDMBOA-Glc from untreated, washed, and antibiotic treated nematodes after 3 incubation days (Figure 3A.) comparing biotransformation of HDMBOA-Glc by nematodes wash collected after incubating nematodes for 24hrs to the same nematode wash but after filtering out microbes collected (Figure 3C.). zooming in with a smaller scaled graph into the different concentration of MBOA recovered from HDMBOA-Glc biotransformation from nematodes wash (Figure 3B.). Different lettters in the table (a,b,c,etc) denotes significant concentration diffrence among EPN treatments. Each bar represents uniquely treated EPNs (EN01), and the colors and height of each bar represent the different kinds of recovered benzoxazinoids derivates and their concentrations in the matrix respectively. Acronomy HD represent the control treatments with just HDMBOA-Glc.Error Bars: Mse.

Discussion

Our work provides insights into the uptake and metabolization of plant secondary metabolites by nematodes.

The observation that desheathed (EPNs whose outer second cuticle has been removed) nematodes are permeable to HDMBOA-Glc indicates that the nematodes second cuticle is a barrier that partially impedes benzoxazinoid entry. EPN infective juveniles (the third stage (J3) duer juveniles) are ensheathed with a second cuticle which result from incomplete molt of the second stage juveniles (J2). This cuticle thus influences toxin uptake from the environment, as suggested previously (Timper & Kaya, 1989; Campbell & Gaugler, 1992; Donald L. Lee, 2002). Additional barriers are present, as even desheathed nematodes did not take up the other benzoxazinoid, MBOA-Glc.

Benzoxazinoids are metabolized exclusively on the outside of the nematodes. We observed a significant decrease in EPN biotransformation rate of washed EPNs compared to the unwashed one, indicating that surface elements play a role in this process. This was further confirmed by the fact that the EPN-wash alone collected after washing nematodes also showed benzoxazinoid biotransformation activity, albeit at a lower level. The second cuticle surface contains a diverse community of microbes, including *Pseudochrobactrum sp., Comamonas sp., Alcaligenes sp., Klebsiella sp., Acinetobacter sp., and Leucobacter spp* was also observed (Jiménez-Cortés *et al.*, 2016; Goda *et al.*, 2020; Ogier *et al.*, 2020; Ogier *et al.*, 2023), and also, a total of more than 200 nematode-derived proteins (Mendy, 2019). Both these 2 factors (surface proteins and microbes) may have a role in benzoxazinoid metabolization.

Surface microbes play a significant role in benzoxazinoid metabolization. Antibiotic treatment with streptomycin significantly reduced HDMBOA-Glc metabolization, and filtering the microbial wash led to the disappearance of MBOA accumulation. In recent years detoxification of benzoxazinoids by enzymes from fungi such as *Gaeumannomyces graminis var. tritici, G. graminis var. graminis, G. graminis var.avenae, and Fusarium culmorum* has also been reported (Friebe *et al.*, 1998; Fomsgaard *et al.*, 2004; Kettle *et al.*, 2015; Glenn *et al.*, 2016). To add on to that, benzoxazinoid glycosides were com-

pletely degraded to benzoxazinoid aglycones in a wheat- yeast enzyme fermentation experiment (Savolainen *et al.*, 2015). Other scientists revealed that, exposing glucosylated benzoxazinones, benzoxazinones benzoxazolinones and several downstream products to microbes (both fungi and bacteria) resulted in strain- and or species-specific compound modification, and degradation (Schütz *et al.*, 2019). Recently, a collection of native maize bacteria such including *Sphingobium* LSP13 and *Microbacterium* LMB2,, revealed that these bacteria degraded MBOA and formed AMPO (Thoenen *et al.*, 2024). Whether microbes are solely responsible for benzoxazinoid metabolization by EPNs, or whether nematode-derived proteins also play a role, requires further study.

In conclusion, we show that nematodes are protected from benzoxazinoid uptake through their cuticle. At the same time, surface microbes efficiently metabolize benzoxazinoids and thereby modulate the biochemical environment of infective juveniles as they come into contact with these compounds as they infect sequestering insects. The consequences of this metabolization remain to be investigated but are likely important in shaping the nematode surface microbiome as well as the host infection process.

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Author contributions

ME, and CR and CP supervised the research. DM conducted the assays, DM, CR and ME analyzed and interpreted the data. DM wrote the first draft. CR and ME contributed to writing the final version.

Conflicts of interest

The authors declare no conflicts of interest.

Figures and tables information

List of figures

Figure1. Metabolization of benzoxazinoids by nematode (EN01) from both inside its body and on its surface. Biotransformation of HDMBOA-Glc (cyan-colored bar portion) to its breakdown derivatives as analyzed on nematode surface cuticle (Supernatant) as well as from inside nematode bodies of nematode with (Figure 1A.) and without (Figure 1B.) second cuticle. Biotransformation of MBOA-Glc (skyblue-coloured bar portion) as analyzed on nematode surface cuticle (Supernatant) as well as from inside nematode bodies of nematode with (Figure 1C.) and without (Figure 1D.) second cuticle. For surface metabolization analyses were made on only the supernatant obtained after incubation of nematodes in benzoxazinoids for a stipulated time. And for assessment of benzoxazinoids inside the nematodes, the benzoxazinoid incubated nematodes were first crushed and analyzed done from nematode contents. Removal of second cuticle was done by soaking nematodes in 5% for about 15 minutes. Each bar represents uniquely treated EPNs (EN01), and the colors and height of each bar represent the different kinds of recovered benzoxazinoids derivates and their concentrations in the matrix respectively. Acronomy HD and MG respectively.Error Bars: Mse. The red line on Figure 1B. represent an omitted bar for that treated for a control treatment so as to put emphasis on the treatments of interest as they had so much differences in concentration.

Figure 2. Metabolization of benzoxazinoids by nematode (EN01) from differently manipulated EPN surfaces. Graphical presentation of the biotransformation of HDMBOA-Glc from nematodes with double- cuticle (third bars),, nematode-cuticle wash (l5th bars) water washed cuticle nematodes (4th bars), nematodes with boiled cuticle (2nd bars) as well as in control treatments (HD) with just HDMBOA-Glc in tap water. Each bar represents uniquely treated EPNs (EN01), and the colors and height of each bar represent the different kinds of recovered benzoxazinoids derivates and their concentrations in the matrix respectively. Error Bars: Mse. A legnd table at the bottomof the graph, represent a statistical comparison of recovered HDMBOA-Glc and its breakdown derivates among differently treated nematodes. And treatments with different letters (a, b, c etc) had different concentration for the respective compound with the one shaded with the color that represent that respective compound in this work being compound concentration difference from the control treatments of just HDMBOA-Glc in water without nematodes

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References

Bayer, C., Heindl, N.R., Rinke, C., Lücker, S., Ott, J.A., Bulgheresi, S. (2009). Molecular characterization of the symbionts associated with marine nematodes of the genus Robbea. *Environmental microbiology reports* 1, 136–144. DOI: 10.1111/j.1758-2229.2009.00019.x

Boemare, N.E., Akhurst, R.J., Mourant, R.G. (1993). DNA relatedness between *Xenorhabdus* spp. (Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a New Genus, Photorhabdus gen. nov. *International Journal of Systematic Bacteriology* 43, 249–255. DOI: 10.1099/00207713-43-2-249

Callahan, B.J., McMurdie, P.J., Holmes, S.P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME journal* 11, 2639–2643. DOI: 10.1038/ismej.2017.119

Campbell, L.R. & Gaugler, R. (1991). Mechanisms for exsheathment of entomopathogenic nematodes. *International Journal for Parasitology* 21, 219–224. DOI: 10.1016/0020-7519(91)90012-V

Campbell, L.R. & Gaugler, R. (1992). Effect of Exsheathment on Motility and Pathogenicity of Two Entomopathogenic Nematode Species. *Journal of Nematology* 24, 365–370.

Donald L. Lee (2002). Cuticle, Moulting and Exsheathment. In: *The Biology of Nematodes*, CRC Press, pp. 171–209.

