Population Characterization of two Important Nematodes in Southeastern Idaho Agroecosystems; *Heterodera avenae* and *Steinernema feltiae*

A Dissertation

Presented in Partial Fulfillment of the Requirement for the

Degree of Doctor of Philosophy

with a

Major in Plant Science

in the

College of Graduate Studies

University of Idaho

By

Pooria Ensafi

Major Professor: Juliet M. Marshall, Ph.D.

Committee Members: Richard W. Smiley, Ph.D.; Arash Rashed, Ph.D.; James W. Woodhall, Ph.D.

Department Head: Robert R. Tripepi, Ph.D.

December 2018

Authorization to Submit Dissertation

This dissertation of Pooria Ensafi, submitted for the degree of Doctor of Philosophy with a Major in Plant Science and titled "Population Characterization of two Important Nematodes in Southeastern Idaho Agroecosystems; *Heterodera avenae* and *Steinernema feltiae*," has been reviewed in final form. Permission, as indicated by signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____ Date: _____

Juliet M. Marshall, Ph.D.

Committee Members: _____ Date: _____

Richard W. Smiley, Ph.D.

_____ Date: _____

Arash Rashed, Ph.D.

_____ Date: _____

James W. Woodhall, Ph.D.

Department Head: _____ Date: _____

Robert R. Tripepi, Ph.D.

Abstract

Increased prevalence of agricultural pests in cereal fields of Southeastern Idaho is associated with increased population density of virulent pathotypes with high level of resistance to conventional control methods. The situation is exacerbated by continuous planting of susceptible cultivars and failure in implementation of management strategies and is now a serious threat to the Idaho's cereal production. Two major pests are responsible for most of the damage: cereal cyst nematode Heterodera avenae (CCN), and wireworms (the larval stage of various species of click beetles Coleoptera: Elateridae). While the absence of high-yield resistant cultivars and lack of effective registered nematicides are the two main challenges for CCN management, the increase in wireworm populations and their damage is the results of failure in all conventional control strategies. In my four years studies, along with the characterization of the CCN population and assessment of resistance and tolerance of spring wheat cultivars, I successfully isolated populations of entomopathogenic nematodes form southeastern Idaho that could provide effective biological control against wireworms. The results provide a platform for future agricultural studies.

Acknowledgments

I would like to express my sincere gratitude to my advisor Prof. Juliet Marshall for the continuous support of my Ph.D. study, her patience, motivation and scientific support. Her guidance helped me in the research and writing of my dissertation.

Besides my advisor, I would like to thank my scientific committee members Drs. Richard Smiley, Arash Rashed, and James Woodhall for their insightful comments, encouragement, and scientific assistance.

I thank Drs. Pamela Hutchinson, Phillip Wharton, Christopher Rogers, Xi Liang for sharing their lab space and equipment's, and my fellow labmates, Sandesh Dangi, Karin Regina Cruzado, Fabiola Aguilar, and Atoosa Nikoukar for the supports and fun we have had in the last four years.

Last but not the least, I would like to thank Dr. Arash Rashed again for dedicating his molecular lab space, equipment, and materials to my projects.

Dedication

To my grandfather Alireza Hassanpour

Table of Contents

List of Tables

List of Figures

Chapter 1. Literature Review

Cereal Cyst Nematodes (CCNs)

Preface,

Dealing with one of the oldest known plant-parasitic nematodes has always been an exciting challenge to nematologists. Early publications describe all cyst nematodes as multiple species of genus *Heterodera* based on morphological characteristics of the cyst (Franklin 1951). Before the separation of *Globodera*, *Punctodera*, and *Heterodera* by Mulvey and Stone (Mulvey and Stone 1976), cyst nematodes used to be grouped morphologically. Subsequently, taxonomists have continued grouping and proposing "artificial groups" for the genus *Heterodera* by adding more characteristics. They have included molecular analysis, host type (number of cotyledons), and morphology and morphometries of the cyst (shape, fenestration, bullae, underbridge, vulva slit), and J2 (lateral field) (Handoo 2002; Subbotin et al. 2003; Subbotin et al. 2011). One reason for the increased number of groups seems to be the large number of valid species (84) under genus *Heterodera*. These current groupings do not imply pathotype, virulence, or economic importance. They represent taxonomic similarity and comprise seven sub-genus groups under *Heterodera* (Subbotin et al. 2010a).

Commonly used concepts

Avenae group: Considering the morphology of cysts, J2 and host type (Mono- or Dicotyledon), most species within the genus *Heterodera* comprise seven distinct groups: Afenestrata, Avenae, Cyperi, Goettingiana, Humuli, Sacchari, and Schachtii (Baldwin and Mundo-Ocampo 1991). These groups are strongly supported by molecular analysis (Subbotin et al. 2010a) . The Avenae group includes species that are morphologically and molecularly comparable to *Heterodera avenae* Wollenweber 1924; some of these species are difficult to differentiate even through standard molecular analysis (Ferris et al. 1999; Rivoal et al. 2003; Toumi et al. 2013). Currently, members of the Avenae group consisted of 12 species: *H. arenaria*, *H. avenae*, *H. aucklandica*, *H. australis*, *H. filipjevi*, *H. mani*, *H. pratensis*, *H. riparia*, *H. sturhani*, *H. ustinovi*, *H. latipons*, *H. hordecalis*. Although all 12 species parasitize Poaceae, only six of the twelve species that can cause damage to cereal crops are considered as CCNs. These six species are *H. avenae*, *H. australis*, *H. filipjevi*, *H. sturhani*, *H. latipons* and *H. hordecalis* (Smiley et al. 2017).

***Heterodera avenae* species complex:** Subbotin studied and explained the *H. avenae* complex in detail (Subbotin et al. 2003). The complex includes ten species very closely related to *H. avenae*, which before the application of precise molecular techniques and morphological study, they were mistakenly

reported as *H. avenae*. The species complex includes *H. arenaria*, *H. avenae*, *H. aucklandica*, *H. australis*, *H. filipjevi*, *H. mani*, *H. pratensis*, *H. riparia*, *H. sturhani*, and *H. ustinovi* (Subbotin 2015; Subbotin et al. 2003; Turner and Subbotin 2013).

Cereal Cyst Nematodes: A sub-group of Heteroderinae that are under the genus *Heterodera* are so-called "Cereal Cyst Nematode" (CCN), which is a group of six species that economically and efficiently parasitize the small grain cereals. CCNs include four members of *H. avenae* complex (*H. avenae*, *H. australis, H. filipjevi*, and *H. sturhani*), plus *H. latipons* and *H. hordecalis*.

In some publications the protocol for naming of CCNs is species/host related: *H. avenae* is the European Cereal Cyst Nematode; *H. filipjevi* is the Filipjev Cereal Cyst Nematode; *H. sturhani* is the Chinese/Sturhan Cereal Cyst Nematode; *H. latipons* is the Mediterranean Cereal Cyst Nematode; and *H. hordecalis* the Barley Cyst Nematode (Smiley et al. 2017; Subbotin 2015).

CCN Systematics and Molecular Phylogeny

Above family level: After De Ley and Blaxter (De Ley and Blaxter 2002), the CCN classification scheme includes additional morphological features mainly based on reports by Lorenzen (Lorenzen 1981, 1994), and molecular phylogenetic results from SSU rDNA (small subunit ribosomal DNA) (Decraemer and Hunt 2013). Hierarchically, the proposed scheme is: "phylum: Nematoda Pott, 1932. Class: Chromadorea Inglis 1983, subclass: Chromadoria Pearse 1942. Order: Rhabditida Chitwood, 1933, suborder: Tylenchina Throne, 1949, infraorder: Tylenchomorpha De Ley & Blaxter, 2002. Superfamily: Tylenchoidea Örley 1980, family: Hoplolaimidae Filipjev 1934, subfamily: Heteroderinae Filipjev & Schuurmans Stekhoven 1941". In this classification, the overall taxa's position is strongly supported by SSU rDNA sequences (De Ley and Blaxter 2002).

Morphological features of CCNs are defined for each taxonomic level. In all CCNs, the endoderm originates from the posterior blastomeric in the 2-cell embryonic stage (Chromadorea). Phasmids are present, the caudal gland is absent, and amphidal openings are pore-like (shared, synapomorphic characters of Rhabditida). The stegostom has no interradial epithelial cells or monodelphic-prodelphic testis (Tylenchina). The stomatostyle is an armature; the lip region is simple, the dorsal pharyngeal gland outlets in procorpus, and there is no valve in the posterior pharyngeal bulb (Tylenchomorpha) (Blaxter et al. 1998; De Ley and Blaxter 2002; Decraemer 2011).

Phylogenetic analysis of sequences within Tylenchomorpha indicates paraphyly of the superfamily Tylenchoidea Örley, 1880 (Subbotin presentation), and Tylenchomorpha is restricted to Tylenchoidea

(Maggenti et al. 1987). This superfamily comprises very important plant parasitic nematodes including CCNs. Morphological characteristics and phylogenetic analysis from different studies support De Ley and Blaxter that include cyst nematodes' subfamily Heteroderinae Filipjev & Schuurmans Stekhoven, 1941 within the family Hoplolaimidae Filipjev, 1934 (De Ley and Blaxter 2002).

Below family level: After Subbotin, De Ley & Blaxter, and Siddiqi (Siddiqi 2000); with some uncertainty, morphological characteristics may help to identify genera of the Heteroderinae. Regarding species delimitation and in-depth classification, integrative taxonomy based on morphological traits, biological information of the host, biochemical results of proteins and isozyme analysis, and molecular analysis is required for cyst nematodes species identification and taxonomy (Smiley et al. 2017; Subbotin et al. 2010a; Subbotin et al. 2010b). Considering all techniques, Heteroderinae comprises 15 genera in total. Of these, females of 8 genera form hardened cysts with 116 valid species. The genera include *Heterodera* Schmidt, 1871 (84 species), *Globodera* Skarbilovich, 1959 (12 species), *Punctodera* Mulvey & Stone, 1976 (4 species), *Cactodera* Krall & Krall, 1978 (13 species), *Dolichodera* Mulvey & Ebsary, 1980 (1 species), *Betulodera* Sturhan, 2002 (1 species), *Paradolichodera* Sturhan, Wouts & Subbotin, 2007 (1 species), and *Vittatidera* Bernard, Handoo, Powers, Donald & Heinz, 2010 (1 species).

Under Heteroderinae, two genera occur on Poaceae:

1- *Heterodera* which includes 25 species (out of total 84 valid species): *zeae*, *turcomanica*, *avenae*, *arenaria*, *aucklandica*, *mani*, *pratensis*, *sturhani*, *australis*, *filipjevi*, *ustinovi*, *hordecalis*, *latipons*, *sorghi*, *sinensis*, *sacchari*, *goldeni*, *oryzicola*, *cyperi*, *elechista*, *mothi*, *cardiolata*, *bifenestra*, *koreana*, *orientalis*.

2- *Punctodera* which includes two species (out of total four valid species): *chalcoensis*, *punctata*.

Cereals are defined as grasses (Poaceae) cultivated for edible components of the grains, including maize, rice, wheat, barley, sorghum, millets, oats, rye, triticale, fonio, job's tears, and zizania. While 27 species of cyst-forming nematodes utilize the family Poaceae as host, only six are considered CCNs which can economically and efficiently parasitize only a few cereal species.

The most economically important CCN, *H. avenae*, comprises different populations around the world. Based on ITS rRNA, gene sequencing, and PCR-RFLP, so far, three population types are distinguished geographically, including Type A: European & North American; Type B: Asian & African; Type C: French & Chinese populations (Turner and Subbotin 2013).

Morphology of Cereal Cyst Nematodes

CCNs initially were named based on morphology. In general, all Heteroderinae have a heteroderid cyst (Hetero = changing, Deros = skin), which is defined as: "A sac containing eggs or juveniles, which is the persistently tanned body wall of the dead mature female" (Luc et al. 1986). All members of Heteroderinae comprise four juvenile stages. Three stages (J2-J4) become sedentary endoparasites inside roots, and the adult females are semi-endoparasitic.

Morphology of the mature females and cyst: Adult females are sedentary, swollen, and lemon-shaped. Cuticle annulation is limited to the head region. Although patterns and annulation in cysts can be specific for a few cyst-forming species, it is similar in CCN species. The stomatostylet is well developed with three basal knobs of slightly different shapes. The metacorpus is enlarged and fills the neck region. The females' body and cyst have a prominent posterior cone with the terminal vulva slit of different length depending on species. Based on the vulva bridge width, a fenestration in *H. avenae* is ambifenestrate, while the other five species are bifenestrate (wider vulva bridge). The cone's underbridge is absent in 3 species (*avenae*, *australis*, and *sturhani*), while it is strongly present in 3 other species (*filipjevi*, *latipons*, and *hordecalis*). Bullae are present and visible in all species except *H. hordecalis* (maybe present in *H. latipons*) (Decraemer and Hunt 2013; Subbotin et al. 2010b).

The lemon-shaped cysts are covered by a sub-crystalline layer, consisting of two layers. The inner layer is attached to the female cuticle and replicates the cuticle pattern of juveniles (since the mature female shows smooth cuticle). This inner layer consists of n-tetracosanoic acid. The outer layer is thicker in *H. avenae* than the other CCN species and is made of hexacosanoic acid and calcium salt (Brown et al. 1971; Hesling 1978). In addition to variations of the cuticle pattern, the color of the cyst can be variable due to age, size, and condition. The young cyst color is pale, and by aging, the color will turn yellow and dark brown. In *H. avenae* the yellow phase does not occur (Franklin 1951). The egg sac is absent for all six CCN species, and Females' reproductive system is didelphic-prodelphic (Subbotin et al. 2010b).

The eggs in CCNs are cylindrical and approximately 126 × 56 µm. Eggshells consist of 3 layers: a lipoprotein layer derived from a vitelline layer of the oocyte, a chitinous middle layer, and an inner lipid layer (Turner and Subbotin 2013).

Morphology of Juvenile Stage 2: Morphological identification using the J2 will not be useful in separating closely related species. J2s are vermiform with the strongly sclerotized head framework and

offset. The body is slightly ventrally curved. Average body, tail, and hyaline length are diverse. *H. australis* and *H. avenae* are longer, while *H. filipjevi*, *hordecalis*, *latipons*, and *sturhani* are slightly shorter. All CCN species have four incisures (lateral lines). The stylet forms at the J2 development stage and becomes robust with an anterior projection (average projection: *australis*, *avenae*, *sturhani*, *latipons* and *filipjevi*; or strong projection: *hordecalis*). Overlap of esophageal glands on the intestine is ventrolateral.

Morphology of Males: Adults and J4 males are vermiform, non-feeding, and free-living nematodes, with a monodelph-prodelphic testis, two spiculi, a gubernaculum, with no caudal allae (Siddiqi 2000).

Biology and Life Cycle

Reproduction: Reproduction in Heteroderinae is diverse and can be sexual or via parthenogenesis. The majority of *Heterodera* species are sexually dimorphic (females are swollen, males vermiform), and despite other plant-parasitic nematodes with autotokous reproduction, CCNs are amphimictic species. In terms of genetic variability and adaptive power, amphimictic is advantageous (Decraemer 2011). The CCN sex determination is genetic and epigenetic (at unfavorable conditions). During the epigenetic, J3s that are genetically female turn to males through sex reversal, which results in a larger proportion of males and increases the chance of fertilization for almost all eggs inside the successfully developed females.

Sex attractants produced by sedentary females were detected through High-Performance Liquid Chromatography HPLC (for non-volatile) and gas chromatography after derivatizing into volatiles (Barrett and Wright 1998). The female's secretions include a wide range of chemicals, which makes the male response species specific. For two major genera, *Globodera* and *Heterodera*, in-vitro bioassays on nematodes' sex attractants showed that at least six pheromone chemicals are involved in male attractants (Green and Plumb 1970; Green 1980). Each female usually copulates with more than one male within the same species. Andersen studied the interrelationships between some species of *Heterodera* and showed interspecies mating is possible, even between two different pathotypes, for instance, pathotype 11 & 12 (Andersen 1965) and 11 & 31 (Rivoal and Person-Dedryver 1982), although the number of eggs and viable juveniles are fewer than intra-pathotype mating (Green and Plumb 1970).

Copulation in CCN starts with ejaculation of immature spermatozoa from the male's ejaculatory duct to the vagina of the female and develop to mature aberrant amoeboid sperm inside the female's

vagina. The sperm migrates toward the female's spermatheca and stays until the ootid arrives. Simultaneously, after insemination, the primary oocyte undergoes meiosis and ootid forms. Each ootid is fertilized by only one sperm. After fusion, the zygote forms, and the outer layer of egg thickens and becomes part of the eggshell. The egg formation is the start of the life cycle.

Life cycle: CCN species have co-evolved with their host and have a single generation per year (Smiley et al. 2017). Like all nematodes, CCNs have one egg stage, four juvenile stages, and an adult stage. From the point of insemination, it usually takes 40-65 days for females to mature. During this time, immature white female turns yellow (no yellow phase in *H. avenae*), and then brown. After death, the body wall of the dead female becomes persistently tanned and form a lemon-shaped cyst, which protects the developing eggs and juveniles (Franklin 1951; Subbotin et al. 2010b).

Embryogenesis in tylenchs is I-type (in tandem, independent of par genes) (Schulze and Schierenberg 2011). Cleavages result in six stem-cells: AB, MSt, E, C, D, and later the P4. After gastrulation, the embryo grows within the eggshell and movement begins. The result of embryogenesis is the formation of J1 inside the eggshell (Decraemer 2011). The first molting happens inside the J1 cuticle, which is inside the eggshell. After the first molt, at the J2 stage, the stomato-stylet forms. Inside the eggshell, J2 of *Heterodera* species is surrounded by osmotic pressure generated by trehalose that reduces the water content of the diapause J2 (Perry et al. 2013).

Dormancy & Hatching: Two types of dormancy may happen in *Heterodera* species:

1- Obligate diapause (genetically regulated); occurs only once in the nematode life cycle. Newly formed unhatched J2s that are inside the egg undergo obligatory diapause to complete development. It takes two to three months for the J2 of *H. avenae* to complete diapause (Cotten 1962).

2- Facultative diapause (environmentally regulated) induced by environmental stress variables like hot, or cold temperature (Rivoal 1983). CCN J2 may enter facultative diapause more than once, before entering the root.

When J2 is ready to hatch, the eggshell becomes permeable, and dehydrated J2 absorb water and becomes active. A single stylet thrust by a head movement in *H. avenae* cause tearing of the eggshell from which the J2 escapes (Banyer and Fisher 1972). The emergence of CCN J2s, including *H. avenae*, is seasonal, and under the influence of the temperature, and moisture (Evans 1986). Although *H. avenae* and *H. latipons* hatch in considerable numbers when the soil is at the field capacity, very high moisture in the soil may delay hatching and emergence of J2. Most studies show that *H. avenae* and

H. latipons do not respond to root diffusates or other stimuli (Scholz and Sikora 2004; Smiley 2009a). However, it is also reported that, root exudates may increase the hatching rate (Banyer and Fischer 1971). After the summer diapause, a chilling temperature of 5-10°C (for *H. avenae*) for 8 weeks breaks the diapause of J2s, and they may either enter soil or stay inside the cyst. Emerged J2 may find the root and continue the lifecycle, otherwise initiate a facultative diapause to pass the winter's freezing temperature. Four seasons should pass for 90% of all eggs to hatch (Kerry and Jenkinson 1976). In the northern parts of France, Europe and the USA populations have spring hatching cycle after the winter facultative diapause. Maximum hatch may be induced when after eight weeks of 7°C (even possible in the field conditions), encysted eggs are exposed to 20°C (Banyer and Fisher 1971). Daily changes in temperature from 5°C to 20°C prevents facultative diapause (Sharma and Sharma 1998). The *avenae* population from Jiangsu mostly hatch at 15°C, rarely over 25°C. Different hatching and emergence profiles of *H. avenae* populations around the word indicate the relatively good adaptation potential of this nematode.

Survival of *H. avenae* eggs in the cysts that are stored at anhydrobiotic periods of 40% relative humidity at 15°C is 5.5 years, which is short compared to *G. rostochiensis* (25 years) (Curtis et al. 1998; Norton 1978). Soil type may also affect hatching. Imren showed that sand dominated soils give the best hatching and emergence (Toktay et al. 2012). *H. filipjevi* does not have a diapause. Hatching is highest after 290 days when incubation is at 15°C in the lab. But in the field, J2s start emerging at 17°C in October and continues through April when the temperature is 2-17°C. Peak hatching is in October and February (Sahin et al. 2010). J2 populations in soil increase from minimum density at seedling stage and increase to a maximum at tillering stage. Such an increase in J2 populations results in maximum penetration and development of cysts in wheat roots (Li et al. 2012b; Wu et al. 2014). Smiley reports the rapid emergence of J2s in Oregon, USA, at a relatively low temperature of 2-5°C, which is similar to the English *H. avenae* population (Kerry and Jenkinson 1976; Smiley et al. 2005).

CCN-root Interaction

J2 leaves the cyst through any opening (Fenestra, the opening of the neck after detachment from the root, or any physical damage to the cyst), and enter the soil. Active *H. avenae* J2 can survive in soil for one to two months in different geographic areas; studies in Australia show three weeks (Davies and Fisher 1976), and in England one to two months (Kerry and Jenkinson 1976). The mechanism by which J2 locates the root is different. Oat root phytosiderophores (specifically avenic and muginec acids) is attractive to the J2s of *H. avenae* (Lung 1993). CO2, amino acids, and sugars from the root are also

possible factors that J2 rely on for locating the plant roots (Perry and Aumann 1998; Turner and Subbotin 2013). After locating the root, using its stylet, J2 of *H. avenae* and *H. filipjevi* penetrates the root growth zone, behind the root cap, and migrate through the root cortex to reach the differential zone.

The J2 uses the hollow stomatostylet to penetrate the cell wall, without bridging the plasmalemma. Plant parasitic nematodes secrete protein effectors to facilitate parasitizing the host cell. Sub-ventral glands secrete various enzymes that degrade the plant cell wall (Davis et al. 2004). Long et al., found that β-1, 4-endoglucanases hydrolyze the β-1, 4-glycosidic bonds of cellulose during the penetration, and found the Hz-eng-1a gene regulation for this enzyme (Long et al. 2012). J2 then stops moving until the syncytium forms. The syncytium is a permanent stimulated feeding site in the root cortex or endoderm. After 24 hours and by the dissolution of the cell-wall of the neighbor cells, the syncytium becomes multinucleate. At the time of formation of the syncytia, Annexin-like genes have been shown to be expressed by the nematode which encodes a long protein of 326 amino acid with four conserved annexin domains produced inside the sub-ventral gland cells of *H. avenae* J2s; Ha-ANNEXIN protein can suppress programmed cell death (Chen et al. 2015). This procedure causes the syncytium to expand, and the saliva of the nematode carries the necessary enzymes to help syncytium expansion (Gheysen and Jones 2006). The cell contents are then withdrawn back through the stylet. Formation of syncytium is always happening either very early in four days for susceptible cultivars, or after two weeks in resistant ones (Grymaszewska and Golinowski 1991). The resistant cultivar's reaction is closely related to the degradation of the cytoplasm and formation of large vacuoles; a reaction that can kill J2, or force it to leave the root for a better host (Koenning and Sipes 1998).

J2 starts feeding on syncytium, develops and molt to J3. J3s have a developed primordial and rectum. J3 may continue the development, molt and turn to a J4 female, or undergo a morphologic and sex reversal, molt, and turn to vermiform J4 male. The J4 vermiform males retain the 3rd stage cuticle until molting to adult males by emerging from both J3 and J4 cuticles and leaving the root. J4s that are supposed females have a similar shape, with the formation of the female reproductive system. The J4 females molt and turn to young adult females. By enlarging, the sedentary adult female ruptures the root cortex and epidermis, and the vulva cone becomes exposed and available for copulation.

Plant Disease Symptoms

Foliar and root symptoms: Jensen first reported *H. avenae* on oat in the USA in 1975. Initially, he stated the symptoms as possible aluminum toxicity before confirming the formation of cysts and

identification of CCN through morphological traits (Jensen et al. 1975). Progression of the aerial symptoms was described as stunting, purple discoloration of first leaves, late flowering, unfilled heads and significant yield loss. Early in the season, pale green patches of weak plants with yellow lower leaves and few tillers are typical symptoms of CCN damage (Dababat et al. 2011).

The root system is shallow due to penetration of J2 from the tip of the root and stopping the root growth. When the growth stops, many lateral branches of the root are produced especially in the area that syncytia exist. This makes the roots bushy and highly branched. Following damage and development of the cysts, the root develops a knotted appearance. *H. latipons* may cause fewer symptoms on roots since the invasion of the root is not usually very close to the tip (Mor et al. 2008).

Characters for diagnosis: Morphologic identification of the cyst forming nematode requires observation of specific characteristics of females (cyst) and juveniles (J2), including morphometrics of the vulval area (fenestration, the width of the vulva bridge, length of vulva slit, and under-bridge), distance from anus to the tip of the tail and presence of the bullae. Cyst shape is closely related to the presence of the vulval cone; a feature used to differentiate the nematodes at the genus level. For instance, the more prominent “lemon-shaped” vulva cone in *Afenestrata* can easily distinguish this genus from the ones with less projected cones and more spherical cysts present in *Globodera*, *Punctodera*, and *Dolichodera*. A sub-crystalline layer in cysts of some species also has been used for differentiation (Brown et al. 1971). Length, width, and some incisures of the lateral lines are essential diagnostic characteristics for J2. Also, stylet length and shape of the knobs, the position of the excretory pore in comparison with the median esophageal bulb, shape & annulation of the tail, and the distance from dorsal gland orifice to the stylet knob are useful for morphological identification. There is a relative relationship between molecular polymorphism and morphological traits (Reed and Frankham 2001). At the genus level, morphological characteristics may assist differentiation, but for species identification biochemical and molecular approaches are often required. The combination of both techniques will avoid errors in morphologic identification and the often-lengthy processing using visual methods (Smiley et al. 2017; Toumi et al. 2017; Yan and Smiley 2009). PCR RFLP of ITS amplicon using one or more restriction enzymes can distinguish most CCNs. Intraspecific polymorphism in the ITS sequences using PCR RFLP can make identification difficult and more convincing molecular identification technique such as sequencing is often necessary.

Sampling and Identification of CCNs

Both molecular and morphological identification require accurate and reliable soil and tissue sampling, handling and preservation of the samples, and efficient extraction for both juvenile and cyst stages. Smiley reports a co-occurrence of two CCN species of economic concern, which state the importance of accurate species identification (Smiley and Yan 2015; Yan and Smiley 2010).

Soil sampling after harvest is the best for representative sampling. For multiple year studies of CCN, evaluating the initial and the pre-plant population of both cysts and J2 in the soil is essential. The root samples at maximum penetration (boot stage) and maximum occurrence of female cysts (flowering stage) should be considered for evaluation of resistance level (Smiley 2009b; Smiley et al. 2005). Although simple pre-plant sampling may be enough to determine the population of known species in infected areas in the field, any quantitative approach, such as qPCR or population studies require precise, extensive sampling (Li et al. 2014; Smiley et al. 2016).

Depending on the aim of the study, if it is for detection purposes, twenty soil sampling cores from the boundaries of patches with symptoms would be adequate, while for quantification studies, three systematic patterns of large samples, each with 60-70 cores, is necessary. Considering the cost and time, the size of the sample may be different (Smiley and Yan 2015; Van Bezooijen 2006). Samples should be labeled and stored in a cool box with ice during transportation. Although several techniques are available, the Seinhorst Apparatus provides better accuracy for cyst extraction with larger initial soil samples. The wet soil cyst extraction method using the Seinhorst Apparatus results in less mortality of the eggs compared to dry soil, (due to desiccation in dry soil), or centrifugation (Seinhorst 1964).

Direct DNA extraction from soil can eliminate the nematode extraction procedure. Smiley et al. developed species-specific assays to distinguish *H. avenae* and *H. filipjevi* (Smiley et al. 2016; Yan et al. 2013).

Molecular Techniques for Identification or Quantification

Target genes: The nuclear ribosomal genes 18S, 28S, ITS1 (Internal Transcribed Spacer), and ITS2, have been successfully used in the identification of cyst nematode. Since the 18S (small subunit of ribosomal RNA) and 28S (large subunit of ribosomal RNA) are conservative in nucleotide sequences, universal primers can be used to amplify the ITS regions (Blaxter et al. 1998; Ferris et al. 1993; Skantar et al. 2007; Vrain et al. 1992). Higher mutation rates in the ITS regions of RNA provides sufficient variation

for differentiating species. In addition, nuclear protein-coding genes (actin and heat shock protein 90), have been used for cyst identification (Kovaleva et al. 2005; Mundo-Ocampo and Baldwin 1992).

Based on genome sequence results of *Caenorhabditis elegans*, the mitochondrial genome of nematodes is circular, double-stranded molecules of 13,794 nucleotide length. mtDNA encodes 36 genes: 12 protein subunits of the mitochondrial respiratory chain, two ribosomal RNAs, and 22 transfer RNAs (Lemire 2005). Likelihood or parsimony analysis of a traditional set of aligned sequences in mitochondrial genome together with the organization of mitochondrial genomes provide characters that can help resolve the phylogeny of animals (Dowton et al. 2002). Gibson et al. sequenced mitochondrial genome of Soybean Cyst Nematode (SCN) and showed that minor differences exist among Hoplolaimidae and very few shared boundaries between genes in *Globodera* and *Punctodera*. They suggest that during divergence of these genera, dramatic reorganization of the mitochondrial genome occurred, and because of rare large-scale genome changes and subsequent losses or reversions in the mitochondrial genome, rearrangement of a mitochondrial gene can be used for analysis of phylogeny (Gibson et al. 2011). As the mtDNA has a relatively higher rate of mutations, it is suitable for identification of cyst nematode races as well as population genetics studies (Subbotin et al. 2010a).

