Evaluating integrated pest management approaches to control wireworms in cereal crops

A Thesis
Presented in Partial Fulfillment of Requirements for the
Degree of Master of Science
with a
Major in Entomology
in the
College of Graduate Studies
University of Idaho
by
Atoosa Nikoukar

Major Professor: Arash Rashed, Ph.D.
Committee Members: Nilsa A. Bosque-Pérez, Ph.D.; Edwin Lewis, Ph.D.; Xi Liang, Ph.D.
Department Administrator: Edwin Lewis, Ph.D.

December 2019

Authorization to Submit Thesis

This thesis of Atoosa Nikoukar, submitted for the degree of Master of Science with a Major in Entomology and titled "Evaluating integrated pest management approaches to control wireworms in cereal crops", has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: ______________________ Date: ____________
Arash Rashed, Ph.D.

Committee Members: ______________________ Date: ____________
Nilsa A. Bosque-Pérez, Ph.D.

______________________ Date: ____________
Edwin Lewis, Ph.D.

______________________ Date: ____________
Xi Liang, Ph.D.

Department Administrator: ______________________ Date: ____________
Edwin Lewis, Ph.D.

Abstract

Wireworms are considered a major re-emergence pest of many crops including wheat, barely, potato, corn and legumes in the Pacific Northwest and Intermountain regions of the USA. Neonicotinoid seed treatments, the only group of insecticides registered in cereals against wireworms, have failed to provide acceptable levels of protection against wireworms. Thus, there is a need to test alternative methods to be employed as components of an integrated management protocol. Focusing on one of the most damaging species in the PNW, the sugar beet wireworm *Limonius californicus*, we conducted greenhouse studies to evaluate (1) the efficacy of commercially available and locally-collected entomopathogenic nematodes, and (2) the effects of insecticide application in a rotation crop (pea) in reducing wireworm damage in subsequent wheat. The locally-collected nematode *Steinernema feltiae* isolate Kyle-F1 caused significantly higher mortality (64%) in *L. californicus* larvae than commercial nematode strains including *Steinernema carpocapsae* (30%), *Heterorhabtidis bacteriophora* (6.6%) and *S. feltiae* (10%). The results suggest that field collected nematode isolates, which are well-adapted to environmental conditions, can be potential candidates against wireworms in the field. We also found that an in-furrow application of bifenthrin, a pyrethroid insecticide effective against wireworm in pea, was effective to reduce wireworm damage in subsequent wheat. Bifenthrin-treated pea followed by wheat caused significantly higher mortality (82%) in *L. californicus* larvae than untreated pea. Moreover, 30% higher emergence was observed in wheat following bifenthrin-treated pea. Our results indicate that integration of both chemical approaches and cultural practices (crop rotation) could be a more effective management strategy for wireworm control.

Acknowledgements

I would like to express my sincere gratitude to my advisor Dr. Arash Rashed for the continuous support of my master's study, his patience, motivation and scientific support. His guidance helped me in all times of research and writing of my thesis.

Besides my advisor, I would like to thank my scientific committee members Prof. Nilsa A. Bosque-Pérez., Prof. Edwin Lewis, and Dr. Xi Liang for their insightful comments, encouragement, and scientific assistance.

I thank my fellow labmates, Dr. Pooria Ensafi, Karin Regina Cruzado, Fabiola Aguilar for all the support and time we have had in the last two years.

Dedication

My work is wholeheartedly dedicated to my beloved parents, who have been my source of inspiration and gave me strength when I thought of giving up, who continually provide their moral, spiritual, emotional and financial support.

To my brothers, sister and friends who shared their words of advice and encouragement and were always there for me.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Wireworm Distribution

Wireworms are the larval stage of click beetles (Coleoptera: Elateridae) and one of the most significant subterranean arthropod pests that cause damage in a wide variety of crops (Comstock & Slingerland, 1891; Parker & Howard, 2001). Larvae of some click beetle species are herbivores, living in the soil and feeding on agricultural crops; some species are omnivorous, living in forest habitats or decaying plant material and feeding on small invertebrates and decaying organisms (Johnson, 2002). The Elateridae comprise a large group of beetles with 10,000 species in 400 genera worldwide (Johnson, 2009). In the Nearctic region, nearly 1,000 species of wireworm have been identified (Johnson, 2002); 965 species in 91 genera have been found in North America. The family Elateridae has been listed as the seventh-most species rich family in North America (Marske & Ivie, 2003). One hundred species have been reported from the Holarctic region that includes Europe, North Africa, Middle East, Northern Asia (North China, Japan, etc.) and North America (Vernon et al., 2013a). In Canada and Alaska, 369 species of click beetles have been reported, with 30 species being economically important pests of crops (Benefer et al., 2012; Bousquet, 1991). Based on the Burghause and Schmitt (2011) survey, three wireworm species, *Agriotes lineatus* (Linnaeus 1767), Agriotes obscurus (Linnaeus 1758) and *Agriotes sputator* (Linnaeus 1758), are the most predominant wireworm species in Europe (Burghause & Schmitt, 2011). These wireworm species are also known as major pests in Europe and Canada in various crops, including corn, strawberries, potato and organic vegetables (LaGasa et al., 2005). These species also have been reported in Mongolia, Kazakhstan, Israel, Iran and Korea (Ritter & Richter, 2013). *Limonius canus* (LeConte) also known as the Pacific Coast wireworm, *L. californicus* (Mannerheim) or sugar beet wireworm, *L. infuscatus* (Motschulsky) known as western field wireworm, and *L. subauratus* (LeConte) known as Colombia Basin wireworm, are the most common wireworm species in irrigated land in the Pacific Northwest coast of North America (Andrews et al., 2008; Mail, 1932). In studies of wireworm species In the Pacific Northwest, distribution and ecology has been intensified for the past several years (Milosavljević et al., 2017; Rashed et al., 2015). In their surveys, a total of 14 pest species of wireworms were identified from more than 250 fields distributed in 20 counties in Idaho and Washington state. Three wireworm species *L. infuscatus*, *L. californicus*, and *Selatosomus pruininus* were reported as the most predominant wireworm species in Washington and Idaho (Milosavljević et al., 2017; Rashed et al., 2015).

Biology and Life Cycle

Adult click beetles are elongated, hard bodied, between 15 to 33 mm long, brown to black in color with spotted patterns (Rashed et al., 2015). The posterior corner of the pronotum in these beetles prolongate backward into the spines (Triplehorn & Johnson, 2005). Adult click beetles are characterized for their unusual click mechanisms. A spine on their prosternum can be fitted in the corresponding groove on the mesosternum, which can be released in a sudden action, accompanied with a click sound, driving the beetle into the air.

The motion and its associated click sound helps the beetle to avoid predation and/or right itself when placed on its back (Triplehorn & Johnson, 2005). Female click beetles lay eggs singly or in the clusters of 100 to 300 in May to June. Eggs are small and spherical and are placed near the soil surface (Parker & Howard, 2001). Grassland is more favorable for beetles for depositing eggs because it can cover the eggs and protect them against desiccation, while providing a rich food source for the adult females (Evans & Gough, 1942). The time for hatching eggs is variable among species depending on temperature and it may take between 4 to 6 weeks. In *H. bicolor*, *Ctenicera aeripennis* and *Ctenicera destructor*, eggs take 13-14 days to hatch at 24.5°C (Doane, 1969). Furlun (1998) recorded three incubation times of 45, 14, and 13 days, at different temperatures of 15, 25 and 29°C, for *Agriotes ustulatus* (Furlan, 1998).

Newly hatched larvae are around 1.5 mm in length and can grow up to 25 mm, depending on the species, when fully developed. Click beetle larvae are, slender, wire-shaped, white to brownish in color. The morphology in the last abdominal segment of the larval stage is often used to identify species. The presence or absence of a “caudal notch” and “urogomphi”, appendages formed on the last abdominal segment, are two of the key morphological traits used in wireworm identification. The line pattern on the larval body, “antero-lateral carina”, “prosternum” which is the triangle plate between the head and first pair of legs, are other characteristics for wireworm identification (Etzler, 2013; Rashed et al., 2015).

Larval development varies depending on environmental conditions and species. Stone (1941) recoded 10 to 13 instars in *L. californicus* females and males, respectively under laboratory condition (Stone, 1941). The life cycle of *L. californicus* may take 7 to 11 years. On the other hand, *Agriotes* spp., with 14 instars, complete their life cycle in a much shorter, 2 to 5-year period. Soil moisture and temperature are the two main factors influencing the first molting but the second and third molting only depend on soil temperature (Parker & Howard, 2001).

Fully developed larvae usually pupate between July and September. The larva burrows a deep hole in the soil and makes a pupation cell 3-5 cm below the soil surface (Comstock & Slingerland, 1891). Pupal stage takes 3 to 4 weeks and the emerged beetles usually stay in soil and use the pupation cell for hibernation (Comstock & Slingerland, 1891; Parker & Howard, 2001).

There are differences in the phenology of various species. Doane (1969) reported that *Selatosomus aeripennis* starts its activity from mid-April to mid-July, with the sex ratio of 6:1 (M: F). *Hypnoidus bicolor* starts its activity late April and stays active until July. *H. bicolor* sex ratio has been estimated at 1:2 (M: F). In the Pacific Northwest, Milosavljevic et al. (2016) reported that the two most predominant wireworm species, *L. californicus* and *L. infuscatus*, do not have similar activity patterns. *L. infuscatus* actively feed on plants in April and May and become inactive the rest of the summer while *L. californicus* is active through the growing season from April to August (Milosavljević et al., 2016).

Factors influencing Growth, Development and Movement of Wireworm

Environmental factors such as soil temperature and moisture affect wireworm growth, development and movement (Campbell, 1937). The most favorable soil moisture for most wireworm species ranges between 9 and 12%, however, some wireworm species prefer dry soil such as *H. bicolor* and *Aoelus mellillus*. The wireworm muscular system is highly affected by soil moisture and their movement increases in dry soil; reduced wireworm movement has been reported in association with wet and saturated soils. Wireworms suffer suffocation when they are exposed to saturated soil conditions and this results in death (Campbell, 1937; Lefko et al., 1998). Food (plant tissue) is a source of moisture for wireworms (Campbell, 1937). Preferred soil temperatures by wireworms is in the range of 10-25°C (Fisher et al., 1975). Soil temperature drives vertical movement of wireworms in soil. Increasing temperature in soil surface in mid-summer signals wireworms to move downward into deeper soil. Dropping temperature in fall, lead wireworms to move upward to soil surface. The same occurs when the soil temperature decreases in late October and wireworms migrate deeper into the soil for overwintering (Fisher et al., 1975). In early summer or mid-June as the temperature of soil rises, *H. bicolor* larvae move down in the soil. They are relatively more sensitive to heat and desiccation compared to the prairie grain wireworm, *Selatosomus aeripennis destructor* (Brown) which mostly co-occurs with *H. bicolor* (King et al., 1933). *Limonius californicus* can survive in dry soil at low temperature but at high temperature they move down in the soil (Zacharuk, 1963). *L. californicus* however prefers higher moisture soils and is primarily associated with irrigated fields. At 10-25°C soil temperature, wireworms stay at 8-10 cm depth; when soil temperature increases over 25 °C or falls below 10°C, they migrate vertically down below 10cm in the soil to find the best temperatures (Fisher et al., 1975). Later instar larvae of *S. aeripennis* stay in 10cm depth soil regardless of temperature but the younger larvae drive themselves deep below 15 cm in soil when the temperature falls down (in early August) (King et al., 1933). Generally, the activity of older larvae is more than younger larvae and they show better movement in the soil which result in aggregation and it may be the reason of patchy damage under field conditions (Doane, 1969).

Feeding, larval molting, cultivation practices and soil type affect wireworm movement in soil (Vernon et al., 2014). Feeding behavior of wireworms is also affected by prior soil moisture and soil temperature (Zacharuk, 1963), so field collected wireworms should be kept in laboratory condition prior to using them in any experiments (van Herk et al., 2013). Sandy soils have been reported as a highly favorable habitat for wireworms, which is also associated with higher rate of damage when compared to organic-rich soil (Hermann et al., 2013; Milosavljević et al., 2017; Rashed et al., 2017). Thus, it is hard to predict the wireworm population in the field by sampling because wireworm movement depends on food source, soil moisture and temperature. For the better results, sampling should be done at peak larval activity period (Doane, 1969).