Fomsgaard, I.S., Mortensen, A.G., Carlsen, S.C.K. (2004). Microbial transformation products of benzoxazolinone and benzoxazinone allelochemicals: A review. *Chemosphere* 54, 1025–1038. DOI: 10.1016/j.chemosphere.2003.09.044

Forst, S., Dowds, B., Boemare, N., Stackebrandt, E. (1997). *Xenorhabdus* and *Photorhabdus* spp.: Bugs that kill bugs. *Annual review of microbiology* 51, 47–72. DOI: 10.1146/annurev.micro.51.1.47

Friebe, A., Vilich, V., Hennig, L., Kluge, M., Sicker, D. (1998). Detoxification of benzoxazolinone allelochemicals from wheat by *Gaeumannomyces graminis* var. tritici, *G. graminis* var. graminis, *G. graminis* var. avenae, and *Fusarium culmorum*. *Applied and environmental microbiology* 64, 2386–2391. DOI: 10.1128/AEM.64.7.2386-2391.1998

Glenn, A.E., Davis, C.B., Gao, M., Gold, S.E., Mitchell, T.R., Proctor, R.H., Stewart, J.E., Snook, M.E. (2016). Two Horizontally Transferred Xenobiotic Resistance Gene Clusters Associated with Detoxification of Benzoxazolinones by *Fusarium* Species. *PLoS ONE* 11, e0147486. DOI: 10.1371/journal.pone.0147486

Goda, N., Mirzae, M., Brunetti, M. (2020). Potentially entomopathogenic nematode isolated from *Popillia japonica*: bioassay, molecular characterization and the associated microbiota. *Bulletin of Insectology, 73*(2), 295-301.

Grewal, P. & Georgis, R. (1999). Entomopathogenic nematodes. *Biopesticides: Use and Delivery* 5, 271–300. DOI: 10.1385/0-89603-515-8:271

Jiménez-Cortés, J.G., Canales-Lazcano, J., Lara-Reyes, N., Rosenblueth, M., Martínez-Romero, E., Contreras-Garduño, J. (2016). Microbiota from Rhabditis regina may alter nematode entomopathogenicity. *Parasitology research* 115, 4153–4165. DOI: 10.1007/s00436-016-5190-3

Kaya, H.K. & Gaugler, R. (1993). Entomopathogenic nematodes. *Annual review of entomology* 38, 181–206. DOI: 10.1146/annurev.en.38.010193.001145

Kettle, A.J., Batley, J., Benfield, A.H., Manners, J.M., Kazan, K., Gardiner, D.M. (2015). Degradation of the benzoxazolinone class of phytoalexins is important for virulence of *Fusarium pseudograminearum* towards wheat. *Molecular plant pathology* 16, 946–962. DOI: 10.1111/mpp.12250

Li, J., Liu, X., Don, F., Xu, J., GUO, L., Kong, Z., Tian, Y., Wu, Y., Zheng, Y. (2013). A Simple Method for the Isolation and Purification of 2,4-Dihydroxy-7-Methoxy-2H-1,4-Benzoxazin-3(4H)-One (DIMBOA) from Maize (*Zea mays* L.) Seedlings. *Journal of Integrative Agriculture* 12, 95–102. DOI: 10.1016/S2095-3119(13)60209-9

Macías, F.A., Marín, D., Oliveros-Bastidas, A., Chinchilla, D., Simonet, A.M., Molinillo, J.M.G. (2006). Isolation and synthesis of allelochemicals from gramineae: benzoxazinones and related compounds. *Journal of agricultural and food chemistry* 54, 991–1000. DOI: 10.1021/jf050896x

Mendy, B. (2019). Studies on nematode factors activating the plant immune system. PhD diss., Dissertation, Bonn, Rheinische Friedrich-Wilhelms-Universität, 2019, 2019.

Ogier, J.-C., Akhurst, R., Boemare, N., Gaudriault, S. (2023). The endosymbiont and the second bacterial circle of entomopathogenic nematodes. *Trends in microbiology* 31, 629–643. DOI: 10.1016/j.tim.2023.01.004

Ogier, J.-C., Pagès, S., Frayssinet, M., Gaudriault, S. (2020). Entomopathogenic nematode-associated microbiota: from monoxenic paradigm to pathobiome. *Microbiome* 8, 25. DOI: 10.1186/s40168-020-00800-5

Robert, C.A., Zhang, X., Machado, R.A., Schirmer, S., Lori, M., Mateo, P., Erb, M., Gershenzon, J. (2017a). Sequestration and activation of plant toxins protect the western corn rootworm from enemies at multiple trophic levels. *eLife* 6. DOI: 10.7554/eLife.29307

Robert, C. am, Zhang, X., Machado, R.A., Schirmer, S., Lori, M., Mateo, P., Erb, M., Gershenzon, J. (2017b). Sequestration and activation of plant toxins protect the western corn rootworm from enemies at multiple trophic levels. *eLife* 6. DOI: 10.7554/eLife.29307

Savolainen, O., Pekkinen, J., Katina, K., Poutanen, K., Hanhineva, K. (2015). Glycosylated Benzoxazinoids Are Degraded during Fermentation of Wheat Bran. *Journal of agricultural and food chemistry* 63, 5943–5949. DOI: 10.1021/acs.jafc.5b00879

Schütz, V., Bigler, L., Girel, S., Laschke, L., Sicker, D., Schulz, M. (2019). Conversions of Benzoxazinoids and Downstream Metabolites by Soil Microorganisms. *Frontiers in Ecology and Evolution* 7. DOI: 10.3389/fevo.2019.00238

Sheehy, L., Cutler, J., Weedall, G.D., Rae, R. (2022). Microbiome Analysis of Malacopathogenic Nematodes Suggests No Evidence of a single bacterial symbiont responsible for Gastropod mortality. *Frontiers in immunology* 13, 878783. DOI: 10.3389/fimmu.2022.878783

Sicker, D., Schneider, B., Hennig, L., Knop, M., Schulz, M. (2001). Glycoside carbamates from benzoxazolin-2(3H)-one detoxification in extracts and exudates of corn roots. *Phytochemistry* 58, 819–825. DOI: 10.1016/S0031-9422(01)00299-0

Tan, L. & Grewal, P.S. (2001). Infection behavior of the rhabditid nematode phasmarhabditis hermaphrodita to the grey garden slug *Deroceras reticulatum*. *Journal of Parasitology* 87, 1349–1354. DOI: 10.1645/0022-3395(2001)087[1349:IBOTRN]2.0.CO;2

Taylor, M.J., Bandi, C., Hoerauf, A. (2005). *Wolbachia* bacterial endosymbionts of filarial nematodes. *Advances in parasitology* 60, 245–284. DOI: 10.1016/S0065-308X(05)60004-8

Thoenen, L., Kreuzer, M., Pestalozzi, C., Florean, M., Mateo, P., Züst, T., Wei, A., Giroud, C., Rouyer, L., Gfeller, V. *et al.* (2024). The lactonase BxdA mediates metabolic specialisation of maize root bacteria to benzoxazinoids. *Nature communications* 15, 6535. DOI: 10.1038/s41467-024-49643-w

Timper, P. & Kaya, H.K. (1989). Role of the second-stage cuticle of entomogenous nematodes in preventing infection by nematophagous fungi. *Journal of Invertebrate Pathology* 54, 314–321. DOI: 10.1016/0022-2011(89)90115-8

Tomar, P., Thakur, N., Yadav, A.N. (2022). Endosymbiotic microbes from entomopathogenic nematode (EPNs) and their applications as biocontrol agents for agro-environmental sustainability. *Egyptian Journal of Biological Pest Control* 32. DOI: 10.1186/s41938-022-00579-7

General discussion

This thesis explores the impact of plant toxins (benzoxazinoids) that are sequestered by an insect herbivore (the western corn rootworm) on its natural enemies(entomopathogenic nematodes, EPNs). We determined the infectivity success phenotype of different EPN strains from all over the world then later adapted the susceptible strains to benzoxazinoids in a laboratory evolutionary experiment. This involved continuous exposure of EPNs to WCR larvae that contain benzoxazinoids for 5 successive generations. We then explored the metabolization of benzoxazinoids by different EPNs and the possible correlation between EPN infectivity and benzoxazinoid breakdown. Lastly, we explored the role played by entomopathogenic nematode associated microbes in benzoxazinoid metabolization.