DNA-based diagnostics

PCR (Polymerase Chain Reaction) based techniques used in nematodes

PCR-RFLP: PCR initially amplify certain part of the DNA to a high level, and then amplicon will be cut by restriction enzymes. Since the high amounts of fragments are amplified through PCR, the restriction result can be visualized directly after electrophoresis, and blotting and hybridization are avoided. Because of greater variation, PCR-RFLP of ITS1 region has been shown more successful than ITS2 for distinguishing several species of the genus *Heterodera* (Blok et al. 1998; Ferris et al. 1994; Ferris et al. 1993; Szalanski et al. 1997). Subbotin et al. successfully used ITS1- 5.8 -ITS2 of ribosomal RNA through PCR-RFLP (Subbotin et al. 1997; Subbotin et al. 2000; Subbotin et al. 1999). The utilization of restriction enzymes *Alu*I, *Bsu*RI, *Bsh*1236I, *cfo*I, and *ScrF*I, generated relatively more polymorphic profiles than other enzymes. For cyst-forming nematodes, one of the *Alu*I, *Ava*I, *Bsu*RI, *Bsh*1236I, *cfo*I, *Mva*I, and *Rsa*I can differentiate most species. Using the restriction enzymes *Hinf*I, *Ira*I, *Pst*I, *Taq*, *Tru*9 resulted in greater differentiation of the *H. avenae* species complex (Amiri et al. 2002; Bekal et al. 1997; Ferris et al. 1999; Gäbler et al. 2000; Maafi et al. 2003; Rivoal et al. 2003; Subbotin et al. 2003).

Referring to studies by Subbotin and Toumi, PCR-RFLP of ITS sequences cannot differentiate *H. avenae* (Type A) from *H. arenaria; H. cruciferae* from *H. carotae*; and three species of *H. trifolii*, *H. daverti* and *H. ciceri* from each other (Subbotin et al. 2010b; Toumi et al. 2015).

Heterogeneity of ITS-rRNA can be visualized as bands on PCR-RFLP gels that can complicate the identification due to different rates of amplification (low or high stability) in PCR. The complexity of an RFLP profile generating several fragments while using a single digestion enzyme does not necessarily help to differentiate intra or inter-population variation, because the number of fragments and their intensity can be constant; although single enzyme *Mva*I for differentiation *H. schachtii* population is reportedly reliable (Amiri et al. 2002; Subbotin et al. 2010b).

PCR with specific primer: CCN detection in a mixed species sample is possible through a single PCR using oligonucleotide primers complementary to a conserved region of the nematode's genome of an individual taxon or group of taxa (Subbotin et al. 2010b).

Species-specific primers have been designed for some cyst-forming nematodes; for *G. rostochiensis* & *G. pallida* through amplifying ITS rRNA (Bulman and Marshall 1997; Mulholland et al. 1996), or through SCAR (Fullaondo et al. 1999); for *H. schachtii* by amplifying ITS rRNA (Amiri et al. 2002); for *H. glycines* by targeting ITS rRNA (Subbotin et al. 2001) or SCAR (Ou et al. 2008); for *H. avenae* and *H. filipjevi* targeting ITS rDNA (Yan et al. 2013), or targeting mitochondrial *co*I (Toumi et al. 2013); for *H. filipjevi* using SCAR markers (Peng et al. 2013); and for *H. latipons* by targeting an actin sequence (gene from alternative non-multi-copy DNA region) (Toumi et al. 2013). Fast and accurate species detection for *avenae* from 8 species and 32 populations, using RAPD and SCAR using single-step PCR, is proposed by Qi Xiao-li et al. (Qi et al. 2012).

Real-time PCR: Quantitative PCR estimates the copy number of nematodes in a sample by measuring the DNA quantity present in a sample. The increase in the amount of target DNA is visualized as it is amplified, by continuous measurement of PCR product in early amplification phases. Real-time PCR monitors the fluorescence emitted during the reaction at the end of each cycle instead of endpoint detection with gel electrophoresis.

To date, the cyst nematodes *G. pallida*, *G. rostochiensis* (Bačić et al. 2008; Toyota et al. 2008), *G. tabacum*, *G. artemisiae* (Bates et al. 2002; Christoforou et al. 2014; Nakhla et al. 2010; Quader et al. 2008), *H. schachtii* (Madani et al. 2005), and *H. glycines* have been tested and quantified through qPCR and the protocols have been developed (Goto et al. 2009; Ye 2012).

Likewise, qPCR assays for quantitative detection of *Heterodera* species are developed, specifically for *H. avenae* and *H. latipons* (Toumi et al. 2015). Intraspecific polymorphism and genetic variability within *H. latipons* due to ITS heterogeneity from the uncompleted concerted evolution of multicopy gene families have also been reported by Rivoal (Rivoal et al. 2003; Waeyenberge et al. 2009). This heterogeneity is reported unsuitable for primer development specific for *latipons* (Toumi et al. 2015). In some species like *avenae*, the ITS region is similar to other members of the group which makes it difficult for designing species-specific primers. Toumi has also targeted the mitochondrially encoded *Cox*1 gene to avoid putative problems in developing primers by using species-specific primers in his study (Toumi et al. 2013; Toumi et al. 2015).

Control

Economic importance: CCNs are the most economically important nematode on wheat (Nicol et al. 2011). The most damaging species of CCN (*H. avenae*, *filipjevi*, and *latipons*) are dispersed worldwide, with pathotypes of these species challenging the management strategies especially for the development of resistant cultivars. *H. avenae* is the most widely distributed CCN species on wheat cultivars of temperate agriculture (Nicol and Rivoal 2008). *H. latipons* is Mediterranean CCN, and *H. filipjevi* (formerly Gotland strain of *H. avenae*) more frequently found in continental climates. Other species of the *Heterodera* found on wheat are not economically significant globally (Nicol and Rivoal 2008).

Environmental variables, specifically water stress, increase the severity of the damage caused by CCN. In 1994, Smiley reported up to 50% reduction in yield from winter wheat plantings in heavily infested fields in Union County, Oregon, USA (Smiley et al. 1994), and 24% decrease on spring wheat (Smiley et al. 2005). Annual yield loss is reported as 15-20% in Pakistan, 23-50% in Australia, or 24% in PNW. Yield loss value in *H. avenae* can reach £ 3million in Europe or 72 million in Australian dollars. The annual yield loss caused by *H. latipons* is not precisely known, although there are reports of 50% loss in areas of Cyprus and 55% in Iran. *H. filipjevi* in Turkey cause 42% reduction and in Iran 48% (Hajihasani et al. 2010b). The threshold value for *H. avenae* in rain-fed is three eggs+J2s per gram of soil in Northwestern USA (Smiley et al. 2017), or 4.6-10.6 eggs+J2s for Rajasthan, India (Mathur et al. 1986); for *H. latipons*, 28 eggs+J2 *latipons* per gram of soil in barley and durum wheat (Hajihasani et al. 2010a). Under drought conditions, the losses from *H. filipjevi* significantly increases (Nicol et al. 2011).

Resistance to cereal cyst nematodes: Identifying an efficient single gene for resistance, that can be used effectively in commercial breeding, and with durability for different pathotypes and races are the

challenges for researchers (Atkinson et al. 2003). Resistance to *H. avenae* has been tested in controlled-environment studies (Smiley et al. 2013; Smiley et al. 2011a), and the stability of resistance in the field is an essential factor that should be examined (Smiley et al. 2013). Continuous planting of resistant varieties with the same source of resistance should be avoided, as in an oat cultivar in France that resulted in the development of virulent CCN populations (Lasserre et al. 1996).

Resistant cultivars can reduce the CCN populations up to 85% annually (Andersen and Andersen 1982). In Australia, the resistant cultivar 'Galleon' (derived from Egyptian CI 3876) controlled the CCN population for wheat planting in next year (Sparrow and Dube 1981). Although the cultivars derived from AUS 10894 in Australia successfully controlled CCN, low yield compared to susceptible cultivars in the absence of nematode pressure had been an issue for marketing (Brown 1987).

Significant studies on the virulence of *H. avenae* to widely produced wheat and barley cultivars has been done in Sweden (Ireholm 1994), the former USSR (including on *H. filipjevi*) (Subbotin et al. 1996), and the United States (*H. avenae* & *H. filipjevi*) (Marshall and Smiley 2016; Smiley and Marshall 2016; Smiley et al. 2013). Resistance genes in cereal cultivars of barley, wheat, and oat have been tested in Northern Europe for Ha11 and Ha12 pathotypes, and in Australia for Ha13 pathotype (Anand et al. 1998). In addition, resistance gene sources for wheat have also found in Afghanistan, Iran and North Africa.

Sources of resistance and genetics of virulence:

Barley: *Rha* resistance genes:

Rha1: Nilsson documented a CCN resistance gene (*Rha1*) for the first time (Nilsson-Ehle 1920) in barley cultivars 'Chevallier,' 'Hanchen,' 'Primus,' 'Drost,' 'Brage,' 'Amsel' and 'Fero' in Germany and Norway. *Rha1* confers resistance to pathotype group Ha1. The location was determined to be on the 2H chromosome (Kretschmer et al. 1997).

Rha2 and *Rha3*: Rha2 was identified by Andersen (Andersen, 1961) on the 2H chromosome at a different locus from *Rha1* in barley cultivar LP191 (origin North Africa). In Morocco, the gene was determined to be induced from more than a single locus, and at an allele linked to Rha2 (Cook and York, 1982). North African cultivars such as CI 3876 from Egypt were used for breeding in Australia (Sparrow and Dube 1981). Barleys carrying *Rha3* and most *Rha2* alleles prevent CCN reproduction in the PNW (Smiley et al. 2011a).

Rha4: is one of the best sources that is mapped to Ha4 locus on chromosome 5H (Kretschmer et al. 1997).

Wheat: *Cre* resistance genes: The cultivar Loros was the first source of resistance in wheat that was reported in Denmark by Nielsen (Nielsen 1966). According to Brown, from the Australian collection, spring wheat *T. aestivum* L., AUS 10894 was used as a parent in backcross program in Australia (Brown 1984), and AUS 10894 was the world's first wheat cultivar bred specifically for resistance to *H. avenae*, now known as *H. australis* (Brown and Young 1982)

Cre1 (Cereal eel worm-locus 1): AUS 10894 (parent1) and 'Loros' (parent2) have *Cre1* on the long arm of chromosome 2B (O'Brien et al. 1980; Williams et al. 1994; Wu et al. 2016). This gene confers resistance to *H. australis* Ha13 and European Ha11 and Ha 12 pathotypes of *H. avenae*. *Cre1* is not effective in all conditions for virulent CCN (Rivoal and Cook 1993). *Cre1*-carrying cultivars show susceptibility to Swedish pathotypes. Also, the stable, resistant cultivars 'Morocco,' *A. sterilis* I376 and Loros have been reported susceptible to *H. filipjevi* (Cook and Rivoal 1998). A study by Smiley et al. shows that cultivars with *Cre1* or *Cre8* that provide resistance against *H. australis* fails to provide resistance or tolerance against *H. avenae* pathotypes in Union County, Oregon, USA. (Smiley et al. 2005). *Cre1* is not sufficiently effective against *H. filipjevi* in Turkey (Toktay et al. 2012).

Cre2: In *Aegilops ventricosa* (Rivoal et al. 1986), *Cre2* is on the 6MV (6D) chromosome that was transferred to a CCN tolerant wheat line (H10-15) and confers high levels of resistance to several European pathotypes and *H. australis* (Delibes et al. 1993; Ogbonnaya et al. 2001). There is no resistance report for *Cre2* against any *Heterodera* species in Turkey (Imren et al. 2013). Jahier indicates that *Cre2* can suppress *H. avenae* pathotypes Ha41 and Ha12 up to 90% and the reduce the size and number of cysts (Jahier et al. 1996).

Cre3 (*Ccn-D1*): In *A. tauschii*, *Cre3* is located and mapped on the distal region of long arm 2D chromosome (2DL) (Eastwood et al. 1994; Eastwood et al. 1991). French wheat cultivar VPM1 is shown to carry *Cre5* (Jahier et al. 2001). High level of resistance response of *Cre3* cultivars has been reported from Saudi Arabia (Al-Doss et al. 2010), Australia against *H. australis Ha13* (Vanstone et al. 2008), and *H. latipons* in Turkey (Imren et al. 2013).

Cre4 (*Ccn-D2*): *Cre4* transferred from *A. tauschii* is located and loosely linked to the proximal region of the 2D chromosome (2DL) in wheat (Eastwood et al. 1994), and provides good resistance against *H. australis* (Nicol et al. 2001).

Cre5: is originally located on chromosome 2NS of *A. ventricosa* and transferred to a 2AS segment of chromosome 6MV (6D) of French wheat line VPM1, which is a translocation of the chromosome 6S.6L-2S (Bonhomme et al. 1995) from *A. ventricosa*. French wheat line VPM1 has the *Cre5* (Jahier et al. 2001; Jahier et al. 1996). The presence of 2NS chromosome segment of *A. ventricosa* is also detected in 'Madsen' (Washington State University) which is highly resistant to *H. filipjevi* (Li et al. 2012a). *Cre5* gives partial resistant to *H. australis* Ha13 in Australia (Ogbonnaya et al. 2001). VPM1 provides intermediate resistance for *H. australis* Ha12 (French) (Rivoal et al. 1986).

Cre6: was derived from *A. ventricosa* where it is located on chromosome 5NV. It is independent of *Cre2* and provides significant resistance to *H. australis* Ha13 Australian pathotype. In wheat cultivar, Moisson and VPM1 partial resistance have been reported on Australian pathotype Ha13 (Ogbonnaya et al. 2001).

Cre7: was derived from *A. triuncialis*, to the wheat cultivar Almatense (H-10-15) (Jayatilake et al. 2015; Romero et al. 1998). *Cre7* provides resistance to *H. avenae* and *H. latipons* in Turkey (Imren et al. 2013).

Cre8: The wheat cultivar 'Festiguay' has *Cre8* on the 6BL chromosome (Williams et al. 2003; Williams et al. 2006). The gene *Cre8* was first observed by Paull et al. and named *Cref* (Paull et al. 1998), then assigned to wheat chromosome 6B by Williams. Festiguay has resistance to Ha13 Australian pathotype. Cultivars with *Cre8* are found resistant to *H. filipjevi* only in Turkey (Imren et al. 2013).

CreR: Cook and York in 1987 reported the gene *CreR* in rye (*Secale cereale*) (Anand et al. 1998), and then it was mapped as *CreR* from the long arm of chromosome 6R, the gene confers strong resistance to Ha13 in Australia and is available to wheat breeders in the form of Chinese Spring 6R(-6D) substitution line (Asiedu et al. 1990; Taylor et al. 1998). This gene confers resistance to *H. filipjevi* and *H. latipons* in Turkey (Imren et al. 2013).

Cre1, *Cre2*, *Cre3*, and *Cre4* are localized on wheat chromosomes that have homologous loci in *A. tauschii* and *A. ventricosa* (Jahier et al. 1996). *Cre1*, *Cre2*, *Cre3*, *Cre7*, and *Cre8* are ineffective against *H. filipjevi* Hfc-1 pathotype in China. *CreR* from rye is reported useful in China (Wu et al. 2016), but in the PNW, except *Cre1* (effective), and *Cre5* (partially effective), the other resistance are not effective against the *H. avenae* population in PNW (Smiley et al. 2013).

All cultivars derived from the French wheat line VPM1 (cross of *A. ventricosa* /*T. persicum*//3*'Marne') have been initially used for resistance against eyespot (Jahier et al. 2001). Chromosome 2AS of VPM1 has the resistant genes *Yr17*, *Lr37* and *Sr38* controlling stripe rust, leaf rust and stem rust (Bariana and

McIntosh 1994), respectively. Cultivars with VPM1 in their pedigree (like Madsen) provide a different level of resistance to *H. avenae* associated with *Cre5* (Jahier et al. 2001; Ogbonnaya et al. 2001). In VMP1 and near-isogenic line testing, lines carrying *Yr17* produce very few *H. avenae* cysts, and linkage between *Yr17* and *Cre5* is incomplete (Jahier et al. 2001). This segment (VPM1) also carries *pm4b* gene derived from *T. persicum* Var. Ex Zhuk that provides resistance to powdery mildew (*Blumeria graminis f.sp. tritici*), although resistance levels varied with environmental conditions and genetic background (Bariana and McIntosh 1994).

Under field conditions, cultivars with the *Cre1* gene ('Ouyen' and 'Chara') have reduced *H. avenae* in the PNW. On 23 entries of the International Test Assortment for Defining Cereal Cyst Nematode Pathotypes, Smiley et al. studied populations from selected local areas in PNW. Smiley reported that CCN pathotypes in the Pacific Northwest (PNW) are closer to Group 2 but were not clearly grouped with Ha12 or Ha22, and do not correspond to any of the 11 pathotypes defined in the Test Assortment. In general, wheat cultivars carrying *Cre1* provide strong suppression of *H. avenae*, but the cultivars in PNW do not appear to have the *Cre1* gene (Marshall and Smiley 2016; Smiley and Marshall 2016; Smiley et al. 2011a).

Oat: resistance genes: A single dominant gene was initially identified by Andersen, 1961, in *Avena sativa* CI 3444 (US1624), which breeders have used in the cultivar Nelson. The gene is resistant to European pathotypes. Another resistant gene originally derives from wild oat *A. sterilis* I376 (Chew et al. 1981). Clamot and Ramoal reported three dominant resistance genes in *Avena sterilis* I. 376; two of them give a high level of resistance, and in the absence of these two genes the third one provides intermediate resistance (Clamot and Rivoal 1984).

As happened in oats, long-term (6 years) planting of the same highly effective resistance genes will lead to the selection a resistance-breaking pathotype and provoke marked genetic modification in a population of *H. avenae* (Lasserre et al. 1996).

Tolerance: Tolerance is defined as no or little reduction in yield in the presence of the nematode, regardless of nematode reproduction and development in roots. Tolerance alone, without the presence of resistance, is not valuable since the population of the nematode will not be reduced or controlled (Brown 1987). Tolerance increases with the plant age and root growth, and root response to nematode invasion may affect tolerance (Fisher 1982). Although valuable, resistance together with

intolerance response could result in significant yield reduction (Smiley and Marshall 2016; Smiley et al. 2013; Yan et al. 2013).

Classification of CCN pathotypes: There are inter- and intra-species virulence/host response variation in CCN populations. CCN pathotypes are defined by interactions with their host as the number of females developing on test differentials of resistant versus susceptible controls (Smiley et al. 2005). Resistant cultivars are the ones that can suppress reproduction of the female in roots or completely prevent it.

Following Nielsen (1972), Andersen and Andersen proposed a simple classification to differentiate CCN pathotypes and virulence genes only for barley based on three cultivars ('Drost', 'KVL 191', and 'Morocco'), with the resistant gene of each one named Ha1, Ha2 and Ha3, respectively (Andersen and Andersen 1982). This early set of test differentials was not sufficient to differentiate common pathotypes.

Ireholm, Cook, and Rivoal extended the classification to a flexible scheme of differentials which was not genetically based, so the heterogeneity of the pathotypes was unknown (the reason for using virulence pathotype in their scheme). The proposed test by Cook and Rivoal (1998) using barley, oat, and wheat can distinguish different pathotypes (Cook and Rivoal 1998). Very often in *H. avenae*, more than one pathotype occurs in one location, and it is difficult to calculate the percentage of each pathotype (Holgado et al. 2009).

CCN management and control: Three significant factors result in fluctuations of the population dynamics of *H. avenae*: the host susceptibility, population density-dependent factors, and the degree of fungal parasitism of cyst. All of these factors should be considered for management strategies. Increases in the population of *H. avenae* to more than 100 J2 per gram of soil would be a rapid increase (Rivoal et al. 1995) often due to adding a susceptible cereal into the rotation. In a long-term susceptible cropping situation, fungal parasites may reduce the population of *H. avenae* (Kerry and Hague 1974).

Association with other pathogens: Cereal Cyst Nematodes in disease complex are poorly documented. Cooccurrence of *Rhizoctonia solani* and *H. australis* in Australia caused severe symptoms in wheat cultivars in light soils (Brown 1984), and resulted in a reduction of root size, plant height and tillers (Meagher et al. 1978) . On winter wheat, low population of *H. avenae* together with *Gaeumannomyces graminis* var. tritici reduces the yield significantly higher from the time each of the pathogens occurs

alone. Cooccurrence of avenae with *Pythium* spp. also result in root size reduction (Smiley et al. 1994). *Fusarium culmorum* and *H. filipjevi* also reduce the winter wheat yield (Hajihassani et al. 2013).

Rotation: CCN hosts are limited to Poaceae (Subbotin et al. 2010b). Crop rotation with non-grass plants effectively controls nematode population, the efficacy of which is closely related to soil type and CCN population density (Smiley et al. 1994). For spring wheat on dryland production in the PNW of the United States, the rotation to non-cereal or dicot crops has not been economically viable, and summer fallow, although useful in dryland cereals, is not recommended due to the wind, soil erosion and other environmental concerns (Smiley et al. 2005).

According to Dababat, rotation with non-cereal or grass-free alternatives is effective in suppressing nematode populations in Egypt (Dababat et al. 2011). A break between winter wheat results in healthy and productive crops compared to annually produced wheat, and early planting of winter wheat in Australia increases yield (Brown 1984). A cropping system for controlling CCN using resistant cultivars always should preserve the balance or stability of the cereal agroecosystems (Rivoal et al. 1995).

Biological control: More than 150 fungal species parasitize the CCN cysts, of which only eight species parasite eggs. In previous studies two fungi reported for biological control of H. avenae which could provide good control (Kerry 1988). In a study in China, out of 42 fungi isolated from *H. avenae* cysts, only five offered good field control, including *Chaetomium* spp., *Fusarium solani*, *Penicillium oxalicum*, *Stemphylium solani* and *F. proliferatum* (Feiyue and Honglian 2011). The obligate parasite *Nematophthora gynophila* infects the cysts, and the facultative parasite *Pochonia chlamydosporia* (Goddard) Zare & Gams (syn *Verticillium chlamydosporium*) parasitizes the eggs (Kerry 1988; Kerry et al. 1982; Kerry and Hirsch 2011).

Pochonia was first reported as a parasite of eggs on *H. avenae* in the UK (Willcox and Tribe 1974), and was widely distributed with limited growth in soil. Although Smiley et. al. reported that 50% of the eggs were infested with the fungi; but in his study at Union County Oregon biological control did not suppress the population below the damaging threshold levels in all fields (Smiley et al. 2005). In addition, fermentation techniques for producing *N. gynophila* is not possible since the fungus is an obligate parasite. Anyways, biological control using fungi is closely related to soil moisture and rain (Kerry et al. 1980).

Trichoderma longibrachiatum has been shown capable of controlling *H. avenae*, and seed treatment with this fungus at the solution rate of $1.5\times10^4 – 1.5\times10^5$ spores per ml significantly increases the spring

wheat plant height, root length, and plant biomass. The CCN invasion decreases while enhancing the plant chlorophyll content and resistance-related enzymes (peroxidase, polyphenol oxidase, phenylalanine ammonia lyase) (Zhang et al. 2014).

Two bacterial strains, *Achromobacter xylosoxidans* and *Bacillus cereus* isolated from *H. filipjevi* cysts in Hanan, China, caused high mortality in J2s and reduced egg hatching. Field and greenhouse studies showed a significant reduction (more than 70%) in white females in the greenhouse and more than 40% in the field. This field reduction of CCN resulted in a greater than 13% yield increase (Zhang et al. 2016).

Paecilomyces carneus and *Cylindrosporium destructans* are also reported effective against CCN (Boag and Lopez-Llorca 1989). Studies in China show that occurrence of hyperparasites have a strong effect on population density and variation and reduce the rotation effect (Riley et al. 2010). Since the positive effects of fungal parasitism could take five years, biological control must be a long-term practice.

Chemical control: Several of effective nematicides are now off the market because of health and safety concerns (Whitehead 1997). For cyst nematodes with one generation per year, like *H. avenae*, nematicides were used successfully, while for cyst nematodes with multiple generations per year chemical control would not be successful or economical (Anonymous 2011).

Hatching Factors & Soil treatment: Any agrochemical that affects membrane permeability (such as thiocarbamate herbicide) is a potential hatching factor. Thiocarbamates such as Dilate stimulate hatch of *H. schachtii* (Kraus and Sikora 1981), and helps the nematicides like Temik that have hatching inhibitory effect on nematodes (Feyaerts and Coosemans 1992).

Aldicarb (Temik): Aldicarb can control CCN populations in soil and can increase the yield by as much as 24% in spring wheat cultivars in soils moderately infested by *H. avenae*, or 40-60% in winter wheat (Brown 1973; Smiley et al. 1994). In-furrow application of Temik do not provide control on winter wheat in Union County, where the growing season is 10 months long (Smiley et al. 2005). However, in Australia and Israel, where growing season is 5 months, this procedure has been effective on spring wheat planted during the autumn (Brown 1973). Smiley reported that the efficacy of the Temik depends on the presence of hatching factors, and yield increases will not be significant if *Rhizoctonia solani* occur in the soil (Smiley et al. 1994). Other plant-parasitic nematodes (PPNs) including *P. neglectus* and *P. thornei* can be controlled at rates ≥2.5 kg aldicarb/ha, by 70–90%, resulting in up to

23% greater yield for the wheat variety 'Machete' (Taylor et al. 1999). Temik has a high toxicity to mammals and has been detected in groundwater, which resulted in the removal from the market.

These early generation of nematicides such as Temik, are no longer available for application due to environmental concerns and safety issues, and effective chemical alternatives have not been registered in the USA (Smiley et al. 2005).

Seed treatment: Aldoxycarb insecticide improves winter wheat yield by 10% in CCN infested soil (Smiley et al. 2005). Seed treatments with Abamectin (Avicta) and *Bacillus firmus* (Votivo) improved plant growth by reducing the damage from soil pathogenic nematodes *H. avenae* and *Pratylenchus*, although the effect on grain yield is not significant (Smiley et al. 2012). Seed treatment with Carbosulfan was effective in India (Bhan and Kanwar 2012). Methylene thiocyanate together with thiamethoxam, or with fipronil-chlorpyrifos as a seed treatment in an experiment in China decreased the *H. avenae* population and increased wheat yield (Cui et al. 2017).

Foliar spray: An extensive study by Smiley in 2011 showed that Movento (Spirotetramat) could reduce the post-harvest density of *H. avenae* in Washington and Idaho up to 90% and 35% respectively, but did not significantly increase the yield, even after a 3-fold concentration application (Smiley et al. 2011b).

Entomopathogenic Nematodes and Wireworm Control

Entomopathogenic nematodes (EPNs) are obligatory parasites of insects that occur naturally in soil.

EPNs Systematics and Molecular Phylogeny

Focusing on the two-major family Steinernematidae and Heterorhabditidae, like other nematodes, by increasing the number of species, the classical identification based on the morphology and morphometrics will not be able to discriminate species, especially for Heterorhabditid nematodes. There are useful characters that are proposed for initial identification including adult male characteristics. Those traits include spicules and gubernaculum (Nguyen and Smart Jr 1996), presence or absence of caudal mucron, the position of copulatory papillae, spermatozoon morphology (Spiridonov and Subbotin 2016), cuticular projections status, or epiptygmata of the virginal female entrance.

Systematics and Morphology of Steinernematids

Gotthold Steiner (1886-1961) discovered and described the first steinernematid, *Aplectana kraussei* Steiner 1923, from hymenopter sawfly *Lyda potropohica* Hartig (= *Cephaleia abietis* L.), which had been

previously collected by Dr. Krausse in 1917 (Krausse 1917). He later described *Neoaplectana glaseri* Steiner 1929, the first nematode that for the first time was employed for biological control of white grubs (Hunt 2007).

Above family level: After Delay and Blaxter (Blaxter et al. 1998; De Ley and Blaxter 2002), Blaxter et al., 1998), the phylogenetic and taxonomic scheme based on molecular phylogenetic results from SSU rDNA (Decraemer and Hunt 2013). Hierarchically, the proposed scheme is: "phylum Nematoda Pott, 1932, class Chromadorea Inglis 1983, subclass Chromadoria Pearse 1942, order Rhabditida Chitwood, 1933, suborder Tylenchina Throne, 1949, infraorder Panagrolaimorpha De Ley & Blaxter, 2002, superfamily Strongyloidoidea Chitwood and McIntosh, 1934, family Steinernematidae Filipjev 1934.

In all Steinernematids, as in CCNs, endoderm originates from posterior blastomeric in 2-cell stage (Chromadorea). Phasmids are present, but the caudal gland is absent, and amphidal openings are pore-like (shared, synapomorphic characters of Rhabditida). Stegostom has no interradial epithelial cells, and monodelphic-prodelphic testis (Tylenchina). All share didelphy, zoopathogenicity, and presence of dauer stage as putative synapomorphies (Panagrolaimorpha) (De Ley and Blaxter 2002).

Two major genera *Steinernema* and *Neosteinernema* comprise more than 83 and one valid species respectively.

Characters required for the morphological identification: The family of Steinernematidae are parasites of insects and other arthropods. Infective juveniles are non-feeding, carrying symbiotic entomopathogenic bacteria. First and second generation of the adults represent amphimictic reproduction; Adams & Nguyen (Adams and Nguyen 2002) will be referred for morphology of all stages.

Morphology of adults: After Nguyen (Nguyen 2007); EPN adults are plump nematodes of medium to large size. Six labial sensilla and four cephalic sensilla both papillae form. Lateral field not clear. Stoma funnel-shaped posteriorly. Procorpus cylindrical, Metacorpus slightly enlarged, the basal bulb is round. Excretory pore is anterior to nerve ring.

Males reproductive system: Gonad is monarchic, reflexed, with two spiculi of the distinct capitulum. Gubernaculum large and distinctly bifurcate ventrally. No bursa. Tail round.

Females: the reproductive system is a didelphic-amphidelphic reflex. The vulva is median and transverse slit. Tail short and conoid in *Steinernema* or longer than the anal diameter in *Neosteinernema*.

Infective Juvenile of the 3rd stage is vermiform, with annulated cuticle, and closed mouth and anus. Stoma collapsed, and pharynx reduced at this stage. Symbiotic bacteria are visible in intestinal pouch posterior to the basal bulb. Nine incisures in lateral lines are visible.

Systematics and Morphology of Heterorhabditids

Although *Rhabditis hambletoni* Pereira, 1937 (Pereira 1937) was the first described heterorhabditid, it is now considered as inquirenda, and the EPN proposed by Poinar *Heterorhabditis bacteriophora* Poinar, 1976 is regarded as type species (Poinar Jr 1979).

Above family level: After Delay and Blaxter (Blaxter et al. 1998; De Ley and Blaxter 2002), the phylogenetic and taxonomic scheme is molecular-based, results from SSU rDNA (Decraemer and Hunt 2013). Hierarchically, the proposed scheme for this group is: “phylum Nematoda Pott, 1932, class Chromadorea Inglis 1983, subclass Chromadoria Pearse 1942, order Rhabditida Chitwood, 1933, suborder Rhabditina Chitwood, 1933, infraorder Rhabditomorpha De Ley & Blaxter, 2002, superfamily Strongyloidea Bird, 1853, family Heterorhabditidae Poinar, 1975.