Wireworms as a crop pest

Wireworms have been reported to be attracted to volatile organic compounds (VOC) and carbon dioxide released by germinating seeds. As polyphagous larvae and having biting and chewing mouth parts, they are reported as a major pest for various crops (Doane, 1969; Gfeller et al., 2013; Traugott et al., 2015). Wireworms were identified as agricultural pests in the 19th century in USA (Comstock & Slingerland, 1891). Due to their species diversity, long larval stage, poorly known taxonomy and life history, and cryptic habitat, they are very difficult to detect and manage (Traugott et al., 2015). They cause serious damage to forage, fruit and vegetable crops (Vernon et al., 2000). Some primary examples include corn, small grains, potato, sugar beet and strawberries (Parker & Howard, 2001; Reddy et al., 2014). Cropping history of a field plays an important role in the risk and extent of wireworm damage. Wireworm infestation is expected to be higher in fields with 1 to 5 years of cereals, on managed fallow (grassy weeds)/pasture and in soil with high organic matter (Adhikari & Reddy, 2017; Schepl & Paffrath, 2005). Wheat and barley are economically important crops grown in the Pacific Northwest and Intermountain regions of the USA. In the PNW cropping systems, wheat and barley are mostly rotated with a wide range of crops such as pulses, potato, sugar beet, all of which can also be damaged by wireworms (Esser et al., 2015a; Milosavljević et al., 2017).

The rate of damage caused by wireworms depends on various factors including plant density, growth stages, vigor, plant and wireworm species and wireworm density in soil (Adhikari & Reddy, 2017). Larvae mostly feed on underground parts such as germinating seed, root, seedling stems and tubers, causing crop thinning and serious yield loss (Vernon et al., 2008a). In cereals, failed germination occurs when wireworms feed on planted and sprouting seeds. Feeding on seedlings can initially be characterized by the presence of dead central leaves which ultimately leads to wilted or dead seedlings (Rashed et al., 2017). Wireworm damage to stems at later stages of plant development may appear as delayed growth or reduced head formation. Patches of wilting and/or dead seedlings in a field may be indicators of wireworm damage that could result in yield reduction (Barsics et al., 2013; Ritter & Richter, 2013). Plants are weakened by wireworm damage and fields can be taken over by weeds competing for the same resources (Antwi et al., 2018; Thomas, 1940). In small grains, wireworm damage can reach 65% on standing crops which may require replanting of crops. Also, wireworms are serious pests of corn which is the third most important agricultural crop worldwide (FAO 2005), causing up to 35% crop damage. Moreover, wireworms feed on beans, peas, carrots, tomatoes, as well as brassicas in seedling and later stages (Adhikari & Reddy, 2017).

Wireworm Management

Chemical Control

Wireworms were considered severe crop pests of cereals and other crops since the early 20th century (Comstock & Slingerland, 1891). At the time, organochlorine and organophosphate insecticides were used to control them (Vernon et al., 2001). However, because of their persistence in soil, toxicity to humans, non-target species, and negative effects on the environment, some of these pesticides were later banned. Lindane is one example of those effective chemistries which its use was limited in 2002 and banned in 2009 by the Environmental Protection Agency. After that, organophosphates (broad spectrum insecticides) were used to contain wireworm damage (Adhikari & Reddy, 2017; Horne & Horne, 1991). However, some inconsistent results were reported from various organophosphate insecticides likely due to variation in wireworm's heterogenous distribution, wireworm's species, soil type and application methods (Furlan et al., 2010).

Newer classes of insecticides such as neonicotinoids and phenylpyrazole provided only moderate control against wireworms (Kuhar & Alvarez, 2008).

Currently, imidacloprid, thiamethoxam and clothianidin are the available neonicotinoid insecticides to control wireworms in cereal crops. Van Herk and Vernon (2007) reported that wireworms that fed on neonicotinoid treated seeds became intoxicated but later recovered and restarted their feeding activity. Thus, neonicotinoids do not inflict high levels of mortality in wireworms and primarily serve as a feeding deterrent (Van Herk et al., 2008). This nonlethal effect would not reduce populations and may promote later resistance to the insecticide. Also, there are some increasing concerns related to potential detrimental effects of noenicitinoid treatments on pollinators in the environment (Godfray et al., 2014).

Wireworm species can vary in their ecology and feeding activity. Some wireworm species start their activity early in the spring and remain active throughout the season, while others may start feeding later into the growing season (Milosavljević et al., 2017; Traugott et al., 2015). Therefore, neonicotinoids applied at planting, as seed treatments, may not offer an effective strategy to manage the wireworm species that are active throughout, or later into, the growing season (Esser et al., 2015a; Milosavljević et al., 2017). The inconsistency in the efficacy of neonicotinoid seed treatments against wireworms makes evaluating and developing alternative, and integrated approaches essential to offer a sustainable management solution for these pests (Andrews et al., 2008).

Biological Control

Wireworms are subterranean organisms, and within their habitat, they are exposed to various soil-living natural enemies including entomopathogenic fungi and nematodes. They do not provide immediate control but once the agents are established in the environment, it can offer an inexpensive, and possibly long-term management of wireworms. Two genera of fungi *Beauveria* and *Metarhizium* were shown to be able to reduce wireworm damage in the field (Jansson & Seal, 1994). A field study conducted in Europe showed that the commercial strain of *B. bassiana* was able to reduce wireworm population compared to the untreated control plots (Ladurner et al., 2008). Similarly, *B. bassiana* under laboratory conditions inflicted significantly higher mortality rates (50%) compared to untreated controls (13%) (Sufyan et al., 2017). Reddy et al. (2014) reported significant differences among the application methods of entomopathogenic fungi rather than the species of fungi in their effects against wireworms. *M. brunneum* F52, *B. bassiana* GHA and *M. robertsii* DWR 346 applied as formulated granules or in-furrow, resulted in higher seed emergence and yields than the fungus-coated seed treatments or the untreated control in wheat (Reddy et al., 2014).

Entomopathogenic nematodes (EPNs), which mostly belong to the two families Steinernematidae and the Heterorhabditidae, are obligate parasites of insects (Burnell & Stock, 2000). EPN foraging behavior varies among different species which can have direct influence on their parasitism efficacy (Griffin et al., 2005). Based on their foraging strategies, entomopathogenic nematodes are classified as cruise (active), ambush (sit-and-wait) and intermediate foragers (Huey & Pianka, 1981; Lewis et al., 2006; McLaughlin, 1989). There are some reports that showed parasitic nematodes have been applied successfully to control some important lepidopteran, dipteran and coleopteran pests in different economically important crops (Burnell & Stock, 2000; Grewal et al., 2005a). However, susceptibility of wireworms to EPN appears to be variable depending on the species of nematode. *Steinernema carpocapsae* (Weiser) has shown some promising results to control wireworms when used with resistant varieties or some insecticides (Schalk et al., 1993). However, the results of Toba et al. (1983) showed the non-effectiveness of S. *carpocapsae* against *L. californicus.* Likewise, Ensafi et al. (2018) have found less than 20% efficacy of *S. carpocapsae* to control sugar beet wireworm. In a laboratory study conducted by Ansari et al. (2009), different strains of *Heterorhabditis bacteriophora* caused 17% to 67% mortality in *Agriotes lineatus* larvae, while *Steinernema feltiae* was not effective in infecting this species. However, the native field-collected strains of *Steinernema feltiae* in Spain caused 7% and 9% mortality in *A. sordidus*, in 5 and 2 days (Campos-Herrera & Gutiérrez, 2009). The efficacy of entomopathogenic nematodes in biological control is highly dependent on factors such as host species, host developmental stage, nematode species and environmental conditions (Grewal et al., 2005b; Lewis et al., 2006). In the case of wireworms, some physical deterrents in their body including dense hairs in the oral cavity, muscular tissues that keep the anus closed, as well as the heavily sclerotized cuticle reduce their susceptibility to entomopathogenic nematodes (Eidt & Thurston, 1995). However, field-collected EPN species, which are adapted to the local environmental conditions and have been exposed to wireworms for a long time, could be promising candidates for controlling wireworms affecting local crops. This area needs to be further explored in wireworms which will be the focus of this study.

Cultural Control

There are few cultural practices that have been recommended to reduce wireworm pressure. Increased seeding density to compensate for wireworm damage is one of the cultural practices adopted by some wheat and barley producers. However, the effectiveness of such cultural practices for reducing wireworm damage has yet to be evaluated. There is one report by Bryson (1930) who found greater rate of damaged plants in a wireworm infested corn field planted early in April compared to those planted in late May (Bryson, 1930). Some commonly-recommended practices to improve soil conditions and to maximize profit, such as no-tillage, lack of proper rotation, continuous cropping, and even irrigation, make field conditions favorable for wireworms, and thus may contribute to increased wireworm abundance (Adhikari & Reddy, 2017).

Wireworm populations are influenced by cropping history and crop rotation. Mustard and cabbage are rich in glucosinolate and rotation to these crops have provided effective control of soil-borne pests (Lichtenstein et al., 1964). Using *Brassica* meal in potted potato reduced wireworm damage (Furlan et al., 2004). Four to five years rotation with alfalfa in Idaho has also been reported to reduce wireworm populations in heavily infested fields (Shirck, 1945). Despite reports of the effectiveness of crop rotation in wireworm management, this practice may not be suitable or profitable in some cropping systems. Alfalfa is not economically competitive with cereals in the Pacific Northwest and wheat and barley are commonly rotated with a wide range of crops such as pea, bean, potato and sugar beet, only where possible.

To date, current management practices, *per se*, failed to provide acceptable levels of wireworm control. They may be effective in protecting crops from wireworm damage for short time; however, they are not able to reduce wireworm populations considerably. So, there is a need to develop integrated management approaches to control wireworms. To evaluate the effectiveness of integrated management practices for wireworm management, Esser et al. (2015) conducted a long-term field experiment applying neonicotinoid seed treatment and crop rotation to control the damage by two wireworm species, *L. californicus* and *L. infuscatus*, in east-central Washington cereals. They found that applying thiamethoxam in the fields highly infested with *L. californicus*, significantly increased crop yield, however, it did not reduce the wireworm population. Conversely, *L. infuscatus* abundance was decreased due to thiamethoxam treatment, even though there was no increase in crop yield. These results might be affected by either different phenology of the two species or variation in susceptibility to insecticide among the wireworm species (Esser et al., 2015b; Vernon et al., 2008b). They also found that no-till summer fallow in rotation with winter wheat may help to reduce wireworm populations in both species of *L. californicus* and *L. infuscatus* (Esser et al., 2015b). Reduction in food availability for wireworms in fallow rotation may result in low abundance of wireworm in subsequent crops. Moreover, collecting small larvae in the solar bait traps, which may be related to eggs laid in the previous year, could be an evidence for the hypothesis that fallow fields may be less favorable for oviposition sites for adults than sites planted with spring wheat (Esser et al., 2015a; Willis et al., 2010). This study's results provided crucial evidence that IPM (Integrated Pest Management) for wireworms may be the most effective control approach. There are insecticides with mortality effects on wireworms that are registered for wireworm management in rotation crops such as legumes, potato and sugar beet, are not labelled for cereals. The application of insecticides, with efficacy against wireworms, in rotation crops may help to reduce wireworm damage in subsequent cereals. The effectiveness on integrating chemical and cultural (i.e., rotation) in managing wireworm pressure in wheat and barley is an area that needs further exploration.

The purpose of this study was to assess alternative and integrated management approaches to reduce damage by the sugar beet wireworm, *L. californicus*, under controlled laboratory and greenhouse conditions. In Chapter 2, I evaluated the efficacy of native nematodes against wireworms and compared the effectiveness of these native EPNs to commercially available EPNs.

In Chapter 3, I studied whether applying an insecticide with mortality effect against wireworms, in pea (as a rotation crop) will help to reduce damage in a subsequent wheat crop. In the final chapter, Chapter 4, I summarized my findings and provided a brief conclusion based on my results.

Literature Cited

Adhikari A & Reddy GV (2017) Evaluation of trap crops for the management of wireworms in spring wheat in Montana. J Arthropod-Plant Interactions 11: 755-766.

Andrews N, Ambrosino MD, Fisher GC & Rondon SI (2008) Wireworm: biology and nonchemical management in potatoes in the Pacific Northwest. PNW Ext. Pub.

Ansari M, Evans M & Butt T (2009) Identification of pathogenic strains of entomopathogenic nematodes and fungi for wireworm control. J Crop protection 28: 269-272.

Antwi FB, Shrestha G, Reddy GV & Jaronski ST (2018) Entomopathogens in conjunction with imidacloprid could be used to manage wireworms (Coleoptera: Elateridae) on spring wheat. J The Canadian Entomologist 150: 124-139.

Barsics F, Haubruge E & Verheggen F (2013) Wireworms' management: an overview of the existing methods, with particular regards to Agriotes spp.(Coleoptera: Elateridae). J Insects 4: 117-152.