The following paragraphs will give a more general discussion of the results we got, more specifically on the success of artificial selection in improving EPN effectiveness, EPNs adaptation mechanisms to plant toxins as well as the impact of EPN symbiosis relationships. I will also discuss the limitations and future perspectives of this work.

Engineering benzoxazinoid tolerant EPNs: Artificial selection

Our research work started with screening the infectivity success of different strains from all over the world. We found that some strains are more, and others are less virulent towards WCR . Previous studies have also reported variation in infectivity towards WCR (Zhang *et al.*, 2019; Bruno *et al.*, 2020). The sequestration of benzoxazinoids, which are known to be toxic and also repel EPNs, likely contributed to this variation (Robert *et al.*, 2017). One of the interesting discoveries of this work was strain EN01, a well-known commercial strains, was also among the list of benzoxazinoid-sensitive strains.

Now the question is how EPN efficacy can be improved or maintained in the presence of benzoxazinoid-sequestering WCRs. So, through this work we managed to show that in such a situation, we can select for resistant EPNs through continually exposing the initially susceptible strains to benzoxazinoids (artificial selection) for at least 3 generations. Besides benzoxazinoid adaptation, artificial selection has also been

successfully used to improve EPN performance under stress in the past, including benzoxazinoid resistance (Zhang *et al.*, 2019), desiccation tolerance (Strauch *et al.*, 2004) and temperature tolerance (Griffin & Downes, 1994; Grewal *et al.*, 1996; Ehlers *et al.*, 2005). This form of forward revolution is effective, as EPNs have short generation time, small genome size and can be cultured easily (Hiltpold *et al.*, 2010). A comparison of genome and transcriptomes before and after selection can be done as further steps to elucidate the genetic pathways/markers involved in benzoxazinoid tolerance or resistance.

Natural selection comes with limitations which need attention. These include the selected trait heritability (h^2) which measures the probability of the selected trait being passed to the next generation (Falconer, 1960). One other set back in selection programs is deterioration of selected trait after removal of the stress (Chaston *et al.*, 2011; Anbesse *et al.*, 2013). And lastly, sometimes a change in some trait may also come with a loss/ cost of some other desirable trait(s), therefore, proper testing and careful consideration must be done before taking any further steps.

Nematode mechanisms of adapting to plant toxins

Besides scientific approaches like artificial selection (Chapter 1) and genetic engineering, EPNs themselves have evolved inherent unique structural, biochemical, and behavioral adaptations (Glazer, 2002; Perry & Wharton, 2011; Perry *et al.*, 2012). Adaptation strategies to plant metabolites such as benzoxazinoids have been explored and classified into tolerance and resistant mechanisms (Jeckel *et al.*, 2022). In this work we have explored some of the putative mechanisms employed by EPNs to avoid the negative effects of benzoxazinoids.

Avoidance

In this work observed that, EPNs have evolved some *behavioural* tolerance in benzoxazinoid environments. We managed to use MBOA-Glc, a reported EPN repellent (Robert *et al.*, 2017), to show that some strains (resistant isolates) have naturally evolved behavioural tolerance to this repellent compound even before the section experiment (chapter 2). This may mean that, benzoxazinoid infectivity success may be linked to

nematode behaviour. However, correlation does not mean causation. Thus, further tests need to be done first to confirm this observation.

Metabolization

We found that EPNs *metabolise* benzoxazinoids (chapter 3 and chapter 4) irrespective of the infectivity success phenotype in chapter 2 and geographical origin. Metabolization can help to detoxify plant toxins into harmless products and, is normally accompanied by either sequestration or excretion of the metabolization products (Jeckel *et al.*, 2022). However, in the case of benzoxazinoid, metabolization may result in the formation of equally active or even more active products, similar to what is known from other compartmentalized defense metabolites ((Morant *et al.*, 2008; Pentzold *et al.*, 2014). Thus, further work is required to determine whether the observed metabolization is beneficial for the EPNs.

Exclusion

Another additional mechanism to resist plant toxins is exclusion, i.e. the avoidance of uptake. We observed that all EPN strains are impermeable to benzoxazinoid entry (chapter 3) but become permeable after removal of the outer cuticle (Chapter 4). This reveals to us that IJs are structurally protected from benzoxazinoids. It has been reported that IJs second cuticle likely plays a role in environmental stress tolerance (Timper & Kaya, 1989; Campbell & Gaugler, 1992; Donald L. Lee, 2002). This may mean that outside their host, EPNs are well protected from most benzoxazinoids impacts but will be challenged inside their host as they remove this outer protective cuticle upon host entry.

Activation/inhibition of responsible enzymes

The last mechanism we intended to explore but didn't conclude was the upregulated or down regulated of responsible proteins. We are aware that benzoxazinoid degradation involve enzymes from microbes (Friebe *et al.*, 1998; Fomsgaard *et al.*, 2004; Kettle *et al.*, 2015; Glenn *et al.*, 2016) and also some other plant enzymes were also reported (Sue *et al.*, 2011; Schulz *et al.*, 2016). Our attempt to explore this was inconclusive due to some of the following reason. Firstly, no reported benzoxazinoid degradation nematode enzyme yet. we had to search for plant enzymes in the nematodes genomes. Secondly, some responsible factors like surface microbes genomic data were missing to. We only had

EPN and endosymbiont bacteria genome. Though, we found some hit in the endosymbiont genome it's difficult to conclude from this point, as we didn't observe metabolization inside the nematode. Further studies with all necessary information and data may help to understand EPN survival mechanisms in benzoxazinoid stressful environment.

Influence of EPN-associated microbes

We found that, EPN surface associated microbes also play a role in EPN benzoxazinoid metabolization . Past report have most reported the mutual benefits with the endosymbiotic bacteria which serve as their pathogenic weapons as well as food during host infection (Boemare *et al.*, 1993; Kaya & Gaugler, 1993; Forst *et al.*, 1997; Grewal & Georgis, 1999; Tomar *et al.*, 2022). Some researchers have also shown that some of the associated microbes on the surface are also entomopathogenic (Ogier *et al.*, 2020). To what extent benzoxazinoid metabolization by surface microbes is beneficial for EPNs remains to be elucidated.

Limitations and future perspectives

Injective juveniles are morphologically and biologically different from parasitic ones

Most of the experiment of chapter 3 and 4 and some parts of chapter 2 were done using dauer juveniles which are the free-living form of EPNs. The answers here may be only relevant to outside host environments and may not apply inside the host where we have different stage and/or state of EPNs. Infective juveniles are quite different from their parasitic state. Some of the differences include the already discussed covering sheath. Other differences include that the infective juveniles are in a dauer state (developmental arrest) with a closed digestive system while the parasitic ones have an open and working digestive system (Timper & Kaya, 1989; Bedding *et al.*, 1993; Kaya & Gaugler, 1993).

Natural environments vs laboratory experiments

We worked with one compound at a time, which allows for controlled results. However, in nature, diverse forms of benzoxazinoids can occur at the same time and place, For example, in one benzoxazinoid extraction study, 15 unique benzoxazinoids ranging from

hydroxamic acids, lactams, benzoxazolinones to aminophenoxazinones (Mwendwa *et al.*, 2021) were reported. Even the study by (Robert *et al.*, 2017)of sequestered benzoxazinoid detected a total of 2-3 unique benzoxazinoids accumulated in WCR. Thus, it will be interesting to have experiments which start with more than one compound in one sample and study their interactions.