In all Heterorhabditids, as in CCNs, endoderm originates from posterior blastomeric in 2-cell stage (Chromadorea). Phasmids are present, the caudal gland is absent, and amphidal openings are pore-like (shared, synapomorphic characters of Rhabditida). Stegostom has short, muscular part with less than four muscle sets in juvenile stages, and present bursa with rays in males (Rhabditomorpha) (De Ley and Blaxter 2002). According to De Ley & Blaxter, within Strongyloidea, morphological diversity is in contrast, and there is little diversity in SSU rRNA (De Ley and Blaxter 2002; Zarlenga et al. 1994).The only genus is *Heterorhabditis* Poinar, 1976, which includes more than 21 valid and confirmed species.

Morphology and Diagnosis characters: Heterorhabditids are obligate parasite of insect and arthropods. Infective juveniles (IJs) are non-feeding, carrying symbiotic entomopathogenic bacteria. Reproduction is hermaphroditic for adults of the first generation, and amphimictic for the second generation; for the morphology of all stages, Adams & Nguyen will be referenced (Adams and Nguyen 2002).

Morphology of adults: After Nguyen (Nguyen 2007), six conoid lips with a single terminal papilla are on the cephalic region. Small amphid, wide and shallow stoma is characteristics of the adults. The

pharynx is short, procorpus cylindrical without enlarging metacorpus. The metacorpus is spherical. Excretory pore is posterior to the basal bulb.

Male: two slender, straight spicules. Gubernaculum is linear. Bursa is present with nine pairs of genital papillae or rays. Tail medium conoid, tapering and pointed.

Female: Transverse slit vulva at mid-body region. Female that are hermaphrodite have didelphic-amphidelphic ovotestes, while the amphimictic females have didelphic-amphidelphic reflexed ovaries. Tail in the female is short and conoid.

Infective Juvenile: at 3rd stage, juveniles are vermiform and inside the J2's cuticle. They are dauer which is non-feeding, closed mouth and anus stage, with an apical dorsal tooth in cephalic region. The lateral field has two ridges with non-visible middle incisure through light microscopy (LM). The stoma collapsed, and pharynx reduced in this stage. Symbiotic bacteria remain in the intestine. Excretory pore, posterior to nerve ring.

Biology of EPN-Bacterium

Infective dauer stage (J3) is contained inside the J2 cuticle (Poinar Jr 1979), carrying the symbiont bacteria in the intestine. Upon detecting its host, the Infective Juvenile (IJ) enters the host through mouth, anus, spiracles or possible damage and locate in hemocoel. Although very rarely, heterorhabditid infective juveniles may also penetrate through integument using the dorsal tooth (Mracek et al. 1965). The bacterium transfers into the hemolymph of the larva and kills the host in 48 hours. The nematode undergoes two or three generation until emerging from the host, and infective juveniles try to find a new host. The relationship between bacteria and EPN is mutualistic (Akhurst and Brooks 1984).

Although environmental extremes increase the rate of mortality in the soil, EPNs can survive low temperature and slow desiccation, and better survive in sandy soils (Kung et al. 1990; Molyneux 1985; Schmidt and All 1979). Aeration may drastically reduce the population in soil (Kung et al. 1990).

Efficacy of EPN Species Against Wireworm

To date, several studies focused on the EPN characteristics and their potential pathogenicity (Ensafi et al. 2018; Půža and Mráček 2010). In case of wireworms, cuticle permeability of J3 in protecting the IJs, motility and scavenging behavior, and presence of the dorsal tooth are traits that need further investigation, as findings from previous studies are contradicting (Lortkipanidze et al. 2016; Toba et al. 1983).

Physical Deterrents to EPN Infection in Wireworm: Explained by Eidt & Thurston, the physical deterrents include morphological barriers and behavioral responses (Eidt and Thurston 1995). In 16 wireworm species, the spiracles are close to EPN IJs. Either primary orifices are tight, or the secondary orifices (which are lined densely by hairs with narrow slit opening) makes the penetration of IJs very difficult (Lanchester 1939). Preoral cavity with dense projecting brushed hairs which originate from hypopharynx, together with preoral digestion, and pharyngeal pump create a proper filtering system against both EPNs with larger diameters (Eidt 1958; Poinar Jr and Georgis 1990). Although the anus does not have a sclerotized structure or barrier, the firmly closed anal opening also prevent possible entry by IJs (Eidt 1958). The intersegment membrane is thick and sclerotized which makes the EPN entry tough (Morris 1985).

Previous studies show that despite these physical barriers, some species of wireworm are susceptible to nematode infection. For instance, *Limonius californicus* showed susceptibility to *S. feltiae* infection only when the nematode was present at high concentration. However, 100% mortality was not achieved even at the highest concentration of 393 EPN per cm^2 in the laboratory; there, 27% mortality was observed after seven days of exposure to 5000 IJs per wireworm larva (Toba et al. 1983). In the field, 29% mortality was achieved by applying 310 EPNs per cm^2 soil surface. Toba and colleagues (1983) also found their set of EPNs failed in infecting summer generations of *L. californicus* and concluded that a minimum of 1.6 million EPN must be present per square meter of soil for successful control. A study by Laumond showed both major EPN species effective (Eidt and Thurston 1995).

All recent studies on EPN efficacy against wireworm target wireworm species that are not problematic in southeastern Idaho (Kleespies et al. 2013; Lortkipanidze et al. 2016; Reddy et al. 2014; Tangtrakulwanich et al. 2014).

Behavioral deterrents in wireworm: Wireworms show active defense and/or behavioral deterrents against *S. carpocapsae*. Fire ants (*Solenopsis invicta*), and scarabaeid larvae actively groom EPNs and inhibit penetration of EPN by removal (Bedding and Molyneux 1982; Drees et al. 1992). There is no report of active defense for wireworm.

Conclusion

Previous studies on the two major cereal pests, *Heterodera avenae* and *Limonius californicus*, have documented the control strategies that are either not applicable, or fail to suppress the population and reduce the damage in southeast Idaho. Complexity of the identification of *H. avenae* and the fact

that species of this nematode require specific resistant/tolerant cultivars make the study of the occurring species in southeastern Idaho very important. It is well documented that different species require different management strategy, and the control methods requires a clear evaluation of species name (identification), precise assessment of possible occurrence of the species in the field (detection), and disease diagnostics of the CCNs. Morphological approach is not enough to distinguish the CCN species with economical damage from each other. Previous molecular approaches such as PCR-RFLP for distinguishing CCN species are time consuming and require a lot of effort, besides, such techniques are not accurate enough to distinguish the closely related species (Avenae species complex). Therefore, applying new molecular approach for identification, detection, and evaluation of population density are inevitable. Ordinary management strategies fail to provide control on the population of wireworms. Biological control strategies using entomopathogenic nematodes, although examined for other species of wireworms, should be assessed against *Limonius californicus*. Because of the intraspecific variability and complexity of identification of effective EPNs, regional approach using molecular identification, as the first step, and the study of pathogenicity of local species should be considered.

References

Adams, B. J., and Nguyen, K. B. 2002. Taxonomy and systematics. Pages 1-33 in: Entomopathogenic Nematology. R. Gaugler, ed. CABI, UK.

Akhurst, R. J., and Brooks, W. M. 1984. The distribution of entomophilic nematodes (Heterorhabditidae and Steinernematidae) in North Carolina. Journal of Invertebrate Pathology 44:140-145.

Al-Doss, A., Al-Hazmi, A. S., Dawabah, A. A., Abdel-Mawgood, A. A., Al-Rehiayani, S. M., Al-Otayk, S., Moustafa, K. A., and Motawei, M. 2010. Impact of *Cre* and Peroxidase genes of selected new wheat lines on cereal cyst nematode (*Heterodera avenae* Woll) resistance. Australian Journal of Crop Science 4:737-743.

Amiri, S., Subbotin, S. A., and Moens, M. 2002. Identification of the beet cyst nematode *Heterodera schachtii* by PCR. European Journal of Plant Pathology 108:497-506.

Anand, S., Cook, R., and Dale, M. 1998. Development of resistant and tolerant varieties. Pages 293-321 in: The Cyst Nematodes. Springer.

Andersen, S. 1965. Heredity of race 1 or race 2 in *Heterodera avenae*. Nematologica 11:121-124.

Andersen, S., and Andersen, K. 1982. Suggestions for determination and terminology of pathotypes and genes for resistance in cyst-forming nematodes, especially *Heterodera avenae*. EPPO Bulletin 12:379-386.

Anonymous. 2011. Plant nematology. 2 ed. CABI, Boston.

Asiedu, R., Fisher, J., and Driscoll, C. 1990. Resistance to *Heterodera avenue* in the rye genome of triticale. TAG Theoretical and Applied Genetics 79:331-336.

Atkinson, H. J., Urwin, P. E., and McPherson, M. J. 2003. Engineering plants for nematode resistance. Annual Review of Phytopathology 41:615-639.

Bačić, J., Stare, B. G., Sĭrca, S., and Urek, G. 2008. Analyses of *Globodera rostochiensis* and *G. pallida* populations from Serbia by morphometrics and real-time PCR. Russian Journal of Nematology 16:63-65.

Baldwin, J. G., and Mundo-Ocampo, M. 1991. Heteroderinae, Cyst-and non-Cyst-forming Nematodes. Pages 275-362 in: Manual of Agricultural Nematology. W. R. Nickle, ed. Marcel Dekker, Inc., New York.

Banyer, R., and Fischer, J. 1971. Effect of temperature on hatching of eggs of *Heterodera avenae*. Nematologica 17:519-534.

Banyer, R., and Fisher, J. 1971. Seasonal variation in hatching of eggs of *Heterodera avenae*. Nematologica 17:225-236.

Banyer, R., and Fisher, J. 1972. Motility in relation to hatching of eggs of *Heterodera avenae*. Nematologica 18:18-24.

Bariana, H., and McIntosh, R. 1994. Characterisation and origin of rust and powdery mildew resistance genes in VPM1 wheat. Euphytica 76:53-61.

Barrett, J., and Wright, D. J. 1998. Intermediary metabolism. Physiology and Biochemistry of Free-Living and Plant-Parasitic Nematodes:331-353.

Bates, J. A., Taylor, E. J. A., Gans, P. T., and Thomas, J. E. 2002. Determination of relative proportions of *Globodera* species in mixed populations of potato cyst nematodes using PCR product melting peak analysis. Molecular Plant Pathology 3:153-161.

Bedding, R., and Molyneux, A. 1982. Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp.(Heterorhabditidae: Nematoda). Nematologica 28:354-359.

Bekal, S., Gauthier, J., and Rivoal, R. 1997. Genetic diversity among a complex of cereal cyst nematodes inferred from RFLP analysis of the ribosomal internal transcribed spacer region. Genome 40:479-486.

Bhan, B., and Kanwar, R. 2012. Compatibility of carbosulfan and *Azotobacter chroococcum* as seed treatment for management of *Heterodera avenae* in Wheat. Annals of Plant Protection Sciences 20:456-458.

Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T., and Thomas, W. K. 1998. A molecular evolutionary framework for the phylum Nematoda. Nature 392:71-75.

Blok, V., Malloch, G., Harrower, B., Phillips, M., and Vrain, T. 1998. Intraspecific variation in ribosomal DNA in populations of the potato cyst nematode *Globodera pallida*. Journal of Nematology 30:262-274.

Boag, B., and Lopez-Llorca, L. V. 1989. Nematodes and nematophagous fungi associated with cereal fields and permanent pasture in eastern Scotland. Crop Research 29:1-10.

Bonhomme, A., Gale, M., Koebner, R., Nicolas, P., Jahier, J., and Bernard, M. 1995. RFLP analysis of an *Aegilops ventricosa* chromosome that carries a gene conferring resistance to leaf rust (*Puccinia recondita*) when transferred to hexaploid wheat. Theoretical and Applied Genetics 90:1042-1048.

Brown, G., Callow, R., Green, C., Jones, F., Rayner, J., Shepherd, A. M., and Williams, T. 1971. The structure, composition and origin of the subcrystalline layer in some species of the genus *Heterodera*. Nematologica 17:591-599.

Brown, R. H. 1973. Chemical control of the cereal cyst nematode (*Heterodera avenae*)-a comparison of methods and rates of application of two systemic nematicides. Animal Production Science 13:587-592.

Brown, R. H. 1984. Ecology and control of cereal cyst nematode (*Heterodera avenae*) in southern Australia. Journal of Nematology 16:216-222.

Brown, R. H. 1987. Control strategies in low-value crops. Pages 351-387 in: Principles and Practice of Nematode Control in Crops. R. H. Brown and B. R. Kerry, eds. Academic Press Australia, Sydney N.S.W.

Brown, R. H., and Young, R. M. 1982. Katyil, a new wheat resistant to cereal cyst nematode. Agnote, Victoria Department of Agriculture 1875/82:38.

Bulman, S., and Marshall, J. 1997. Differentiation of Australasian potato cyst nematode (PCN) populations using the polymerase chain reaction (PCR). New Zealand Journal of Crop and Horticultural Science 25:123-129.

Chen, C., Liu, S., Liu, Q., Niu, J., Liu, P., Zhao, J., and Jian, H. 2015. An ANNEXIN-like protein from the cereal cyst nematode Heterodera avenae suppresses plant defense. PLOS One 10:e0122256-e0122256.

Chew, B., Cook, R., and Thomas, H. 1981. Investigations on resistance of oats to *Heterodera avenae*: location of resistance genes. Euphytica 30:669-673.

Christoforou, M., Pantelides, I. S., Kanetis, L., Ioannou, N., and Tsaltas, D. 2014. Rapid detection and quantification of viable potato cyst nematodes using qPCR in combination with propidium monoazide. Plant Pathology 63:1185-1192.

Clamot, G., and Rivoal, R. 1984. Genetic resistance to cereal cyst nematode *Heterodera avenae* Woll. in wild oat *Avena sterilis* I. 376. Euphytica 33:27-32.

Cook, R., and Rivoal, R. 1998. Genetics of Resistance and Parasitism. Pages 322-352 in: The Cyst Nematodes. S. B. Sharma, ed. Kluwer Academic Publishers, Dortdrekht, The Netherlands.

Cotten, J. 1962. Effect of temperature on hatching in the cereal root eelworm. in: Nature Nature Publishing Group.

Cui, J.-K., Huang, W.-K., Peng, H., Lv, Y., Kong, L.-A., Li, H.-X., Luo, S.-J., Wang, Y., and Peng, D.-L. 2017. Efficacy evaluation of seed-coating compounds against cereal cyst nematodes and root lesion nematodes on wheat. Plant Disease 101:428-433.

Curtis, R. H. C., Dunn, J., Robinson, M. P., Martins, F., and Evans, K. 1998. Serological identification and quantification of potato cyst nematodes from clean cysts and processed soil samples. Annals of applied biology 133:65-79.

Dababat, A. A., Pariyar, S., Nicol, J. M., and Duveiller, E. 2011. Cereal Cyst Nematodes: An unnoticed threat to global cereal production. Page 2, France. Available at: http://hdl.handle.net/10883/3991.

Davies, K. A., and Fisher, J. 1976. Duration of infectivity of second stage larvae of *Heterodera avenae*. Nematologica 22:163-168.

Davis, E. L., Hussey, R. S., and Baum, T. J. 2004. Getting to the roots of parasitism by nematodes. Trends in Parasitology 20:134-141.

De Ley, P., and Blaxter, M. 2002. Systematic position and phylogeny. Pages 1-30 in: The Biology of Nematodes. D. L. Lee, ed. Taylor and Francis, London.

Decraemer, W. 2011. Nematode morphology. Academia Press / Gent.

Decraemer, W., and Hunt, D. J. 2013. Structure and classification. Pages 3-37 in: Plant Nematology. R. N. Perry and M. Moens, eds. CAB International, UK.

Delibes, A., Romero, D., Aguaded, S., Duce, A., Mena, M., Lopez-Braña, I., Andrés, M. F., Martin-Sanchez, J., and García-Olmedo, F. 1993. Resistance to the cereal cyst nematode (*Heterodera avenae* Woll.) transferred from the wild grass *Aegilops ventricosa* to hexaploid wheat by a "stepping-stone" procedure. Theoretical and Applied Genetics 87:402-408.

Dowton, M., Castro, L., and Austin, A. 2002. Mitochondrial gene rearrangements as phylogenetic characters in the invertebrates: the examination of genome'morphology'. Invertebrate Systematics 16:345-356.

Drees, B. M., Miller, R. W., Vinson, B. S., and Georgis, R. 1992. Susceptibility and behavioral response of red imported fire ant (Hymenoptera: Formicidae) to selected entomogenous nematodes (Rhabditida: Steinernematidae & Heterorhabditidae). Journal of Economic Entomology 85:365-370.

Eastwood, R. F., Lagudah, E. S., and Appels, R. 1994. A directed search for DNA sequences tightly linked to cereal cyst nematode resistance genes in *Triticum tauschii*. Genome 37:311-319.

Eastwood, R. F., Lagudah, E. S., Appels, R., Hannah, M., and Kollmorgen, J. F. 1991. *Triticum tauschii*: a novel source of resistance to cereal cyst nematode (*Heterodera avenae*). Crop and Pasture Science 42:69-77.

Eidt, D. C. 1958. Anatomy and histology of the full-grown larva of *Ctenicera aeripennis* destructor (Brown)(Coleoptera: Elateridae). Canadian Journal of Zoology 36:317-361.

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

Ensafi, P., Crowder, D. W., Esser, A. D., Zhao, Z., Marshall, J. M., and Rashed, A. 2018. Soil type mediates the effectiveness of biological control against *Limonius californicus* (Coleoptera: Elateridae). Journal of Economic Entomology 111:2053-2058.

Evans, A. A. F. 1986. Diapause as a survival strategy. Page 591 in: 25th Annual Meeting of Society of Nematologists Journal of Nematology, Sheraton Twin Towers, Orlando, Florida.

Feiyue, Y. H. C. L. Z., and Honglian, L. (2011) 'Isolation and identification of fungal parasites of cyst nematodes in *Heterodera avenae* group'. Available at: http://en.cnki.com.cn/Article_en/CJFDTotal-ZWBF201101011.htm.

Ferris, V., Ferris, J., Faghihi, J., and Ireholm, A. 1994. Comparisons of isolates of *Heterodera avenae* using 2-D PAGE protein patterns and ribosomal DNA. Journal of Nematology 26:144-151.

Ferris, V. R., Ferris, J. M., and Faghihi, J. 1993. Variation in spacer ribosomal DNA in some cyst-forming of plant parasitic nematodes. Fundamental and Applied Nematology 16:177-184.

Ferris, V. R., Subbotin, S. A., Ireholm, A., Spiegel, Y., Faghihi, J., and Ferris, J. M. 1999. Ribosomal DNA sequence analysis of *Heterodera filipjevi* and *H. latipons* isolates from Russia and comparisons with other nematode isolates. Russian Journal of Nematology 7:121-125.

Feyaerts, H., and Coosemans, J. 1992. Influence of the thiocarbamate herbicide cycloate on the attraction of beet cyst nematodes (*Heterodera schachtii* Schmidt) by their host plants. in: Internationaal Symposium over Fytofarmacie en Fytitrie, Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent (Belgium).

Fisher, J. 1982. Problems with the Use of Resistance in Wheat to the Australian Pathotype of *Heterodera avenae*. EPPO Bulletin 12:417-421.

Franklin, M. T. 1951. The cyst-forming species of *Heterodera*. Commonwealth Agricultural Bureau, Great Britain.

Fullaondo, A., Barrena, E., Viribay, M., Barrena, I., Salazar, A., and Ritter, E. 1999. Identification of potato cyst nematode species *Globodera rostochiensis* and *G. pallida* by PCR using specific primer combinations. Nematology 1:157-163.

Gäbler, C., Sturhan, D., Subbotin, S. A., and Rumpenhorst, H. J. 2000. *Heterodera pratensis* sp n., a new cyst nematode of the *H. avenae* complex (Nematoda : Heteroderidae). Russian Journal of Nematology 8:115-126.

Gheysen, G., and Jones, J. T. 2006. Molecular Aspects of Plant-Nematode Interactions. Plant Nematology:234-254.

Gibson, T., Farrugia, D., Barrett, J., Chitwood, D. J., Rowe, J., Subbotin, S., and Dowton, M. 2011. The mitochondrial genome of the soybean cyst nematode, *Heterodera glycines*. Genome 54:565-574.

Goto, K., Sato, E., and Toyota, K. 2009. A novel detection method for the soybean cyst nematode *Heterodera glycines* Ichinohe using soil compaction and real-time PCR. Nematological Research (Japanese Journal of Nematology) 39:1-7.

Green, C., and Plumb, S. C. 1970. The interrelationships of some *Heterodera* spp. indicated by the specificity of the male attractants emitted by their females. Nematologica 16:39-46.

Green, C. D. 1980. Nematode sex attractants. Helmintology Abstracts, Seris A, Animal and Human Helminthology 49:327-339.

Grymaszewska, G., and Golinowski, W. 1991. Structure of syncytia induced by *Heterodera avenae* Woll. in roots of susceptible and resistant wheat (*Triticum aestivum* L.). Journal of Phytopathology 133:307-319.

Hajihasani, A., Maafi, Z. T., Nicol, J. M., and Seraji, A. 2010a. Relationships between population densities of the cereal cyst nematode, *Heterodera latipons* and yield losses of winter wheat in microplots. Australasian Plant Pathology 39:530-535.

Hajihasani, A., Maafi, Z. T., Nicol, J. M., and Rezaee, S. 2010b. Effect of the cereal cyst nematode, *Heterodera filipjevi*, on wheat in microplot trials. Nematology 12:357-363.

Hajihassani, A., Maafi, Z. T., and Hosseininejad, A. 2013. Interactions between *Heterodera filipjevi* and *Fusarium culmorum*, and between *H. filipjevi* and *Bipolaris sorokiniana* in winter wheat. Journal of Plant Diseases and Protection 120:77-84.

Handoo, Z. A. 2002. A key and compendium to species of the *Heterodera avenae* group (Nematoda : Heteroderidae). Journal of Nematology 34:250-262.

Hesling, J. J. 1978. Cyst nematodes: morphology and identification of *Heterodera*, *Globodera* and *Punctodera*. Pages 125-155 in: Plant Nematology. J. F. Southey, ed. HM Stationery Office, London.

Holgado, R., Andersson, S., Rowe, J., Clark, I., and Magnusson, C. 2009. Management strategies for cereal cyst nematodes *Heterodera* spp. in Norway. Pages 154-159 in: International Cereal Cyst Nematode Initiative, I. T. Riley, J. M. Nicol and A. A. Dababat, eds. CIMMYT, Turkey, Antalya.

Hunt, D. J. 2007. Overview of taxonomy and systematics. Pages 27-57 in: Entomopathogenic Nematodes: Systematics, phylogeny and bacterial symbionts, vol. 5. K. B. Nguyen and D. J. Hunt, eds. Brill, Leiden-Boston.

Imren, M., Toktay, H., Bozbuga, R., Dababat, A., and Elekcioglu, İ. H. 2013. Pathotype determination of the cereal cyst nematode, *Heterodera avenae* (Wollenweber, 1924) in the Eastern Mediterranean Region in Turkey. Turkish Journal of Entomology 37:13-19.

Ireholm, A. 1994. Characterization of pathotypes of cereal cyst nematodes, *Heterodera* spp., in Sweden. Nematologica 40:399-411.

Jahier, J., Tanguy, A., Abelard, P., and Rivoal, R. 1996. Utilization of deletions to localize a gene for resistance to the cereal cyst nematode, *Heterodera avenue*, on an *Aegilops ventricosa* chromosome. Plant Breeding 115:282-284.

Jahier, J., Abelard, P., Tanguy, A. M., Dedryver, F., Rivoal, R., Khatkar, S., and Bariana, H. S. 2001. The *Aegilops ventricosa* segment on chromosome 2AS of the wheat cultivar 'VPM1' carries the cereal cyst nematode resistance gene Cre5. Plant Breeding 120:125-128.

Jayatilake, D. V., Tucker, E. J., Brueggemann, J., Lewis, J., Garcia, M., Dreisigacker, S., Hayden, M. J., Chalmers, K., and Mather, D. E. (2015) 'Genetic mapping of the *Cre8* locus for resistance against cereal cyst nematode (*Heterodera avenae* Woll.) in wheat'. Available at: https://doi.org/10.1007/s11032-015-0235-3.

Jensen, H., Eshtiaghi, H., Koepsell, P., and Goetze, N. 1975. The oat cyst nematode, *Heterodera avenae*, occurs in oats in Oregon. Plant Disease Reporter 59:1-3.

Kerry, B. R. 1988. Fungal Parasites of Cyst Nematodes. Agriculture, Ecosystems & Environment 24:293-305.

Kerry, B. R., and Hague, N. G. M. 1974. The invasion and development of the cereal cyst nematode, *Heterodera avenae* in the roots of autumn and spring sown cereals. Annals of Applied Biology 78:319-330.

Kerry, B. R., and Jenkinson, S. C. 1976. Observations on emergence, survival and root invasion of second-stage larvae of the cereal cyst nematode, *Heterodera avenae*. Nematologica 22:467-474.

Kerry, B. R., and Hirsch, P. R. 2011. Ecology of *Pochonia chlamydosporia* in the rhizosphere at the population, whole organism and molecular scales. Pages 171-182 in: Biological Control of Plant-Parasitic Nematodes: . K. G. Davies and Y. Spiegel, eds. Springer.

Kerry, B. R., Crump, D. H., and Mullen, L. A. 1980. Parasitic fungi, soil-moisture and multiplication of the cereal cyst nematode *Heterodera avenae*. Nematologica 26:57-68.

Kerry, B. R., Crump, D. H., and Mullen, L. A. 1982. Studies of the ceral cyst nematode, *Heterodera avenae* under continuous cereals, 1974-1978 .I. Plant-growth and nematode multiplication. Annals of Applied Biology 100:477-487.

Kleespies, R. G., Ritter, C., Zimmermann, G., Burghause, F., Feiertag, S., and Leclerque, A. 2013. A survey of microbial antagonists of *Agriotes* wireworms from Germany and Italy. Journal of Pest Science 86:99-106.

Koenning, S. R., and Sipes, B. S. 1998. Biology. Pages 156-191 in: The Cyst Nematodes, vol. 1. S. B. Sharma, ed. Kluwer Academic publications.

Kovaleva, E. S., Subbotin, S. A., Masler, E. P., and Chitwood, D. J. 2005. Molecular characterization of the actin gene from cyst nematodes in comparison with those from other nematodes. Comparative Parasitology 72:39-49.

Kraus, R., and Sikora, R. 1981. Die Wirkung des Herbizides Diallat auf den Befall von *Heterodera schachtii* an Zuckerrüben/Effect of the herbicide diallate on *Heterodera schachtii* development on sugar beet. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection 88:210-217.

Krausse, A. 1917. Forstentomologische Exkursionen ins Eggegebirge zum Studium der Massenvermehrung der *Cephaleia abietis* L. Pages 46-49 Archiv für Naturgeschichte, Berlin.

Kretschmer, J. M., Chalmers, K. J., Manning, S., Karakousis, A., Barr, A. R., Islam, A. K. M. R., Logue, S. J., Choe, Y. W., Barker, S. J., Lance, R. C. M., and Langridge, P. 1997. RFLP mapping of the Ha 2 cereal cyst nematode resistance gene in barley. Theoretical and Applied Genetics 94:1060-1064.

Kung, S. P., Gaugler, R., and Kaya, H. K. 1990. Soil Type and Entomopathogenic Nematode Persistence. Journal of Invertebrate Pathology 55:401-406.

Lanchester, H. P. 1939. The External Anatomy of the Larva of the Pacific Coast Wireworm. United States Department of Agriculture, Economic Research Service. Available at: http://EconPapers.repec.org/RePEc:ags:uerstb:168465.

Lasserre, F., Gigault, F., Gauthier, J. P., Henry, J. P., Sandmeier, M., and Rivoal, R. 1996. Genetic variation in natural populations of the cereal cyst nematode (*Heterodera avenae* Woll.) submitted to resistant and susceptible cultivars of cereals. Theoretical and Applied Genetics 93:1-8.

Lemire, B. 2005. Mitochondrial genetics. WormBook : the online review of C. elegans biology:1-10.

Li, H., Cui, L., Li, H., Wang, X., Murray, T. D., Conner, R. L., Wang, L., Gao, X., Sun, Y., Sun, S., and Tang, W. 2012a. Effective resources in wheat and wheat-thinopyrum derivatives for resistance to *Heterodera filipjevi* in China. Crop Science 52:1209-1217.

Li, X.-h., Ma, J., and Chen, S.-l. 2012b. Effect of temperature on the penetration and development of *Heterodera avenae*. Triticeae Crops 32:977-981.

Li, Y., Lawrence, G. W., Lu, S., Balbalian, C., and Klink, V. P. 2014. Quantitative field testing *Heterodera glycines* from metagenomic DNA samples isolated directly from soil under agronomic production. PloS One 9:e89887.

Long, H., Peng, H., Huang, W., Wang, G., Gao, B., Moens, M., and Peng, D. 2012. Identification and molecular characterization of a new β-1, 4-endoglucanase gene (Ha-eng-1a) in the cereal cyst nematode *Heterodera avenae*. European journal of plant pathology 134:391-400.

Lorenzen, S. 1981. Entwurf eines phylogenetischen systems der freilebenden nematoden. Kommissionsverlag F. Leuwer.

Lorenzen, S. 1994. Phylogenetische systematik gestern, heute und morgen. Biologie in unserer Zeit 24:200-206.

Lortkipanidze, M., Gorgadze, O., Gabroshvili, N., Burjanadze, M., and Kuchava, M. 2016. Efficacy evaluation of *Heterorhabditis bacteriophora* against click beetle (coleoptera: elateridae). Pages 119-122 Microbial and Nematode Control of Invertebrate Pests, IOBC-WPRS.

Luc, M., Weischer, B., Stone, A., and Baldwin, J. 1986. On the definition of heteroderid cysts. Revue de nématologie 9:418-421.

Lung, G. 1993. The role of phytosiderophores as an attractive substance of root exudates from several cereals for second stage juveniles of *Heterodera avenae*. Mededelingen van de Faculteit landbouwwetenschappen 58.

Maafi, Z. T., Subbotin, S. A., and Moens, M. 2003. Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences. Nematology 5:99-111.