Benefer CM, Knight ME, Ellis JS, Hicks H & Blackshaw RP (2012) Understanding the relationship between adult and larval Agriotes distributions: the effect of sampling method, species identification and abiotic variables. J Applied soil ecology 53: 39-48.

Bousquet Y (1991) Checklist of beetles of Canada and Alaska. Agriculture Canada Ottawa.

Bryson HR (1930) A study of field practices as related to wireworm infestations (Elateridae). J Journal of economic entomology 23: 303-315.

Burghause F & Schmitt M (2011) Monitoring of the clickbeetle genus Agriotes (Elateridae, Coleoptera) in Rhineland-Palatinate in the years 2008 to 2010. J Gesunde Pflanzen 63: 27-32.

Burnell AM & Stock SP (2000) Heterorhabditis, Steinernema and their bacterial symbionts-lethal pathogens of insects. J Nematology 2: 31-42.

Campbell RE (1937) Temperature and moisture preferences of wireworms. J Ecology 18: 479-489.

Campos-Herrera R & Gutiérrez C (2009) Screening Spanish isolates of steinernematid nematodes for use as biological control agents through laboratory and greenhouse microcosm studies. J Journal of invertebrate pathology 100: 100-105.

Comstock JH & Slingerland MV (1891) Wireworms. Cornell University.

Doane JF (1969) A method for separating the eggs of the prairie grain wireworm, Ctenicera destructor, from soil. J The Canadian Entomologist 101: 1002-1004.

Eidt D & Thurston GS (1995) Physical deterrents to infection by entomopathogenic nematodes in wireworms (Coleoptera: Elateridae) and other soil insects. J The Canadian Entomologist 127: 423-429.

Ensafi P, Crowder DW, Esser AD, Zhao Z, Marshall JM & Rashed A (2018) Soil Type Mediates the Effectiveness of Biological Control Against Limonius californicus (Coleoptera: Elateridae). J Journal of economic entomology 111: 2053-2058.

Esser AD, Milosavljević I & Crowder DW (2015a) Effects of neonicotinoids and crop rotation for managing wireworms in wheat crops. J Journal of economic entomology 108: 1786-1794.

Esser AD, Milosavljević I & Crowder DWJJoee (2015b) Effects of neonicotinoids and crop rotation for managing wireworms in wheat crops. 108: 1786-1794.

Etzler FE (2013) Identification of economic wireworms using traditional and molecular methods: Montana State University-Bozeman, College of Agriculture.

Evans A & Gough H (1942) Observations on some factors influencing growth in wireworms of the genus Agriotes Esch. J Annals of Applied Biology 29: 168-175.

Fisher J, Keaster A & Fairchild M (1975) Seasonal vertical movement of wireworm larvae in Missouri: influence of soil temperature on the genera Melanotus Escholtz and Conoderus Escholtz. J Annals of the Entomological Society of America 68: 1071-1073.

Furlan L (1998) The biology of Agriotes ustulatus Schäller (Col., Elateridae). II. Larval development, pupation, whole cycle description and practical implications. Journal of Applied Entomology 122: 71-78.

Furlan L, Bonetto C, Patalano G & Lazzeri L (2004) Potential of biocidal meals to control wireworm populations, Vol. 31: Proceedings of the first International Symposium on "Biofumigation: a possile alternative to methyl bromide (ed., pp. 313-316.

Furlan L, Bonetto C, Finotto A, Lazzeri L, Malaguti L, Patalano G & Parker W (2010) The efficacy of biofumigant meals and plants to control wireworm populations. J Industrial Crops Products 31: 245-254.

Godfray HCJ, Blacquiere T, Field LM, Hails RS, Petrokofsky G, Potts SG, Raine NE, Vanbergen AJ & McLean AR (2014) A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. J Proceedings of the Royal Society B: Biological Sciences 281: 20140558.

Grewal P, Koppenhöfer A & Choo H (2005a) Lawn, turfgrass and pasture applications. J Nematodes as biocontrol agents: 115-146.

Grewal PS, Ehlers R-U & Shapiro-Ilan DI (2005b) Nematodes as biocontrol agents. CABI.

Griffin C, Boemare NE & Lewis EE (2005) Biology and behaviour. J Nematodes as biocontrol agents: 47-64.

Hermann A, Brunner N, Hann P, Wrbka T & Kromp B (2013) Correlations between wireworm damages in potato fields and landscape structure at different scales. J Journal of pest science 86: 41-51.

Horne PA & Horne JA (1991) The life-history and control of Hapatesus hirtus Candeze (Coleoptera: Elateridae) in Victoria. J Australian Journal of Agricultural Research 42: 827-834.

Huey RB & Pianka ER (1981) Ecological consequences of foraging mode. J Ecology 62: 991-999.

Jansson RK & Seal D (1994) Biology and management of wireworm on potato. Advances in Potato Pest Biology Management. APS Press, St. Paul, MN: 31-53.

Johnson P (2009) Classification of the Nearctic genera of Elateridae.

Johnson PJ (2002) 58. Elateridae Leach 1815. J American Beetles 2: 160-173.

King K, Arnason A & Glen R (1933) The wireworm problem in field crops of western Canada. J Saskatoon Entomol. Branch Lab. Leafl. 86: -.

Kuhar TP & Alvarez JM (2008) Timing of injury and efficacy of soil-applied insecticides against wireworms on potato in Virginia. J Crop protection 27: 792-798.

Ladurner E, Benuzzi M, Fiorentini F & Franceschini S (2008) Beauveria bassiana strain ATCC 74040 (Naturalis®), a valuable tool for the control of the cherry fruit fly (Rhagoletis cerasi): In Proceeding of Ecofruit-13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: 93-97. Weinsberg/Germany.

LaGasa EH, Loucks J & Spurrier L (2005) 2004 Western Washington Delimiting Survey for *Agriotes obscurus* and *A. lineatus* (Coleoptera: Elateridae), Exotic Wireworm Pests New to the United States. Washington State Department of Agriculrure.Pub. 805:144p.

Lefko SA, Pedigo LP, Batchelor WD & Rice ME (1998) Spatial modeling of preferred wireworm (Coleoptera: Elateridae) habitat. J Environmental entomology 27: 184-190.

Lewis EE, Campbell J, Griffin C, Kaya H & Peters A (2006) Behavioral ecology of entomopathogenic nematodes. J Biological control 38: 66-79.

Lichtenstein E, Morgan D & Mueller CFC (1964) Insecticides in nature, naturally occurring insecticides in Cruciferous crops. J Journal of Agricultural 12: 158-161.

Mail AG (1932) Ph and wireworm incidence. J Journal of economic entomology 25: 836-840.

Marske KA & Ivie MA (2003) Beetle fauna of the United States and Canada. J The Coleopterists Bulletin 57: 495-504.

McLaughlin RL (1989) Search modes of birds and lizards: evidence for alternative movement patterns. J The American Naturalist 133: 654-670.

Milosavljević I, Esser AD & Crowder DW (2016) Effects of environmental and agronomic factors on soil-dwelling pest communities in cereal crops. Agriculture, Ecosystems & Environment 225: 192-198.

Milosavljević I, Esser AD & Crowder DW (2017) Seasonal population dynamics of wireworms in wheat crops in the Pacific Northwestern United States. J Journal of pest science 90: 77-86.

Parker WE & Howard JJ (2001) The biology and management of wireworms (Agriotes spp.) on potato with particular reference to the UK. J Agricultural Forest Entomology 3: 85-98.

Rashed A, Etzler F, Rogers C & Marshall J (2015) Wireworms in Idaho cereals: Monitoring and identification. J University of Idaho Extension Bulletin 898.

Rashed A, Rogers CW, Rashidi M & Marshall JM (2017) Sugar beet wireworm Limonius californicus damage to wheat and barley: evaluations of plant damage with respect to soil media, seeding depth, and diatomaceous earth application. J Arthropod-Plant Interactions 11: 147-154.

Reddy GV, Tangtrakulwanich K, Wu S, Miller JH, Ophus VL, Prewett J & Jaronski ST (2014) Evaluation of the effectiveness of entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat. J Journal of invertebrate pathology 120: 43-49.

Ritter C & Richter E (2013) Control methods and monitoring of Agriotes wireworms (Coleoptera: Elateridae). J Journal of Plant Diseases Protection 120: 4-15.

Schalk J, Bohac J, Dukes P & Martin W (1993) Potential of non-chemical control strategies for reduction of soil insect damage in sweetpotato. J Journal of the American Society for Horticultural Science 118: 605-608.

Schepl U & Paffrath A (2005) Strategies to regulate the infestation of wireworms (Agriotes spp.) in organic potato farming: Results. J IOBC–Bulletin 28: 101-104.

Shirck FH (1945) Crop rotations and cultural practices as related to wireworm control in Idaho. Journal of Economic Entomology 38: 627-633.

Stone MW (1941) Life history of the sugar-beet wireworm in southern California. US Dept. of Agriculture.

Sufyan M, Abbasi A, Gogi MD, Arshad M, Nawaz A & Neuhoff D (2017) Efficacy of Beauveria bassiana for the management of economically important wireworm species (Coleoptera: Elateridae) in organic farming. J Gesunde Pflanzen 69: 197-202.

Thomas CA (1940) The biology and control of wireworms. A review of the literature. Bulletin of the Pennsylvania Agricultural Experiment Station.

Toba HH, Lindegren JE, Turner JE & Vail PV (1983) Susceptibility of the Colorado potato beetle and the sugarbeet wireworm to Steinernema feltiae and S. glaseri. J Journal of nematology 15: 597.

Traugott M, Benefer CM, Blackshaw RP, van Herk WG & Vernon RS (2015) Biology, ecology, and control of elaterid beetles in agricultural land. J Annual Review of Entomology 60: 313-334.

Triplehorn C & Johnson N (2005) Borror and DeLong's Introduction to the Study of Insects. 2005. J Brooks Cole. Belmont, CA.

Van Herk W, Vernon R, Tolman J & Ortiz Saavedra H (2008) Mortality of a wireworm, Agriotes obscurus (Coleoptera: Elateridae), after topical application of various insecticides. J Journal of economic entomology 101: 375-383.

van Herk WG, Vernon RS & McGinnis S (2013) Response of the dusky wireworm, Agriotes obscurus (Coleoptera: Elateridae), to residual levels of bifenthrin in field soil. J Journal of pest science 86: 125-136.

Vernon B, Lagasa E & Philip H (2001) Geographic and temporal distribution of Agriotes obscurus and A. lineatus (Coleoptera: Elateridae) in British Columbia and Washington as determined by pheromone trap surveys. J Journal of the Entomological Society of British Columbia 98: 257-266.

Vernon R, Van Herk W, Tolman J, Ortiz Saavedra H, Clodius M & Gage B (2008a) Transitional sublethal and lethal effects of insecticides after dermal exposures to five economic species of wireworms (Coleoptera: Elateridae). J Journal of economic entomology 101: 365-374.

Vernon R, Van Herk W, Tolman J, Ortiz Saavedra H, Clodius M & Gage BJJoEE (2008b) Transitional sublethal and lethal effects of insecticides after dermal exposures to five economic species of wireworms (Coleoptera: Elateridae). 101: 365-374.

Vernon RS, Kabaluk T & Behringer A (2000) Movement of Agriotes obscurus (Coleoptera: Elateridae) in strawberry (Rosaceae) plantings with wheat (Gramineae) as a trap crop. J The Canadian Entomologist 132: 231-241.

Vernon RS, van Herk WG, Clodius M & Harding C (2013) Crop protection and mortality of Agriotes obscurus wireworms with blended insecticidal wheat seed treatments. J Journal of pest science 86: 137-150. doi:10.1007/s10340-011-0392-z.

Vernon RS, Van Herk WG, Clodius M & Harding C (2014) Further studies on wireworm management in Canada: damage protection versus wireworm mortality in potatoes. J Journal of economic entomology 106: 786-799.

Willis RB, Abney MR, Holmes GJ, Schultheis JR & Kennedy GG (2010) Influence of preceding crop on wireworm (Coleoptera: Elateridae) abundance in the coastal plain of North Carolina. J Journal of economic entomology 103: 2087-2093.

Zacharuk R (1963) Comparative food preferences of soil-, sand-, and wood-inhabiting wireworms (Coleoptera, Elateridae). J Bulletin of entomological research 54: 161-165.