Time and resources

If time and resources were infinite, the following tests would improve the conclusions

- Do infectivity assays of my F0s and F5s from my Chapter 1 selection experiment at the same time. And then compare the two from one experiment.
- Do mortality assay from hatching juvenile from WCR to be closer to the natural situation.
- Asses alternative methods to surface sterilize EPNS without removal of the sheath.
- Asses other effects of EPN associated microbes on nematode biological and biochemical processes (health, life span etc.).
- Asses benzoxazinoid metabolization by individual endogenous and surface microbes alone.
- Continue searching for tolerance and resistance mechanisms underlying the infectivity differences between resistant vs susceptible or adapted vs non-adapted nematode groups, excluding the already tested hypothesis (metabolism, exclusion, avoidance, enzyme activation or inhibition). Start by testing excretion, sequestration, insensitivity or compensation differences etc

Conclusion

In conclusion, this thesis highlights the capacity of EPNs to rapidly adapt to and metabolize plant chemical defenses, thus providing new insights into enhancing the efficacy of biocontrol strategies against BX-sequestering pests like WCR. To what extent the ability of EPNs to metabolize BXs affects their performance and fitness is currently unclear, but it seems unlikely that metabolization is NOT a major factor that contributes to EPN BX resistance and biocontrol success against BX sequestering insects such as the

western corn rootworm. We additionally showed that nematodes are protected from benzoxazinoid uptake through their cuticle. At the same time, surface microbes efficiently metabolize benzoxazinoids and thereby modulate the biochemical environment of infective juveniles as they come into contact with these compounds as they infect sequestering insects. The consequences of this metabolization remain to be investigated but are likely important in shaping the nematode surface microbiome as well as the host infection process. EPNs encounter benzoxazinoid stressful situation both outside and outside their host but have or can evolve mechanisms to avoid benzoxazinoid toxicity.

Publication bibliography

Ahmad, Munir; Muhammad, Wali; Sajjad, Asif (2020): Ecological Management of Cotton Insect Pests. In Shakeel Ahmad, Mirza Hasanuzzaman (Eds.): Cotton Production and Uses. Singapore: Springer Singapore, pp. 213–238.

Alemanno, Alberto (2013): The science, law and policy of neonicotinoids and bees: A new test case for the precautionary principle. In *Eur. j. risk regul.* 4 (2), pp. 191–207. DOI: 10.1017/S1867299X00003342.

Alouw, J. C.; Miller, N. J. (2015): Effects of benzoxazinoids on specialist and generalist *Diabrotica* species. In *J. Appl. Entomol.* 139 (6), pp. 424–431. DOI: 10.1111/jen.12194.

Anbesse, Samuel; Sumaya, Nanette Hope; Dörfler, Anna Verena; Strauch, Olaf; Ehlers, Ralf-Udo (2013): Selective breeding for desiccation tolerance in liquid culture provides genetically stable inbred lines of the entomopathogenic nematode *Heterorhabditis bacteriophora*. In *Applied microbiology and biotechnology* 97 (2), pp. 731–739. DOI: 10.1007/s00253-012-4227-5.

Andersen, Douglas C. (1987): Below-ground herbivory in natural communities: A Review emphasizing fossorial animals. In *The Quarterly Review of Biology* 62 (3), pp. 261–286. DOI: 10.1086/415512.

Andow, David A.; Pueppke, Steven G.; Schaafsma, Arthur W.; Gassmann, Aaron J.; Sappington, Thomas W.; Meinke, Lance J. et al. (2016): Early detection and mitigation of resistance to Bt maize by western corn rootworm (Coleoptera: Chrysomelidae). In *J Econ Entomol* 109 (1), pp. 1–12. DOI: 10.1093/jee/tov238.

Baumeler, A.; Hesse, M.; Werner, C. (2000): Benzoxazinoids-cyclic hydroxamic acids, lactams and their corresponding glucosides in the genus *Aphelandra* (Acanthaceae). In *Phytochemistry* 53 (2), pp. 213–222. DOI: 10.1016/S0031-9422(99)00508-7.

Bažok, Renata; Lemić, Darija; Chiarini, Francesca; Furlan, Lorenzo (2021): Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in Europe: Current status and sustainable pest management. In *Insects* 12 (3). DOI: 10.3390/insects12030195.

Bedding, R. A.; Akhurst, R. J.; Kaya, H. K. (1993): Nematodes and the biological control of insect pests: CSIRO Publishing.

Blake, Robin (2018): EU neonicotinoid ban removes vital tools in global fight against pests. In *outlook pest man* 29 (5), pp. 197–200. DOI: 10.1564/v29_oct_02.

Blandino, Massimo; Ferracini, Chiara; Rigamonti, Ivo; Testa, Giulio; Saladini, Matteo A.; Jucker, Costanza et al. (2017): Control of western corn rootworm damage by application of soil insecticides at different maize planting times. In *Crop Protection* 93, pp. 19–27. DOI: 10.1016/j.cropro.2016.11.006.

Blossey, Bernd; Hunt-Joshi, Tamaru R. (2003): Belowground herbivory by insects: influence on plants and aboveground herbivores. In *Annual review of entomology* 48, pp. 521–547. DOI: 10.1146/annurev.ento.48.091801.112700.

Boemare, N. E.; Akhurst, R. J.; Mourant, R. G. (1993): DNA relatedness between *Xenorhabdus* spp. (Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a New Genus, Photorhabdus gen. nov. In *International Journal of Systematic Bacteriology* 43 (2), pp. 249–255. DOI: 10.1099/00207713-43-2-249.

Boriani, M.; Agosti, M.; Kiss, J.; Edwards, C. R. (2006): Sustainable management of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), in infested areas: experiences in Italy, Hungary and the USA. In *EPPO Bulletin* 36 (3), pp. 531–537. DOI: 10.1111/j.1365-2338.2006.01055.x.

Branson, Terry F.; Johnson, Rorerta D. (1973): Adult western corn rootworms: oviposition, fecundity, and longevity in the laboratory123. In *J Econ Entomol* 66 (2), pp. 417–418. DOI: 10.1093/jee/66.2.417.

Brown, Valerie K.; Gange, Alan C. (1990): Insect herbivory insect below ground. In : *Advances in Ecological Research* Volume 20, vol. 20: Elsevier (Advances in Ecological Research), pp. 1–58.

Bruijn, Wouter J. C. de; Gruppen, Harry; Vincken, Jean-Paul (2018): Structure and biosynthesis of benzoxazinoids: Plant defence metabolites with potential as antimicrobial scaffolds. In *Phytochemistry* 155, pp. 233–243. DOI: 10.1016/j.phytochem.2018.07.005.

Bruno, Pamela; Machado, Ricardo A. R.; Glauser, Gaétan; Köhler, Angela; Campos-Herrera, Raquel; Bernal, Julio et al. (2020): Entomopathogenic nematodes from Mexico that can overcome the resistance mechanisms of the western corn rootworm. In *Scientific reports* 10 (1), p. 8257. DOI: 10.1038/s41598-020-64945-x.

Campbell, L. Rickert; Gaugler, R. (1992): Effect of exsheathment on motility and pathogenicity of two entomopathogenic nematode species. In *Journal of Nematology* 24 (3), pp. 365–370.

Chaston, John M.; Dillman, Adler R.; Shapiro-Ilan, David I.; Bilgrami, Anwar L.; Gaugler, Randy; Hopper, Keith R.; Adams, Byron J. (2011): Outcrossing and crossbreeding recovers deteriorated traits in laboratory cultured *Steinernema carpocapsae* nematodes. In *International journal for parasitology* 41 (7), pp. 801–809. DOI: 10.1016/j.ijpara.2011.02.005.

Ciche, Todd A.; Darby, Creg; Ehlers, Ralf-Udo; Forst, Steven; Goodrich-Blair, Heidi (2006): Dangerous liaisons: The symbiosis of entomopathogenic nematodes and bacteria. In *Biological Control* 38 (1), pp. 22–46. DOI: 10.1016/j.biocontrol.2005.11.016.

Darlington, Molly; Reinders, Jordan D.; Sethi, Amit; Lu, Albert L.; Ramaseshadri, Partha; Fischer, Joshua R. et al. (2022): RNAi for Western Corn Rootworm Management:

Lessons learned, challenges, and future directions. In *Insects* 13 (1). DOI: 10.3390/insects13010057.

Degrugillier, Maurice E.; Degrugillier, Sheila S.; Jackson, Jan J. (1991): Nonoccluded, cytoplasmic virus particles and rickettsia-like organisms in testes and spermathecae of *Diabrotica virgifera*. In *Journal of invertebrate pathology* 57 (1), pp. 50–58. DOI: 10.1016/0022-2011(91)90040-W.

Deguine, Jean-Philippe; Aubertot, Jean-Noël; Bellon, Stéphane; Côte, François; Lauri, Pierre-Eric; Lescourret, Françoise et al. (2023): Agroecological crop protection for sustainable agriculture. In, vol. 178: Elsevier (*Advances in Agronomy*), pp. 1–59.