Madani, M., Subbotin, S. A., and Moens, M. 2005. Quantitative detection of the potato cyst nematode, *Globodera pallida*, and the beet cyst nematode, *Heterodera schachtii*, using real-time PCR with SYBR green I dye. Molecular and Cellular Probes 19:81-86.

Maggenti, A. R., Luc, M., Raski, D. J., Fortuner, R., and Geraert, E. 1987. A reappraisal of Tylenchina (Nemata). 2. Classification of the suborder Tylenchina (Nemata: Diplogasteria). Revue de Nématologie 10:135-142.

Marshall, J. M., and Smiley, R. W. 2016. Spring barley resistance and tolerance to the cereal cyst nematode *Heterodera avenae*. Plant Disease 100:396-407.

Mathur, B., Handa, D., Swarup, G., Sethi, C., Sharma, G., and Yadav, B. 1986. On the loss estimation and chemical control of 'Molya' disease of wheat caused by *Heterodera avenae* in India. Indian Journal of Nematology 16:152-159.

Meagher, J., Brown, R., and Rovira, A. 1978. The effects of cereal cyst nematode (*Heterodera avenae*) and *Rhizoctonia solani* on the growth and yield of wheat. Australian Journal of Agricultural Research 29:1127-1137.

Molyneux, A. S. 1985. Survival of infective juveniles of *Heterorhabditis* spp., and *Steinernema* spp.(Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects. Revue de Nématologie 8:165-170.

Mor, M., Spiegel, Y., and Oka, Y. 2008. Histological study of syncytia induced in cereals by the Mediterranean cereal cyst nematode *Heterodera latipons*. Nematology 10:279-287.

Morris, O. 1985. Susceptibility of 31 species of agricultural insect pests to the entomogenous nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora*. The Canadian Entomologist 117:401-407.

Mracek, Z., Hanzal, R., and Kodrik, D. 1965. Sites of penetration of juvenile steinernematids and heterorhabditids (Nematoda) into the larvae of G*alleria mellonella* (Lepidoptera). Invertebrate Pathology 52:477-478.

Mulholland, V., Carde, L., ODonnell, K. J., Fleming, C. C., and Powers, T. O. 1996. Use of the polymerase chain reaction to discriminate potato cyst nematode at the species level. Bcpc Symp Ser:247-252.

Mulvey, R., and Stone, A. 1976. Description of *Punctodera matadorensis* n. gen., n. sp.(Nematoda: Heteroderidae) from Saskatchewan with lists of species and generic diagnoses of *Globodera* (n. rank), *Heterodera*, and *Sarisodera*. Canadian Journal of Zoology 54:772-785.

Mundo-Ocampo, M., and Baldwin, J. 1992. Comparison of host response of *Ekphymatodera thomasoni* with other Heteroderinae. Fundamental and Applied Nematology 15:63-70.

Nakhla, M. K., Owens, K. J., Li, W., Wei, G., Skantar, A. M., and Levy, L. 2010. Multiplex real-time PCR assays for the identification of the potato cyst and tobacco cyst nematodes. Plant Disease 94:959-965.

Nguyen, K. B. 2007. Methodology, morphology and identification. Pages 59-119 in: Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbiont, vol. 5. K. B. Nguyen and D. J. Hunt, eds. Brill, Leiden.

Nguyen, K. B., and Smart Jr, G. C. 1996. Identification of entomopathogenic nematodes in the Steinernematidae and Heterorhabditidae (Nemata: Rhabditida). Journal of Nematology 28:286.

Nicol, J. M., and Rivoal, R. 2008. Global knowledge and its application for the integrated control and management of nematodes on wheat. Pages 251-294 in: Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes. A. Ciancio and K. G. Mukerji, eds. Springer, The Netherlands.

Nicol, J. M., Rivoal, R., Trethowan, R. M., Van Ginkel, M., Mergoum, M., and Singh, R. P. 2001. CIMMYT's approach to identify and use resistance to nematodes and soil-borne fungi, in developing superior wheat germplasm. Pages 381-389 in: Wheat in a Global Environment. Z. Bedö and L. Láng, eds. Springer.

Nicol, J. M., Turner, S. J., L., C. D., Den Nijs, L. J., Hockland, S., and Maafi, Z. T. 2011. Current nematode threats to world agriculture. Pages 21-43 in: Genomics and Molecular Genetics of Plant-Nematode Interactions. J. Jones, G. Gheysen and C. Fenoll, eds. Springer, Dordrecht Heidelberg London New York.

Nielsen, C. H. 1966. Untersuchungen Über Die Vererbung Der Resistenz Gegen Den Getreidenematoden (*Heterodera avenae*) Beim Weizen. Nematologica 12:575-578.

Nilsson-Ehle, H. 1920. Über Resistenz gegen *Heterodera Schachti* bei gewissen Gerstensorten, ihre Vererbungsweise und Bedeutung für die Praxis. Hereditas 1:1-34.

Norton, D. C. 1978. Ecology of plant-parasitic nematodes. John Wiley & Sons, Inc.

O'Brien, P., Fisher, J., and Rathjen, A. 1980. Inheritance of resistance in two wheat cultivars to an Australian population of *Heterodera avenae*. Nematologica 26:69-74.

Ogbonnaya, F., Seah, S., Delibes, A., Jahier, J., Lopez-Brana, I., Eastwood, R., and Lagudah, E. 2001. Molecular-genetic characterisation of a new nematode resistance gene in wheat. Theoretical and Applied Genetics 102:623-629.

Ou, S., Peng, D., Li, Y., and Wang, Y. 2008. Restriction fragment length polymorphism and sequences analysis of rDNA-ITS region of cereal cyst nematode (*Heterodera avenae*) on wheat from Zhengzhou. Acta Phytopathologica Sinica 38:407-413.

Paull, J., Chalmers, K., Karakousis, A., Kretschmer, J., Manning, S., and Langridge, P. 1998. Genetic diversity in Australian wheat varieties and breeding material based on RFLP data. Theoretical and Applied Genetics 96:435-446.

Peng, H., Qi, X., Peng, D., Long, H., He, X., Huang, W., and He, W. 2013. Sensitive and direct detection of *Heterodera filipjevi* in soil and wheat roots by species-specific SCAR-PCR assays. Plant Disease 97:1288-1294.

Pereira, C. 1937. *Rhabditis hambletoni* n. sp., nema apparentemente semiparasito da 'broca do algodoeiro' (*Gasterocercodes brasiliensis*). Pages 215-230 Archivo do Instituto Biologico.

Perry, R. N., and Aumann, J. 1998. Behaviour and sensory responses. in: The Physiology and Biochemistry of Free-living and Plant-parasitic Nematodes. R. N. Perry and D. J. Wright, eds. CABI Publishing, UK.

Perry, R. N., Wright, D. J., and Chitwood, D. J. 2013. Reproduction, Physiology and Biochemistery. Pages 219-245 in: Plant Nematology. R. N. Perry and M. Moens, eds. CAB International, USA.

Poinar Jr, G. O. 1979. Nematodes for Biological Control of Insects. CRC Press, Inc., Boca Raton, FL.

Poinar Jr, G. O., and Georgis, R. 1990. Characterization and field application of *Heterorhabditis bacteriophora* strain HP88 (Heteorhabditidae: Rhabditida). Revue de Nématologie 13:387-393.

Půža, V., and Mráček, Z. 2010. Does scavenging extend the host range of entomopathogenic nematodes (Nematoda: Steinernematidae)? Journal of Invertebrate Pathology 104:1-3.

Qi, X., Peng, D., Peng, H., Long, H., Huang, W., and He, W. 2012. Rapid molecular diagnosis based on SCAR marker system for cereal cyst nematode. Scientia Agricultura Sinica 45:4388-4395.

Quader, M., Nambiar, L., and Cunnington, J. 2008. Conventional and real-time PCR-based species identification and diversity of potato cyst nematodes (*Globodera* spp.) from Victoria, Australia. Nematology 10:471-478.

Reddy, G. V., Tangtrakulwanich, K., Wu, S., Miller, J. H., Ophus, V. L., Prewett, J., and Jaronski, S. T. 2014. Evaluation of the effectiveness of entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat. Journal of Invertebrate Pathology 120:43-49.

Reed, D. H., and Frankham, R. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095-1103.

Riley, I. T., Hou, S., and Chen, S. 2010. Crop rotational and spatial determinants of variation in *Heterodera avenae* (cereal cyst nematode) population density at village scale in spring cereals grown at high altitude on the Tibetan Plateau, Qinghai, China. Australasian Plant Pathology 39:424-430.

Rivoal, R. 1983. Biologie d' *Heterodera avenae* Wollenweber en France. III. Evolution des diapauses des races Fr1 et Fr4 au cours de plusieurs années consécutives; influence de la température. Revue de Nématologie 6:157-164.

Rivoal, R., and Person-Dedryver, F. 1982. Caractérisation des pathotypes d' *Heterodera avenae* en France: influence de la période de culture slir le pouvoir discriminant de cultivars d' *Avena sativa* et différences dans la capacityéàfôrmer des femelles1. EPPO Bulletin 12:387-391.

Rivoal, R., and Cook, R. 1993. Nematode Pests of Cereals. Pages 259-303 in: Plant Parasitic Nematodes in Temperate Agriculture. K. Evans, D. L. Trudgill and J. M. Webster, eds. CABI.

Rivoal, R., Lasserre, F., and Cook, R. 1995. Consequences of long-term cropping with resistant cultivars on the population dynamics of the endoparasitic nematodes *Heterodera avenae* and *Pratylenchus neglectus* in a cereal production ecosystem. Nematologica 41:516-529.

Rivoal, R., Valette, S., Bekal, S., Gauthier, J.-P., and Yahyaoui, A. 2003. Genetic and phenotypic diversity in the graminaceous cyst nematode complex, inferred from PCR-RFLP of ribosomal DNA and morphometric analysis. European Journal of Plant Pathology 109:227-241.

Rivoal, R., Dosba, F., Jahier, J., Pierre, J.-S., Penard, P., Tanguy, A.-M., Abelard, P., and Querrien, M.-T. 1986. Les lignées d'addition blé-*Aegilops ventricosa* Tausch. VI.--Etude de la localisation chromosomique de la résistance à l'égard d'H*eterodera avenae* Woll. Agronomie 6:143-148.

Romero, M., Montes, M., Sin, E., Lopez-Brana, I., Duce, A., Martin-Sanchez, J., Andres, M., and Delibes, A. 1998. A cereal cyst nematode (*Heterodera avenae* Woll.) resistance gene transferred from *Aegilops triuncialis* to hexaploid wheat. Theoretical and Applied Genetics 96:1135-1140.

Sahin, E., Nicol, J. M., Elekcioglu, I. H., and Rivoal, R. 2010. Hatching of *Heterodera filipjevi* in controlled and natural temperature conditions in Turkey. Nematology 12:193-200.

Schmidt, J., and All, J. N. 1979. Attraction of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) to common excretory products of insects. Environmental Entomology 8:55-61.

Scholz, U., and Sikora, A. R. 2004. Hatching behaviour and life cycle of *Heterodera latipons* Franklin under Syrian agro-ecological conditions. Nematology 6:245-256.

Schulze, J., and Schierenberg, E. 2011. Evolution of embryonic development in nematodes. EvoDevo 2:16.

Seinhorst, J. 1964. Methods for the extraction of *Heterodera* cysts from not previously dried soil samples. Nematologica 10:87-94.

Sharma, S. B., and Sharma, R. 1998. Hatch and emergence. Pages 191-216 in: The Cyst Nematodes. S. B. Sharma, ed. Springer Science & Business Media, Boston.

Siddiqi, M. R. 2000. Tylenchida: parasites of plants and insects. 2 ed. CABI Publishing, Wallingford, Oxon OX10 8DE, UK.

Skantar, A. M., Handoo, Z. A., Carta, L. K., and Chitwood, D. J. 2007. Morphological and molecular identification of *Globodera pallida* associated with potato in Idaho. Journal of Nematology 39:133-144.

Smiley, R., Dababat, A. A., Iqbal, S., Jones, M. G., Tanha Maafi, Z., Peng, D., Subbotin, S. A., and Waeyenberge, L. 2017. Cereal Cyst Nematodes: A complex and destructive group of *Heterodera s*pecies. Plant Disease 101:1692-1720.

Smiley, R. W. 2009a. Water and temperature parameters associated with winter wheat diseases caused by soilborne pathogens. Plant Disease 93:73-80.

Smiley, R. W. 2009b. Occurrence, distribution and control of *Heterodera avenae* and *H. filipjevi* in the western USA. Pages 35-40 in: Cereal cyst nematodes: status, research and outlook, I. T. Riley, J. M. Nicol and A. A. Dababat, eds. CIMMYT, Ankara, Turkey.

Smiley, R. W., and Yan, G. 2015. Discovery of *Heterodera filipjevi* in Washington and comparative virulence with *H. avenae* on wheat. Plant Disease 99:376-386.

Smiley, R. W., and Marshall, J. M. 2016. Detection of dual *Heterodera avenae* resistance plus tolerance traits in spring wheat. Plant Disease 100:1677-1685.

Smiley, R. W., Yan, G., and Pinkerton, J. N. 2011a. Resistance of wheat, barley and oat to *Heterodera avenae* in the Pacific Northwest, USA. Nematology 13:539-552.

Smiley, R. W., Marshall, J. M., and Yan, G. 2011b. Effect of foliarly applied spirotetramat on reproduction of *Heterodera avenae* on wheat roots. Plant Disease 95:983-989.

Smiley, R. W., Ingham, R. E., Uddin, W., and Cook, G. H. 1994. Crop Sequences for managing cereal cyst nematode and ungal pathogens of winter wheat. Plant Disease 78:1142-1149.

Smiley, R. W., Whittaker, R. G., Gourlie, J. A., Easley, S. A., and Ingham, R. E. 2005. Plant-parasitic nematodes associated with reduced wheat yield in Oregon: *Heterodera avenae*. Journal of Nematology 37:297-307.

Smiley, R. W., Machado, S., Rhinhart, K. E. L., Reardon, C. L., and Wuest, S. B. 2016. Rapid quantification of soilborne pathogen communities in wheat-based long-term field experiments. Plant Disease 100:1692-1708.

Smiley, R. W., Gourlie, J. A., Rhinhart, K. E. L., Marshall, J. M., Anderson, M. D., and Yan, G. 2012. Influence of nematicides and fungicides on spring wheat in fields infested with soilborne pathogens. Plant Disease 96:1537-1547.

Smiley, R. W., Marshall, J. M., Gourlie, J. A., Paulitz, T. C., Kandel, S. L., Pumphrey, M. O., Garland-Campbell, K., Yan, G., Anderson, M. D., Flowers, M. D., and Jackson, C. A. 2013. Spring Wheat tolerance and resistance to *Heterodera avenae* in the Pacific Northwest. Plant Disease 97:590-600.

Sparrow, D., and Dube, A. 1981. Breeding barley cultivars resistant to cereal cyst nematode in Australia. Pages 410-417 in: Proceedings 4th International Barley Genetics Symposium, M. J. C. Asher, R. P. Ellis, A. M. Hatter and R. N. H. Whitehouse, eds., Edinburgh, Scotland.

Spiridonov, S. E., and Subbotin, S. A. 2016. Phylogeny and phylogeography of Heterorhabditis and Steinernema. Advances in Taxonomy and Phylogeny of Entomopathogenic Nematodes of the Steinernematidae and Heterorhabditidae 12:413-427.

Subbotin, S. A. 2015. *Heterodera sturhani* sp. n. from China, a new species of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae). Russian Journal of Nematology 23:145-152.

Subbotin, S. A., Rumpenhorst, H. J., and Sturhan, D. 1996. Morphological and electrophoretic studies on populations of the *Heterodera avenae* complex from the former USSR. Russian Journal of Nematology 4:29-38.

Subbotin, S. A., Waeyenberge, L., and Moens, M. 2000. Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda : Heteroderidae) based on the ribosomal DNA-RFLP. Nematology 2:153-164.

Subbotin, S. A., Mundo-Ocampo, M., and Baldwin, J. 2010a. Systematics of Cyst Nematodes (Nematoda: Heteroderinae), Part B. 8B ed. Brill, Boston.

Subbotin, S. A., Mundo-Ocampo, M., and Baldwin, J. G. 2010b. Systematics of Cyst Nematodes (Nematoda: Heteroderinae), Part A. 8A ed. Brill, Boston.

Subbotin, S. A., Sturhan, D., Waeyenberge, L., and Moens, M. 1997. *Heterodera riparia* sp. n.(Tylenchida: Heteroderidae) from common nettle, *Urtica dioica* L., and rDNA-RFLP separation of species from the *H. humuli* group. Russian Journal of Nematology 5:143-157.

Subbotin, S. A., Waeyenberge, L., Molokanova, I. A., and Moens, M. 1999. Identification of *Heterodera avenae* group species by morphometrics and rDNA-RFLPs. Nematology 1:195-207.

Subbotin, S. A., Sturhan, D., Rumpenhorst, H. J., and Moens, M. 2003. Molecular and morphological characterisation of the *Heterodera avenae* species complex (Tylenchida : Heteroderidae). Nematology 5:515-538.

Subbotin, S. A., Vera, I. C. D. P., Mundo-Ocampo, M., and Baldwin, J. G. 2011. Identification, phylogeny and phylogeography of circumfenestrate cyst nematodes (Nematoda: Heteroderidae) as inferred from analysis of ITS-rDNA. Nematology 13:805-824.

Subbotin, S. A., Vierstraete, A., De Ley, P., Rowe, J., Waeyenberge, L., Moens, M., and Vanfleteren, J. R. 2001. Phylogenetic relationships within the cyst-forming nematodes (Nematoda, Heteroderidae) based on analysis of sequences from the ITS regions of ribosomal DNA. Molecular Phylogenetics and Evolution 21:1-16.

Szalanski, A. L., Sui, D. D., Harris, T., and Powers, T. O. 1997. Identification of cyst nematodes of agronomic and regulatory concern with PCR-RFLP of ITS1. Journal of Nematology 29:255.

Tangtrakulwanich, K., Reddy, G. V., Wu, S., Miller, J. H., Ophus, V. L., and Prewett, J. 2014. Efficacy of entomopathogenic fungi and nematodes, and low risk insecticides against wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae). Journal of Agricultural Science 6:1-9.

Taylor, C., Shepherd, K. W., and Langridge, P. 1998. A molecular genetic map of the long arm of chromosome 6R of rye incorporating the cereal cyst nematode resistance gene, *CreR*. Theoretical and Applied Genetics 97.

Taylor, S., Vanstone, V. A., Ware, A., McKay, A. C., Szot, D., and Russ, M. H. 1999. Measuring yield loss in cereals caused by root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) with and without nematicide. Crop and Pasture Science 50:617-627.

Toba, H. H., Lindegren, J. E., Turner, J. E., and Vail, P. V. 1983. Susceptibility of the Colorado potato beetle and the sugarbeet wireworm to *Steinernema feltiae* and *S. glaseri*. Journal of Nematology 15:597-601.

Toktay, H., Yavuzaslanoğlu, E., İmren, M., Nicol, J., Elekcioğlu, İ. H., and Dababat, A. 2012. Screening for resistance to *Heterodera filipjevi* (Madzhidov) Stelter (Tylenchida: Heteroderidae) and *Pratylenchus thornei* (Sher & Allen)(Tylenchida: Pratylenchidae) sister lines of spring wheat. Turkiye Entomoloji Dergisi 36:455-461.

Toumi, F., Waeyenberge, L., Viaene, N., Dababat, A., Nicol, J. M., Ogbonnaya, F., and Moens, M. 2013. Development of two species-specific primer sets to detect the cereal cyst nematodes *Heterodera avenae* and *Heterodera filipjevi*. European Journal of Plant Pathology 136:613-624.

Toumi, F., Waeyenberge, L., Viaene, N., Dababat, A. A., Nicol, J. M., Ogbonnaya, F., and Moens, M. 2015. Development of qPCR assays for quantitative detection of *Heterodera avenae* and *H. latipons*. European Journal of Plant Pathology 143:305-316.

Toumi, F., Waeyenberge, L., Viaene, N., Dababat, A. A., Nicol, J. M., Ogbonnaya, F., and Moens, M. 2017. Cereal cyst nematodes: importance, distribution, identification, quantification, and control. European Journal of Plant Pathology:1-20.

Toyota, K., Shirakashi, T., Sato, E., Wada, S., and Min, Y. Y. 2008. Development of a real-time PCR method for the potato-cyst nematode *Globodera rostochiensis* and the root-knot nematode *Meloidogyne incognita*. Soil Science and Plant Nutrition 54:72-76.

Turner, S. J., and Subbotin, S. A. 2013. Cyst Nematodes. Pages 109-143 in: Plant Nematology. R. N. Perry and M. Moens, eds. CABI, Nosworthy Way, Wallingford, Oxfordshire OX10 8DE, UK.

Van Bezooijen, J. 2006. Sampling. Pages 2-11 in: Methods and Techniques for Nematology. Wageningen University Wageningen.

Vanstone, V., Hollaway, G., and Stirling, G. 2008. Managing nematode pests in the southern and western regions of the Australian cereal industry: continuing progress in a challenging environment. Australasian Plant Pathology 37:220-234.

Vrain, T., Wakarchuk, D., Levesque, A., and Hamilton, R. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology 15:563-573.

Waeyenberge, L., Viaene, N., Subbotin, S., and Moens, M. 2009. Molecular identification of *Heterodera* spp., an overview of fifteen years of research. Pages 109-114 in: Cereal cyst nematodes: status, research and outlook, I. T. Riley, J. M. Nicol and A. A. Dababat, eds.

Whitehead, A. G. 1997. Plant nematode control. CAB international, New York.

Willcox, J., and Tribe, H. 1974. Fungal parasitism in cysts of *Heterodera*: I. preliminary investigations. Transactions of the British Mycological Society 62:585-IN583.

Williams, K., Fisher, J., and Langridge, P. 1994. Identification of RFLP markers linked to the cereal cyst nematode resistance gene (Cre) in wheat. Theoretical and Applied Genetics 89:927-930.

Williams, K., Lewis, J., Bogacki, P., Pallotta, M., Willsmore, K., Kuchel, H., and Wallwork, H. 2003. Mapping of a QTL contributing to cereal cyst nematode tolerance and resistance in wheat. Crop and Pasture Science 54:731-737.

Williams, K. J., Willsmore, K. L., Olson, S., Matic, M., and Kuchel, H. 2006. Mapping of a novel QTL for resistance to cereal cyst nematode in wheat. Theoretical and Applied Genetics 112:1480-1486.

Wu, H. Y., He, Q., Liu, J., Luo, J., and Peng, D. L. 2014. Occurrence and development of the cereal cyst nematode (*Heterodera avenae*) in Shandong, China. Plant Disease 98:1654-1660.

Wu, L., Cui, L., Li, H., Sun, L., Gao, X., Qiu, D., Sun, Y., Wang, X., Murray, T., and Li, H. 2016. Characterization of resistance to the cereal cyst nematode in the soft white winter wheat 'Madsen'. Plant Disease 100:679-685.

Yan, G., and Smiley, R. W. 2009. Discovery of *Heterodera filipjevi* on wheat in the USA. Pages 94-99 in: Cereal cyst nematodes: status, research and outlook, I. T. Riley, J. M. Nicol and A. A. Dababat, eds. CIMMYT, Ankara, Turkey.

Yan, G., and Smiley, R. W. 2010. Distinguishing *Heterodera filipjevi* and *H. avenae* Using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism and cyst morphology. Phytopathology 100:216-224.

Yan, G., Smiley, R. W., Okubara, P. A., and Skantar, A. M. 2013. Species-Specific PCR assays for differentiating *Heterodera filipjevi* and *H. avenae*. Plant Disease 97:1611-1619.

Ye, W. 2012. Development of Prime Time-Real-Time PCR for species identification of soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) in North Carolina. Journal of Nematology 44:284-290.

Zarlenga, D. S., Stringfellow, F., Nobary, M., and Lichtenfels, J. R. 1994. Cloning and characterization of ribosomal RNA genes from three species of *Haemonchus* (Nematoda: Trichostrongyloidea)

and identification of PCR primers for rapid differentiation. Experimental Parasitology 78:28-36.

Zhang, J., Li, Y., Yuan, H., Sun, B., and Li, H. 2016. Biological control of the cereal cyst nematode (*Heterodera filipjevi*) by *Achromobacter xylosoxidans* isolate 09X01 and *Bacillus cereus* isolate 09B18. Biological Control 92:1-6.

Zhang, S., Gan, Y., and Xu, B. 2014. Efficacy of *Trichoderma longibrachiatum* in the control of *Heterodera avenae*. BioControl 59:319-331.

Chapter 2. Resistance and Tolerance Screening of Spring Wheat Cultivars to Cereal Cyst Nematode *Heterodera avenae*

Pooria Ensafi, Richard W. Smiley, Margaret T. Moll, Arash Rashed, James W. Woodhall, Chad A. Jackson, Juliet M. Marshall.

Abstract:

Increased populations of Heterodera avenae in southeastern Idaho is associated with increased planting of susceptible cultivars and lack of crop rotation. Identifying high-yield, resistant spring wheat cultivars carrying tolerance requires testing new genotypes and susceptibility assessment of marketed cultivars. We conducted two experiments to determine whether cultivars with putative resistance could maintain acceptable yield in the presence of CCN. We also evaluated their tolerance response in relation to previously tested cultivars. Seven spring wheat cultivars were planted in two irrigated commercial fields that were naturally infested with high populations of *Heterodera avenae*. Measures of resistance, tolerance and grain yield were assessed in aldicarb-treated versus non-treated plots. Aldicarb significantly increased the yield of the susceptible cultivars Snow Crest, WestBred 936, WB9411, Patwin-515 and WB9668 in both trials. The effect of aldicarb on moderately resistant WB-Rockland and moderately susceptible Expresso was limited in the second trial which experienced water stress. WB-Rockland with the *Cre5* resistance gene maintained its standard yield, while none of the other cultivars in the experiment showed resistance or tolerance. Our results indicated that aldicarb can improve the grain yield in irrigated crop production systems and supports the hypothesis that high yield susceptible cultivars can mask the effect of CCN on grain yield when managed properly.

Introduction:

Cereal cyst nematodes are the most economically important pathogenic nematodes of wheat worldwide (Nicol et al. 2011). Annual yield loss of 23-50% with the estimated value of AU$72 million has been reported in Australian wheat production (Brown 1984). Comparable to the reports from other countries, in Oregon and Washington field trials, Smiley reported 50% yield reduction in the irrigated winter wheat cultivars that were planted annually in the fields that were heavily infested with *H. avenae* (Smiley et al. 1994). In another study, Smiley observed 24% improvement in spring wheat yield after application of aldicarb, which was comparable to previous reports from Australia (Smiley et al. 2005). From his observations on known infested fields with damaging CCN populations, Smiley roughly estimated US$3.4 million yield loss resulted from roughly 21,000 tons of reduced yield in the PNW (Smiley 2009).

Idaho is a key state in wheat production with approximately 471,459 ha of wheat in 2017, valued at US$415,657,000 (USDA/NASS 2018). With such extensive planting, *H. avenae* management becomes crucial. Three significant factors result in population fluctuations of *H. avenae*: the host susceptibility, population density-dependent factors, and the degree of fungal parasitism of cyst. Although all these factors should be considered in management strategies, successful conventional techniques that can be applied in southeastern Idaho are very limited. Studies in Union County Oregon show that fungal parasitism fails to suppress the population below the damaging threshold in the field (Smiley et al. 2005). Rotation with non-grass plants, although effective (Dababat et al. 2011), has not been economically viable in PNW; summer fallow has environmental concerns due to soil erosion, and chemical control either fails (seed treatment) or is not applicable due to the chemical registration regulations and economics (Smiley et al. 2013; Smiley et al. 2011; Smiley et al. 2005). Considering a potential 85% annual reduction of CCN population by resistant cultivars (Andersen and Andersen 1982), while other conventional techniques fail to offer a viable solution in Idaho, management strategies based on resistant/tolerant cultivars become particularly important.

In 1966, Nielsen from Denmark reported the first source of CCN resistance in wheat cultivar 'Loros' C.I. No: 3779 (Nielsen 1966). The spring wheat *T. aestivum* L. cv. 'AUS 10894' is the world's first cultivar explicitly bred for resistance to *H. avenae* in Australia (Brown and Young 1982) for the single resistant gene *Cre1* (Cereal resistance to eelworm locus 1). Soon after, the resistance genes *Cre2* (Rivoal et al. 1986), *Cre5* (Bonhomme et al. 1995) and *Cre6* (Ogbonnaya et al. 2001) from *Aegilops ventricosa*; *Cre3* (Eastwood et al. 1991) and *Cre4* (Eastwood et al. 1994) from *A. tauschii*; *Cre7* (Romero et al. 1998) from *A. triuncialis*; and *Cre8* (Paull et al. 1998) from wheat cultivar 'Festiguay' were reported and mapped. However, due to the occurrence of different *H. avenae* pathotypes, none of the *Cre* genes could provide complete resistance worldwide. Smiley assessed the selected entries with *Cre1* from the International Test Assortment Cultivars in PNW field trials and showed that single-dominant *Cre1* gene, and to some extent *Cre8,* effectively reduced the multiplication of the PNW pathotype of *H. avenae* (Smiley 2009). Other cultivars with *Cre* genes provided either no resistance or moderate resistance in PNW; none of the locally prevalent spring wheat cultivars in PNW were found to have resistance (Smiley et al. 2017). Therefore, examining the stability and durability of the resistant cultivars against local pathotypes and tolerance or the ability of the cultivars to withstand and/or recover from nematode damage (Smiley et al. 2013) needs additional research. Identifying effective genes for resistance that can be used in commercial breeding (Atkinson et al. 2003), and avoiding the continuous

planting of resistant cultivars (Lasserre et al. 1996) are standard practices that should be implemented for a sustainable successful management.

The purpose of this study was to assess resistance and tolerance levels of the spring wheat cultivars that are widely planted in the area against *H. avenae* pathotype prevalent in southeastern Idaho. To that end, we (1) evaluated the CCN population density in the soil before planting and after harvest to assess the *H. avenae* multiplication rate, (2) assessed the host resistance at flowering by counting the gravid females per root, (3) assessed the tolerance level of the selected cultivars by measuring the improvement in their grain yield after suppressing the CCN population in the field, and (4) compared the previously tested cultivars for the possible development of virulent CCN populations. The trial was repeated in following year at a different field location.

Materials and Methods

Study location.