Chapter 2: Efficacy Evaluation of Naturally Occurring and Commercially Available Entomopathogenic Nematodes for Managing Sugar Beet Wireworm

Abstract

Wireworms are a major resurging pest of many crops, including wheat, barley, potato, corn and legumes in the Pacific Northwest (PNW) and Intermountain regions of the USA. Neonicotinoid seed-treatments are the only group of insecticides registered for application in cereals. These seed treatments, however, have not been consistently effective in reducing wireworm damage. Thus, there is a need for alternative control methods to be developed and subsequently implemented as a component of an integrated management approach. To date, several studies have been conducted to identify entomopathogenic organisms that may effectively reduce wireworm populations. Focusing on one of the most damaging species in the PNW, the sugar beet wireworm *Limonius californicus* (Coleptera: Elateridae), we conducted a greenhouse study to evaluate the efficacy of commercially available entomopathogenic nematodes, *Steinernema carpocapsae* and *Heterorhabtidis bacteriophora*, and locally collected *Steinernema feltiae* from two different dryland barley fields. locally collected nematodes caused significantly higher mortality (64%) in wireworms than either *S. carpocapsae* (30%) or *H. bacteriophora* (6.6%) ($P = 0.010$). *H. bacteriophora* was not effective against sugar beet wireworms. Later, we also compared efficacies of the commercial and locally collected *S. feltiae*. Field-collected strains caused significantly higher mortality (56.67%) than the commercially available *S. feltiae* (10%) (P=0.002). The results of

this chapter suggest that local entomopathogenic strains, which are well-adapted to environmental conditions can be promising candidates for a biocontrol approach against wireworms in the field.

Introduction

Wireworms, the larval stage of click beetles (Coleoptera: Elateridae), are generalist herbivores that feed on a wide range of crops including potato, onion, sugar beet, carrot, wheat and barley. They feed on different plant tissues and can cause serious damage which may lead to delayed growth, and plant death. In addition, wounds resulting from their feeding on plants can facilitate secondary infections by opportunistic pathogens (Traugott et al., 2015; Vernon et al., 2008a).

Wheat and barley are economically important crops grown in the Pacific Northwest and Intermountain regions of the USA. These crops' contribution to the regional economy is estimated at $1.95 billion in 2018 (USDA NASS 2018). Until early 2000s, wireworms were not considered as serious pests due to the availability of broad-spectrum insecticides which were effective in reducing populations (Toba et al., 1985). The availability of those inexpensive and effective insecticides masked the importance of identifying alternative management approaches to reduce wireworm pressure for more than 40 years (Vernon et al., 2013a). Those chemistries are now banned and removed from the market because of their potential detrimental effects on human health, non-target organisms and the environment. Subsequently, in the absence of an effective replacement, wireworms re-emerged as severe pest especially in cereal crops following degradation of the previously-used persistent conventional insecticides in the soil combined with practices that favor wireworm survival, such as increased no/minimum tillage (Adhikari & Reddy, 2017; Jedlička & Frouz, 2007; Vernon et al., 2008a). Currently, growers rely on neonicotinoid seed treatments as the only class of insecticide that is registered for application in cereals to control wireworms. However, neonicotinoids do not inflict high levels of mortality in wireworms and primarily serve as a feeding deterrents (Van Herk et al., 2008). Also, there are some increasing concerns related to potential detrimental effects of noenicitinoid treatments on pollinators in the environment (Godfray et al., 2014).

There are different wireworm species that can damage cereals (Rashed et al. 2015). Wireworm species can vary in their ecology and feeding activity. Some wireworm species start their activity early in the spring and remain active throughout the season, while others may actively feed later into the growing season (Milosavljević et al., 2017; Traugott et al., 2015). Therefore, neonicotinoid seed treatments may not be an effective strategy to manage wireworm species that are active throughout, or later into, the growing season (Esser et al., 2015a; Milosavljević et al., 2017). The inconsistency in the efficacy of neonicotinoid seed treatments against wireworms further highlights the importance of evaluating and developing alternative, and integrated approaches, to offer a sustainable management solution for these pests (Andrews et al., 2008).

Wireworms are subterranean organisms, and within their habitat, they are exposed to various soil-living natural enemies including entomopathogenic fungi and nematodes. In relation to this, entomopathogenic fungi such as *Metarhizium* spp. and *Beauveria bassiana*, have been reported to cause considerable mortality in wireworms under field and laboratory conditions, and may be promising as biological control agents against this pest (Ester & Huiting, 2007; Kabaluk et al., 2007; Reddy et al., 2014; Sufyan et al., 2017).

Entomopathogenic nematodes (EPNs), which mostly belong to the families Steinernematidae and the Heterorhabditidae, are obligate parasites of insects. These soil-dwelling nematodes are associated with endosymbiont bacteria which are required for killing and digesting the insect's tissues as well as providing nutrients for nematode growth and development (Burnell & Stock, 2000). The infective stage of entomopathogenic nematodes is the non-feeding third stage juvenile (IJ) that occurs free in the soil and seek out invertebrate prey to infect (Lewis et al., 2006). Insects' movements and carbon dioxide are the main cues for infective juveniles to locate their host (Bedding, 2006). Once the infective juveniles get into insects' hemocoel, their symbiont bacteria start to multiply and release toxins and enzymes that digest the host tissue, which results in death of the insect. Nematode development resumes inside the host body and new infective juveniles emerge into the soil (Burnell & Stock, 2000). EPN foraging behavior vary among different species and have direct influence on their parasitism efficacy (Griffin et al., 2005). Based on their foraging strategies, entomopathogenic nematodes are classified as cruise (active), ambush (sit-and-wait) and intermediate foragers (Huey & Pianka, 1981; Lewis et al., 2006; McLaughlin, 1989). Cruise foragers actively move through the soil and seek cues to find their hosts. Among commercially available nematodes, *Heterorhabditis bacteriophora* are cruisers and particularly effective against sedentary hosts. Ambush foragers, on the other hand, have less mobility in the environment and are effective to find active insects. *Steinernema carpocapsae* is the extreme ambusher among commercially-available EPNs. Some EPN species such as *Steinernema feltiae* have intermediate foraging behavior and can be effective against a wide range of sedentary to mobile hosts (Griffin et al., 2005; Lewis et al., 2006).

To date, parasitic nematodes have been applied successfully to control some important lepidopteran, dipteran and coleopteran pests in different economically important crops (Burnell & Stock, 2000; Grewal et al., 2005a). However, susceptibility of wireworms to EPN appears to be variable depending on the species of nematode. It has been reported that applying different strains of *Heterorhabditis bacteriophora* caused 17% to 67% mortality in *Agriotes lineatus* larvae, while *Steinernema feltiae* was not effective to infect *A. lineatus* (Ansari et al., 2009). The Spanish strain of *S. feltiae* collected from Rioja, Spain caused as low as 7% mortality in 2 days in *A. sordidus* larvae under laboratory conditions while *S. carpocapsae* showed lower virulence, 4-5% mortality within 4-7 days, in this insect larvae (Campos-Herrera & Gutiérrez, 2009). The efficacy of parasitic nematodes in biological control highly depends on factors such as host species, host developmental stage, nematode species and abiotic environmental conditions (Grewal et al., 2005b; Lewis et al., 2006). However, native field-collected EPN species that are adapted to the local environmental conditions, have been reported as the better candidates to control the endemic insects (Campos-Herrera & Gutiérrez, 2009; Morton & Garcia-del-Pino, 2017); this area needs to be further explored in wireworms.

The aim of this study was to examine the virulence of some naturally occurring entomopathogenic nematodes and commercially available EPN species against the sugar beet wireworm, *Limonius californicus,* in a controlled laboratory setup. Sugar beet wireworm is a predominant wireworm species, and most damaging in wheat and barley crops, in the Pacific Northwest (PNW) and intermountain regions of the USA. The sugar beet wireworm is known to be active throughout the growing season (Milosavljević et al., 2017; Rashed et al., 2015).

Methods and Materials

Source of Wireworm and Study Location

This study was conducted in the Eastern Idaho Entomology Laboratory, at the University of Idaho, Aberdeen Research and Extension Center, Aberdeen, ID, between August 2107 and June 2018. The average daily temperature record in the laboratory was 23±2 °C. Wireworms were collected from a dryland wheat field located near Ririe, ID (43°340 56.500 N 111°320 21.500 W), using multiple solar bait traps. Solar bait traps consisted of a mixture of soaked untreated wheat and barley seeds, buried 6 inches deep in the ground and covered with soil and a black plastic bag; sprouting seeds in the soil release CO_2 and other chemical compounds that attract wireworms to the bait. After two weeks, germinated seeds with the surrounding soil were recovered and transported to the laboratory to collect the captured wireworms (Rashed et al., 2015). Collected wireworms were placed individually in 5×5×10 cm (W×L×H) plexiglass containers filled with sand, and two barley seeds as their feeding source. Containers were kept at room temperature and the sand surface was kept moist until wireworms were used in experiments.

Soil Media Preparation

The soil media used for this experiment was a mixture of sieved sand and peatmoss (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada) in the ratio of 75% and 25%, respectively. The mixed soil also contained 370g fertilizer (Osmocote, Scott- Sierra Horticultural Products Co., Marysville, OH) and 112g vermiculite (Therm-o-Rock West Inc., Chandler, AZ) per each load, which is around 22,300g of mixed soil. Before mixing the soil, all ingredients were sterilized in an oven to remove all pathogens and microorganisms. Peat-moss and vermiculate were put in oven-safe containers, covered with aluminum foil and were baked in 82°C for 30 minutes. For sterilizing sand, the oven was preheated to 120°C, and the covered sand container with foil was baked for 4 hours. The soil mix was homogenized in a soil mixer before placing into the pots.

Entomopathogenic Nematode Source

Three commercially available species of entomopathogenic nematodes as well as one local isolate of entomopathogenic nematode (collected from two fields, see below), were compared in this experiment. Local nematodes were recovered from the soil, collected from two wireworm infested fields in Soda Springs, ID, (42°4554.2 N 111°4057.6 W and 42°4208.9 N 111°3352.7 W) (Table 1). The *Galleria* white trap method was used to extract the pathogenic nematodes from the soil (Bedding & Akhurst, 1975). Briefly, multiple samples of soil collected from each field were mixed. Then, one hundred grams of soil samples from each location were placed in the plastic containers, and seven waxworms, *Galleria mellonella* (Lepidoptera: Pyralidae) (waxworm; Timberline Fisheries, Marion, IL), were put in each container. Containers were kept in the dark room at room temperature for 2-3 days. The infected larvae were rinsed with tap water and moved to a white trap to recover EPNs. In white traps, seven infected waxworm larvae were placed on filter paper on 50 mm petri dishes inside the 9 cm petri dishes that were filled with tap water (White, 1927). After approximately 10 days the infective juveniles (IJs) emerged from dead waxworm bodies and swam into the water. Emerged nematodes were identified morphologically and then identifications were later confirmed molecularly. For morphological identification, some morphometric features were measured which included body length, body width, tail length, nerve ring and the distance from anterior body to the secretory-excretory pore. Molecular identification/confirmation was performed based on partial ribosomal RNA gene complex sequencing (internal transcribed spacer; ITS1 and ITS2) and D2D3 expansions of 28S (See Ensafi 2018, for more details). The collected nematodes from both fields were determined to be *Steinernema feltiae* (Ensafi 2018). Thereafter, *S. feltiae* collected from the two fields are referred to as *S. feltiae* isolate Kyle-F1 and *S. feltiae* isolate Curtis-F2.

The two species of commercially available EPN in this experiment were *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* obtained through ARBICO Organics Co. (Oro Valley, OR). The first set of experiments did not include the commercial *Steinernema feltiae* and were conducted to compare the efficacies of the field collected isolates versus those of commercial *S. carpocapsae* and *H. bacteriophora*. Following species confirmation (see results), the efficacy of the field-collected nematodes was compared with the commercially available *S. feltiae*, through a second set of experiments. Local and commercial nematodes were all reared in last-instar waxworm, *G. mellonella* (Lepidoptera: Pyralidae), and newly emerged juveniles were stored in tap water at 7°C for one week, prior to each assay. Before inoculations, infective juveniles were acclimated at room temperature (23°C) for an hour and their movement and activity were verified under a stereomicroscope.

Experimental Design and Treatments

Commercial and Local EPN Evaluation

The experimental pots were cone-shaped and 4.2 × 20.32 cm (Diameter × Height) in size. Treatments were arranged in a completely randomized design. The experiment was conducted three times. The first two time-blocks were carried out during August 2017, each one week apart. The third time-block was conducted during June 2018. A total of five treatments, including the non-treated wireworm control, were assessed per time-block. There were 10 pot-replicates per treatment per time-block (Table 1). Each cone-shaped pot was filled with the soil mix moistened with tap water. A single *L. californicus* was placed in each pot, 7 cm deep, in the center of the pot. Three wheat seeds were placed in each pot as wireworm feeding source. Entomopathogenic nematodes were suspended in 100ml tap water and inoculated onto the soil surface. One nematode dose of 250 IJ per square cm (3100 IJ per pot) was applied for all treatments. Control pots were inoculated with tap water without any IJ. Experimental pots were maintained in the laboratory at 23±2°C and L16:D8 for 12 days. Insects were checked for mortality after 12 days, and dead insects were removed and dissected under a stereomicroscope to confirm nematode presence. Live insects were placed individually in containers filled with autoclaved sand for one week and were checked daily to record mortality.