Donald L. Lee (2002): Cuticle, moulting and exsheathment. In : The biology of nematodes: CRC Press.

Ehlers, Ralf-Udo; Oestergaard, Jesko; Hollmer, Sonja; Wingen, Michael; Strauch, Olaf (2005): Genetic selection for heat tolerance and low temperature activity of the entomopathogenic nematode–bacterium complex *Heterorhabditis Bacteriophora–Photorhabdus Luminescens*. In *BioControl* 50 (5), pp. 699–716. DOI: 10.1007/s10526-005-5079-z.

Ehrlich, Paul R.; Ehrlich, Anne H.; Daily, Gretchen C. (1993): Food security, population and environment. In *Population and Development Review* 19 (1), p. 1. DOI: 10.2307/2938383.

Erb, Matthias; Robert, Christelle am (2016): Sequestration of plant secondary metabolites by insect herbivores: Molecular mechanisms and ecological consequences. In *Current Opinion in Insect Science* 14, pp. 8–11. DOI: 10.1016/j.cois.2015.11.005.

European Commission (2013): Bee health: EU-wide restrictions on pesticide use to enter into force.

Falconer, Douglas S. (1960): Introduction to quantitative genetics. New York, USA: Ronald Press Comp.

FAO (2010): The State of Food Insecurity in the World 2010. Economic crises–impacts and lessons learned.

FAO (2025): Plant production and protection. Sustainable plant pest and disease management.

Ferracini, Chiara; Blandino, Massimo; Rigamonti, Ivo E.; Jucker, Costanza; Busato, Enrico; Saladini, Matteo A. et al. (2021): Chemical-based strategies to control the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. In *Crop Protection* 139, p. 105306. DOI: 10.1016/j.cropro.2020.105306.

Fess, Tiffany L.; Kotcon, James B.; Benedito, Vagner A. (2011): Crop Breeding for Low Input Agriculture: A sustainable response to feed a growing world population. In *Sustainability* 3 (10), pp. 1742–1772. DOI: 10.3390/su3101742.

Fomsgaard, Inge S.; Mortensen, Anne G.; Carlsen, Sandra C. K. (2004): Microbial transformation products of benzoxazolinone and benzoxazinone allelochemicals--a

review. In *Chemosphere* 54 (8), pp. 1025–1038. DOI: 10.1016/j.chemosphere.2003.09.044.

Forst, S.; Dowds, B.; Boemare, N.; Stackebrandt, E. (1997): *Xenorhabdus* and *Photorhabdus* spp.: Bugs that kill bugs. In *Annual review of microbiology* 51, pp. 47–72. DOI: 10.1146/annurev.micro.51.1.47.

Friebe, A.; Vilich, V.; Hennig, L.; Kluge, M.; Sicker, D. (1998): Detoxification of benzoxazolinone allelochemicals from wheat by *Gaeumannomyces graminis* var. tritici*, G. graminis* var. graminis, *G. graminis* var. avenae, and *Fusarium culmorum*. In *Applied and environmental microbiology* 64 (7), pp. 2386–2391. DOI: 10.1128/AEM.64.7.2386-2391.1998.

Gassmann, Aaron J. (2012): Field-evolved resistance to Bt maize by western corn rootworm: Predictions from the laboratory and effects in the field. In *Journal of invertebrate pathology* 110 (3), pp. 287–293. DOI: 10.1016/j.jip.2012.04.006.

Glazer, I. (2002): Survival biology. In R. Gaugler (Ed.): Entomopathogenic nematology. UK: CABI Publishing, pp. 169–187.

Glenn, Anthony E.; Davis, C. Britton; Gao, Minglu; Gold, Scott E.; Mitchell, Trevor R.; Proctor, Robert H. et al. (2016): Two horizontally transferred *Xenobiotic* resistance gene clusters associated with detoxification of benzoxazolinones by *Fusarium* Species. In *PLoS ONE* 11 (1), e0147486. DOI: 10.1371/journal.pone.0147486.

Goda, N.;. Mirzae, M.; Brunetti, M. (2020): Potentially entomopathogenic nematode isolated from *Popillia japonica*: bioassay, molecular characterization and the associated microbiota. *Bulletin of Insectology*, *73*(2).

Grant, R. H.; Seevers, K. P. (1989): Local and long-range movement of adult western corn rootworm (Coleoptera: Chrysomelidae) as evidenced by washup along southern lake of Michigan shores. In *Environ Entomol* 18 (2), pp. 266–272. DOI: 10.1093/ee/18.2.266.

Gray, Michael E.; Sappington, Thomas W.; Miller, Nicholas J.; Moeser, Joachim; Bohn, Martin O. (2009): Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. In *Annual review of entomology* 54, pp. 303–321. DOI: 10.1146/annurev.ento.54.110807.090434.

Grewal, P. S. (2012): Entomopathogenic nematodes as tools in integrated pest management. In D. P. Abrol, U. Shankar (Eds.): Integrated pest management: principles and practice. UK: CABI, pp. 162–236.

Grewal, P. S.; Gaugler, R.; Shupe, C. (1996): Rapid changes in thermal sensitivity of entomopathogenic nematodes in response to selection at temperature extremes. In *Journal of invertebrate pathology* 68 (1), pp. 65–73. DOI: 10.1006/jipa.1996.0059.

Grewal, Parwinder; Georgis, Ramon (1999): Entomopathogenic nematodes. In *Biopesticides: Use and Delivery* 5, pp. 271–300. DOI: 10.1385/0-89603-515-8:271.

Griffin, C. T.; Downes, M. J. (1994): Selection of *Heterorhabditis* sp. (Nematoda: Heterorhabditidae) for improved infectivity at low temperature.*Nematologica* 37, pp. 83-91.

Heather, M. Kelly; Sebe, Brow. (2017): Belowground (soil) insect pests: Crops pest guides. University of Tennessee.

Hill, Roscoe E. (1975): Mating, oviposition patterns, fecundity and longevity of the western corn rootworm12. In *J Econ Entomol* 68 (3), pp. 311–315. DOI: 10.1093/jee/68.3.311.

Hillel, Daniel. (2010): Dawn of agriculture.

Hiltpold, Ivan; Baroni, Mariane; Toepfer, Stefan; Kuhlmann, Ulrich; Turlings, Ted C. J. (2010): Selection of entomopathogenic nematodes for enhanced responsiveness to a volatile root signal helps to control a major root pest. In *The Journal of experimental biology* 213 (Pt 14), pp. 2417–2423. DOI: 10.1242/jeb.041301.

Hoffmann, Amanda M.; French, B. Wade; Jaronski, Stefan T.; Gassmann, Aaron J. (2014): Effects of entomopathogens on mortality of western corn rootworm (Coleoptera: Chrysomelidae) and fitness costs of resistance to Cry3Bb1 maize. In *J Econ Entomol* 107 (1), pp. 352–360. DOI: 10.1603/EC13247.

Hoffmann, M. P.; Kirkwyland, J. J.; Gardner, J. (2000): Impact of western corn rootworm (Coleoptera: Chrysomelidae) on sweet corn and evaluation of insecticidal and cultural control options. In *J Econ Entomol* 93 (3), pp. 805–812. DOI: 10.1603/0022-0493-93.3.805.

Horlings, L. G.; Marsden, T. K. (2011): Towards the real green revolution? Exploring the conceptual dimensions of a new ecological modernisation of agriculture that could 'feed the world'. In *Global Environmental Change* 21 (2), pp. 441–452. DOI: 10.1016/j.gloenvcha.2011.01.004.

Hummel, H. E. (2003): Introduction of *Diabrotica virgifera virgifera* into the old world and its consequences: A recently acquired invasive alien pest species on *Zea mays* from North America. In *Communications in agricultural and applied biological sciences* 68 (4 Pt A), pp. 45–57.

Hummel, Hans E. (2007): *Diabrotica virgifera virgifera* LeConte: Inconspicuous leaf beetle--formidable challenges to agriculture. In *Communications in agricultural and applied biological sciences* 72 (2), pp. 7–32.