The experimental plots were set up and planted on two different fields near St. Anthony, Fremont County Idaho; the first trial was planted on April 25, 2016 (latitude 43.921507, longitude -111.627094), and the second trial on April 24, 2017 (latitude 43.916356, longitude -111.640508). Both fields are in a semi-arid continental climate with cold winters and warm-dry summers of about 1,520-meter altitude. Soil sampling was performed at the time of planting and right after harvesting for evaluation of population density of *H. avenae* and multiplication rates respectively (Table 2-1).

Table 2-1 Location and field properties of spring wheat cultivar trials for assessment of resistance/tolerance to Heterodera avenae in southeastern Idaho

	2016	**2017**
Latitude (N)	43.921507	43.916356
Longitude (W)	-111.627094	-111.640508
Altitude (m)	1521	1521
Precipitation (mm)	352	352
Water (Irrigated system)	Central Pivot	Hand-move solid set
Cropping	Wheat/Fallow	Barley
Soil Type	Gravelly sandy loam	Gravelly sandy loam
Initial Population	1013-8703	4516-11013
Planting date	April 25, 2016	April 24, 2017
Root sampling date	July 7, 2016	June 26, 2017
Harvest date	August 26, 2016	August 29, 2017

The lowest and highest initial populations of juveniles plus eggs per kg soil associated with plots are presented.

Selection of the wheat cultivars

'WB-Rockland' (Accession number (ACNO): PI-659487, Experimental designation (EXPT.DES): SJ908-247, released in 2010 by Monsanto Technology LLC, Arizona) is an HRSW (class: hard red spring wheat) that was selected from the cross 'Expresso'/2*Solano; a relatively low-yield (5918.09 kg ha^{-1}) cultivar that is adapted to the PNW (WestBred 2016). It has *Yr15* and *Yr17* markers and confers resistance to stripe rust (Jackson 2011). WB-Rockland was previously tested in southeastern Idaho and identified resistant and tolerant to CCN *Heterodera avenae* (Smiley and Marshall 2016; Smiley et al. 2013).

'Expresso' (ACNO: PI 651616 PVPO, EXPT.DES: DA984-034SSR, released in 2006 by WestBred LLC Yuma, Az); is an HRSW selected from the cross 'Express'*6/Yr15'Avocet'//Express*6/'Madsen'. The isogenic line 'Avocet' is the donor of *YR15* and Madsen is the donor of LR37/SR38/YR17 genes. Previous studies show that Madsen has the *Cre5* CCN resistance gene received from 'VPM1' (Allan et al. 1989; Jahier et al. 1996; Smiley et al. 2013), Therefore, we included Expresso in the trials to study Expresso's

resistance and tolerance response in southeastern Idaho as a potential to be integrated into breeding programs for CCN management.

'WB9668' (ACNO: PI 671994, EXPT.DES: BZ908-552, released in 2014 by WestBred-Monsanto Technology LLC. Bozeman MT.); is an HRSW that originated from the cross Expresso/WB-Idamax (USDA 2014; WestBred 2016). WB9668 had a high level of resistance to stripe rust and was reported as a highly resistant cultivar against CCN. This high-yielding and high-protein cultivar has been considered as a replacement for WestBred 936, and is widely grown in the PNW (Marshall et al. 2018).

'WB9411' (ACNO: PI 674346, EXPT.DES: BZ908-418, released in 2015 by Monsanto Technology LLC. Bozeman, MT); is an HRSW derived from a cross of 'Volt'/ WestBred 936 (USDA 2015). Volt is a German hard red spring wheat with resistance to Fusarium head blight (FHB). WB9411 was widely grown in Idaho for several years between 2010 and 2016. While resistant to stripe rust and having lower susceptibility to scab (Fusarium head blight), its response to CCN is not clear (Marshall et al. 2018).

'WestBred 936' (ACNO: PI 587200, EXPT.DES: PH986-61, released in 1992 by WestBred-Monsanto); is a HRSW developed from a male-sterile facilitated, recurrent selection population '906 alpha-84'. Although having excellent end-use quality, WestBred 936 is very susceptible to many of the frequently occurring diseases, including stripe rust, black chaff, scab and CCN (Marshall et al. 2015). WestBred 936 tested as very susceptible and intolerant to CCN in southeastern Idaho (Smiley and Marshall 2016; Smiley et al. 2013). The cultivar was included in the trial as a susceptible check.

'Snow Crest' (ACNO: PI 642376, EXPT.DES: BZ998-247W, released in 2005 by WestBred-Monsanto LLC. Bozeman, MT); is a HWSW (hard white spring wheat) developed from the cross KLASIC/IDO-377-S//PH-992-265-W. Snow Crest is a high quality, high yield cultivar which is also very susceptible to stripe rust and CCN, with a moderately intolerant response to CCN in southeastern Idaho (Smiley and Marshall 2016).

'Patwin-515' (ACNO: PI 666962, EXPT.DES: UC1680, released in 2013 by the University of California, Davis); is an HWSW derived from 'Patwin' (UC1419) (Madsen/2*Expresso). The presence of 2NS-2AS translocation that carries resistance genes: *YR17* (stripe rust), *Lr37* (leaf rust), and *Sr38* (stem rust) are confirmed in Patwin-515, which therefore was expected to have reasonable resistance levels against CCN. The cultivar has not been planted in southeastern Idaho.

Experiment design and procedure

The experimental procedure and method were conducted similarly to the previous studies in the PNW and southeastern Idaho (Marshall and Smiley 2016; Smiley and Marshall 2016; Smiley et al. 2013). Six replicates of the seven spring wheat entries (42 main plots of 20 × 5 m) were planted as a split-plot RCBD (Randomized Complete Block Design) in a previously known heavily infested location of the field near St. Anthony, Idaho. Each of the six repeated blocks contained one of the seven cultivars (main plots) randomly distributed within the block. Each main plot (cultivar) was split randomly to aldicarb treated/nontreated sub-plots. The trial border separated the experiment from the owner's crop (10m width). Main plots within each replicate block aligned side by side with no border, while a 30cm border (not planted) was set between block replicates.

Plots were seeded to a constant rate of 404,686 seeds per ha and were planted with a Hege500 Seeder without or mixed with nematicide aldicarb (Temik 15G, Bayer CropScience, rate: 4.2 kg ha^{-1}). Fertilizer, seed treatment, and pest or disease control were not applied during the experiment. Plots were irrigated by the cooperating producer.

Figure 2-1 Main plots of each cultivar sub plotted to aldicarb (Temik) treated and nontreated subplots

Soil & Plant sampling methods

The soil was sampled following Viaene et al. (2012); in brief, one composite soil sample from 10 cores in each plot was collected through a systematic pattern, both before planting and after harvest, using a 2.5cm diameter soil probe. The soil samples were labeled and transferred to the University of Idaho Aberdeen Research and Extension Center and kept at 4°C until processed. Following Cobb's decanting and sieving method, the CCN J2s, and other free-living soilborne nematodes were collected from 300 g soil subsample through a series of 1000, 700, 250, 150, 60 and 32µm sieves (Cobb 1918). Specimens with stomato-stylet were transferred to a staining block and temporarily fixed for identification (De

Grisse 1969). Cysts were extracted from 300 g wet soil subsamples using a calibrated Seinhorst apparatus (Seinhorst 1964), details in Appendix-1.

From each non-treated plot (21 plots total), six plants were carefully dug with a shovel at approximately the flowering stage (Feekes growth stage 10.5.1) and transferred to the lab. Roots were washed, and the debris was collected over a 250µm sieve. In addition to the collected cysts, young females that were partially exposed from the root were stained with acid fuchsin for evaluation under the stereomicroscope, the staining step provide accuracy in the assessment of young females. All plots were harvested using a Wintersteiger plot combine (Wintersteiger Inc., Salt Lake City, UT) on August 26, 2016 and August 29, 2017, respectively.

Evaluations

Identification of CCNs was based on the morphology of the cyst terminal region including vulva cone underbridge, bullae, and fenestration type, plus the morphometrics of J2s (Subbotin et al. 2010). The species level identification was later confirmed by amplification and sequencing of two partial genomic DNA segment: 1) ribosomal RNA (rRNA) gene including partial region of small subunit 18S, complete inter transcribed spacer 1 (ITS1), complete 5.8 subunit, complete ITS2, and partial region of large subunit 28S; and 2) partial mitochondrial cytochrome oxidase I gene (*CTC-1* syn: *COI* mtDNA). Briefly, we extracted cysts from wet soil using Seinhorst apparatus and transferred cysts to a glass petri dish with distilled water. After 3 days (at room temperature), a single cyst was cut opened and one hatched second stage juvenile (J2) was transferred to a 2ml hard body tube containing a single 2.8 mm ceramic bead (Omni international) and 18 µl of worm lysis buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$, 1 mM dithiothreitol, 0.45% Tween 20). The content homogenized for 30 seconds at high speed using Bead Ruptor 12 (Omni international). The tube was transferred to -80°C for 10 min, thawed, 2 µl of proteinase K (600 µg ml^{-1}) was added, and then incubated at 65°C for 45 min. The proteinase K was inactivated by 10 min exposure to 95°C. The tube was centrifuged for 2 min at 11600 × *g*, then the supernatant was transferred to 1.5 ml tube and stored at -20°C.

Qiagen *Taq* PCR Core Kit with forward primer TW81 (5'-GTTTCCGTAGGTGAACCTGC-3'), and reverse primer AB28 (5'- ATATGCTTAAGTTCAGCGGGT-3') were used for amplification of the ITS rRNA gene (Subbotin et al. 2000), and forward primer Het-coxiF (5'-TAGTTGATCGTAATTTTAATGG-3'), and reverse primer Het-coxiR (5'-CCTAAAACATAATGAAAATGWGC-3') were used for amplification of partial *COI* mtDNA (Subbotin 2015). Before amplification, T_m (Melting temperature) for the annealing stage of the

PCR were assessed through the temperature gradient PCR and adjusted to T_m: 55 °C for TW81 & AB28, and T_m: 45 °C for Het-coxiF & Het-coxiR.

A master mixture (per reaction; double-distilled water: 18.85 µl, 10X PCR buffer: 2.5 µl, 0.5 mM $MgCl_2$: 0.75 µl, 10 mM dNTP-Mix: 0.5 µl, 1.5 µM forward primer: 0.15 µl, 1.5 µM reverse primer: 0.15 µl, *Taq* DNA polymerase 5 units μl^{-1}: 0.1 µl) was prepared and 23 µl was transferred to a 0.2 µl PCR tube, 2 µl DNA (minimum concentration: 20 ng μl^{-1}) suspension was added to the tube and the mixture was run at a temperature profile of: 2 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at the related T_m, 2 min at 72 °C, and 10 min at 72 °C. PCR reaction was verified through AGE (Agarose gel electrophoresis); 2 µl loading buffer mixed with three µl PCR product and run in previously prepared 1% agarose gel prepared in 0.5% TBE buffer and stained with GelRed™ at 72 volts for one hour. DNA bands were visualized by AlphaImager® MINI (Protein Simple). Verified PCR product was quantified using Nanodrop 2000C (ThermoFisher Scientific) and sent to Molecular Research Core Facility of Idaho State University (Pocatello, ID) for purification and sequencing.

Resistance and tolerance assessment

CCN population density was calculated based on a total number of cysts in each sample and the number of eggs+J2s contained in 30% of randomly picked cysts. Resistance was quantified based on the newly formed white females per plant and categorized as VR (very resistant with ≤ 1 white female per plant), R (resistant: 1.1 to 3), MR (moderately resistant: 3.1 to 6), MS (moderately susceptible: 6.1 to 12), S (susceptible: 12.1 to 25), and VS (very susceptible >25) groups (Ireholm 1994; Smiley and Marshall 2016).

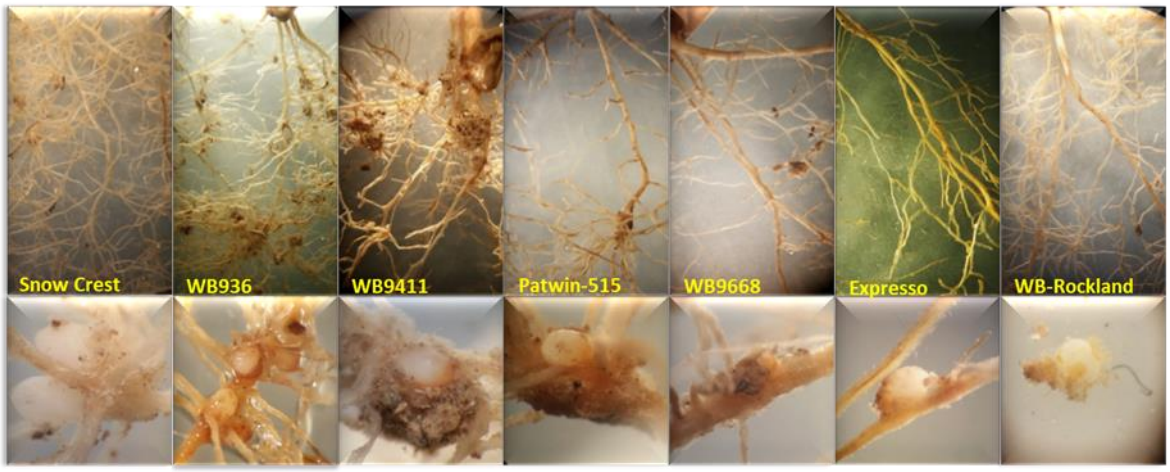

Figure 2-2 Spring wheat root damage tested in St. Anthony, ID for resistance response to CCN infection

Following Smiley and Marshall (Smiley and Marshall 2016; Smiley et al. 2013), grain yield and test weight at harvest were measured and used as good indicators for incidence and severity of root injury. So, the tolerance assessment for each cultivar was scaled based on the relative increase in grain yield and test weight of aldicarb treated vs. non-treated subplots. The scale includes: VT (very tolerant ≤5% yield increase after nematicide), T (tolerant 5.1 to 10%), MT (moderately tolerant 10 to 15%), MI (moderately intolerant 15.1 to 30%), I (intolerant 30.1 to 50%), or VI (very intolerant >50%).

To evaluate the tolerance, Statistical analysis of two years data for the grain yield and test weight was performed in SAS® 9.4 (SAS Institute Inc., Cary, NC, USA) with consideration of 'cultivar': main plot, 'Aldicarb treatment': sub-plot, 'replicates': blocks nested in year, and 'year': environment/time block. Only non-treated sub-plots were used for the incidence evaluation of white females and multiplication rate, and tolerance analysis was also performed for each year individually.

Results

Nematode characteristics

The cyst-forming nematodes in both study areas were identified as *H. avenae*. The initial population (Pi) of cyst and J2+Eggs at the time of planting is provided in Table 2-2. The population density was evaluated for each plot before planting and after the harvest. In 2016 trial, cereal cyst nematode (J2+eggs) was evenly distributed through main plots, and no significant differences between replicates (blocks) and cultivars were detected (df = 4 & 6, P = 0.5819 & 0.2783 respectively). The mean Pi values for Patwin515, Snow Crest, WB9668, WestBred 936, Expresso, WB-Rockland and WB9411 were 8703, 7216, 7059, 5317, 4614, 4611 and 1013 J2+eggs/kg wet soil, respectively.

Nematode development success

The successfully developed white females were assessed at the flowering stage when the juveniles stage 4 females had molted, been fertilized, and become gravid females (Li et al. 2012; Smiley et al. 2017; Wu et al. 2014) (dates in Table 2-1). The effect of interaction of cultivar x year (P = 0.938) on the development of white females in each cultivar was not significant. Therefore the two-year analysis results are included in the calculations. Main plot (cultivar) significantly affected the development of *H. avenae* females (df = 6, P = 0.001). Mean values for newly developed cyst for different cultivars was: Snow Crest = 147.3 (VS), WestBred 936 = 112.9 (VS), WB9411 = 60.6 (VS), Patwin515 = 20.4 (S), WB9668 = 17.2 (S), Expresso = 12.4 (MS), and WB-Rockland 4.4 (MR). The population of brown cysts, produced during the previous year, were not significantly different between cultivars; values for Snow

Crest = 37.4, WestBred 936 = 47.0, WB9411 = 47.9, Patwin515 = 63.3, WB9668 = 35.8, Expresso = 36.1, and WB-Rockland = 45.7 brown cysts per plot.

Table 2-2 Changes in population density following spring wheat cultivars during 2016 near St. Anthony ID. Standard errors are presented

	Pre-plant		Post-harvest		Change (%)	
Cultivar	**Cyst**	**J2+Eggs**	**Cyst**	**J2+Eggs**	**Cyst**	**J2+Egg**
Expresso	186.7 ± 23.7	4641.2 ± 1309.7	183.3 ± 40.8	3824.8 ± 1219.6	-1.79	-17.6
Patwin-515	172.0 ± 19.1	8703.0 ± 2131.7	196.1 ± 31.0	4161.7 ± 1561.7	14.02	-52.2
Snow Crest	145.3 ± 28.8	7215.6 ± 2328.4	182.8 ± 27.1	4393.7 ± 2056.4	25.76	-39.1
WestBred 936	127.3 ± 27.3	5316.9 ± 2353.7	163.3 ± 25.7	4140.0 ± 2638.5	28.27	-22.1
WB-Rockland	136.0 ± 33.2	4611.1 ± 1548.6	146.7 ± 25.1	1852.7 ± 700.5	7.84	-59.8
WB9411	108.7 ± 21.5	1012.7 ± 218.6	130.0 ± 24.4	1904.0 ± 679.9	19.63	88.0
WB9668	164.0 ± 31.6	7058.7 ± 1073.8	165.0 ± 19.2	5724.4 ± 2618.6	0.61	-18.9

Cultivar tolerance assessment

The effects of the main plot (cultivar) on the grain yield for both years was significant ($P = 0.0001$). Year in our trials also had a significant impact on grain yield, which prevents the combination of two-year data for statistical analysis. The relative increase in the yield after treatment with aldicarb is presented in Table 2-3.

In 2016, cultivars Snow Crest and WB9668 were tolerant (9% & 8% increase in grain yield respectively) and Expresso, Patwin515, WestBred 936, WB-Rockland, and WB9411 were very tolerant (1.5, 1, -6, -0.5, -2% increase). In 2017, WB-Rockland (19%), Expresso (21%), and WB9668 (29%) fit in the moderately intolerant group, and WestBred 936 (38%), Patwin515 (43%), WB9411 (43%), and Snow Crest (45%) were categorized as intolerant. In general, the adjusted p-value for the two years indicates that aldicarb could significantly increase the grain yield for the four cultivars Snow Crest ($P < 0.001$), WB9668 ($P < 0.001$), WB9411 ($P = 0.004$), and Patwin-515 ($P < 0.001$), its effect on WestBred 936 ($P = 0.2266$), WB-Rockland ($P = 0.3739$), and Expresso ($P = 0.1264$) was not enough to improve the yield significantly.

The combination of two-year assessment on grain yield would give us the results as follow: Snow crest MI (19%), WestBred 936 MI (16%), WB9411 MI (16%), Patwin515 MT (15%), WB9668 MT (15%), Expresso T (9%), and WB-Rockland T (7%).

Table 2-3 Resistance (as measured by the ability of CCN to reproduce on roots) and tolerance (maintenance of yield under high CCN population pressure) of 7 spring wheat cultivars to *Heterodera avenae* in southeastern Idaho. Standard errors are presented. White cysts are those counted Feeks 10.5.1, Brown cysts were extracted from the soil after harvest. Control had not been treated, whereas the treated plots had aldicarb (4.2 kg ha^{-1}) mixed with seed at planting. Change is percent change in yield after treatment with aldicarb to reduce nematode populations at planting

		Resistance Factors		Grain Yield (kg ha^{-1})		%
	Cultivar	White cysts	Brown cysts	Control	Treated	Change
2016	Expresso	9.5 ± 2.7	37.3 ± 6.1	6187.3 ±445.7	6279.2 ±377.7	1.5
	Patwin-515	18.4 ± 4.6	57.6 ± 9.9	6105.0 ±452.9	6165.2 ±476.6	1.0
	Snow Crest	148.6 ± 42.6	43.1 ± 5.6	4808.9 ±162.6	5265.0 ±90.7	9.5
	WestBred 936	95.5 ± 20.6	47.2 ± 11.5	2632.3 ±162.1	2471.7 ±220.8	-6.1
	WB-Rockland	4.36 ± 1.1	50.3 ± 8.5	6274.7 ±278.9	6253.5 ±156.3	-0.3
	WB9411	53.1 ± 34.1	51.7 ± 9.4	6370.8 ±120.1	6231.4 ±207.1	-2.2
	WB9668	19.4 ± 3.8	34.9 ± 3.6	6878.6 ±375.8	7410.8 ±286.2	7.7
2017	Expresso	15.2 ± 2.2	35.0 ± 7.7	3424.8 ±145.2	4645.9 ±247.4	21.5
	Patwin-515	22.6 ± 1.8	68.9 ± 15.0	3197.2 ±247.1	4566.2 ±118.5	42.8
	Snow Crest	146.0 ± 14.1	31.8 ± 4.7	2417.6 ±105.7	3518.3 ±246.6	45.5
	WestBred 936	130.3 ± 16.6	46.8 ± 11.1	2592.7 ±203.8	3588.4 ±90.7	38.4
	WB-Rockland	4.5 ± 0.9	41.1 ± 7.2	4147.9 ±303.0	4924.9 ±270.1	18.7
	WB9411	68.1 ± 16.3	44.1 ± 7.7	3123.5 ±346.7	4469.5 ±309.9	43.1
	WB9668	15.0 ± 1.3	37.0 ± 6.7	3573.1 ±335.6	4604.4 ±262.5	28.9

Host Resistance and CCN Multiplication rate

We measured the number of cysts per 1L soil after harvest. In the WB-Rockland plots, the number of cysts decreased by 7.5%, and Expresso by 2%. While WB9668 maintained the population almost equal to Pi (+0.6%), for other cultivars the cyst population increased more than 10%; including Patwin515 (14%), WB9411 (20%), Snow Crest (26%), and WestBred 936 (28%). Host resistance was characterized by the number of cysts formed per plant after acid fuchsin.

Discussion

Selecting the right spring wheat for southeastern Idaho as available high yield cultivars are being planted in heavily infested fields resulting in significant yield loss at a low incidence of root invasion. The resistant cultivars either have not provided high yield or are not tolerant, and therfore are no longer commercially available. Growers therefore face the risk of exceeding the economic threshold for CCN when planting currently available varieties. Identification of cultivars with both resistance and tolerance traits will help with significantly reducing the yield loss associated with this pathogen.

In the PNW, the initial population (Pi) density responsible for the economic threshold of 10% grain yield loss was previously defined as 1300 J2+Eggs/kg soil in rainfed spring wheat, and 16,800 J2+Eggs/ kg soil in irrigated winter wheat cultivars (Smiley et al. 2017; Smiley et al. 2013). Our estimates of Pi were higher than the threshold level of 1300 J2+Eggs/kg soil for rainfed spring wheat but 50% lower than that of the irrigated winter wheat. Since the number of successful white females in our reference cultivars, WB-Rockland (R) and Snow Crest (S) were higher than previous records in the region, we assumed the Pi as sufficient to induce yield reduction.

Considering two-year average evaluations of resistance, we observed that environmental variables (year, location, cropping system and irrigation type), significantly influenced the grain yield but not the development success and incidence of white females in root systems of each cultivar. Different from the previous study in the same region where WB-Rockland had been assessed as resistant, with fewer than 3 females per root (Smiley and Marshall 2016; Smiley et al. 2013), we observed a weaker resistance response of WB-Rockland with an average of 4 females per root system, which put this cultivar in the moderately resistant group. Similarly, we found Snow Crest very susceptible (147.3 females per root) while it was previously reported as susceptible (average 35.9 females per root). The difference could not be caused by the high initial population pressure since we observed lower Pi in our plots compared to Smiley's studies. The evaluation of Pi in the previous studies was at the scale of the complete trial area. Our evaluations were done at the scale of each plot for all cultivars. Therefore, it seems the reason for the difference could be the acid fuchsin staining and stereomicroscope examination procedure that we applied to detect partially attached females that were not completely washed off the roots. That level of detail was not conducted in previous studies in the PNW, which led to an apparent underestimate of white females produced on these cultivars during the first generation studies reported previously (Smiley 2009). We therefore recommend that the enumeration of white females in future studies include the acid fuchsin stain to detect gravid white females that were not

dislodged from roots during the washing process. It appears that the additional step, while time consuming, improves the accuracy of the counting and possibly improves the differentiation of levels of resistance for cultivars evaluated in naturally-infested commercial fields.

This study had some limitations including the different irrigation systems between the two locations, application of aldicarb mixed with the seed in treated plots, setting up the experimental plots in two different rotations, wheat & barley, during 2016 and 2017, and co-occurrence of stripe rust in the 2016 trial. These limitations required separate data analysis and discussion for each year.

In 2016, the trial was set up in a wheat field with an efficient central pivot irrigation system. As Smiley reported, efficient irrigation and management could mask the effect of *H. avenae* on the grain yield of spring wheat cultivars and lead to miscalculation of tolerance (Fard et al. 2015; Smiley et al. 2017). In 2016, we observed two major patterns in average grain yields; WB-Rockland, Expresso, WB9668, and Patwin515 expressed high levels of tolerance with exceptional grain yield, and no significant losses in nontreated plots. There, no improvement in the grain yield was observed from aldicarb because all cultivars except WestBred 936 (due to stripe rust) resulted in high yields. In contrast, a second pattern was observed in the very susceptible but high yielding Snow Crest, where even highly efficient irrigation and management could not mask grain yield loss, as demonstrated by a 10% improvement of grain yield when aldicarb was applied.

In the 2017 trial, we observed several challenging factors that all could reduce the grain yield. The lower water input management system (for barley planted in the surrounding field) with the less efficient hand-move solid set irrigation system resulted in 20% to 50% lower yields compared to the 2016 trial. Aldicarb improved the grain yield in this higher stress situation. Yield in untreated plots during 2017, as compared to 2016, was reduced for WB-Rockland (34%), Expresso (38%), WB9668 (48%), Patwin515 (48%), Snow Crest (50%), and WB9411 (51%). During 2017, aldicarb treatment improved the grain yield in each cultivar; WB-Rockland (19%), Expresso (22%), WB9668 (29%), Patwin515 (43%), WB9411 (43%), and Snow Crest (45%). It was reported previously that *H. avenae* and other nematodes cause a more pronounced reduction of grain yield when affected plants were subjected to water stress (Smiley et al. 2017). This phenomenon was anecdotally supported during our two years of field trials at nearby locations in fields naturally infested with *H. avenae*.

Compared to resistance, the mechanism underlying the tolerance response is not clearly defined, which makes the experimental design and assessment strategy very challenging. The key finding of this

study relevant to our hypothesis was that the tolerance level from exposure to an economically damaging population of *H. avenae* exhibit strong variation due to environmental variables. This result could explain the tolerance variations repeatedly observed in previous field studies. We documented a significant effect of aldicarb in suppressing the *H. avenae* damage in a water-stressed situation even in irrigated systems, which indirectly confirms earlier studies (Brown 1987; Smiley et al. 1994).

WB-Rockland is considered a low yielding cultivar (5918 kg ha^{-1} average yield under irrigation) (Smiley and Yan 2015) but that averaged was exceeded by 6% in our 2016 trial. Considering the Pi exceeded the economic threshold in WB-Rockland plots and an average number of developed white females was also higher, our results support the previous report of dual resistance/tolerance characteristics of this cultivar when adequately managed in irrigated fields (Smiley and Marshall 2016). However, only the resistance response could be detected in WB Rockland in the 2017 field having water stress. This is an important finding that must be considered in *H. avenae* management recommendations and in future research. Since WB-Rockland contains *Cre5*, together with *Yr15* and *Yr17* markers that confer resistance to stripe rust, it is recommended to use in wheat breeding programs that need to include resistance to *H. avenae* in southeastern Idaho. Expresso (moderately susceptible), WB9668 and Patwin-515 (susceptible) were not able to suppress the reproduction of *H. avenae* but had much lower numbers of white females than the remaining three cultivars in our study. The utility of Expresso, WB9668 and Patwin-515 in cropping systems will depend upon results of more definitive studies of tolerance to *H. avenae*. Three other cultivars in our study conferred high grain yield but were so susceptible to *H. avenae* that they should not be included in cereal rotations of infested fields.

Acknowledgments

Special thanks to D. Daw (St. Anthony, ID) for the land and crop management assistance; T. Shelman and L. Jones (University of Idaho, Aberdeen) for technical assistance; This material is based upon work supported by the Idaho Wheat Commission, the Idaho Barley Commission, and the U.S. Department of Agriculture ARS Specific Cooperative Agreement No. 58-5348-4-040.

Author and Co-authors' Contribution

Pooria Ensafi: Investigation (performing the experiments, data/evidence collection); Formal analysis (application of statistical method); Data Curation; Writing (original draft preparation)

Richard W. Smiley: Writing (review and editing including critical review, pre-publication revision)

Margaret T. Moll: Investigation (help with monitoring and data collection)

Arash Rashed: Formal analysis (help with application of statistical analysis); Writing (review of manuscripts)

James W. Woodhall: Resources (provision of molecular analysis); Writing (review of manuscripts)

Chad A. Jackson: Investigation (help with performing the experiment, data collection)

Juliet M. Marshall: Supervision (oversight and leadership responsibility); Funding (acquisition of financial support); Resources (provision of study materials and analysis); Writing (review and editing manuscripts)

References

Allan, R. E., Peterson, C. J., Rubenthaler, G. L., Line, R. F., and Roberts, D. E. 1989. Registration of 'Madsen' wheat. Crop science 29:1575.

Andersen, S., and Andersen, K. 1982. Suggestions for determination and terminology of pathotypes and genes for resistance in cyst-forming nematodes, especially *Heterodera avenae*. EPPO Bulletin 12:379-386.

Atkinson, H. J., Urwin, P. E., and McPherson, M. J. 2003. Engineering plants for nematode resistance. Annual Review of Phytopathology 41:615-639.

Bonhomme, A., Gale, M., Koebner, R., Nicolas, P., Jahier, J., and Bernard, M. 1995. RFLP analysis of an *Aegilops ventricosa* chromosome that carries a gene conferring resistance to leaf rust (*Puccinia recondita*) when transferred to hexaploid wheat. Theoretical and Applied Genetics 90:1042-1048.

Brown, R. H. 1984. Ecology and control of cereal cyst nematode (*Heterodera avenae*) in southern Australia. Journal of Nematology 16:216.