Virulence Evaluation of Commercial and Local Isolates of *S. Feltiae*

This study was conducted to evaluate virulence of commercial and native strains of *Steinernema feltiae* under laboratory conditions in May 2019 at the University of Idaho, Moscow, ID. Previously described cone-shaped pots were used for this experiment. Treatments were arranged in a completely randomized design in three time-blocks, each 3 days apart. The two field-collected *S. feltiae* isolates, *S. feltiae* isolate Kyle-F1 and *S. feltiae* isolate Curtis-F2, and one commercially purchased *S. feltiae* strain were assessed in this experiment. Non-treated wireworm control was included as a treatment. However, this treatment was excluded from the analyses since no mortality was observed. Cone-shaped pots were filled with the soil mix (described previously). A single sugar beet wireworm larva of *L. californicus* larva was placed 7 cm deep in each pot. A dose of 250 IJs/sq. cm was applied. The number of nematodes was recorded in 100 μl of suspension under a stereomicroscope, and 3100 IJs were suspended in 10 ml water and were inoculated onto the soil surface of each pot. Three wheat seeds as the wireworm food source were placed in each pot. Pots were kept at room temperature (23±2 °C) and L15:D9 in the laboratory and were moistened with tap water daily. After 12 days, insects were checked for mortality and dead larvae were removed. Live larvae were individually placed in small containers filled with autoclaved sand and checked daily for mortality for one week.

Statistical analysis

Statistical analyses were conducted using SAS version 9.4. Generalized Linear Mixed Model (GLIMMIX) was used to evaluate the fixed effect of treatment (nematode species), time-block (random effect) and treatment × time-block interaction on wireworm mortality. This model assumed a binomial distribution with a logit link function. Time-block and interaction were included in the initial model and were removed in a stepwise approach when their effect was not significant.

Results

Efficacy of the Commercial and Field-Collected Nematode Species

The results of this study indicated significant differences in the efficacies of the evaluated EPN species against *L. californicus* ($F=6.39$; $df = 3,116$; $P< 0.001$). Non-treated positive control did not cause any mortality and was not included in the statistical analyses. The locally collected nematodes *S. feltiae* Kyle-F1 caused significantly higher mortality (64%) in wireworms than either commercial species, *S. carpocapsae* (30%) or *H. bacteriophora* (6.6%). The lowest mortality was observed in *H. bacteriophora* (6.6%) (Fig. 1). The virulence of two field-collected local nematode isolates on *L. californicus* was significantly different ($t= 3.02$; $df = 3,116$; $P= 0.003$). Time-block, and the interaction between time-block and treatment were not statistically significant and were excluded from the final model.

Virulence Evaluation of Commercial and Local Isolates of *S. feltiae*

Our results indicated that percent wireworm mortality caused across the commercial isolate and the two field-collected isolates of *S. feltiae* was significantly different ($F= 6.71$; $df= 2, 87$; $P= 0.002$). Similar to our previous assays, *S. feltiae* isolate Kyle-F1 caused significantly higher mortality (56.67%) in *L. californicus* larvae than either commercial strain (10%) or the other local isolate, *S. feltiae* Curtis-F2 (26.67%) (Fig. 2). There was no significant difference between mortality rates due to the application of the commercial isolate of *S. feltiae*, and one of the naturally collected nematode isolates, *S. feltiae* Curtis-F2 against sugar beet wireworm. No significant effects of time-block or the interaction between time-block and treatment were detected, and these two factors were excluded from the final model.

Discussion

Generally, some physical deterrents in wireworm's body including dense hairs in the oral cavity, muscular tissues that keep the anus closed and, heavily sclerotized cuticle affect their susceptibility to entomopathogenic nematode infection and make them resistant to nematode's infection (Eidt & Thurston, 1995). However, the results of this study show variability in the susceptibility of *L. californicus* to entomopathogenic nematodes, with one isolate of the native field-collected *S. feltiae* inflicting as high as 69% mortality on sugar beet wireworms.

Soil characteristics are known to influence the rate of wireworm damage in different crops. Sandy soils have been reported as a highly favorable habitat for wireworms, which is also associated with higher rate of damage when compared to organic-rich soil (Hermann et al., 2013; Milosavljević et al., 2017; Rashed et al., 2017). The effect of soil texture is not just reflected in wireworm damage rate. Application of the EPN *S. carpocapsae* in sand-dominated soil has been shown to reduce damage caused by *L. californicus* in wheat in a potted greenhouse study (Ensafi et al., 2018), however, the EPN efficacy is reduced in the soil mix containing higher proportion of peatmoss. Although sand-dominated soil was also used in this experiment, mortality rates by *S. carpocapsae* in our experiment (30%) were only slightly improved compared to the earlier study by Ensafi et al. (2018) (10% to 20%). Albeit small, this improvement in efficacy most likely can be attributed to the use of small cone-shaped pots. Overall, however, *S. carpocapsae* does not appear to be a good candidate for biological control of the sugar beet wireworm since its efficacy remained relatively low, even in small pots, which were used with the intention of maximizing the would-be host exposure to the pathogen. The observed low efficacy of *S. carpocapsae* against *L. californicus* supports Toba et al. (1983) conclusion stating the non-effectiveness of *S. carpocapsae* against *L. californicus* as a biological control agent.

In laboratory studies carried out by Ansari et al. (2009), *H. bacteriophora* applied to *Agriotes lineatus* larvae provided acceptable level of control against *Agriotes lineatus*, causing 67% mortality in larvae. While, in a laboratory experiment by Morton et al. (2017), *H. bacteriophora* was not effective to control *A. obscurus* and inflicted 11.1% mortality in *A. obscurus* larvae.

In contrast, *H. bacteriophora* inflicted the least mortality (6.6 %) on *L. californicus* larvae in our study. This inconsistency can be explained by differences in wireworm species and possibly less susceptibility of the last instar *L. californicus* to the strains of EPNs which we used in our experiments. Our results, however, supported those by Morris (1985) who found that neither *H. bacteriophora* nor *S. carpocapsae* were effective in controlling *Ctenicera destructor* (Brown).

Other studies also have reported poor efficacy of *S. feltiae* against different wireworm species including *A. lineatus*, *A. obscurus*, *A. fuscicollis*, *A. sordidus* (Ansari et al., 2009; Morton & Garcia-del-Pino, 2017; Zhao et al., 1996). Likewise, our study results indicating that the commercial strain of *S. feltiae* was not effective in controlling *L. californicus*, agrees with those findings. However, local isolates of *S. feltiae* collected from the cereal fields provided acceptable control against sugar beet wireworms, by causing more than 55% larval mortality across both our laboratory assays.

The efficacy of EPNs also depends on various factors such as nematode strain, environmental conditions and the host physiology and developmental stage (Grewal et al., 2005b; Lewis et al., 2006). Environmental conditions may affect EPN's virulence and reproduction especially in non-native EPNs which may not be well-adapted to new conditions and result in a reduction in their efficacy against endemic pests. However, local nematodes can be better candidates for the biocontrol approach against particular insects living in the same habitat (Campos-Herrera & Gutiérrez, 2009; Gaugler, 2002). Likewise, in our study, locally collected *S. feltiae* proved more effective in reducing wireworm numbers compared to the commercially obtained *S. feltiae*.

In conclusion, we determined that sugar beet wireworm is relatively more susceptible to field-collected EPN isolates than the commercially obtained species. It can be explained because local nematodes have been exposed to wireworms in their field of collection for a long time and are thus adapted for penetrating into the wireworm's body more effectively than the non-native commercially obtained nematodes. Future field studies are required to confirm laboratory results under field conditions. Identifying the effective dose of nematode, timing of application, the method of application and how to formulate them as biocontrol agents are factors that need to be investigated further to improve field efficacy.

Literature Cited

Adhikari A & Reddy GV (2017) Evaluation of trap crops for the management of wireworms in spring wheat in Montana. J Arthropod-Plant Interactions 11: 755-766.

Andrews N, Ambrosino MD, Fisher GC & Rondon SI (2008) Wireworm: biology and nonchemical management in potatoes in the Pacific Northwest. PNW Ext. Pub.

Ansari M, Evans M & Butt T (2009) Identification of pathogenic strains of entomopathogenic nematodes and fungi for wireworm control. J Crop Protection 28: 269-272.

Bedding R & Akhurst R (1975) A simple technique for the detection of insect paristic rhabditid nematodes in soil. J Nematologica 21: 109-110.

Bedding RA (2006) Entomopathogenic nematodes from discovery to application. J Biopesticides International 2: 87-119.

Burnell AM & Stock SP (2000) Heterorhabditis, Steinernema and their bacterial symbionts-lethal pathogens of insects. J Nematology 2: 31-42.

Campos-Herrera R & Gutiérrez C (2009) Screening Spanish isolates of steinernematid nematodes for use as biological control agents through laboratory and greenhouse microcosm studies. J Journal of invertebrate pathology 100: 100-105.

Eidt D & Thurston GS (1995) Physical deterrents to infection by entomopathogenic nematodes in wireworms (Coleoptera: Elateridae) and other soil insects. J The Canadian Entomologist 127: 423-429.

Ensafi P, Crowder DW, Esser AD, Zhao Z, Marshall JM & Rashed A (2018) Soil Type Mediates the Effectiveness of Biological Control Against Limonius californicus (Coleoptera: Elateridae). J Journal of economic entomology 111: 2053-2058.

Esser AD, Milosavljević I & Crowder DW (2015a) Effects of neonicotinoids and crop rotation for managing wireworms in wheat crops. J Journal of economic entomology 108: 1786-1794.

Ester A & Huiting H (2007) Controlling wireworms (Agriotes spp.) in a potato crop with biologicals. J IOBC wprs Bulletinv 30: 189.

Gaugler R (2002) Entomopathogenic Nematology CABI Publishing. J Wallingford Oxon OX10 8DE, UK: 1-388.

Godfray HCJ, Blacquiere T, Field LM, Hails RS, Petrokofsky G, Potts SG, Raine NE, Vanbergen AJ & McLean AR (2014) A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. J Proceedings of the Royal Society B: Biological Sciences 281: 20140558.

Grewal P, Koppenhöfer A & Choo H (2005a) Lawn, turfgrass and pasture applications. J Nematodes as biocontrol agents: 115-146.

Grewal PS, Ehlers R-U & Shapiro-Ilan DI (2005b) Nematodes as biocontrol agents. CABI.

Griffin C, Boemare NE & Lewis EE (2005) Biology and behaviour. J Nematodes asBiocontrol Agents: 47-64.

Hermann A, Brunner N, Hann P, Wrbka T & Kromp B (2013) Correlations between wireworm damages in potato fields and landscape structure at different scales. J Journal of Pest Science 86: 41-51.

Huey RB & Pianka ER (1981) Ecological consequences of foraging mode. J Ecology 62: 991-999.

Jedlička P & Frouz J (2007) Population dynamics of wireworms (Coleoptera, Elateridae) in arable land after abandonment. J Biologia 62: 103-111.

Kuhar TP & Alvarez JM (2008) Timing of injury and efficacy of soil-applied insecticides against wireworms on potato in Virginia. J Crop Protection 27: 792-798.

Lewis EE, Campbell J, Griffin C, Kaya H & Peters A (2006) Behavioral ecology of entomopathogenic nematodes. J Biological control 38: 66-79.

McLaughlin RL (1989) Search modes of birds and lizards: evidence for alternative movement patterns. J The American Naturalist 133: 654-670.

Milosavljević I, Esser AD & Crowder DW (2017) Seasonal population dynamics of wireworms in wheat crops in the Pacific Northwestern United States. J Journal of pest science 90: 77-86.

Morton A & Garcia-del-Pino F (2017) Laboratory and field evaluation of entomopathogenic nematodes for control of Agriotes obscurus (L.)(Coleoptera: Elateridae). J Journal of Applied Entomology 141: 241-246.

Rashed A, Etzler F, Rogers C & Marshall J (2015) Wireworms in Idaho cereals: Monitoring and identification. J University of Idaho Extension Bulletin 898.

Rashed A, Rogers CW, Rashidi M & Marshall JM (2017) Sugar beet wireworm Limonius californicus damage to wheat and barley: evaluations of plant damage with respect to soil media, seeding depth, and diatomaceous earth application. J Arthropod-Plant Interactions 11: 147-154.