Jeckel, Adriana Moriguchi; Beran, Franziska; Züst, Tobias; Younkin, Gordon; Petschenka, Georg; Pokharel, Prayan et al. (2022): Metabolization and sequestration of plant specialized metabolites in insect herbivores: Current and emerging approaches. In *Frontiers in physiology* 13, p. 1001032. DOI: 10.3389/fphys.2022.1001032.

Jiménez-Cortés, Jesús Guillermo; Canales-Lazcano, Jorge; Lara-Reyes, Nancy; Rosenblueth, Mónica; Martínez-Romero, Esperanza; Contreras-Garduño, Jorge (2016): Microbiota from Rhabditis regina may alter nematode entomopathogenicity. In *Parasitology research* 115 (11), pp. 4153–4165. DOI: 10.1007/s00436-016-5190-3.

Journey, A. M.; Ostlie, K. R. (2000): Biological control of the western corn rootworm (Coleoptera: Chrysomelidae) using the entomopathogenic nematode: *Steinernema carpocapsae*. In *Environ Entomol* 29 (4), pp. 822–831. DOI: 10.1603/0046-225X-29.4.822.

Kathage, Jonas; Castañera, Pedro; Alonso-Prados, José Luis; Gómez-Barbero, Manuel; Rodríguez-Cerezo, Emilio (2018): The impact of restrictions on neonicotinoid and fipronil insecticides on pest management in maize, oilseed rape and sunflower in eight European Union regions. In *Pest management science* 74 (1), pp. 88–99. DOI: 10.1002/ps.4715.

Kaya, H. K.; Gaugler, R. (1993): Entomopathogenic nematodes. In *Annual review of entomology* 38 (1), pp. 181–206. DOI: 10.1146/annurev.en.38.010193.001145.

Kettle, Andrew J.; Batley, Jacqueline; Benfield, Aurelie H.; Manners, John M.; Kazan, Kemal; Gardiner, Donald M. (2015): Degradation of the benzoxazolinone class of phytoalexins is important for virulence of Fusarium pseudograminearum towards wheat. In *Molecular plant pathology* 16 (9), pp. 946–962. DOI: 10.1111/mpp.12250.

Khudoykulov, Azamjon; Anorbaev, Azimjon; Kholikova, Gulnoza (2021): Bioecological properties of underground pests in cultivation of potatoes planted after grain. In *E3S Web Conf.* 244, p. 2033. DOI: 10.1051/e3sconf/202124402033.

Khush, Gurdev S. (1999): Green revolution: preparing for the 21st century. In *Genome* 42 (4), pp. 646–655. DOI: 10.1139/g99-044.

Kluser, S.; Neumann, P.; Chauzat, M. P.; Pettis, J. S.; Peduzzi, P.; Witt, R. et al. (2010): Disorders of bee colonies around the world and other threats to insect pollinators.

Knolhoff, L. M.; Onstad, D. W.; Spencer, J. L.; Levine, E. (2006): Behavioral differences between rotation-resistant and wild-type *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). In *Environ Entomol* 35 (4), pp. 1049–1057. DOI: 10.1603/0046-225X-35.4.1049.

Kogan, Marcos.; Paxton, Jack.: (1983). Natural inducers of plant resistance to insects. *Plant resistance to insects*, *208*, pp. 153–171. DOI: 10.1021/bk-1983-0208.ch009.

Krogh, Stine S.; Mensz, Susanne J. M.; Nielsen, Susan T.; Mortensen, Anne G.; Christophersen, Carsten; Fomsgaard, Inge S. (2006): Fate of benzoxazinone allelochemicals in soil after incorporation of wheat and rye sprouts. In *J. Agric. Food Chem.* 54 (4), pp. 1064–1074. DOI: 10.1021/jf051147i.

Kuhlmann, U.; Toepfer, S.; Feng Zhang, Feng Zhang: Is classical biological control against western corn rootworm in Europe a potential sustainable management strategy, pp. 263–284. DOI: 10.1079/9780851998176.0263.

Kuhlmann, U.; van der Burgt, W.A.C.M. (1998): Possibilities for biological control of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in Central Europe. In *Biocontrol News and Information* 19, pp. 59–68.

Kurtz, B.; Toepfer, S.; Ehlers, R.-U.; Kuhlmann, U. (2007): Assessment of establishment and persistence of entomopathogenic nematodes for biological control of western corn rootworm. In *Journal of Applied Entomology* 131 (6), pp. 420–425. DOI: 10.1111/j.1439-0418.2007.01202.x.

Laurino, D.; Porporato, M.; Patetta, A.; Manino, A. (2011): T oxicity of neonicotinoid insecticides to honey bees: laboratory tests. *BULLETIN OF INSECTOLOGY* 64, 107–113.

Lemic, Darija; Mikac, Katarina M.; Ivkosic, Stephanie A.; Bažok, Renata (2015): The temporal and spatial invasion genetics of the western corn rootworm (Coleoptera: Chrysomelidae) in Southern Europe. In *PLoS ONE* 10 (9), e0138796. DOI: 10.1371/journal.pone.0138796.

Loulou, Ameni; Mastore, Maristella; Caramella, Sara; Bhat, Aashaq Hussain; Brivio, Maurizio Francesco; Machado, Ricardo A. R.; Kallel, Sadreddine (2023): Entomopathogenic potential of bacteria associated with soil-borne nematodes and insect immune responses to their infection. In *PLoS ONE* 18 (1), e0280675. DOI: 10.1371/journal.pone.0280675.

Machado, Ricardo A. R.; Thönen, Lisa; Arce, Carla C. M.; Theepan, Vanitha; Prada, Fausto; Wüthrich, Daniel et al. (2020): Engineering bacterial symbionts of nematodes improves their biocontrol potential to counter the western corn rootworm. In *Nature biotechnology* 38 (5), pp. 600–608. DOI: 10.1038/s41587-020-0419-1.

Meinke, Lance J.; Siegfried, Blair D.; Wright, Robert J.; Chandler, Laurence D. (1998): Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. In *J Econ Entomol* 91 (3), pp. 594–600. DOI: 10.1093/jee/91.3.594.

Meinke, Lance J.; Souza, Dariane; Siegfried, Blair D. (2021): The use of insecticides to manage the western corn rootworm, *Diabrotica virgifera virgifera*, LeConte: History, field-evolved resistance, and associated mechanisms. In *Insects* 12 (2). DOI: 10.3390/insects12020112.

Meissle, M.; Mouron, P.; Musa, T.; Bigler, F.; Pons, X.; Vasileiadis, V. P. et al. (2010): Pests, pesticide use and alternative options in European maize production: current status and future prospects. In *Journal of Applied Entomology* 134 (5), pp. 357–375. DOI: 10.1111/j.1439-0418.2009.01491.x.

Meissle, Michael; Romeis, Jörg; Bigler, Franz (2011): Bt maize and IPM in Europe. In *outlook pest man* 22 (6), pp. 257–261. DOI: 10.1564/22dec04.

Miller, Nicholas; Estoup, Arnaud; Toepfer, Stefan; Bourguet, Denis; Lapchin, Laurent; Derridj, Sylvie et al. (2005): Multiple transatlantic introductions of the western corn rootworm. In *Science* 310 (5750), p. 992. DOI: 10.1126/science.1115871.

Miller, Thomas A.; Krysan, James L. (1986): Methods for the study of pest *Diabrotica*. New York, NY: Springer New York.

Morant, Anne Vinther; Jørgensen, Kirsten; Jørgensen, Charlotte; Paquette, Suzanne Michelle; Sánchez-Pérez, Raquel; Møller, Birger Lindberg; Bak, Søren (2008): Beta-glucosidases as detonators of plant chemical defense. In *Phytochemistry* 69 (9), pp. 1795–1813. DOI: 10.1016/j.phytochem.2008.03.006.

Mwendwa, James M.; Weston, Paul A.; Weidenhamer, Jeffrey D.; Fomsgaard, Inge S.; Wu, Hanwen; Gurusinghe, Saliya; Weston, Leslie A. (2021): Metabolic profiling of benzoxazinoids in the roots and rhizosphere of commercial winter wheat genotypes. In *Plant Soil* 466 (1-2), pp. 467–489. DOI: 10.1007/s11104-021-04996-9.