Brown, R. H. 1987. Control strategies in low-value crops. Pages 351-387 in: Principles and Practice of Nematode Control in Crops. R. H. Brown and B. R. Kerry, eds. Academic Press Australia, Sydney N.S.W.

Brown, R. H., and Young, R. M. 1982. Katyil, a new wheat resistant to cereal cyst nematode. Agnote, Victoria Department of Agriculture 1875/82:38.

Cobb, N. A. 1918. Estimating the nematode population of the soil, with special reference to the sugar-beet and root-gall nemas, *Heterodera schachtii* Schmidt and *Heterodera radicicola* (Greef) Muller, and with a description of *Tylencholaimus aeqaualis* n. sp. USDA, Agricultural Technology Circular 1:47.

Dababat, A. A., Pariyar, S., Nicol, J. M., and Duveiller, E. 2011. Cereal Cyst Nematodes: An unnoticed threat to global cereal production. Page 2, France. Available at: http://hdl.handle.net/10883/3991.

De Grisse, A. T. 1969. Redescription ou modifications de quelques technique utilisees dan l'etude des nematodes phytoparasitaires. Pages 351-369 Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent Belgium.

Eastwood, R. F., Lagudah, E. S., and Appels, R. 1994. A directed search for DNA sequences tightly linked to cereal cyst nematode resistance genes in *Triticum tauschii*. Genome 37:311-319.

Eastwood, R. F., Lagudah, E. S., Appels, R., Hannah, M., and Kollmorgen, J. F. 1991. *Triticum tauschii*: a novel source of resistance to cereal cyst nematode (*Heterodera avenae*). Crop and Pasture Science 42:69-77.

Fard, H. K., Pourjam, E., and Maafi, Z. T. 2015. Assessment of grain yield reduction caused by *Heterodera filipjevi* in wheat cultivars under normal irrigation and drought stress in natural field conditions. Pages 115-120 in: Nematodes of Small Grain Cereals: current status and research, A. A. Dababat, R. W. Muminjanov and R. W. Smiley, eds. FAO, Ankara, Turkey.

Ireholm, A. 1994. Characterization of pathotypes of cereal cyst nematodes, *Heterodera* spp., in Sweden. Nematologica 40:399-411.

Jackson, L. (2011) 'Wheat cultivars for California'. Available at: https://smallgrains.ucdavis.edu/cereal_files/WhtCVDescLJ11.pdf.

Jahier, J., Tanguy, A., Abelard, P., and Rivoal, R. 1996. Utilization of deletions to localize a gene for resistance to the cereal cyst nematode, *Heterodera avenue*, on an *Aegilops ventricosa* chromosome. Plant Breeding 115:282-284.

Lasserre, F., Gigault, F., Gauthier, J., Henry, J., Sandmeier, M., and Rivoal, R. 1996. Genetic variation in natural populations of the cereal cyst nematode (*Heterodera avenae* Woll.) submitted to resistant and susceptible cultivars of cereals. Theoretical and Applied Genetics 93:1-8.

Li, X.-h., Ma, J., and Chen, S.-l. 2012. Effect of temperature on the penetration and development of *Heterodera avenae*. Triticeae Crops 32:977-981.

Marshall, J. M., and Smiley, R. W. 2016. Spring barley resistance and tolerance to the cereal cyst nematode *Heterodera avenae*. Plant Disease 100:396-407.

Marshall, J. M., Jackson, C., Shelman, T., Jones, L., and O'Brien, K. 2015. 2014 small grain report. Page 132. University of Idaho, college of agricultural and life sciences. Available at: http://www.extension.uidaho.edu/publishing/pdf/RES/RES186.pdf.

Marshall, J. M., Jackson, C., Shelman, T., Jones, L., Arcibal, S., and O'Brien, K. 2018. 2017 small grain report. Page 150. University of Idaho, college of agricultural and life sciences. Available at: http://www.extension.uidaho.edu/publishing/pdf/RES/RES191.pdf.

Nicol, J. M., Turner, S. J., L., C. D., Den Nijs, L. J., Hockland, S., and Maafi, Z. T. 2011. Current nematode threats to world agriculture. Pages 21-43 in: Genomics and Molecular Genetics of Plant-Nematode Interactions. J. Jones, G. Gheysen and C. Fenoll, eds. Springer, Dordrecht Heidelberg London New York.

Nielsen, C. H. 1966. Untersuchungen über die vererbung der resistenz gegen den getreide nematoden (*Heterodera avenae*) beim weizen. Nematologica 12:575-578.

Ogbonnaya, F., Seah, S., Delibes, A., Jahier, J., Lopez-Brana, I., Eastwood, R., and Lagudah, E. 2001. Molecular-genetic characterisation of a new nematode resistance gene in wheat. Theoretical and Applied Genetics 102:623-629.

Paull, J., Chalmers, K., Karakousis, A., Kretschmer, J., Manning, S., and Langridge, P. 1998. Genetic diversity in Australian wheat varieties and breeding material based on RFLP data. Theoretical and Applied Genetics 96:435-446.

Rivoal, R., Dosba, F., Jahier, J., Pierre, J.-S., Penard, P., Tanguy, A.-M., Abelard, P., and Querrien, M.-T. 1986. Les lignées d'addition blé-*Aegilops ventricosa* Tausch. VI.--Etude de la localisation chromosomique de la résistance à l'égard d'H*eterodera avenae* Woll. Agronomie 6:143-148.

Romero, M., Montes, M., Sin, E., Lopez-Brana, I., Duce, A., Martin-Sanchez, J., Andres, M., and Delibes, A. 1998. A cereal cyst nematode (*Heterodera avenae* Woll.) resistance gene transferred from *Aegilops triuncialis* to hexaploid wheat. Theoretical and Applied Genetics 96:1135-1140.

Seinhorst, J. 1964. Methods for the extraction of *Heterodera* cysts from not previously dried soil samples. Nematologica 10:87-94.

Smiley, R., Dababat, A. A., Iqbal, S., Jones, M. G., Tanha Maafi, Z., Peng, D., Subbotin, S. A., and Waeyenberge, L. 2017. Cereal Cyst Nematodes: A complex and destructive group of *Heterodera s*pecies. Plant Disease 101:1692-1720.

Smiley, R. W. 2009. Occurrence, distribution and control of *Heterodera avenae* and *H. filipjevi* in the western USA. Pages 35-40 in: Cereal cyst nematodes: status, research and outlook, I. T. Riley, J. M. Nicol and A. A. Dababat, eds. CIMMYT, Ankara, Turkey.

Smiley, R. W., and Yan, G. 2015. Discovery of *Heterodera filipjevi* in Washington and comparative virulence with *H. avenae* on wheat. Plant Disease 99:376-386.

Smiley, R. W., and Marshall, J. M. 2016. Detection of dual *Heterodera avenae* resistance plus tolerance traits in spring wheat. Plant Disease 100:1677-1685.

Smiley, R. W., Marshall, J. M., and Yan, G. P. 2011. Effect of foliarly applied spirotetramat on reproduction of *Heterodera avenae* on wheat roots. Plant Disease 95:983-989.

Smiley, R. W., Ingham, R. E., Uddin, W., and Cook, G. H. 1994. Crop sequences for managing cereal cyst nematode and fungal pathogens of winter wheat. Plant Disease 78:1142-1149.

Smiley, R. W., Whittaker, R. G., Gourlie, J. A., Easley, S. A., and Ingham, R. E. 2005. Plant-parasitic nematodes associated with reduced wheat yield in Oregon: *Heterodera avenae*. Journal of Nematology 37:297-307.

Smiley, R. W., Marshall, J. M., Gourlie, J. A., Paulitz, T. C., Kandel, S. L., Pumphrey, M. O., Garland-Campbell, K., Yan, G., Anderson, M. D., Flowers, M. D., and Jackson, C. A. 2013. Spring wheat tolerance and resistance to *Heterodera avenae* in the Pacific Northwest. Plant Disease 97:590-600.

Subbotin, S. A. 2015. *Heterodera sturhani* sp. n. from China, a new species of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae). Russian Journal of Nematology 23:145-152.

Subbotin, S. A., Waeyenberge, L., and Moens, M. 2000. Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda : Heteroderidae) based on the ribosomal DNA-RFLP. Nematology 2:153-164.

Subbotin, S. A., Mundo-Ocampo, M., and Baldwin, J. 2010. Systematics of Cyst Nematodes (Nematoda: Heteroderinae), Part B. 8B ed. Brill, Boston.

USDA (2014) 'WB9668'. Available at: https://apps.ams.usda.gov/cms/adobeimages/201400235.pdf.

USDA (2015) 'WB9411'. Available at: https://apps.ams.usda.gov/cms/adobeimages/201500198.pdf.

USDA/NASS (2018) '2017 State Agricultural Overview for Idaho'. Available at: https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=IDAHO.

WestBred. 2016. WB9668 Hard Red Spring.

Wu, H. Y., He, Q., Liu, J., Luo, J., and Peng, D. L. 2014. Occurrence and development of the cereal cyst nematode (*Heterodera avenae*) in Shandong, China. Plant Disease 98:1654-1660.

Chapter 3. Occurrence of Natural Populations of Entomopathogenic Nematode *Steinernema feltiae* in Cereal Fields of Southeast Idaho

Pooria Ensafi, James W. Woodhall, Arash Rashed, Richard W. Smiley, Juliet M. Marshall

Abstract:

In a targeted survey for detecting local population of entomopathogenic nematodes (EPNs) in south and southeast Idaho, USA, six natural populations of EPNs were isolated from the soils of cereal fields that were heavily infested with larval stage of click beetles (Coleoptera: Elateridae). The consensus sequence from forward and reverse reads of the partial ribosomal RNA gene complex including internal transcribed spacers (ITS1 and ITS2) and D2-D3 expansions of the 28S large subunit confirmed the occurrence of *Steinernema feltiae* (Filipjev, 1934). Phylogenetic relationships inferred from maximum likelihood (ML) analysis of ITS-rRNA sequences distinguished the isolated EPNs in the Feltiae clade, and cytochrome c oxidase I (*COI*) of mitochondrial DNA resolved the phylogenetic relationships within *S. feltiae* subclade. *S. feltiae* is an effective biological control agent, with active nictation, cruiser locomotion and infection dynamics that can be variable at intraspecific level. Therefore, identification of this nematode must be made with in-depth molecular analysis. This study is the first report and characterization of *Steinernema feltiae* in southeast Idaho. We suggest additional studies on the infection dynamics and bacterial symbiosis for these isolates.

Introduction:

Steinernema Travassos 1927 (Travassos 1927) comprises more than 55 entomopathogenic nematode species (Nguyen et al. 2007) that share mutualistic symbiosis with the pathogenic bacteria *Xenorhabdus* (Thomas and Poinar JR 1979). In this relationship, the Gram-negative bacteria release endo- and exotoxins which suppress insect's immune system and cause septicemia in different life stages of the insect host, which provides food for the infective nematode juveniles (IJs) to complete their lifecycle (Poinar JR 1990). Unique characteristics of *Steinernema* including long dauer stage, soil-dwelling with active locomotion, and cruiser or ambusher traits of the IJs (Campbell and Gaugler 1997) make them a great biological control agent for large-scale integrated pest management (IPM) (Bedding and Akhurst 1975; Morton and Garcia-del-Pino 2017; Toba et al. 1983).

In southeast Idaho, IPM strategies are important since conventional chemical controls fail to reduce the population of wireworms (the larval stage of the click beetles; Coleoptera: Elateridae), mainly because of the biology of wireworms which live in the soil for several years and move up to 1.5 m deep

in the soil to avoid the unfavorable environmental conditions (Andrews et al. 2008; Rashed et al. 2017). In our previous study on the efficacy of market-based biological control agents against sugar beet wireworm (*Limonius califonicus*), although mediated by soil type, the ambusher EPN *Steinernema carpocapsae* (Ennis et al. 2010) provided comparatively good control under the greenhouse conditions (Ensafi et al. 2018). Different studies have also documented the efficacy of Steinernematids against wireworms (Barsics et al. 2013; Furlan et al. 2009) under controlled environments, but were not very promising under field.

It has been well studies and documented that different environmental variables could result in intraspecific variations in the biology and infection potential of entomopathogenic nematodes. Campos-Herrera and colleagues have studied the patterns of traits associated with the infection of different populations of *S. feltiae*. They showed that these patterns differ among populations with genetic variability because of the adaptation to a different habitat (Campos-Herrera and Gutiérrez 2014); traits such as sex ratio (more females in crop edge and agricultural groves), or juveniles' mortality and locomotion. Currently, efforts have been focused on the identification of local isolates and study of the genetic variability within, and between *S. feltiae* populations (Abate et al. 2018; Kuwata et al. 2006; Tumialis et al. 2016).

In this study, we conducted an extensive survey in cereal fields of south and southeast Idaho, where the Eastern Idaho Entomology Laboratory is monitoring for the incidence and a population density of wireworm species, to find, isolate and identify the EPNs that are locally adapted and can be potentially virulent to the wireworms. To that end, we (1) monitored and sampled the soil from 48 cereal fields around southeast Idaho, and isolated the existing EPN populations, (2) initiated the phylogenetic relationships between the isolates and previously submitted accessions, and (3) studied the inter- and intraspecific variations between the identified species.

Materials and Methods:

Study locations:

From September 2017 to April 2018, 48 cereal fields in the south, and southeastern Idaho were monitored for the possible occurrence of local entomopathogenic nematode (EPN) populations. From three wireworms trapping locations per field, three composite soil samples from 5 cores inside 4 m^2 of each location were collected using a 5 cm diameter soil probe at 20 cm depth. The soil samples were

labeled and transferred to the Aberdeen Research and Extension Center and kept at 10 °C until processed.

Table 3-1 Sampling location details with the positive occurrence of EPNs in the cereal fields of southeastern Idaho, with soil type for each location adapted from Web Soil Survey, National Resources Conservation Service; United States Department of Agriculture

Isolate	Location	Sampling Coordinates		Irrigation	Soil type	Sample date
Ben-F1	Swan Valley	43.411571	-111.249813	Rainfed	Silt loam	10.25.2017
Ben-F4	Swan Valley	43.425037	-111.318941	Rainfed	Sandy loam	10.25.2017
CurtisF2	Soda Springs	42.765041	-111.682655	Rainfed	Silty clay loam	10.16.2017
Gordon	Ririe	43.584797	-111.547692	Rainfed	Silt loam	10.22.2017
Hans-F1	Arbon Valley	42.631807	-112.512676	Rainfed	Silt loam	10.17.2017
Kyle-F1	Soda Springs	42.702469	-111.564643	Irrigated	Silty clay loam	10.16.2017

EPN isolation from soil and multiplication:

We recovered nematodes from the soil samples using the trapping method by Bedding and Akhurst. Briefly, each sample was mixed, the debris was removed, and three subsamples of 250 ml soil were separated from each composite and transferred to a 470 ml plastic container. Seven larvae of *Galleria mellonella* L. Lepidoptera: Pyralidae (Waxworm; Timberline Fisheries, Marion, IL) were transferred to the top of the soil of each container. The container closed with the lid and put upside down in the dark room at room temperature (Bedding and Akhurst 1975).. Every 2-3 days, the waxworms were checked, dead larvae were removed and rinsed using sterile water. EPNs recovered from waxworms using the modified white trap. Briefly; seven larvae were put on a filter paper on the lid of a 50 mm small petri dish, inside a 9 cm petri dish that was filled with 20 ml water. After 10 days Infective juveniles (IJs) emerged from the dead waxworms into the surrounding water inside the petri dish, which then collected in a flask (White 1927).

Taxonomic study:

For the taxonomic study of each isolate, we prepared two separate 9 cm petri dishes with moistened filter paper containing seven waxworms per petri dish. Isolated IJs were inoculated at the rate of 100 IJs per waxworm and stored in a dark room at 20-25 °C. Time of death and mortality rate were recorded, and dead larvae were transferred to white traps. After collecting newly emerged IJs, dead larvae from one petri dish were dissected under a stereomicroscope, while the second petri dish

remained at the dark room for the collection of the second-generation adults (Malan et al. 2016). For the light microscopy, we used Glycerol-Ethanol protocol (De Grisse 1969). Briefly; from each population, 20 specimens of IJs and adults were picked and transferred to a staining block and killed by adding 0.5 ml De Grisse solution-I (99-part 4% formaldehyde pH>7 and one-part glycerin; heated to 70 °C). The uncovered staining blocks were transferred to a closed desiccator filled to one-tenth of its volume with 96% ethanol in an oven at 40 °C temperature. After 12 h., the staining blocks were removed, the volume of the ethanol was reduced, and then the standing blocks were topped up with the De Grisse solution-II (95-part ethanol 96% plus 5-part glycerin). The blocks were partially covered with the lid (3/4) and were put in the oven with the same temperature. Few drops of the solution-II were added to the blocks every 2 h., four times. At the final stage, a few drops of the solution III (50:50 of 96% ethanol plus glycerin) were added to the blocks and left in the oven for 12 h. The fixed specimens were picked and transferred onto glass slides and were permanently fixed with a cover glass. Commonly used morphometrics of the features that are required for morphological identification were obtained from imaging using a Nikon DS-F12 digital camera. The ImageJ 1.52a software (Wayne Rasband, NIH, USA) were used for measurements.

Molecular Systematics:

DNA extraction, amplification, purification, and sequencing:

DNA Extraction: We extracted the DNA using a modified method described by Joyce et al. Briefly, a single live first-generation female was transferred to a 2ml hard body tube containing 20 µl worm lysis buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$, 0.45% Tween 20). The nematode was crushed, and the content was homogenized for 30 seconds at high speed using Bead Ruptor 12 (Omni International). The tube was kept in -80 °C for 10 min, thawed at 8 °C, 2 µl Qiagen Proteinase K (600 µg ml^{-1} stock) was added to the tube, and then incubated at 65 °C for 45 min. The proteinase K was inactivated by 10 min exposure to 95 °C. The tube was centrifuged for 2 min at 11600 × *g,* and the supernatant was transferred to a 1.5 ml tube and stored at -20 °C (Joyce et al. 1994)..

DNA amplification and sequencing: Using Qiagen *Taq* PCR Core Kit, polymerase chain reactions (PCR) were carried out in a 0.2 ml thin wall PCR tube containing 23 µl master mix (per reaction; ultra-pure nuclease-free water: 14.6 µl, Q-solution: 5 µl, 10X PCR buffer: 2.5 µl, 10 mM dNTP-Mix: 0.5 µl, 1.5 µM forward primer: 0.15 µl, 1.5 µM reverse primer: 0.15 µl, *Taq* DNA polymerase 5 units $µl^{-1}$: 0.1 µl). 2 µl of DNA (minimum concentration: 20 ng $µl^{-1}$) suspension was added to the tube and the mixture was

run at a temperature profile of 4 min at 94 °C, 35 cycles of 1 min at 94 °C, 1.5 min at the related T_m, 2 min at 72 °C, and 10 min at 72 °C in a thermocycler (Eppendorf AG 2231, Hamburg).

PCR product was verified through AGE (Agarose gel electrophoresis); 2 µl loading buffer mixed with three µl PCR product and run in previously prepared 1% agarose gel prepared in 0.5% TBE buffer and stained with GelRed™ at 72 volts for one hour. DNA bands were visualized by AlphaImager® MINI (Protein Simple). The PCR products were purified using Applied Biosystems™ ExoSAP-IT™ Express PCR Product Cleanup Reagent (Thermo Fisher Scientific) and sent to Eurofins Genomics LLC (Louisville, KY, USA) for sequencing. The new sequences obtained from the amplification of ribosomal RNA were deposited in the GenBank under accession numbers MK131018 – MK131021. The primer sets for amplification and melting temperatures are presented in Table 3-2.

Study of the consensus sequences:

We used Chromas 2.6.5 (Technelysium Pty Ltd) software to visualize the raw sequences. Consensus sequences from forward read and reverse reads were obtained using MEGA-X (Version 10.0.5). For the search of homology, we assessed the similarity of each sequence with known collected sequences in GenBank (National Centre for Biotechnology Information, U.S. National Library of Medicine; Rockville Pike, Bethesda MD) using BLAST (Basic Local Alignment Search Tool). From the hit list, the sequences with highest total Score and lowest expected value were retrieved from the GenBank and downloaded in 'Fasta' format for the study of Phylogeny.

Table 3-2 Universal PCR primers used for molecular study of Steinernematid isolates

	Primer name, Direction, - Sequence (5' – 3')	T_m(°C)	Size (kbp)	Source
ITS-rRNA	18S FW- TTGATTACGTCCCTGCCCTTT 26S RV- TTTCACTCGCCGTTACTAAGG	60	1 - 1.1	(Vrain et al. 1992)
	TW81 FW- GTTTCCGTAGGTGAACCTGC AB28 RV- ATATGCTTAAGTTCAGCGGGT	55	0.9 - 1	(Hominick et al. 1997)
D2 D3	D2F FW- CCTTAGTAACGGCGAGTGAAA 536 RV- CAGCTATCCTGAGGGAAAC	55	1 – 1.1	(Nguyen et al. 2006) (Stock et al. 2001)
CO1	FW- CCTACTATGATTGGTGGTTTTGGTAATTG COI-R2 RV-GTAGCAGCAGTAAAATAAGCACG	45	0.8 – 0.9	(Kanzaki and Futai 2002)

Phylogenetic study of the EPNs:

Target genes: In our study we analyzed data from three different genomic regions: 1) ribosomal RNA gene including partial sequence of 18S small subunit, complete sequence of (ITS1-5.8-ITS2), and partial sequence of 28S large subunit; 2) partial sequence of D2 and D3 expansions fragments from large subunit of rRNA; 3) partial sequence of cytochrome c oxidase subunit 1 (*CO1*) gene. From the blast hit list, and with the consideration of phylogenetic groups and distribution, only accession numbers that had previously been used for the phylogenetic construction of the genus *Steinernema* were retrieved.

Choice of accessions: Following two publications (Spiridonov and Subbotin 2016; Stock et al. 2001), for the rRNA ITS region, we included the KJ696685, AY171268, AB243441, DQ105794, FJ235074, EU421129, DQ310469, JF728856, AF121050, Ay230185, AY171265, FJ666052, EF431959, AY171255, AY171264, AY230176, JN171593, AF122019, EF152568, AY355441 accessions from the Feltiae clade , plus one major taxa from remaining groups of the genus *Steinernema*, which include DQ375757, AB243440, AY171288, FJ410327, AF122021, AY171276, FJ860033, DQ835613, AF122018, AY171298, AY171290, and three outgroups JN636060, X03680, JN636079.

For the D2 and D3 expansions, we included the accessions 'GU569059, Eu421130, AF331906, GU569051, GU569053, AF331896, AF331891, EF152569, KF289902, AF331897, AF331908, FJ666054, GU569048, KJ950292, AF331893, FJ263674, GU569042, AF331909', plus three outgroups NR_000055, JN636137, KF732844.

To study the phylogeny from the mitochondrial gene (mtDNA), the accessions were retrieved from the GenBank directly, including all accessions for the genus *Steinernema* excluding unknown species and duplicates, plus two outgroups X5423.1, and X5452.1.

Multiple sequence alignment and phylogenetic analysis:

We used weighted Clustal (ClustalW) in MEGA-X (Version 10.0.5) for the complete multiple sequence's alignment and establishment of positional homology between nucleotides. After the alignment, the sequences were trimmed to the primers borders and saved in MEG-X format. The best model for the statistical method of maximum likelihood for all nucleotide sites was assessed in MEGA-X, and the model with the lowest BIC score (Bayesian information criterion) for the substitution pattern was chosen for the construction of phylogenetic tree (Kumar et al. 2018; Spiridonov and Subbotin 2016).

The phylogenetic relationships were estimated using maximum likelihood (ML) statistical method. Following the results from the best fit model for the aligned sequences, the consensus trees were generated using an appropriate substitution model at the two discrete Gamma categories among sites in MEG-X.

Results

Morphometric taxonomy and in vivo life cycle

For all isolates, inoculated infective juveniles (IJs) were able to cause 100% mortality within 48H from the time of exposure. Thirteen days after the inoculation, IJs started to emerge. *Steinernema* isolate (Kyle-F1) was used for the morphological identification; specimens were reared on waxworm (*Galleria mellonella*) and dissected from the cadaver. *Steinernema feltiae* isolate Kyle-F1 has similar IJs' morphometric to *Steinernema feltiae* (Filipjev, 1934). The body length (L) is 824.9 ±49 µm and body width (BW) is 27.5 ± 1.7 µm. Distance from the anterior body to the secretory-excretory pore (EP) is 55.6 ± 3.9 µm, tail length (T) is 78.9 ± 6.1 µm, and nerve ring (NR) is at 82.6 ± 5.3 µm anteriorly. The ratios a (L/BW), b (L/EP), and c (L/T) are 30.1, 6.4, and 10.5 respectively. Compared to *S. feltiae*; first-generation males are slightly shorter 1232.7 ± 164.0 µm. Body diam is 141.7 ± 15.8 µm; EP is 82.5 ± 10.1 µm; T is 28.7 ± 2.5 µm; spicule length is 70.9 ± 3.4 µm; and gubernaculum length is 46.2 ± 4.1 µm.

Molecular phylogenetic analysis:

From the studied locations, six EPN populations were successfully isolated and propagated. Amplification of ribosomal RNA (partial 18S and 28S, and complete ITS1, 5.8S, and ITS2 regions) of four populations were found reliable for the molecular analysis were submitted to the GenBank. Isolates 'Curtis-F2, Gordon, Hans-F1, and Kyle-F1' were registered under accessions numbers MK131018 to MK131021 respectively. For the search of homology, each isolate's sequence was blasted in the GenBank, and two accessions with the highest score and lowest E-values were considered as similar taxa presented in Table 3-3. In addition to search of homologous sequences, primer position and borders of each full sequence region (ITS1&2, and 5.8S) were assessed. The ITS1-5.8S-ITS2 includes 730 base pairs (ITS1: 275, 5.8S: 157, and ITS2: 298).

Table 3-3 Homology search results from the nucleotide blast in GenBank

	Isolate	Accession #	GenBank aligned accessions		# of *feltiae* hits	Score
ITS1-5.8S-ITS2	Curtis-F2	MK131018	AF121050.2	JF728856.1	105	1491
	Gordon	MK131019	AF121050.2	JF728856.1	104	1491
	Hans-F1	MK131020	AF121050.2	AY230185.1	99	1681
	Kyle-F1	MK131021	AF121050.2	JF728856.1	105	1491
D2 D3 expansion of 28S	Ben-F1	-	AF331906.1	JF920966.1	14	1659
	Ben-F4	-	AF331906.1	JF920966.1	14	1635
	Curtis-F2	-	AF331906.1	JX436334.1	15	1544
	Gordon	-	AF331906.1	GU569049.1	15	1472
	Hans-F1	-	AF331906.1	JF920966.1	15	1628
	Kyle-F1	-	AF331906.1	GU569049.1	15	1406
CO1 mitochondrial DNA	Ben-F1	-	LM608088.1	LM608090.1	52	1290
	Ben-F4	-	LM608090.1	DQ285544.1	52	1284
	Curtis-F2	-	LM608088.1	LM608090.1	52	1295
	Gordon	-	LM608088.1	LM608090.1	52	1295
	Hans-F1	-	LM608088.1	LM608090.1	52	1284
	Kyle-F1	-	LM608088.1	LM608090.1	52	1275

The accession numbers in the table are the result of nucleotide Blast with highly similar sequences (mega Blast), maximum target of 500 at expected threshold of 10.

Results from the model test specific for ITS rRNA showed that HKY+G (Hasegawa-Kishino-Yano) model represents lowest BIC. Therefore, the ancestral states were inferred using the Maximum Likelihood method in HKY+G (Hasegawa et al. 1985) with 1000 bootstrap replications and 6 discrete gammas (G) distributed rates among sites. The original phylogenetic tree is presented as Figure 3-1.

For the D2 and D3 expansions of 28S rRNA, GTR+G (General time reversible using discrete gamma distribution) model was estimated to have the lowest BIC. The phylogenetic relationships were constructed at 1000 bootstrap replications and 6 discrete gammas which is presented in Figure 3-2.

Maximum Likelihood fit of GTR+G+I (General Time Reversible using a discrete gamma distribution model with 2 rates categories, assuming that a certain fraction of sites is evolutionarily invariable). The phylogenetic relationships inferred from 1000 bootstraps is presented in Figure 3-3.

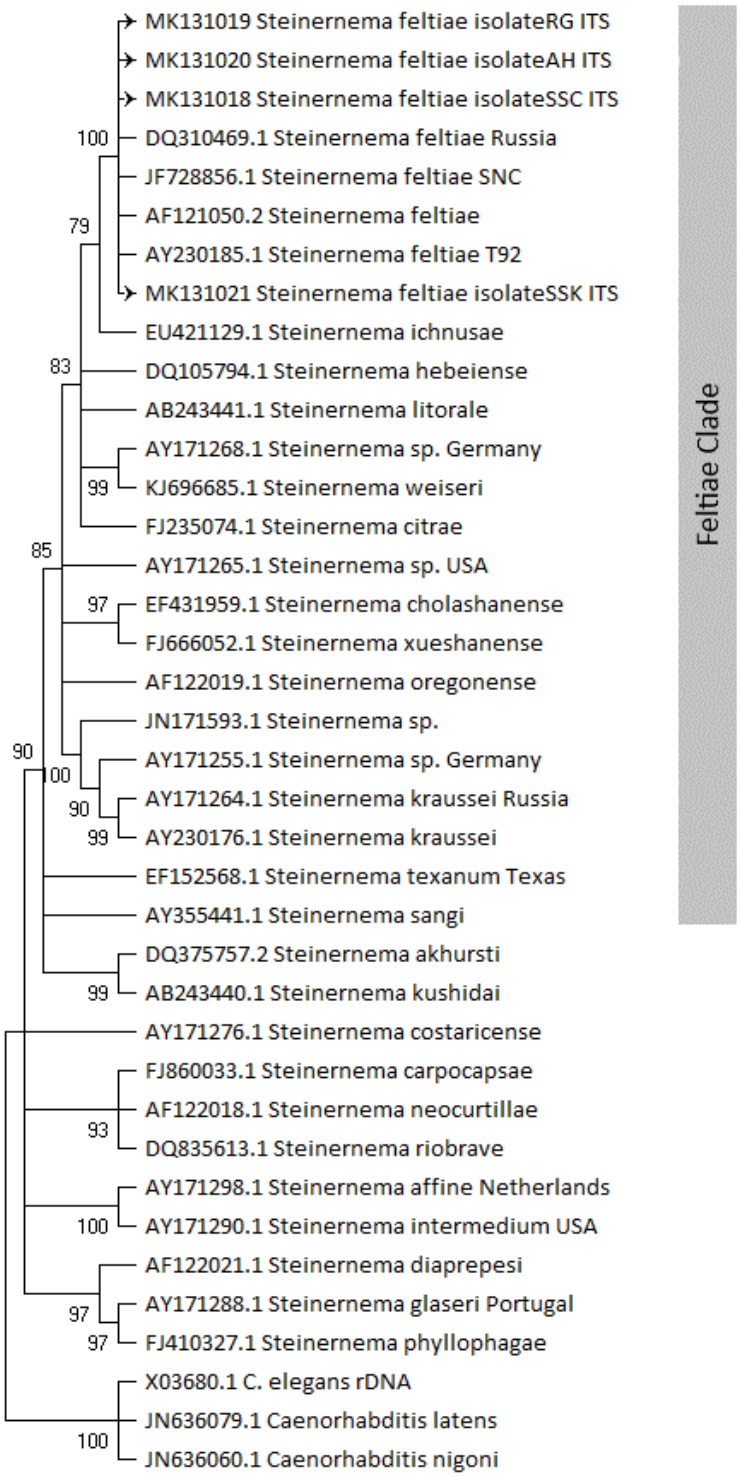

Figure 3-1 Phylogenetic relationships inferred from maximum likelihood analysis of ITS rRNA gene using HYK+G model of DNA evolution with 1000 bootstraps and Gamma distribution 2. Probabilities lower than 70% are not given. Local EPN isolates are marked with arrow. The Feltiae Clade marked in the figure is obtained from Spiridonov & Subbotin 2016 and presented for the reference.