Reddy GV, Tangtrakulwanich K, Wu S, Miller JH, Ophus VL, Prewett J & Jaronski ST (2014) Evaluation of the effectiveness of entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat. J Journal of Invertebrate Pathology 120: 43-49.

Sufyan M, Abbasi A, Gogi MD, Arshad M, Nawaz A & Neuhoff D (2017) Efficacy of Beauveria bassiana for the management of economically important wireworm species (Coleoptera: Elateridae) in organic farming. J Gesunde Pflanzen 69: 197-202.

Toba HH, Lindegren JE, Turner JE & Vail PV (1983) Susceptibility of the Colorado potato beetle and the sugarbeet wireworm to Steinernema feltiae and S. glaseri. J Journal of nematology 15: 597.

Traugott M, Benefer CM, Blackshaw RP, van Herk WG & Vernon RS (2015) Biology, ecology, and control of elaterid beetles in agricultural land. J Annual Review of Entomology 60: 313-334.

Van Herk W, Vernon R, Tolman J & Ortiz Saavedra H (2008) Mortality of a wireworm, Agriotes obscurus (Coleoptera: Elateridae), after topical application of various insecticides. J Journal of economic entomology 101: 375-383.

Vernon R, Van Herk W, Tolman J, Ortiz Saavedra H, Clodius M & Gage B (2008a) Transitional sublethal and lethal effects of insecticides after dermal exposures to five economic species of wireworms (Coleoptera: Elateridae). J Journal of economic entomology 101: 365-374.

Vernon RS, Blackshaw RP, van Herk WG & Clodius M (2014) Mass trapping wild Agriotes obscurus and Agriotes lineatus males with pheromone traps in a permanent grassland population reservoir. J Agricultural Forest Entomology 16: 227-239.

Zhao K, Zhang L, Song J, Zhang L, Li G, Wang X & Wang Y (1996) On the application of steinernematid nematodes against eight lepidopterous and coleopterous insect pests. J Acta Phytophylacica Sinica 23: 20-24.

Table 2.1. Sampling location details with positive occurrence of EPNs in cereal fields of southeastern Idaho. Soil type for each location is adapted from Web Soil Survey, National Resources Conservation Service; United States Department of Agriculture

Isolate	Location	Sampling Coordinates		Irrigation	Soil type	Sample date
CurtisF2	Soda Springs	42.765041	-111.682655	Rainfed	Silty clay loam	10.16.2017
Kyle-F1	Soda Springs	42.702469	-111.564643	Irrigated	Silty clay loam	10.16.2017

Table 2.2. List of treatments including entomopathogenic nematode (EPN) species and wireworm species used in the experiment.

Order	Pot Replicate	EPN Species	Wireworm Species
Treatment1	10	*S. carpocapsae*	*L. californicus*
Treatment2	10	*H. bacteriophora*	*L. californicus*
Treatment3	10	*S. feltiae* Kyle-F1	*L. californicus*
Treatment4	10	*S. feltiae* Curtis-F2	*L. californicus*
Treatment5	10	--------	*L. californicus*

Table 2.3. List of treatments including entomopathogenic nematode (EPN) species and wireworm species used in the experiment.

Order	Pot Replicate	EPN Species	Wireworm Species
Treatment1	10	*S. feltiae* Commercial	*L. californicus*
Treatment2	10	*S. feltiae* Kyle-F1	*L. californicus*
Treatment3	10	*S. feltiae* Curtis-F2	*L. californicus*
Treatment4	10	--------	*L. californicus*

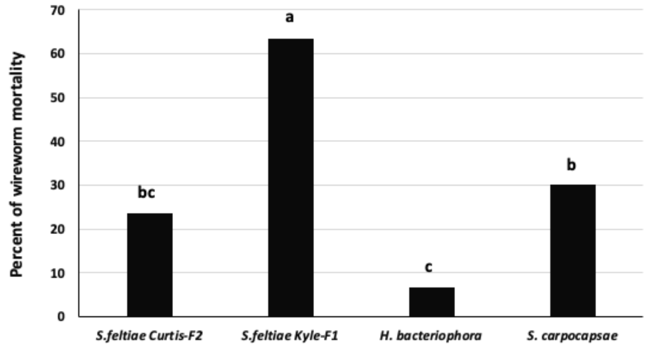

Fig. 2.1. Percentage of wireworm mortality caused by each EPN species. Field-collected isolate, *S. feltiae* Kyle-F1 caused significantly higher mortality than commercially available EPN strains.

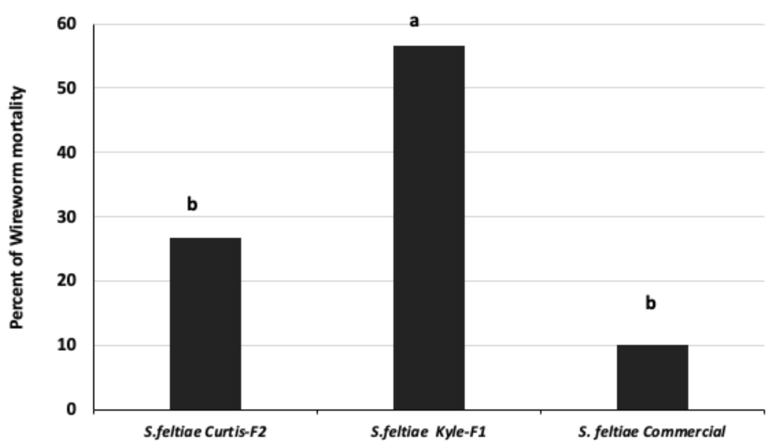

Fig. 2.2. Percentage of wireworm mortality caused by each field-collected *S. feltiae* isolate and commercially strain of *S. feltiae*. Field-collected isolate, *S. feltiae* Kyle-F1 caused significantly higher mortality than both commercial strain and locally-collected *S. feltiae* Curtis-F2.

Chapter 3: The Effect of In-Furrow Application of Pyrethroid in Reducing Wireworm Damage in Subsequent Wheat

Abstract

The term “wireworm” is used to describe the larval stage of click beetles (Coleoptera: Elateridae). Wireworms are a major pest of many crops, including cereals and vegetables grown in the Pacific Northwest (PNW). Neonicotinoid seed-treatments are the only chemical control option registered for cereal application. The seed treatments, however, has not been effective in reducing wireworm damage in cereals. Thus, there is a need to test alternative methods, to be employed as components of an integrated management protocol. Focusing on one of the most damaging species in the PNW, the sugar beet wireworm *Limonius californicus*, we conducted a greenhouse study to evaluate the effect of in-furrow application of the pyrethroid bifenthrin, in pea, a commonly planted rotation crop in the PNW, in reducing wireworm damage in the subsequent wheat crop. In the treatment where bifenthrin-treated pea was followed by thiamethoxam-treated wheat, up to 82% mortality was reported in wireworms. This mortality rate was significantly higher than those observed in treatments where untreated pea was followed by untreated wheat (30%). Germination success was relatively higher in wheat that followed pea treated with bifenthrin compared to the wheat treatments which followed untreated peas.

Introduction

The term “wireworm” is used to describe the click beetle larvae (Coleoptera: Elateridae) which are major pests of various crops including cereals and vegetables grown in the Pacific Northwest and Intermountain region of the USA. Adult click beetles emerge in spring, and after mating, females lay their eggs in the soil, typically in grassy and weedy areas likely to minimize risk of desiccation of eggs and neonates. Eggs may be oviposited either singly or in small clusters (Fox, 1973; Parker & Howard, 2001). Newly hatched larvae live in the soil for several years and feed actively on plant tissue as well as soil organic matter (Andrews et al., 2008; Traugott et al., 2015). Larvae depends on environmental conditions and species can stay in soli between 3-11 years (Becker & Dogger, 1991).wireworms feed on various crops and can cause damage. In cereals, failed germination occurs when wireworms feed on planted and sprouting seeds. (Rashed et al., 2017). Feeding on plant stem can result in delayed growth and in the mature fields, different patches of green plants may be indicate wireworm damage. (Barsics et al., 2013; Ritter & Richter, 2013). Plants are weakened by wireworm damage and fields can be taken over by weeds competing for same resources (Antwi et al., 2018; Thomas, 1940).

Wireworms’ long-life cycle, underground habitat, ability to survive in both agricultural and natural ecosystems, and their resilience make it difficult to find practical management strategies against wireworms (Adhikari & Reddy, 2017). For the past 30 years, wireworm have been controlled using broad-spectrum organochlorine insecticides such as Lindane (Vernon et al., 2008a). These insecticide had been banned due to their long lasting toxic effects in the soil as well as detrimental effects on human health and non-target organism (Toba et al., 1985; Vernon et al., 2008a). Recently, wireworms resurged as a severe pest especially in cereal crops. This resurgence has been attributed to both degradation of the conventional insecticide residues in the soil, as well as an increase in no-till farming practices that is known to support wireworm development (Adhikari & Reddy, 2017; Parker & Howard, 2001). Currently, producers rely on neonicotinoid seed treatments to protect their crops against wireworms. Once wireworms feed on those treated seeds, they get sick for a while and after recovering they continue to attack crops (Van Herk et al., 2007). Although chemical applications may reduce the wireworm pressure for a short time in cereal fields, they are not able to eliminate wireworm population from the field (Vernon et al., 2008a).

Cultural practices have been evaluated and recommended for reducing wireworm damage. Increased seed densities can compensate for the wireworm damage in cereal crops. Delayed spring planting is another strategy that can protect susceptible seedlings from wireworm exposure (Adhikari & Reddy, 2017). However, there is not enough scientific research to support the effectiveness of these practices. As, wireworms are polyphagous herbivores and feed on cultivated crops, non-cultivated plants and soil organic matter, it is difficult to simply find an effective crop rotation for wireworm management that could be implemented in all cropping systems. There are, however, a few reports of crop rotations which are shown to have a negative impact on wireworm populations in cereals. Gibson et al. (1958) found that crop rotation of alfalfa, lettuce, sunflowers and buckwheat was effective in reducing the population of *L. canus* and *L. californicus* larvae in the field (Gibson, 1958). Crop rotation with mustard and cabbage could also be effective in reducing soil-borne pests because of toxic compounds, such as glycosinolates, in their tissues (Lichtenstein et al., 1964). Furlan et al. (2009) showed that applying chopped tissue of the brown mustard *Brassica juncea*, into soil may reduce wireworm population in potted potatoes in the greenhouse. In Idaho, long rotation with alfalfa for four years, decreased wireworm population in a heavily infested field while, in that cropping system, rotation with red clover increased wireworm abundance (Shirck, 1945). However, alfalfa is not economically competitive with wheat and barley in the Pacific Northwest. Griffiths (1974) examined the susceptibility of different crops including pea, bean, white mustard, cabbage and wheat, to damage by *Agriotes* sp. He reported that pea and bean seedlings showed higher emergence success in wireworm-infested plots compared to wheat (Griffiths, 1974). Esser et al. (2015) have evaluated two management strategies for wireworm in four-years field trails. Esser et al. (2015) used the neonicotinoid insecticide, thiamethoxam, applied as a seed treatment combined with summer fallow and winter wheat rotation instead of continuous spring wheat. Their results showed no-till summer fallow in rotation with winter, reduced wireworm populations in two wireworm species, *Limonius californicus* and *L. infuscatus*, in comparison to continuous spring wheat planting. They also found, thiamethoxan was effective to reduce wireworm damage in cereals; however, it did not eliminate wireworm population from the field (Esser et al., 2015a). This study is a successful example of an integrated management approach, combining a preventive insecticide treatment and a cultural practice.

Pea is one of the important rotation crops in the PNW region and is popular in the Intermountain region of the USA (e.g., southeast Idaho). As wireworms can feed on numerous hosts and survive on organic compounds and plant residues in the field, rotation or fallow rotation by itself would not be an effective strategy (Traugott et al., 2015). Neonicotinoid seed treatment, the only class of insecticide that is registered for application in cereals to control wireworms, primarily serves as feeding deterrent and do not eliminate wireworm population from the field (Van Herk et al., 2008). While some of the chemistries with efficacies against wireworms are registered for application in legumes, they are not allowed to apply in cereals. Bifenthrin is a synthetic pyrethroid insecticide and one of the chemicals, which has been recently registered by the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) in 2013 against wireworms in legumes. Pyrethroids are broad-spectrum insecticides that indiscriminately target both pest and non-pest (including beneficial) arthropods and other invertebrates. Bifenthrin is highly persistent in soil because of its low water solubility (solubility = 0.1 mg/l) and high soil adsorption coefficient (Koc= 1.31-3.02$\times10^5$)(Fecko, 1999; van Herk et al., 2013). Depending on soil type and environmental conditions, half-life of bifenthrin in soil may take between 122-345 days , and is virtually stable even under flooded conditions (Fecko, 1999).