Narva, Kenneth E.; Siegfried, Blair D.; Storer, Nicholas P. (2013): Transgenic approaches to western corn rootworm control. In *Advances in biochemical engineering/biotechnology* 136, pp. 135–162. DOI: 10.1007/10_2013_195.

Oerke, E. C. (2006): Crop losses to pests. In *J. Agric. Sci.* 144 (1), pp. 31–43. DOI: 10.1017/S0021859605005708.

Ogier, Jean-Claude; Akhurst, Raymond; Boemare, Noël; Gaudriault, Sophie (2023): The endosymbiont and the second bacterial circle of entomopathogenic nematodes. In *Trends in microbiology* 31 (6), pp. 629–643. DOI: 10.1016/j.tim.2023.01.004.

Ogier, Jean-Claude; Pagès, Sylvie; Frayssinet, Marie; Gaudriault, Sophie (2020): Entomopathogenic nematode-associated microbiota: from monoxenic paradigm to pathobiome. In *Microbiome* 8 (1), p. 25. DOI: 10.1186/s40168-020-00800-5.

Pasquier, Antoine; Monticelli, Lucie S.; Moreau, Adeline; Kaltenbach, Benjamin; Chabot, Candice; Andrieux, Thibault et al. (2021): A promising predator-in-first strategy to control western corn rootworm population in maize fields. In *Agronomy* 11 (10), p. 1984. DOI: 10.3390/agronomy11101984.

Pentzold, Stefan; Zagrobelny, Mika; Rook, Fred; Bak, Søren (2014): How insects overcome two-component plant chemical defence: plant β-glucosidases as the main target for herbivore adaptation. In *Biological reviews of the Cambridge Philosophical Society* 89 (3), pp. 531–551. DOI: 10.1111/brv.12066.

Perry, R. N.; Wharton, D. A. (2011): Molecular and physiological basis of nematode survival. UK: CAB International.

Perry, Roland N.; Ehlers, Ralf-Udo; Glazer, Itamar (2012): A Realistic Appraisal of Methods to Enhance Desiccation Tolerance of Entomopathogenic Nematodes. In *Journal of Nematology* 44 (2), pp. 185–190.

Pilz, C.; Wegensteiner, R.; Keller, S. (2007): Selection of entomopathogenic fungi for the control of the western corn rootworm *Diabrotica virgifera virgifera*. In *Journal of Applied Entomology* 131 (6), pp. 426–431. DOI: 10.1111/j.1439-0418.2007.01179.x.

Pilz, Christina; Keller, Siegfried; Kuhlmann, Ulrich; Toepfer, Stefan (2009): Comparative efficacy assessment of fungi, nematodes and insecticides to control western corn rootworm larvae in maize. In *BioControl* 54 (5), pp. 671–684. DOI: 10.1007/s10526-009-9209-x.

Poinar, George O.; Leutenegger, Ruth (1968): Anatomy of the Infective and Normal Third-Stage Juveniles of Neoaplectana carpocapsae Weiser (Steinernematidae: Nematoda). In *The Journal of Parasitology* 54 (2), p. 340. DOI: 10.2307/3276948.

Popp, József; Pető, Károly; Nagy, János (2013): Pesticide productivity and food security. A review. In *Agron. Sustain. Dev.* 33 (1), pp. 243–255. DOI: 10.1007/s13593-012-0105-x.

Rajendra Prasad (2013): Prasad, R. (2013). Population growth, food shortages and ways to alleviate hunger. *Current Science*, *105*(1), 32–36. http://www.jstor.org/stable/24092674

Reddy, P. Parvatha (2017): Agro-ecological approaches to pest management for sustainable agriculture. (pp. 295-309). Singapore:: Springer Singapore.

Robert, Christelle A. M.; Mateo, Pierre (2022): The chemical ecology of benzoxazinoids. In *Chimia* 76 (11), p. 928. DOI: 10.2533/chimia.2022.928.

Robert, Christelle A.M.; Zhang, Xi; Machado, Ricardo Ar; Schirmer, Stefanie; Lori, Martina; Mateo, Pierre et al. (2017): Sequestration and activation of plant toxins protect the western corn rootworm from enemies at multiple trophic levels. In *eLife* 6. DOI: 10.7554/eLife.29307.

Rondon, Silvia I.; Gray, Michael E. (2004): Ovarian development and ovipositional preference of the western corn rootworm (Coleoptera: Chrysomelidae) variant in east central Illinois. In *J Econ Entomol* 97 (2), pp. 390–396. DOI: 10.1093/jee/97.2.390.

Rudeen, Melissa L.; Jaronski, Stefan T.; Petzold-Maxwell, Jennifer L.; Gassmann, Aaron J. (2013): Entomopathogenic fungi in cornfields and their potential to manage larval western corn rootworm *Diabrotica virgifera virgifera*. In *Journal of invertebrate pathology* 114 (3), pp. 329–332. DOI: 10.1016/j.jip.2013.09.009.

Schulz, Margot; Filary, Barbara; Kühn, Sabine; Colby, Thomas; Harzen, Anne; Schmidt, Jürgen et al. (2016): Benzoxazolinone detoxification by N-Glucosylation: The multi-compartment-network of Zea mays L. In *Plant Signaling & Behavior* 11 (1), e1119962. DOI: 10.1080/15592324.2015.1119962.

Schulz, Margot; Marocco, Adriano; Tabaglio, Vincenzo; Macias, Francisco A.; Molinillo, Jose M. G. (2013): Benzoxazinoids in rye allelopathy: From discovery to application in sustainable weed control and organic farming. In *J Chem Ecol* 39 (2), pp. 154–174. DOI: 10.1007/s10886-013-0235-x.

Sicker, Dieter; Frey, Monika; Schulz, Margot; Gierl, Alfons (2000): Role of natural benzoxazinones in the survival strategy of plants. In : I*nternational review of cytology*, vol. 198: Academic Press, pp. 319–346.

Sicker, Dieter; Schulz, Margot (2002): Benzoxazinones in plants: Occurrence, synthetic access, and biological activity. In Atta-ur-Rahman (Ed.): Studies in natural products chemistry : Bioactive natural products (Part H), vol. 27: *Elsevier*, pp. 185–232.

Singh, Ashish Kumar; Kumar, Manish; Ahuja, Amit; Vinay, B. K.; Kommu, Kiran Kumar; Thakur, Sharmishtha et al. (2022): Entomopathogenic nematodes. In : *Biopesticides*: Elsevier, pp. 73–92.

Souza, Dariane; Peterson, Julie A.; Wright, Robert J.; Meinke, Lance J. (2020): Field efficacy of soil insecticides on pyrethroid-resistant western corn rootworms (*Diabrotica virgifera virgifera* LeConte). In *Pest management science* 76 (2), pp. 827–833. DOI: 10.1002/ps.5586.

St Clair, Coy R.; Gassmann, Aaron J. (2021): Linking land use patterns and pest outbreaks in Bt maize. In *Ecological applications : a publication of the Ecological Society of America* 31 (4), e02295. DOI: 10.1002/eap.2295.

St Clair, Coy R.; Norris, Edmund J.; Masloski, Kenneth E.; Coats, Joel R.; Gassmann, Aaron J. (2020): Evaluation of pyrethroids and organophosphates in insecticide mixtures for management of western corn rootworm larvae. In *Pest management science* 76 (11), pp. 3871–3878. DOI: 10.1002/ps.5938.

Strauch, O.; Oestergaard, J.; Hollmer, S.; Ehlers, R.-U (2004): Genetic improvement of the desiccation tolerance of the entomopathogenic nematode *Heterorhabditis bacteriophora* through selective breeding. In *Biological Control* 31 (2), pp. 218–226. DOI: 10.1016/j.biocontrol.2004.03.009.

Sue, Masayuki; Nakamura, Chihiro; Nomura, Taiji (2011): Dispersed benzoxazinone gene cluster: molecular characterization and chromosomal localization of glucosyltransferase and glucosidase genes in wheat and rye. In *Plant Physiol* 157 (3), pp. 985–997. DOI: 10.1104/pp.111.182378.

Sushma Naithani (2021): The Origins of Agriculture: Oregon State University (History and Science of Cultivated Plants).