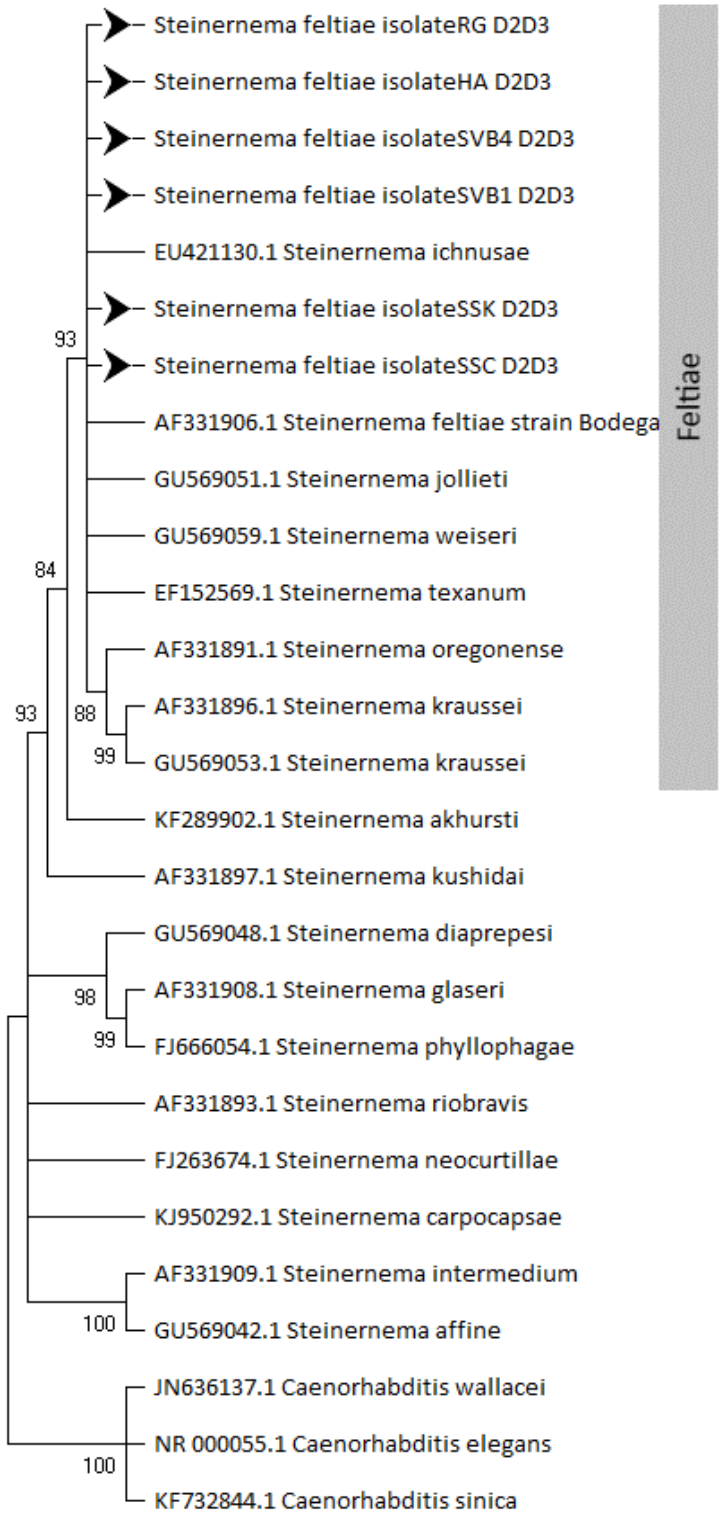

Figure 3-2 Phylogenetic relationships inferred from maximum likelihood of D2 and D3 sequences of 28S rRNA using GTR+G+I model of DNA evolution. Probabilities lower than 70% are not shown. The local EPN isolates are marked with arrow. The Feltiae Clade obtained from Spiridonov and Subbotin 2016, Presented for the reference

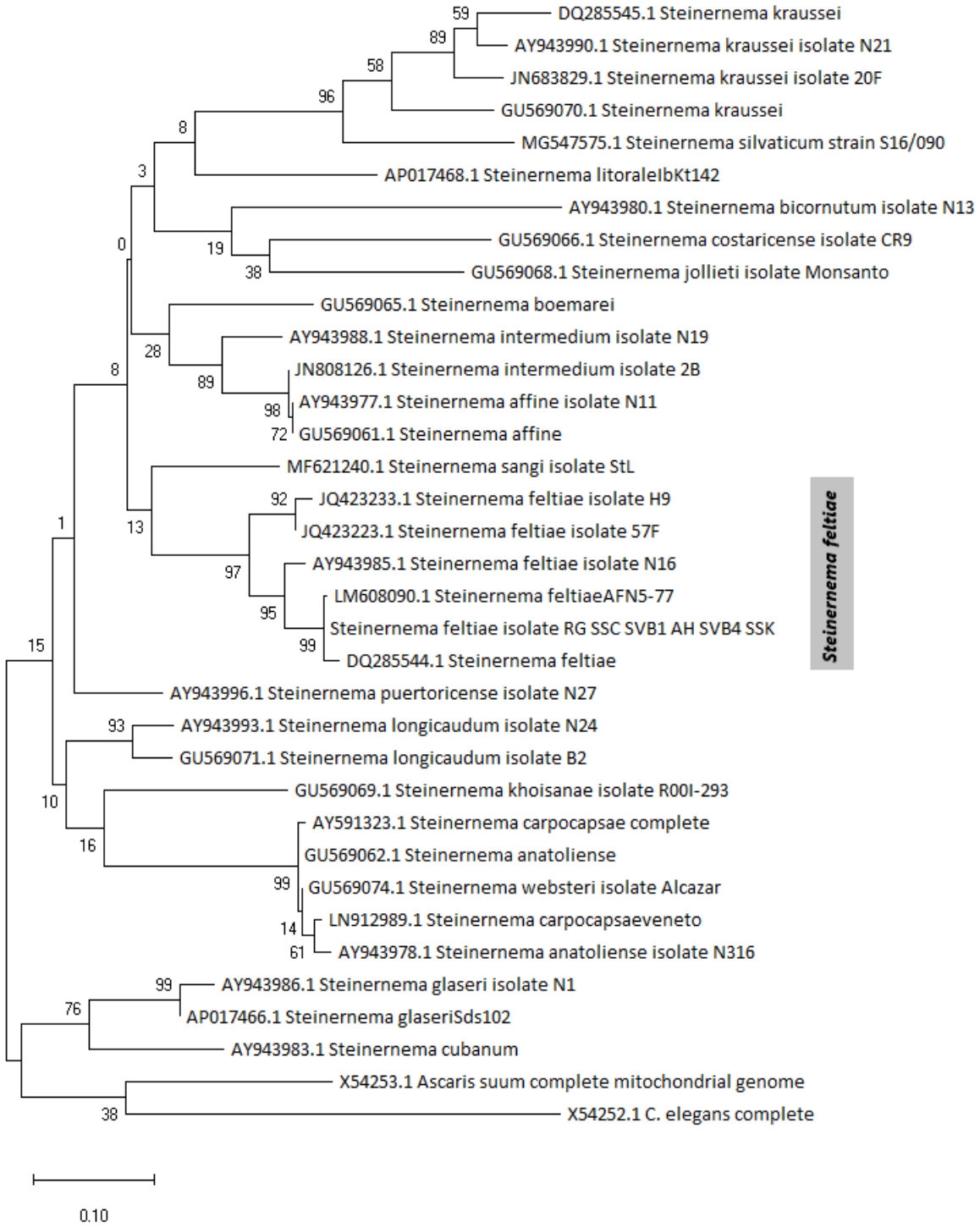

Figure 3-3 Phylogenetic relationships inferred from maximum likelihood analysis of CO1 gene of the mitochondrial DNA using GTR+G+I model of evolution. The S. feltiae species that are obtained from the GenBank are marked

Discussion

Characterizing regional populations of entomopathogenic nematodes (EPNs), especially Steinernematids, is critical because the main traits associated with pathogenicity of this group are greatly variable at inter- or intraspecific level (Campbell and Gaugler 1997). Furthermore, accurate identification of pathogenic sources in nematode population improves the understanding of dispersal and may lead to an increase in efficacy of the agricultural pest management (Hominick et al. 1997). Our study is the first report of *Steinernema feltiae* (Filipjev, 1934) in Idaho, and in fact, the first extensive survey in Southeastern Idaho to study the occurrence and phylogenetic relationships of EPNs in cereal fields that are naturally infested with wireworm larvae.

Morphological identification of the *S. feltiae* isolates in our study was only useful for characterization to the genus level. Adding extra morphological features misled our identification because the IJs morphometrics resembled type species *S. feltiae* while the morphometrics of the first-generation male was significantly smaller. Such differences could be the result of errors in measurements, application of different techniques or inconsistent terminology (Hominick et al. 1997; Spiridonov and Subbotin 2016). Our study confirms the report by Spiridonov, that morphometrics of different stages of *S. feltiae* have little effect on identification of Steinernematids and does not affect the molecular reconstruction of phylogenetic relationships, with the exception of very few characteristics which are only available through transmission electron microscopy (such as sperm morphology and occurrence of membranous organelles) (Slos et al. 2015; Spiridonov et al. 2004; Spiridonov and Subbotin 2016).

Before utilization of in-depth molecular approaches, we sequenced the ITS-rRNA that have been reported useful for the identification and application of phylogenetic relationships of the EPNs (Nguyen et al. 2001). Results from the sequencing and search of homology showed more than 95% similarity to *S. feltiae*. We constructed the phylogenetic relationship from maximum likelihood analysis of ITS-rRNA, using both HYK+G and GTR+G+I model, and found that ITS-rRNA could significantly (100%) resolve a clear species-level subclade that includes southeastern Idaho's isolates and the *S. feltiae* accessions from the GenBank. The most recent Feltiae Clade that was proposed by Stock et. al. (2001) was also confirmed in our study which clearly indicates that sequences from ITS-rRNA are still effective for explaining interspecific variability (Stock et al. 2001).

Although variable, ITS-rRNA in our experiment could not delineate intraspecific variation within species of *S. feltiae* subclade, and also failed to significantly resolve the Kushidai clade, which was recently separated from Feltiae clade by Spiridonov (Spiridonov and Subbotin 2016). On the other hand,

reconstruction of the phylogenetic relationship targeting D2-D3 expansion of rRNA clearly resolved the separated Kushidai clade but was not variable enough to differentiate a clear subclade for *S. feltiae* species. These second structures of large subunit of rRNA seems to be applicable as the findings supports previous reports (Nadler et al. 2006; Spiridonov and Subbotin 2016).

From the observations by Campos-Herrera and Gutierrez, we knew that there is considerable intraspecific variability in the infection dynamics and sex ratio of *S. feltiae* population, which they hypothesized as a "possible effect of habitat on the phenotypic plasticity" (Campos-Herrera and Gutiérrez 2014). Besides, Půža and colleagues have also reported that intra-individual variability commonly occurs in ITS-rRNA, and could be seen as haplotype diversity in an individual species population (Půža et al. 2015). In our experiment, the ribosomal gene complex could not resolve such interspecific variations. Therefore, we constructed the phylogenetic relationship of maximum likelihood from cytochrome oxidase subunit I of the mitochondrial gene of the southeast Idaho isolates, and found that *CO1* is a useful gene for the study of intraspecific variations of Steinernematids, but it does not provide enough variation to resolve interspecific phylogenetic relationships, which supports the results of a Japanese study (Kuwata et al. 2006).

In addition to the current study, two other extensive studies have been initiated by the Eastern Idaho Entomology Lab (University of Idaho) team to compare the biological control potential of this study's isolates and Steinernematids from the market against wireworms.

Acknowledgments

We would like to thank F. Aguilar and B. Whalen for their help with various aspects of this experiment. This study project was supported by the Idaho Wheat Commission, the Idaho Barley Commission, and United States Department of Agriculture- National Institute of Food and Agriculture (USDA-NIFA) grant 2016-70006-25829.

Author and Co-authors' Contribution

Pooria Ensafi: Investigation (performing the experiments, data/evidence collection); Formal analysis (application of statistical method); Data Curation; Writing (original draft preparation)

James W. Woodhall: Resources (provision of molecular analysis); Writing (review and editing including critical review of manuscripts)

Arash Rashed: Resources (provision of study materials), Funding (acquisition of financial support); Writing (review of manuscripts).

Richard W. Smiley: Writing (review and editing including critical review, pre-publication revision)

Juliet M. Marshall: Supervision (oversight and leadership responsibility); Funding (acquisition of financial support); Resources (provision of study materials and analysis); Writing (review and editing manuscripts).

References

Abate, B. A., Slippers, B., Wingfield, M. J., Malan, A. P., and Hurley, B. P. 2018. Diversity of entomopathogenic nematodes and their symbiotic bacteria in south African plantations and indigenous forests. Nematology 20:355-371.

Andrews, N., Ambrosino, M., Fisher, G., and Rondon, S. 2008. Wireworm biology and nonchemical management in potatoes in the Pacific Northwest. Oregon State University Extension Service 607:1-18.

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

Bedding, R. A., and Akhurst, R. J. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21:109-110.

Campbell, J. F., and Gaugler, R. 1997. Inter-specifie variation in entomopathogenic nematode foraging strategy: dichotomy or variation along a continuum? Fundamental and Applied Nematology 20:393-398.

Campos-Herrera, R., and Gutiérrez, C. 2014. *Steinernema feltiae* intraspecific variability: infection dynamics and sex-ratio. Journal of Nematology 46:35.

De Grisse, A. T. 1969. Redescription ou modifications de quelques technique utilisees dan l'etude des nematodes phytoparasitaires. Pages 351-369 Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent Belgium.

Ennis, D., Dillon, A., and Griffin, C. 2010. Simulated roots and host feeding enhance infection of subterranean insects by the entomopathogenic nematode *Steinernema carpocapsae*. Journal of Invertebrate Pathology 103:140-143.

Ensafi, P., Crowder, D. W., Esser, A. D., Zhao, Z., Marshall, J. M., and Rashed, A. 2018. Soil type mediates the effectiveness of biological control against *Limonius californicus* (Coleoptera: Elateridae). Journal of Economic Entomology 111:2053-2058.

Furlan, L., Bonetto, C., Costa, B., Finotto, A., and Lazzeri, L. 2009. Observations on natural mortality factors in wireworm populations and evaluation of management options. IOBC/wprs Bull 45:436-439.

Hasegawa, M., Kishino, H., and Yano, T.-a. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160-174.

Hominick, W. M., Briscoe, B. R., del Pino, F. G., Heng, J., Hunt, D. J., Kozodoy, E., Mracek, Z., Nguyen, K. B., Reid, A., Spiridonov, S., Stock, P., Sturhan, D., Waturu, C., and Yoshida, M. 1997. Biosystematics of entomopathogenic nematodes: current status, protocols and definitions. Journal of Helminthology 71:271-298.

Joyce, S. A., Reid, A., Driver, F., and Curran, J. 1994. Application of polymerase chain reaction (PCR) methods to identification of entomogenic nematodes. Pages 178-187 in: COST 812 Biotechnology: Genetics of entomopathogenic nematode-bacterium complexes, A. M. Burnell, R.-U. Ehlers and J. P. Masson, eds. Luxembourg: European Commission, St. Patrick's College, Maynooth, Co. Kildare, Ireland.

Kanzaki, N., and Futai, K. 2002. A PCR primer set for determination of phylogenetic relationships of *Bursaphelenchus* species within the xylophilus group. Nematology 4:35-41.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.

Kuwata, R., Shigematsu, M., Yoshiga, T., Yoshida, M., and Kondo, E. 2006. Intraspecific variations and phylogenetic relationships of steinernematids isolated from Japan based on the sequences of the ITS region of the nuclear rRNA gene and the partial mitochondrial COI gene. Nematological Research (Japanese Journal of Nematology) 36:11-21.

Malan, A., Knoetze, R., and Tiedt, L. 2016. *Steinernema jeffreyense* n. sp.(Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. Journal of Helminthology 90:262-278.

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

Nadler, S. A., Bolotin, E., and Stock, S. P. 2006. Phylogenetic relationships of *Steinernema* Travassos, 1927 (Nematoda: Cephalobina: Steinernematidae) based on nuclear, mitochondrial and morphological data. Systematic Parasitology 63:159-179.

Nguyen, K., Maruniak, J., and Adams, B. 2001. Diagnostic and phylogenetic utility of the rDNA internal transcribed spacer sequences of *Steinernema*. Journal of Nematology 33:73.

Nguyen, K. B., Malan, A. P., and Gozel, U. 2006. *Steinernema khoisanae* n. sp.(Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. Nematology 8:157-175.

Nguyen, K. B., Hunt, D. J., and Mracek, Z. 2007. Steinernematidae: species descriptions. Pages 121-609 in: Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts, vol. 5. K. B. Nguyen and D. J. Hunt, eds.

Poinar JR, G. O. 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae. Pages 23-61 in: Entomopathogenic Nematodes in Biological Control. R. Gaugler and H. K. Kaya, eds. CRC Press, Boca Raton, FL, USA.

Půža, V., Chundelová, D., Nermuť, J., Žurovcová, M., and Mráček, Z. 2015. Intra-individual variability of ITS regions in entomopathogenic nematodes (Steinernematidae: Nematoda): implications for their taxonomy. Biocontrol 60:547-554.

Rashed, A., Rogers, C. W., Rashidi, M., and Marshall, J. M. 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. Arthropod-Plant Interactions 11:147-154.

Slos, D., Ensafi, P., Claeys, M., Yushin, V. V., Decraemer, W., and Bert, W. 2015. Ultrastructure of sperm development in the genus Ditylenchus (Nematoda: Anguinidae). Nematology 17:313-324.

Spiridonov, S. E., and Subbotin, S. A. 2016. Phylogeny and phylogeography of *Heterorhabditis* and *Steinernema*. Pages 413-427 in: Advances in Entomopathogenic Nematode Taxonomy and Phylogeny, D. J. Hunt and K. B. Nguyen, eds. Brill.

Spiridonov, S. E., Reid, A. P., Podrucka, K., Subbotin, S. A., and Moens, M. 2004. Phylogenetic relationships within the genus *Steinernema* (Nematoda: Rhabditida) as inferred from analyses of sequences of the ITS1-5.8 S-ITS2 region of rDNA and morphological features. Nematology 6:547-566.

Stock, P. S., Campbell, J. F., and Nadler, S. A. 2001. Phylogeny of *Steinernema* Travassos, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters. Journal of Parasitology 87:877-889.

Thomas, G. M., and Poinar JR, G. O. 1979. *Xenorhabdus* gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family Enterobacteriaceae. International Journal of Systematic Evolutionary Microbiology 29:352-360.

Toba, H. H., Lindegren, J. E., Turner, J. E., and Vail, P. V. 1983. Susceptibility of the Colorado potato beetle and the sugarbeet wireworm to *Steinernema feltiae* and *S. glaseri*. Journal of Nematology 15:597-601.

Travassos, L. 1927. Uma nova *Capillaria parasita* de peixes be agua doce: *Capillaria sentinosa* n. sp. Boletim Biologico 10:215-217.

Tumialis, D., Łopieńska, M., Mazurkiewicz, A., Pezowicz, E., and Skrzecz, I. 2016. Identification and intraspecific variability of *Steinernema feltiae* (Filipjev, 1934) isolates from different localities in Poland. Helminthologia 53:304-308.

Vrain, T., Wakarchuk, D., Levesque, A., and Hamilton, R. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology 15:563-573.

White, G. 1927. A method for obtaining infective nematode larvae from cultures. Science (Washington) 66:302-303.

Chapter 4. Soil Type Mediates the Effectiveness of Biological Control Against *Limonius californicus* (Coleoptera: Elateridae)

Ensafi, P., Crowder, D. W., Esser, A. D., Zhao, Z., Marshall, J. M., and Rashed, A. 2018. Soil type mediates the effectiveness of biological control against Limonius californicus (Coleoptera: Elateridae). Journal of Economic Entomology 111:2053-2058.

Abstract:

Wireworms, the larval stage of click beetles (Coleoptera: Elateridae), are a considerable threat to cereal and vegetable production in the Pacific Northwest and Intermountain regions of the United States. As insecticides are generally ineffective, alternative controls are needed to improve wireworm management. Wireworms are continuously exposed to a wide range of subterranean pathogenic organisms in the soil; identifying these organisms and determining their impact would contribute to the development of biological control for wireworms. Here, we evaluated the efficacy of an entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae), and a fungus, *Metarhizium brunneum* Petch (strain F52) (Hypocreales: Clavicipitaceae), for control of the Pacific Northwest predominant wireworm species Limonius californicus, in two different soil media. We also examined whether diatomaceous earth (DE) increases the efficacy of entomopathogens through facilitating their penetration into the host integument. Treatments containing *M. brunneum* (F52) resulted in the highest rates of wireworm mortality, indicating that the fungus may be more effective than the nematode at reducing population size. However, results were impacted by soil media. In peatmoss-dominated medium, *M. brunneum*-containing treatments were more effective in reducing feeding damage than treatments containing S. carpocapsae. However, in sand-dominated medium, treatments with *S. carpocapsae* provided relatively better seedling protection. No consistent effect of DE was detected. Our results suggest that the effectiveness of wireworm biological control agents depends on soil media, such that the application of biological control against wireworms must be made with knowledge of field soil type.

Introduction:

The importance of developing integrated pest management (IPM) strategies for pest control is particularly important when conventional chemical approaches fail to offer a solution. Wireworms are one such example of an ongoing pest problem with limited management options in the Pacific Northwest and Intermountain regions of the United States. Wireworms are the larval stage of click beetles (Coleoptera:

Elateridae) and have been a major challenge to a wide range of crops. This is because they live in the soil for several years, continuing to feed on underground plant tissues and moving as deep as 1.5 m into the soil profile when environmental conditions are not favorable (Andrews et al. 2008). In cereals, damage to the sprouting seed can result in emergence failure, while later stage plants can experience delayed growth and yield.

The resurgence of wireworms is largely attributed to bans on persistent conventional pesticides (Vernon et al. 2008). Currently, neonicotinoid seed treatments are the only pesticide option registered in cereal crops; however, such seed treatments often fail to provide acceptable levels of protection (Vernon et al. 2008; Vernon et al. 2009). Moreover, there is uncertainty about the future of neonicotinoid seed treatments due to their potential link with increased bee mortality (Godfray et al. 2014). Thus, while developing IPM strategies for wireworms was historically a low priority given the availability of a diverse set of inexpensive conventional insecticides, there is now an urgent need to rigorously investigate alternative management approaches.

As soil dwelling organisms, wireworms are exposed to a wide range of natural enemies that inhabit the same subterranean environment. Wireworm mortality from entomopathogenic fungi, bacteria, and nematodes has been documented (Barsics et al. 2013; Furlan et al. 2009; Traugott et al. 2015); studies have also investigated the effectiveness of *Metarhizium spp.* (Kabaluk and Ericsson 2007a; Reddy et al. 2014) *Beauveria bassiana* (Ester and Huiting 2007; Kölliker et al. 2011; Ladurner et al. 2009; Sufyan et al. 2017), and the parasitic nematode *Steinernema spp*. (Ester and Huiting 2007; Morton and Garcia-del-Pino 2017; Toba et al. 1983) for managing wireworms. While some of these studies demonstrated substantial wireworm mortality, or reduced crop damage e.g., (Ladurner et al. 2009; Morton and Garcia-del-Pino 2017; Reddy et al. 2014); environmental variables such as temperature, food availability, and exposure time may influence the efficacy of entomopathogenic agents (Kabaluk and Ericsson 2007b).

While the entomopathogenic fungi (EPF) have generally been effective for wireworm (larva) control (Campos-Herrera and Gutiérrez 2009; Půža and Mráček 2010; San-Blas et al. 2012), but see (Morton and Garcia-del-Pino 2017; Toba et al. 1983), *M. brunneum* has also shown efficacy against the adult stage of wireworms, especially when applied with pheromone (Kabaluk et al. 2015; Kabaluk et al. 2013). Moreover, improved effectiveness of the entomopathogenic nematodes (EPN) when they are applied in combination with EPF has also been reported in lepidopterans (Acevedo et al. 2007; Barbercheck and Kaya 1991; Schulte et al. 2009) and coleopterans (Anbesse et al. 2008; Choo et al. 2002). Fungal infections might enhance nematode efficacy by impairing avoidance behaviors of host

organisms, or by increasing the rate of host respiration, which can attract nematodes (Ansari et al. 2008). However, the underlying mechanisms of such synergistic interactions remain poorly understood. Moreover, differences in soil characteristics may explain variability in the observed efficacies of various biological control against wireworms. For instance, wireworm responses to environmental cues are known to be influenced by soil characteristics such as porosity and texture (Hermann et al. 2013; Jones and Shirck 1942; Parker and Seeney 1997; Van Herk and Vernon 2006), which could subsequently affect their movement, behavior, and related ecological interactions with natural enemies in the soil. In addition, the thick integument of the later larval instars in most wireworm species can pose a physical barrier, limiting the ability of the entomopathogenic organisms to penetrate their host. Therefore, presenting wireworms with an environment which increases the likelihood of physical damage to their integument may facilitate entomopathogenic infections. The natural dust particle, diatomaceous earth (DE), is known for its efficacy in damaging waxy cuticles of insects, which would ultimately result in mortality due to desiccation (Ebeling 1971; Kuronic 1998) (However, the effectiveness of DE in facilitating entomopathogenic infections in wireworms has yet to be determined.

Here, we evaluated efficacies of biological control against one of the most damaging wireworm species in the Pacific Northwest (Esser et al. 2015; Milosavljevic et al. 2015) and Intermountain ((Morales-Rodriguez et al. 2014; Rashed et al. 2015) regions of the United States, *Limonius californicus* (Mannerheim) (Coleoptera: Elateridae). Our objectives were to: 1) evaluate effectiveness of two commercially available biological control organisms, the EPN *S. carpocapsae* and the EPF *M. brunneum* (strain F52), in protecting wheat plants; 2) determine whether a combined EPF–EPN application offered synergistic protection against wireworms; and 3) examine whether the addition of DE improved the effectiveness of the biocontrol agents. As both the extent of wireworm damage (Rashed et al. 2017), and the efficacy of EPN (Eidt and Thurston 1995), can be influenced by soil texture, all evaluations were conducted in both sand-dominated and peatmoss-rich media.

Materials and Methods

Wireworm Collection and Study Location

The sugar beet wireworm *L. californicus* was collected in June 2017 from a heavily infested dryland wheat field near Ririe, ID, using solar bait traps as described in Rashed et al. (2015). In summary, each trap consisted of germinating wheat and barley seeds placed at approximately 15-cm depth, topped with soil, and then covered by a dark plastic. Collected wireworms were maintained individually in 5

× 5 × 10 cm (W × L × H) plexiglass containers filled with field soil and two barley seeds. Wireworms were kept in the containers until being used in the experiment within 10 d of collection. The study was carried out in the University of Idaho, Aberdeen Research and Extension Center greenhouses in Aberdeen, ID, from June to July 2017. The average daily temperature in the greenhouse was 26.8°C (SE: 0.3°C).

Soil Media and Experimental Pots

Two soil media were prepared by manipulating sand:peatmoss (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada) ratios. Both media mixes contained fixed amounts of fertilizer (Osmocote, Scott-Sierra Horticultural Products Co., Marysville, OH) and vermiculite (ThermoRock West Inc., Chandler, AZ). The sand-dominated medium consisted of 75% sand and 25% peatmoss; the peatmoss- rich medium was a 50:50 mix. Soils were homogenized prior to placement in pots.

Experimental Design and Treatments

Experiments were conducted in two 'time-blocks', one wk apart, and in two separated greenhouse chambers. There was a total of nine treatments, in each soil medium per time-block (Table 4-1). Thiamethoxam, a commonly used neonicotinoid to manage wire- worm damage, was included as a chemical control treatment. Our treatments also included a non-treated positive (wireworm, with no treatment) and a noninfested negative (no wireworm) control. The remaining treatments of *S. carpocapsae*, *M. brunneum*, and the combination of the two, with or without DE, constituted the remaining experimental treatments (Table 4-1). There were 5 pot- replicates per treatment per time-block (10 pot-replicates total per treatment), arranged in a completely randomized design. Each pot was 22.9 × 22.9 × 24.1 cm (W × L × H) and contained a single *L. californicus* larva that was placed 10 cm below the soil surface; larvae were selected from late instars for uniformity in size and averaged 15.1 (SE = 0.16) mm in length. After wireworms were added to pots, each pot was planted with four spring wheat seeds (var. UI Stone; (Chen et al. 2013)), one in each corner of the pot, at a 2.5-cm depth. All entomopathogenic mixtures were suspended in 473 ml of water and applied across the soil surface, prior to covering the dropped seeds, to simulate at-planting field applications. The 473 ml suspension volume was selected to prevent loss of natural enemies through drainage. *Metarhizium brunneum* strain F52 (Met52 EC), previously classified and incorporated as commercial product *M. anisopliae* by Novozymes Biological Inc. (Rehner and Kepler 2017), was obtained from Evergreen Growers Supply LLC., Clackamas, OR. The product was applied at the rate of 3 ml per pot (EPF), following the highest

rate that is recommended for drench application in pot. *Steinernema carpocapsae* (ARBICO Organics Co., Oro Valley, OR) was applied at the rate of 19 ml (~52,258 EPN) per pot (EPN). The application rate for DE (Perma-Guard, Bountiful, UT) treatment was 0.88 g for each pot (168 kg/ha). Similar to the entomopathogens, DE powder was first suspended in water (or EPF/EPN mix, depending on treatment) and then applied to the soil surface, prior to covering seeds. Thiamethoxam (CruiserMaxx, Syngenta, Greensboro, NC) treatment was applied as seed treatments at the recommended rate of 325 ml/100 kg.