In this study we evaluated whether applying the insecticide bifenthrin in pea would benefit subsequent cereal crops by reducing wireworm populations and/or damage.

Methods and Materials

Source of Wireworm and Study Location

Larvae of the sugar beet wireworm, *Limonius californicus,* which is the predominant wireworm species damaging cereal crops in the PNW (Milosavljević et al., 2017; Rashed et al., 2015), were collected from a dryland wheat field located near Ririe, Idaho (43°340 56.500

N 111°320 21.500 W) between May and June 2018, using solar bait traps. Each bait trap consisted of previously soaked mixture of wheat and barley seeds (1:1), placed in a 6-inch deep hole in the ground, covered with soil and a black plastic bag on top, to retain heat, moisture and CO_2 (Rashed et al., 2015). Traps were recovered after 12 days, and the collected wireworms were placed individually in 5×5×10 cm (W×L×H) plexiglass containers filled with sand and two barley seeds as their feeding source. Wireworm containers were kept in the room temperature ranging between (20 and 25 °C). A potted greenhouse experiment was set up in greenhouses at the University of Idaho, Aberdeen Research and Extension Center in Aberdeen, ID, between June and August 2018. The average daily temperature recorded in greenhouse chambers was 26.8 (0.3) °C.

Soil Media Preparation

A sand-dominated medium, reported as favorable to the sugar beet wireworms (Rashed et al. 2017), and associated with higher rates of damage compared to soil with high organic matter contents (Hermann et al., 2013; Milosavljević et al., 2017; Rashed et al., 2017) was prepared. The soil media used for this experiment was a mixture of sieved sand and peatmoss (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada) in the ratio of 75% and 25%, respectively. The mixed soil also contained 370 g of fertilizer (Osmocote, Scott- Sierra Horticultural Products Co., Marysville, OH) and 112 g of vermiculite (Therm-o-Rock West Inc., Chandler, AZ) per each load. Each mixed load was estimated at approximately 22.3 kg. Mixed media was homogenized in a soil mixer before placing into the 22.9 × 22.9 × 33.1 cm (W × L× H) plastic pots.

Experimental Design and Treatments

The spring pea variety Banner was selected as the “rotation” crop. The Syngenta winter wheat variety SY-Ovation was used as the following cereal crop. Experiments were conducted in two time-blocks, one week apart, each in a separate greenhouse chamber. Each pot was seeded with 4 wheat or pea seeds, depending on the treatment or the stage of the trial. Seeds were planted 5 inches apart, one at each corner of the pot. There were 6 treatments: 1) Pea treated with bifenthrin followed by spring wheat treated with the thiamethoxam neonicotinoid (TP/TW) , 2) Pea treated with bifenthrin followed by untreated spring wheat (TP/UW), 3) Untreated pea followed by spring wheat treated with the thiamethoxam neonicotinoid (UP/TW), 4) Untreated pea followed by un-treated spring wheat (UP/UW), 5) Pea treated with bifenthrin followed by spring wheat treated with the thiamethoxam neonicotinoid- No wireworm (Non-infested control), 6) Untreated pea followed by un-treated spring wheat (control). There were 10 pot-replicates per treatments per time-block (Table1). Bifenthrin (Capture LFR, FMC Corporation, Philadelphia, PA) was applied along the small furrow with seeds on the soil surface, at the company recommended rate of 6.8 oz/acre, prior to covering seeds with soil. Thiamethoxam (Cruiser Maxx, Syngenta, Greensboro, NC), a common neonicotinoid insecticide was used, at the recommended rate of 325 ml/ 100 kg seeds, to treat cereal seeds in “treated-wheat” treatments. Two control treatments, non-treated control (no wireworm, non-treated) and non-infested control (no wireworm), were included to the treatments. A single *L. californicus* larvae was placed in the center of each pot in 12 cm depth two days before planting.

All pots were arranged in a completely randomized design within each time-block. Experimental pots were maintained in two separate greenhouse chambers with the average daily temperature 26.8 $\pm$ 0.3°C and L16: D8 for four weeks. Pots were watered daily using hand water breaker. After four weeks, pea plants were removed at the based on the stem (or "harvested") and four winter wheat seeds (variety SY-Ovation) was planted in each corner of the same pots in 2.5 cm depth. Pots were maintained in greenhouse in the previous condition for four weeks.

Evaluations

All wheat plants were removed from the potted soil four weeks after planting. Emergence success, plant damage (evidence of feeding at the very base of the stem) and plant biomass were recorded in wheat seedlings in each pot. The number of germinated seeds were recorded in each pot. Above- and below-ground plant tissues were removed gently washed and then dried in 60°C for 96 hours. After the 96 hrs., plant biomass for both above and belowground tissues were measured. Wireworm mortality was recorded at the end of the experiment, following the harvest (removal) for the wheat seedlings by screening and sieving the soil in each pot, and if wireworm was not found it was recorded as dead.

Statistical Analysis

Statistical analyses were conducted in SAS (version 9.4). Generalized Linear Mixed Model (GLIMMIX), with time-block as the random effect, and treatment and pot replicate as fixed effects and time-block $\times$ treatment interaction, was used to compare germination success, wireworm mortality and plant biomass among the 6 treatments. A binomial distribution ('0' or '1') was assumed and a logit link function was applied to compare emergence success and wireworm mortality across treatments. Control treatments without wireworm were not included in mortality analyses. Pot, treatments, time-block and the interaction between time-block and treatment were the factors included in the initial model. Time-block, pot and the interaction term were removed, in a stepwise approach, if their effects were not significant.

To compare plant biomass, a normal distribution and an identity link function were used. In a similar way, treatment, pot, time-block and the interaction term were included in the starting model. In the final model, factors with no effected were excluded from the final model in a stepwise approach.

Results

Wireworm Mortality

Wireworm mortality was significantly affected by treatment (F= 3.76; df = 3,72; $P<$ 0.001). Bifenthrin-treated pea followed by thiamethoxam-treated wheat was the treatment with significantly higher rate of wireworm mortality (82%) compared to the untreated-pea followed by untreated-wheat (30%) (Fig.1). Wireworm mortality observed in bifenthrin-treated pea followed thiamethoxam-treated wheat was not considerably higher than bifenthrin-treated pea that was followed by untreated wheat. Wireworm mortality in untreated-pea followed by thiamethoxam-treated wheat and untreated pea followed by untreated-wheat was relatively lower than the remaining treatments, not exceeding 40%. No significant difference was detected between thiamethoxam-treated wheat and untreated wheat that followed untreated peas. No effects of time-block (F= 0.44; df = 1,72; *P*= 0.507*)* and time-block × treatment interaction (F= 0.81; df = 3,72; *P*= 0.490) was present.

Emergence Success in wheat

Emergence success in wheat seedlings was also significantly affected by treatment (F= 3.72; df = 5,108; *P*= 0.004). The percentage of emerged plants in thiamethoxam-treated wheat that followed untreated pea (80%) was significantly lower than those in non-infested control (90.28%). Showing a similar trend, untreated wheat that followed untreated pea showed significantly lower emergence (70.4%) than the non-infested controls (90.28%) (Fig.2). Emergence success in spring wheat treated with thiamethoxam that followed pea treated with bifenthrin did not considerably differ from untreated wheat that followed bifenthrin-treated pea. Overall, applying bifenthrin in pea treatments resulted in higher germination in subsequent wheat treatments compared to untreated wheat that followed untreated pea. Time-block (F= 0.51; df = 1,108; *P*= 0.476) and interaction (F= 1.12; df = 5,108; *P*= 0.355) did not influence germination success and were excluded from our final model.

Wheat Biomass

No significant effect of treatment on wheat biomass was detected (F= 1.36; df = 5,108; *P*= 0.246). However, the average plant biomass in thiamethoxam-treated wheat that followed untreated pea (73.1mg ± 8.9) was significantly lower than the non-infested control (92mg ± 11.1) (t= 1.85; df = 1,108; *P*= 0.067) (Fig.3). The biomass loss in the wheat treatments that followed either treated or untreated peas, were not significantly different. A significant effect of time-block was detected (F=12.83; df =1,108; P< 0.001). Pot-replicate and the interaction term were removed from the final model because of their non-significant effects.

Discussion

Through this greenhouse study we showed that applying chemicals with mortality effect on wireworms in a rotation crop can benefit the subsequent cereal by reducing wireworm numbers. Since neonicotinoid seed treatments, the only group of insecticides registered for cereal application against wireworms, are not effective in reducing wireworm numbers, evaluating alternative integrated approaches would be important to provide affected PNW growers with one additional management tool. In the present study, an integration of chemical and cultural (i.e., crop rotation) methods was evaluated in managing the sugar beet wireworm *L. californicus* in winter wheat. As grasslands are the suitable oviposition sites for the click beetles, crop rotation with broadleaf crops can help to reduce wireworm pressure in fields, and subsequently, in cereal crops that would be planted in those fields (Ritter & Richter, 2013).

Although neonicotinoids have not been effective chemicals in reducing wireworm numbers and protecting cereal crops, there is some documented evidence that points to the effectiveness of other classes of insecticides, such as organophosphate and phenylpyrazole insecticides, against wireworms (Vernon et al., 2013b). Vernon et al. (2013) showed that fipronil (phenylpyrazole) and chlorpyrifos (organophosphate) applied as in-furrow treatments can cause up to 93% and 71% mortality in *Agriotes obscurus* larvae in potato, respectively. Moreover, a field trial conducted by Lilly (1973) showed that both in-furrow and broadcast application of fonofos (organothiophosphate) in potato fields infested with *L. californicus* can be more effective in reducing damage compared the carbamate carbofuran.

In our mortality assessment, we recorded 82% mortality in bifenthrin-treated peas, which confirms the high efficacy of this chemical against sugar beet wireworms. Bifenthrin is currently registered in two of the commonly planted crops, potatoes and legumes, in rotation with cereals in the Pacific North west (Esser et al., 2015a; Milosavljević et al., 2017). We further demonstrated that in-farrow application of bifenthrin can reduce *L. californicus* damage in subsequence winter wheat. In multiple bifenthrin-treated pots, wireworms were observed on the soil surface which is characteristic of insecticide poisoning (Van Herk et al., 2013). Similarly, Van Herk et al. (2013) discovered that wireworms become sick and nearly dead around 2 hours after bifenthrin application and moved to the soil surface. While in our study wireworms desiccated and died on the soil surface, it has been shown that they might recover if transferred to untreated soil (Van Herk et al., 2013). Comparing the mortality effect in thiamethoxam-treated wheat with untreated wheat which both followed untreated pea proved that thiamethoxam seed treatment by itself is not effective in reducing wireworm populations. However, Esser et al. (2015) in a field study found that thiamethoxam seed treatment reduced wireworm population if it was integrated with fallow rotation. Their finding also echoed non-effectiveness of the thiamethoxam seed treatment, by itself, in reducing wireworm population. Our finding also supported that integration of both cultural (crop rotation) and chemical methods can be effective in controlling wireworms than either approach alone.

Wheat treatments that followed untreated pea had lower seed germination than bifenthrin-treated peas. This was likely due to feeding damage by wireworms in non-treated treatments, which would result in failed emergence, a commonly reported damage caused by wireworms (Rashed et al., 2015). For germinated seedlings, however, wheat plant biomass was not affected by insecticide treatments. This lack of significant effect might have resulted from optimal greenhouse conditions supporting the healthy growth of the slightly damaged plants. It is also important to note that some of the control plants (and plants from other treatments) suffered from heterogeneity of lighting coverage in one area of the greenhouse chamber which resulted in their delayed development. Thus, results from biomass comparisons should be interpreted cautiously.

In summary, integration of rotation and chemical application appears to be a promising approach in reducing wireworm pressure in cereals. Proper rotation with dichotomous would reduce wireworm pressure through reduced adult oviposition activity and would also allow for application of insecticides with mortality effect which would reduce wireworm numbers in the field thus benefiting subsequent cereal crops. Further field studies are required to evaluate and confirm the effectiveness of this, and similar, integrated approaches against wireworms in cereals.

Literature Cited

Adhikari A & Reddy GV (2017) Evaluation of trap crops for the management of wireworms in spring wheat in Montana. J Arthropod-Plant Interactions 11: 755-766.

Andrews N, Ambrosino MD, Fisher GC & Rondon SI (2008) Wireworm: biology and nonchemical management in potatoes in the Pacific Northwest.