Sutter, G. R.; Branson, T. F.; Fisher, J. R.; Elliott, N. C.; Jackson, J. J. (1989): Effect of insecticide treatments on root damage ratings of maize in controlled infestations of western corn rootworms (Coleoptera: Chrysomelidae). In *J Econ Entomol* 82 (6), pp. 1792–1798. DOI: 10.1093/jee/82.6.1792.

Sutter, G. R.; Fisher, J. R.; Elliott, N. C.; Branson, T. F. (1990): Effect of insecticide treatments on root lodging and yields of maize in controlled infestations of western corn rootworms (Coleoptera: Chrysomelidae). In *J Econ Entomol* 83 (6), pp. 2414–2420. DOI: 10.1093/jee/83.6.2414.

Tabashnik, B. E. (1994): Evolution of Resistance to Bacillus Thuringiensis. In *Annual review of entomology* 39 (1), pp. 47–79. DOI: 10.1146/annurev.en.39.010194.000403.

Tabashnik, B. E.; Finson, N.; Johnson, M. W.; Moar, W. J. (1993a): Resistance to Toxins from Bacillus thuringiensis subsp. kurstaki Causes Minimal Cross-Resistance to *B. thuringiensis* subsp. aizawai in the Diamondback Moth (Lepidoptera: Plutellidae). In *Applied and environmental microbiology* 59 (5), pp. 1332–1335. DOI: 10.1128/aem.59.5.1332-1335.1993.

Tabashnik, Bruce E.; Finson, Naomi; Chilcutt, Charles F.; Cushing, Nancy L.; Johnson, Marshall W. (1993b): Increasing Efficiency of Bioassays: Evaluating Resistance to *Bacillus thuringiensis* in Diamondback Moth (Lepidoptera: Plutellidae). In *J Econ Entomol* 86 (3), pp. 635–644. DOI: 10.1093/jee/86.3.635.

Timper, Patricia; Kaya, Harry K. (1989): Role of the second-stage cuticle of entomogenous nematodes in preventing infection by nematophagous fungi. In *Journal of invertebrate pathology* 54 (3), pp. 314–321. DOI: 10.1016/0022-2011(89)90115-8.

Toepfer, S.; Gueldenzoph, C.; Ehlers, R.-U.; Kuhlmann, U. (2005): Screening of entomopathogenic nematodes for virulence against the invasive western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in in Europe. *Bulletin of Entomological Research*, *95*(5), 473-482

Toepfer, Stefan; Kurtz, Benedikt; Kuhlmann, Ulrich (2010): Influence of soil on the efficacy of entomopathogenic nematodes in reducing *Diabrotica virgifera virgifera* in maize. In *Journal of pest science* 83 (3), pp. 257–264. DOI: 10.1007/s10340-010-0293-6.

Toepfer, Stefan; Zhang, Feng; Kuhlmann, Ulrich (2009): Assessing host specificity of a classical biological control agent against western corn rootworm with a recently developed testing protocol. In *Biological Control* 51 (1), pp. 26–33. DOI: 10.1016/j.biocontrol.2009.07.003.

Tomar, Preety; Thakur, Neelam; Yadav, Ajar Nath (2022): Endosymbiotic microbes from entomopathogenic nematode (EPNs) and their applications as biocontrol agents for agro-environmental sustainability. In *Egypt J Biol Pest Control* 32 (1). DOI: 10.1186/s41938-022-00579-7.

UN (2022): World population prospects. Edited by Department of Economic and Social Affairs: Population Division.

Vachon, Vincent; Laprade, Raynald; Schwartz, Jean-Louis (2012): Current models of the mode of action of Bacillus thuringiensis insecticidal crystal proteins: a critical review. In *Journal of invertebrate pathology* 111 (1), pp. 1–12. DOI: 10.1016/j.jip.2012.05.001.

Valencia, Arnubio; Alves, Analiza P.; Siegfried, Blair D. (2013): Molecular cloning and functional characterization of an endogenous endoglucanase belonging to GHF45 from the western corn rootworm, Diabrotica virgifera virgifera. In *Gene* 513 (2), pp. 260–267. DOI: 10.1016/j.gene.2012.10.046.

van Dam, Nicole M. (2009): Belowground Herbivory and Plant Defenses. In *Annu. Rev. Ecol. Evol. Syst.* 40 (1), pp. 373–391. DOI: 10.1146/annurev.ecolsys.110308.120314.

Vidal, S.; Kuhlmann, U.; Edwards, C. R. (Eds.) (2005): Western corn rootworm: Ecology and management. UK: CAB International.

War, Abdul Rashid; Paulraj, Michael Gabriel; Ahmad, Tariq; Buhroo, Abdul Ahad; Hussain, Barkat; Ignacimuthu, Savarimuthu; Sharma, Hari Chand (2012):

Mechanisms of plant defense against insect herbivores. In *Plant Signaling & Behavior* 7 (10), pp. 1306–1320. DOI: 10.4161/psb.21663.

War, Abdul Rashid; Paulraj, Michael Gabriel; War, Mohd Yousf; Ignacimuthu, Savarimuthu (2011): Jasmonic Acid-Mediated-Induced Resistance in Groundnut (Arachis hypogaea L.) Against Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae). In *J Plant Growth Regul* 30 (4), pp. 512–523. DOI: 10.1007/s00344-011-9213-0.

Wesseler, J.; Fall, E. H. (2010): Potential damage costs of Diabrotica virgifera virgifera infestation in Europe – the 'no control' scenario. In *Journal of Applied Entomology* 134 (5), pp. 385–394. DOI: 10.1111/j.1439-0418.2010.01510.x.

Wouters, Felipe C.; Blanchette, Blair; Gershenzon, Jonathan; Vassão, Daniel G. (2016): Plant defense and herbivore counter-defense: Benzoxazinoids and insect herbivores. In *Phytochemistry reviews : proceedings of the Phytochemical Society of Europe* 15 (6), pp. 1127–1151. DOI: 10.1007/s11101-016-9481-1.

Wu, Felicia; William, Butz P. (2004): The future of genetically modified crops: Lessons from the green revolution.

Yang, Guang; You, Minsheng; Vasseur, Liette; Zhao, Yiying; Liu, Chunhui (2011): Development of RNAi in Insects and RNAi-Based Pest Control. In Margarita Stoytcheva (Ed.): Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment: IntechOpen.

Zhang, Xi; van Doan, Cong; Arce, Carla C. M.; Hu, Lingfei; Gruenig, Sandra; Parisod, Christian et al. (2019): Plant defense resistance in natural enemies of a specialist insect herbivore. In *Proceedings of the National Academy of Sciences of the United States of America* 116 (46), pp. 23174–23181. DOI: 10.1073/pnas.1912599116.

Zhou, Shaoqun; Richter, Annett; Jander, Georg (2018): Beyond defense: Multiple functions of benzoxazinoids in maize metabolism. In *Plant Cell Physiol* 59 (8), pp. 1528–1537. DOI: 10.1093/pcp/pcy064.

Zikmundová, M.; Drandarov, K.; Bigler, L.; Hesse, M.; Werner, C. (2002): Biotransformation of 2-benzoxazolinone and 2-hydroxy-1,4-benzoxazin-3-one by endophytic fungi isolated from *Aphelandra tetragona*. In *Applied and environmental microbiology* 68 (10), pp. 4863–4870. DOI: 10.1128/AEM.68.10.4863-4870.2002.

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4062 # Declaration of Consent

Declaration of consent

on the basis of Article 18 of the PromR Phil.-nat. 19

Name/First Name: Maushe Dorothy

Registration Number: 21-117-023

Study program: Ecology and Evolution

Bachelor ☐ Master ☐ Dissertation ☑

Title of the thesis: Adaptations of entomopathogenic nematodes to plant-derived benzoxazinoids

Supervisor: Prof. Dr. Christelle Roberts
Prof. Dr. Matthias Erb

I declare herewith that this thesis is my own work and that I have not used any sources other than those stated. I have indicated the adoption of quotations as well as thoughts taken from other authors as such in the thesis. I am aware that the Senate pursuant to Article 36 paragraph 1 litera r of the University Act of September 5th, 1996 and Article 69 of the University Statute of June 7th, 2011 is authorized to revoke the doctoral degree awarded on the basis of this thesis.
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