Antwi FB, Shrestha G, Reddy GV & Jaronski ST (2018) Entomopathogens in conjunction with imidacloprid could be used to manage wireworms (Coleoptera: Elateridae) on spring wheat. J The Canadian Entomologist 150: 124-139.

Barsics F, Haubruge E & Verheggen F (2013) Wireworms' management: an overview of the existing methods, with particular regards to Agriotes spp.(Coleoptera: Elateridae). J Insects 4: 117-152.

Becker E & Dogger J (1991) Elateridae (Elateroidea)(including Dicronychidae, Lissomidae). J Immature insects 2: 410-418.

Esser AD, Milosavljević I & Crowder DW (2015a) Effects of neonicotinoids and crop rotation for managing wireworms in wheat crops. J Journal of economic entomology 108: 1786-1794.

Esser AD, Milosavljević I & Crowder DWJJoee (2015b) Effects of neonicotinoids and crop rotation for managing wireworms in wheat crops. 108: 1786-1794.

Fecko A (1999) Environmental fate of bifenthrin. Department of Pesticide Regulation. Environmental Monitoring and Pest Management Branch, Sacramento.

Fox C (1973) Some feeding responses of a wireworm, Agriotes sputator (L.),(Coleoptera: Elateridae). J Phytoprotection.

Furlan L, Bonetto C, Finotto A, Lazzeri L, Malaguti L, Patalano G & Parker W (2010) The efficacy of biofumigant meals and plants to control wireworm populations. J Industrial Crops Products 31: 245-254.

Gibson KE (1958) Effect of some crop rotations on wireworm populations in irrigated lands. US Dept. of Agriculture.

Griffiths DC (1974) Susceptibility of plants to attack by wireworms (Agriotes spp.). Annals of Applied Biology 78: 7-13.

Lichtenstein E, Morgan D & Mueller CFC (1964) Insecticides in nature, naturally occurring insecticides in Cruciferous crops. J Journal of Agricultural 12: 158-161.

Parker WE & Howard JJ (2001) The biology and management of wireworms (Agriotes spp.) on potato with particular reference to the UK. J Agricultural Forest Entomology 3: 85-98.

Rashed A, Etzler F, Rogers C & Marshall J (2015) Wireworms in Idaho cereals: Monitoring and identification. J University of Idaho Extension Bulletin 898.

Rashed A, Rogers CW, Rashidi M & Marshall JM (2017) Sugar beet wireworm Limonius californicus damage to wheat and barley: evaluations of plant damage with respect to soil media, seeding depth, and diatomaceous earth application. J Arthropod-Plant Interactions 11: 147-154.

Ritter C & Richter E (2013) Control methods and monitoring of Agriotes wireworms (Coleoptera: Elateridae). J Journal of Plant Diseasesn Protection 120: 4-15.

Shirck FH (1945) Crop rotations and cultural practices as related to wireworm control in Idaho. Journal of Economic Entomology 38: 627-633.

Thomas CA (1940) The biology and control of wireworms. A review of the literature. Bulletin of the Pennsylvania Agricultural Experiment Station.

Toba H, O'Keeffe L, Pike K, Perkins E & Miller J (1985) Lindane seed treatment for control of wireworms (Coleoptera: Elateridae) on wheat in the Pacific Northwest. J Crop protection 4: 372-380.

Traugott M, Benefer CM, Blackshaw RP, van Herk WG & Vernon RS (2015) Biology, ecology, and control of elaterid beetles in agricultural land. J Annual Review of Entomology 60: 313-334.

Van Herk W, Vernon R, Clodius M, Harding C & Tolman J (2007) Mortality of five wireworm species (Coleoptera: Elateridae), following topical application of clothianidin and chlorpyrifos. J Journal of the Entomological Society of British Columbia 104: 55-64.

Van Herk W, Vernon R, Tolman J & Ortiz Saavedra H (2008) Mortality of a wireworm, Agriotes obscurus (Coleoptera: Elateridae), after topical application of various insecticides. J Journal of economic entomology 101: 375-383.

van Herk WG, Vernon RS & McGinnis S (2013) Response of the dusky wireworm, Agriotes obscurus (Coleoptera: Elateridae), to residual levels of bifenthrin in field soil. J Journal of pest science 86: 125-136.

Vernon R, Van Herk W, Tolman J, Ortiz Saavedra H, Clodius M & Gage B (2008a) Transitional sublethal and lethal effects of insecticides after dermal exposures to five economic species of wireworms (Coleoptera: Elateridae). J Journal of economic entomology 101: 365-374.

Vernon R, Van Herk W, Tolman J, Ortiz Saavedra H, Clodius M & Gage BJJoEE (2008b) Transitional sublethal and lethal effects of insecticides after dermal exposures to five economic species of wireworms (Coleoptera: Elateridae). 101: 365-374.

Vernon RS, Blackshaw RP, van Herk WG & Clodius M (2014) Mass trapping wild Agriotes obscurus and Agriotes lineatus males with pheromone traps in a permanent grassland population reservoir. J Agricultural forest entomology 16: 227-239.

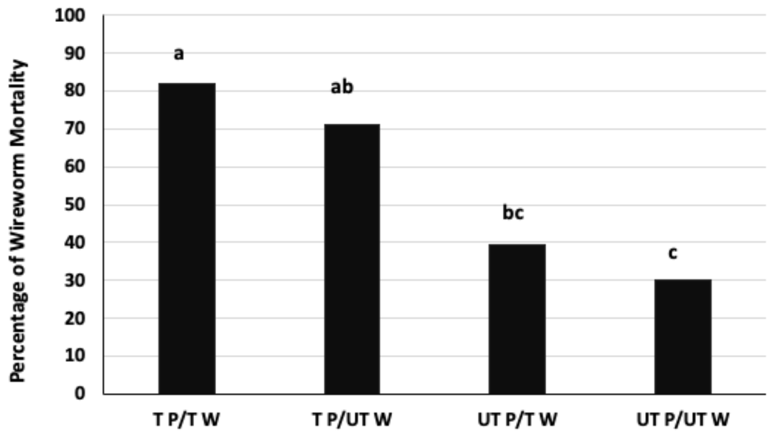

Fig. 3.1. Percentage of wireworm mortality in :1) TP/TW: Wheat treated with Cruiser Maxx (TW) planted after winter pea treated with Capture (TP), 2) TP/UW: Untreated wheat after treated winter pea, 3) UP/TW: Treated wheat after untreated pea, and 4) UP/UW: Untreated wheat after untreated pea. Significant differences among treatments occurred ($F_{3,108}$ = 3.76; P = 0.0144). Relatively higher mortality was associated with pea treatments that treated with Capture.

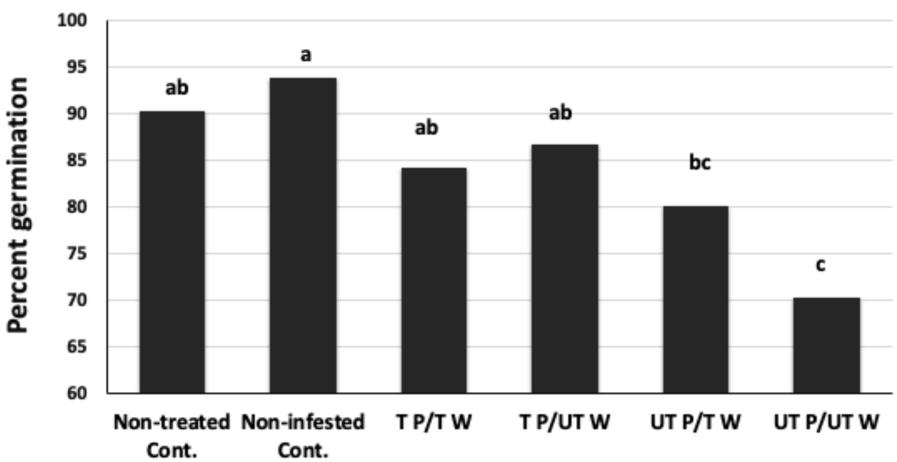

Fig. 3.2. Percentage of success germination in :1) TP/TW: Wheat treated with Cruiser Maxx (TW) planted after winter pea treated with Capture (TP), 2) TP/UW: Untreated wheat after treated winter pea, 3) UP/TW: Treated wheat after untreated pea, 4) UP/UW: Untreated wheat after untreated pea, 5) Untreated wheat after untreated pea without wireworm (Non-Treated control), and 6) Wheat treated with Cruiser Maxx planted after winter pea treated with Capture without wireworm (Non-infested control). Significant differences among treatments occurred ($F_{5,108}$ = 3.72; P = 0.0038). Relatively lower seed germination was associated with untreated treatments

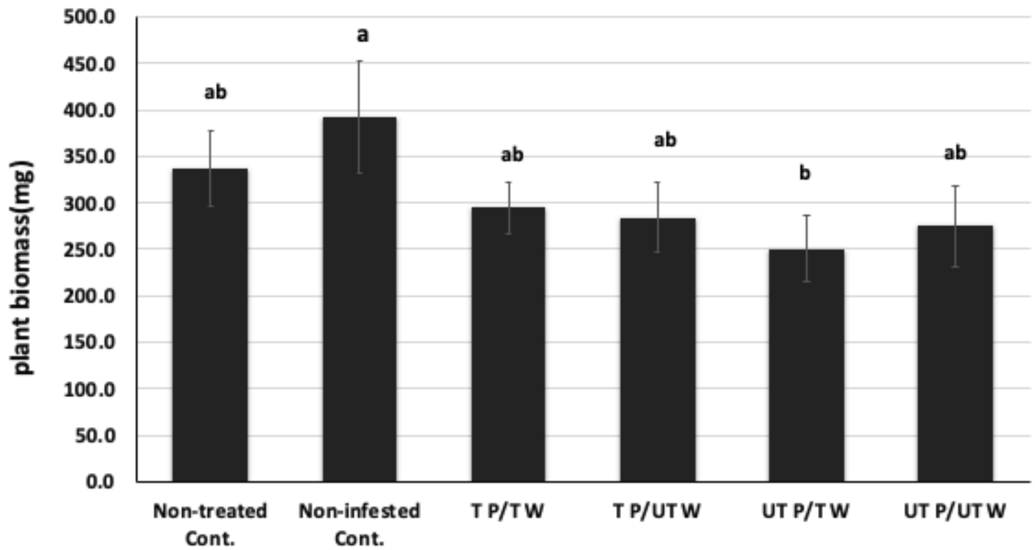

Fig. 3.3. Plant biomass affected by: 1) TP/TW: Wheat treated with Cruiser Maxx (TW) planted after winter pea treated with Capture (TP), 2) TP/UW: Untreated wheat after treated winter pea, 3) UP/TW: Treated wheat after untreated pea, 4) UP/UW: Untreated wheat after untreated pea, 5) Untreated wheat after untreated pea without wireworm (Non-Treated control), and 6) Wheat treated with Cruiser Maxx planted after winter pea treated with Capture without wireworm (Non-infested control). No significant differences among treatments were detected ($F_{5,108} = 1.36$; $P = 0.246$).

Chapter 4: Conclusion

Wireworms reemerged as economic pests of cereals and other crops in the Pacific Northwest and Intermountain regions of USA in recent years. Currently, neonicotinoid seed treatments are the only class of insecticides registered for use in cereals against wireworms. This group of insecticides is not effective in reducing wireworm populations and primarily serve as feeding deterrents. So, developing an alternative integrated approach would be important to reduce wireworm pressure in cereals in PNW. From our studies, it has been demonstrated that entomopathogenic nematodes collected from heavily wireworm infested fields, caused higher mortality than commercially available nematodes in sugar beet wireworm, which is reported as the predominant wireworm species damaging cereals in PNW. Native nematodes, *S. feltiae* Kyle-F1 and *S. feltiae* Curtis-F2, showed better level of protection against *L. californicus* than commercially available strains of *H. bacteriophora*, *S. carpocapsae* and *S. feltiae*. Adaptation of native nematodes to local environmental conditions, including their constant exposure to wireworms in the field, can perhaps explain this observed efficacy. Perhaps they can be extracted and cultured to be used as potential biocontrol agents against *L. californicus* in southern Idaho cropping systems. I also showed that integration of chemical and cultural methods can be a potential effective strategy for wireworm control under greenhouse condition. The pyrethroid bifenthrin caused considerable mortality in wireworms when applied in-furrow in pea. Combined with the observed improved germination in subsequent wheat, the integration of rotation and chemical application appear to be a promising approach to reduce wireworm pressure in cereal crops, at least in the absence of other more sustainable management approaches. However, further field studies are required to evaluate and confirm the efficacy of this integrated method, since the current study was conducted under controlled conditions where wireworm mobility might have been restricted.