Table 4-1 Details of treatments applied in both soil media

	Treatment	Replicate per block	*Metarhizium brunneum* (EPF)	*Steinernema carpocapsae* (EPN)	DE	Seed treatment	*Limonius californicus*
Sand-dominated	1	5	-	-	-	√	√
	2	5	√	√	√	-	√
	3	5	√	√	-	-	√
	4	5	-	√	-	-	√
	5	5	√	-	-	-	√
	6	5	√	-	√	-	√
	7	5	-	√	√	-	√
	8	5	-	-	-	-	√
	9	5	-	-	-	-	-
Peatmoss-dominated	1	5	-	-	-	√	√
	2	5	√	√	√	-	√
	3	5	√	√	-	-	√
	4	5	-	√	-	-	√
	5	5	√	-	-	-	√
	6	5	√	-	√	-	√
	7	5	-	√	√	-	√
	8	5	-	-	-	-	√
	9	5	-	-	-	-	-

Treatments involved combinations of entomopathogens (EPF and/or EPN), DE, and thiamethoxam seed treatment.

Evaluations

Emergence success, probability of plant damage, damage latency (number of days to damage), and plant biomass were recorded in each pot. Plant damage was measured as the presence of wilted or dead central leaf, the presence of point of feeding (just below the soil surface) at harvest, and/or seedling death. Above- and belowground tissues were removed five wk after planting and dried at 40°C for 96 h prior to biomass determination.

After harvest, soil media within each pot was screened twice through #4 and #14 sieves. If the wireworm was not recovered, the soil was inspected by two experimenters, on a white background, to find the wireworm. If wireworm was not found it was scored as dead; wireworm bodies decompose within 3 wk and they cannot be recovered after this point, especially where infected by the EPF (A.R. and P.E., personal observations).

Statistical Analysis

Statistical analyses were performed in R (3.4.1; R Core Team). We used generalized linear mixed models (GLMMs) to explore the fixed effects of soil media, treatment, and the treatment × soil interaction, and the random effect of time-block, on latency (to damage) and plant biomass. Where a significant treatment × soil interaction was present, results from type III Wald chi-square tests are presented. These models assumed a normal distribution of the response variables based on the distributions of the observed data (Shapiro–Wilk normality tests, $P > 0.05$ for both response variables). Total plant biomass was calculated on a per-pot basis. Similar models with the same explanatory variables were used for germination success, the probability of feeding damage, and wireworm mortality, except these models included a binomial error distribution, with a logit link, based on the distribution of the responses. 'Noninfested' (no wireworm) controls were not included in feeding damage analyses.

Results

Wireworm Mortality

Wireworm mortality was affected by treatment (X2 = 14.84, df = 7, $P = 0.038$; Fig. 1). The highest mortality was observed in EPF/ DE (55%), followed by treatments EPN/EPF (50%), EPN/EPF/DE (50%), and EPF (44.5%), all of which included the EPF. Mortality rates in thiamethoxam (30%), EPN (14.3%), and EPN/DE (20%) treatments were not significantly different from the nontreated control (15%) (Fig. 4-1). No significant effects of soil medium, and soil × treatment were detected.

Germination Success, Wireworm Damage Rate, and Latency

Germination success did not vary among treatments (X2 = 8.63, df = 8, $P = 0.37$), ranging from 85 to 98%; the highest rate of emergence occurred in the noninfested controls. No significant effects of soil medium or soil × treatment interaction were detected.

The rate of wireworm damage, however, was affected by both treatment (X2 = 16.76, df = 7, *P* = 0.019) and soil (X2 = 4.06, df = 1, *P* = 0.043), with an overall higher probability of damage associated with sand-dominated than peatmoss-dominated medium. A significant soil × treatment interaction (X2 = 15.29, df = 7, *P* = 0.032) was also detected and results for each soil medium are presented separately (Fig. 4-2).

In both soil media, the lowest rate of damage was associated with the EPN/EPF/DE treatment (Fig. 4-2 A and B). In peatmoss-dominated medium, the lowest predicted probabilities of damage were associated with EPF (0.15) and EPN/EPF/DE (0.12), but not with EPF/ DE (0.32) (Fig. 4-2A). Overall, predicted probabilities of wireworm damage were observed to be relatively lower for EPF, EPF/DE, and EPF/EPN/DE treatments compared to the thiamethoxam (0.35) and EPN/DE (0.42) treatments (Fig. 4-2A).

The predicted probability of damage in sand-dominated medium was observed to be the lowest in EPN/DE (0.15), EPN/EPF (0.22), and EPN/EPF/DE (0.12), which were significantly lower than the nontreated control (0.45), but not different from the thiamethoxam seed treatment (0.25) (Fig. 4-2 B)

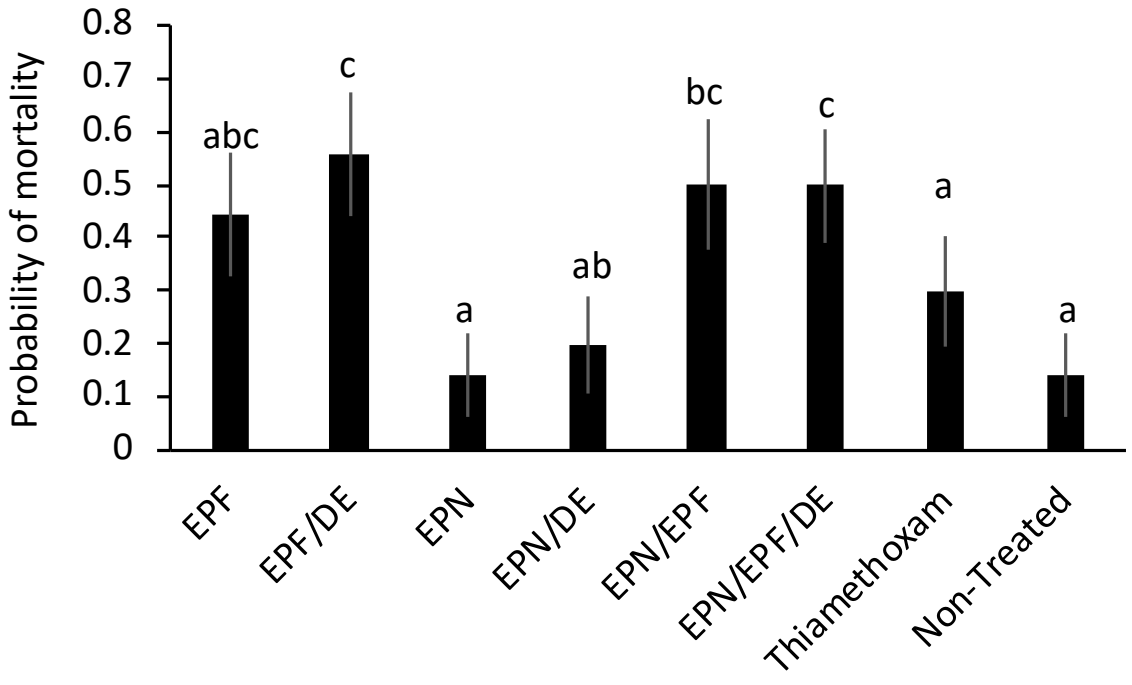

Figure 4-1 The average probability of mortality in each of the biological and chemical treatments including non-infested (no wireworm), non-treated wireworm, seed treatment (CruiserMaxx), entomopathogenic fungus (EPF), entomopathogenic nematode (EPN), and the combination of the two, applied with (EPF/EPN/DE) and without (EPF/EPN) Diatomaceous earth (DE). Error bars represent standard errors (±1)

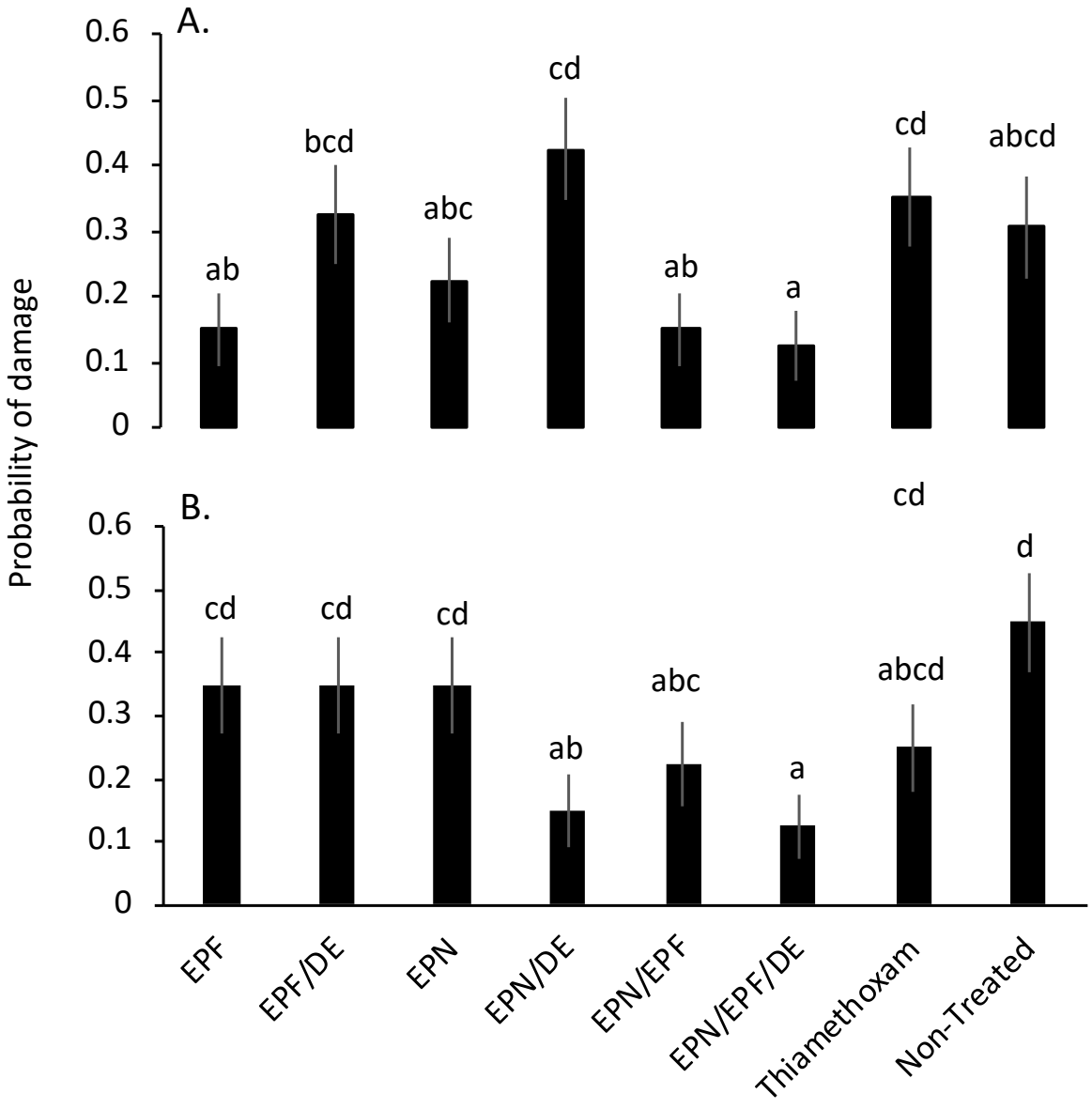

Figure 4-2 The average probability of feeding damage in each of the biological and chemical treatments including non-treated, seed treatment (thiamethoxam), entomopathogenic fungus (EPF), entomopathogenic nematode (EPN), and the combination of the two applied with (EPF/EPN/DE) and without (EPF/EPN) Diatomaceous earth (DE), in peatmoss-dominated (A) and sand-dominated (B) media. Error bars represent standard errors (±1)

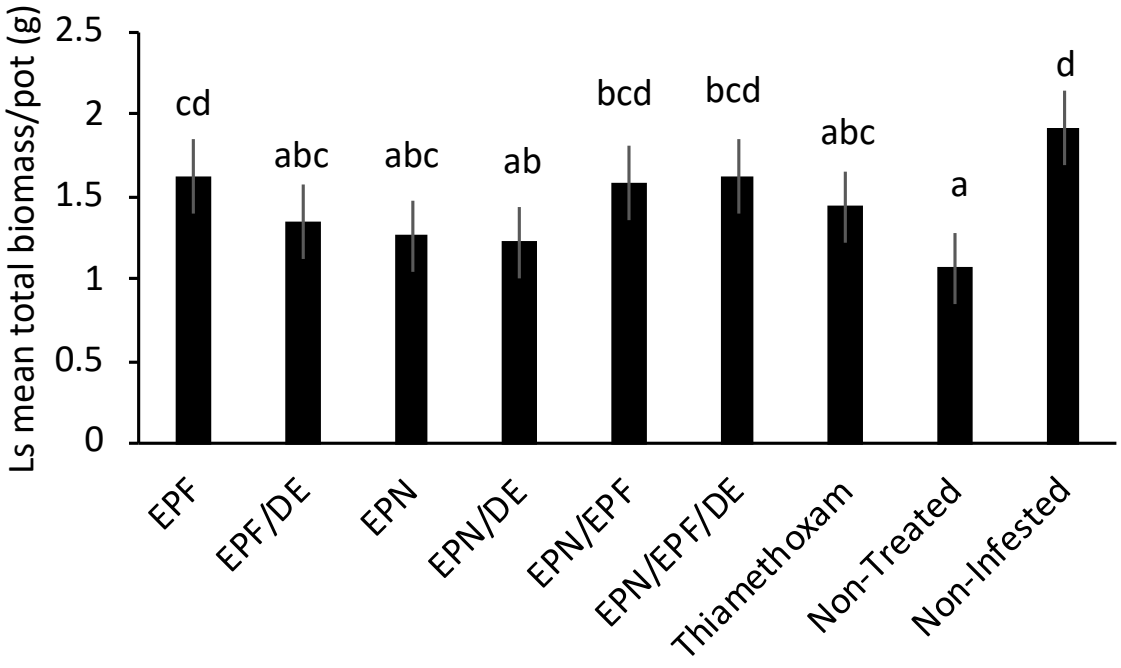

Figure 4-3 Least square means of total plant biomass, per pot, in the evaluate treatments that included non-infested (no wireworm), non-treated, seed treatment (CruiserMaxx), entomopathogenic fungus (EPF), entomopathogenic nematode (EPN), and the combination of the two, applied with (EPF/EPN/DE) and without (EPF/EPN) Diatomaceous earth (DE). Error bars represent standard errors (±1).

Time to damage, i.e., damage latency, was not affected by treatment (X2 = 11.56, df = 7, *P* = 0.12) or soil media (X2 = 0.61, df = 1, *P* = 0.43). In the peatmoss-dominated medium, the average number of days (±SE) to detect wireworm feeding damage in the emerged seedlings was 19.4 (0.87) versus the 18.5 (0.79) recorded for the sand-dominated soil. No significant soil medium × treatment interaction was detected.

Plant Biomass

Total plant biomass was affected by treatment (X2 = 22.58, df = 8, *P* = 0.004) but not by soil media (X2 = 1.27, df = 8, P = 0.26) or the interaction between the two factors (X2 = 11.26, df = 8, *P*= 0.19) (Fig. 4-3). Average plant biomasses in EPF (1.95 ± 0.19), EPN/EPF (1.90 ± 0.22), and EPN/EPF/DE (1.96 ± 0.21) were not statistically different from the noninfested (2.21 ± 0.14) pots but were significantly higher than the nontreated controls (1.24 ± 0.14). Although the observed total plant biomasses in EPF/DE (1.57 ± 0.17), EPN (1.47 ± 0.16), and EPN/DE (1.48 ± 0.16) treatments were relatively higher than that of the nontreated controls, the improvement was not significant (Fig. 4-3).

Discussion

Our results showed variability in the probability of postemergence damage between the EPF- and EPN-containing treatments, and between sand-dominated and peatmoss-dominated media. Wireworm mortality was primarily associated with EPF-containing treatments, regardless of the soil media. However, no synergistic effect on mortality was detected in EPN/EPF, since no statistical difference was present among EPF, EPF/EPN, and EPF/EPN/DE; this also indicates that no antagonism between natural enemies was observed.

While the highest rate of mortality was achieved in the EPF/DE treatment, all EPF-containing treatments resulted in relatively higher mortality rates than the nontreated, EPN, EPN/DE, and thiamethoxam treatments. This supports a study by Ansari et al. (2009), who reported *Agriotes lineatus* mortality rates between 10 and 100% following direct exposure to inoculums of 10 different strains of *M. anisopliae* in laboratory trials. Increases in plant biomass were also associated with the majority of EPF-containing treatments when compared to the nontreated wireworm controls. The observed 30% mortality in our thiamethoxam treatment, and the lack of difference from our nontreated control, is in line with previous studies that show neonicotinoids do not cause considerable wireworm mortality but rather reduce feeding damage by inducing morbidity (Van Herk et al. 2007; Vernon et al. 2008).

Soil physical characteristics are known to affect the extent of wireworm damage to crops (Milosavljević et al. 2016; Rashed et al. 2017), with greater damage often associated with sandy soil (Hermann et al. 2013; Rashed et al. 2017). Similarly, 48.6% and 33.2% of seedlings in our study were affected by wireworms in sand- dominated and peatmoss-rich media, respectively. Quick water depletion in the porous sand has been suggested to stimulate wireworm searching for moisture in succulent underground plant tissues such as potato tubers (Hermann et al. 2013). It is also possible that increased plant residue and organic matter in the peatmoss-dominated medium provided wireworms with an alternate food source (Hemerik and Fluiter 1999), which reduced feeding on the wheat seeds. However, Traugott and colleagues (2007, 2008) indicated soil organic contents only constitute a negligible portion Agriotes spp. diet, making this unlikely. Moreover, wireworm damage latency was not influenced by soil media, indicating that the presence of plant residue in peatmoss- dominated soil did not inhibit wireworm foraging.

Although no effects of soil media or treatment were detected on germination success, significant variations in postemergence feeding damage were present. In the sand-dominated medium, the combination of EPN and EPF, either with (EPN/EPF/DE) or without (EPN/ EPF) DE, and the combination

of EPN and DE (EPN/DE) significantly reduced the rate of damage to wheat seedlings compared to the nontreated wireworm controls and applications of either EPN or EPF alone. However, a synergistic interaction between EPN and EPF is unlikely to explain the effectiveness of EPN/EPF and EPN/ EPF/DE, since the EPN/DE treatment was also effective in reducing damage. Although nonsignificant, the probability of damage in sand- dominated medium was also relatively lower in EPN-treated pots than the nontreated controls (Fig. 4-2). The effectiveness of the EPN in the sand-dominated medium might be due to improved dispersal and survival in more porous sandy soils (Kaya and Gaugler 1993; Moyle and Kaya 1981). Although a previous potted field trial concluded nearly 50% A. obscurus mortality following *S. carpocapsae* application (Morton and Garcia-del-Pino 2017), in our study the average mortality rate due to *S. carpocapsae* did not exceed 20% in the absence of the EPF. The difference between these results might be explained by variation in wireworm species or by the use of primarily late instar larvae in our study; late instar wireworms are hard-bodied and less vulnerable to nematode infestation than early instars. Since no considerable mortality was detected for EPN-only treatments (EPN and EPN/DE), the overall reduced damage in nematode treatments in the sand-dominated medium might have been the result of manipulated wireworm behavior following infestation. While nematode infestation could eventually lead to wireworm mortality over time, the nonlethal effect(s) of nematodes on wireworm behavior and feeding warrants additional studies.

The EPF *M. brunneum* (strain F52) appeared to play a key role in reducing wireworm damage. In the peatmoss-dominated medium, damage rates in the presence of *M. brunneum* (EPF) and both *M. brunneum* and *S. carpocapsae*, with (EPN/EPF/DE) or without (EPN/EPF) the addition of DE, were significantly lower than pots treated with a mixture of *S. carpocapsae* and DE as well as those which were treated with thiamethoxam. Damage rates in EPF, EPN/ EPF, and EPN/EPF/DE treatments were consistently, but non-significantly, lower than the nontreated control. The improved efficacy of EPF-containing treatments in peatmoss-dominated medium can be attributed to its relatively higher moisture retention than the sand- dominated medium. This supports a previous report of increased *M. anisopliae* (= *brunneum*) incidence in soil with relatively higher organic content (Quesada-Moraga et al. 2007).

Our results indicate that soil texture and composition may mediate the effectiveness of *S. carpocapsae* and *M. brunneum* (strain F52). While *M. brunneum* was more effective in protecting plants in the peatmoss- rich soil, *S. carpocapsae* treatments were more effective in the sand- dominated medium. The addition of DE did not appear to improve the efficacy of either natural enemy. However, the

reduced damage observed in EPN/DE and EPN/EPF/DE treatments in sandy media suggests that DE might facilitate nematode infestation of wireworms or interfere with wireworm movement within the sandy soil, where water depletion hap- pens quickly, thus improving DE efficacy. As the mode of action of this product is primarily through damaging the protective cuticle of pests, elevated moisture levels can interfere with DE function (Ebeling 1971; Ebeling and Wagner 1959; Korunic 1998)

Overall, *M. brunneum* (F52) appeared the more effective natural enemy in terms of ability to reduce *L. californicus* numbers. However, soil application of both *S. carpocapsae* and *M. brunneum* (F52) showed potential in reducing *L. californicus* damage to wheat seed- lings, and in some cases were more effective than the chemical seed treatment. Our findings are consistent with those of a field study by (Reddy et al. 2014) reporting efficacies of *B. bassiana*, *M. brunneum*, and *M. robertsii*, against *L. californicus*, as comparable to imidacloprid seed treatment. The observed efficacy of the biological control organisms in reducing feeding damage was not consistent between soil media, indicating that recommendation of biological control agent must be made with knowledge of field soil type. Moreover, in this we focused on late instars of L. californicus which is one of the most difficult species of wireworm to manage in Western, Pacific Northwest, and Intermountain regions of the United States.

While application of the EPN and EPF against wireworms in potted greenhouse trials appeared promising, evaluating efficacies of EPN and EPF under field circumstances would be an essential step as differences in application dose and other environmental variables may impact outcomes. Thus, future field studies in various soil types, and with different species of wireworms, will improve our under- standing of entomopathogenic organism ecology and their overall efficacy against wireworms.

Author and Co-authors' Contribution

Pooria Ensafi: Investigation (performing the experiments, data/evidence collection); Data Curation; Writing (original draft preparation)

David W. Crowder: Formal analysis (help with statistical analysis); Writing (review and editing including critical review, pre-publication revision)

Aaron W. Esser: Writing (review and editing of manuscripts)

Juliet M. Marshall: Writing (review and editing of manuscripts)

Arash Rashed: Supervision (oversight and leadership responsibility); Funding (acquisition of financial support); Resources (provision of study materials and analysis); Writing (review and editing manuscripts).

References:

Acevedo, J. P. M., Samuels, R. I., Machado, I. R., and Dolinski, C. 2007. Interactions between isolates of the entomopathogenic fungus *Metarhizium anisopliae* and the entomopathogenic nematode *Heterorhabditis bacteriophora* JPM4 during infection of the sugar cane borer *Diatraea saccharalis* (Lepidoptera: Pyralidae). Journal of Invertebrate Pathology 96:187-192.

Anbesse, S. A., Adge, B. J., and Gebru, W. M. 2008. Laboratory screening for virulent entomopathogenic nematodes (*Heterorhabditis bacteriophora* and *Steinernema yirgalemense*) and fungi (*Metarhizium anisopliae* and *Beauveria bassiana*) and assessment of possible synergistic effects of combined use against grubs of the barley chafer *Coptognathus curtipennis*. Nematology 10:701-709.

Andrews, N., Ambrosino, M., Fisher, G., and Rondon, S. 2008. Wireworm biology and nonchemical management in potatoes in the Pacific Northwest. Oregon State University Extension Service 607:1-18.

Ansari, M., Shah, F., and Butt, T. 2008. Combined use of entomopathogenic nematodes and *Metarhizium anisopliae* as a new approach for black vine weevil, *Otiorhynchus sulcatus*, control. Entomologia Experimentalis et Applicata 129:340-347.

Barbercheck, M. E., and Kaya, H. K. 1991. Competitive interactions between entomopathogenic nematodes and *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) in soilborne larvae of *Spodoptera exigua* (Lepidoptera: Noctuidae). Environmental Entomology 20:707-712.

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

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

Chen, J., Wheeler, J., Clayton, J., Zhao, W., O'Brien, K., Zhang, J., Jackson, C., Marshall, J., Brown, B., and Campbell, K. 2013. Registration of 'UI Stone'soft white spring wheat. Journal of Plant Registrations 7:321-326.

Choo, H. Y., Kaya, H. K., Huh, J., Lee, D. W., Kim, H. H., Lee, S. M., and Choo, Y. M. 2002. Entomopathogenic nematodes (*Steinernema spp*. and *Heterorhabditis bacteriophora*) and a fungus *Beauveria brongniartii* for biological control of the white grubs, *Ectinohoplia rufipes* and *Exomala orientalis*, in Korean golf courses. BioControl 47:177-192.

Ebeling, W. 1971. Sorptive dusts for pest control. Annual Review of Entomology 16:123-158.

Ebeling, W., and Wagner, R. E. 1959. Rapid desiccation of drywood termites with inert sorptive dusts and other substances. Journal of Economic Entomology 52:190-207.

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

Esser, A. D., Milosavljević, I., and Crowder, D. W. 2015. Effects of neonicotinoids and crop rotation for managing wireworms in wheat crops. Journal of economic entomology 108:1786-1794.

Ester, A., and Huiting, H. 2007. Controlling wireworms (*Agriotes spp*.) in a potato crop with biologicals. IOBC wprs Bulletin 30:189.

Furlan, L., Bonetto, C., Costa, B., Finotto, A., and Lazzeri, L. 2009. Observations on natural mortality factors in wireworm populations and evaluation of management options. IOBC/wprs Bull 45:436-439.

Godfray, H. C. J., Blacquiere, T., Field, L. M., Hails, R. S., Petrokofsky, G., Potts, S. G., Raine, N. E., Vanbergen, A. J., and McLean, A. R. 2014. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proc. R. Soc. B 281:20140558.

Hemerik, L., and Fluiter, R. d. 1999. No preference of the wireworm, Agriotes lineatus (L.), for four grass species.

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

Jones, E., and Shirck, F. 1942. The seasonal vertical distribution of wireworms in the soil in relation to their control in the Pacific Northwest. Journal of Agricultural Research 65:125-142.

Kabaluk, J. T., and Ericsson, J. D. 2007a. Metarhizium anisopliae seed treatment increases yield of field corn when applied for wireworm control. Agronomy Journal 99:1377-1381.

Kabaluk, J. T., and Ericsson, J. D. 2007b. Environmental and behavioral constraints on the infection of wireworms by *Metarhizium anisopliae*. Environmental Entomology 36:1415-1420.

Kabaluk, J. T., Lafontaine, J., and Borden, J. H. J. J. o. p. s. 2015. An attract and kill tactic for click beetles based on *Metarhizium brunneum* and a new formulation of sex pheromone. 88:707-716.

Kabaluk, T., Janmaat, A., Sheedy, C., Goettel, M., and Noronha, C. 2013. 10 Agriotes spp. L., Wireworms and Click Beetles (Coleoptera: Elateridae). Biological Control Programmes in Canada 2001-2012:72.

Kaya, H. K., and Gaugler, R. 1993. Entomopathogenic Nematodes. Annual Review of Entomology 38:181-206.

Kölliker, U., Biasio, L., and Jossi, W. 2011. Potential control of Swiss wireworms with entomopathogenic fungi. IOBC/wprs Bull 66:517-520.

Korunic, Z. 1998. ReviewDiatomaceous earths, a group of natural insecticides. Journal of Stored Products Research 34:87-97.

Kuronic, Z. J. J. S. p. R. 1998. Diatomaceous earths, a group of natural insecticides. Review. Journal of Stored Product Research 34:87-97.

Ladurner, E., Quentin, U., Franceschini, S., Benuzzi, M., and Ehlers, R. 2009. Efficacy evaluation of the entomopathogenic fungus *Beauveria bassiana* strain ATCC 74040 against wireworms (*Agriotes spp*.) on potato. IOBC/wprs Bull 45:445-448.

Milosavljevic, I., Esser, A. D., and Crowder, D. W. 2015. Identifying wireworms in cereal crops. Washington State University Extension.

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

Morales-Rodriguez, A., O'Neill, R. P., and Wanner, K. W. 2014. A survey of wireworm (Coleoptera: Elateridae) species infesting cereal crops in Montana. The Pan-Pacific Entomologist 90:116-125.

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

Moyle, P. L., and Kaya, H. K. 1981. Dispersal and infectivity of the entomogenous nematode, Neoaplectana carpocapsae Weiser (Rhabditida: Steinernematidae), in sand. Journal of Nematology 13:295.

Parker, W., and Seeney, F. 1997. An investigation inot the use of multiple site characteristics to predict the presence and infestation level of wireworms (*Agriotes sup.*, Coleoptera: Elateridae) in individual grass fields. Annals of Applied Biology 130:409-425.

Půža, V., and Mráček, Z. 2010. Does scavenging extend the host range of entomopathogenic nematodes (Nematoda: Steinernematidae)? Journal of Invertebrate Pathology 104:1-3.

Quesada-Moraga, E., Navas-Cortés, J. A., Maranhao, E. A., Ortiz-Urquiza, A., and Santiago-Álvarez, C. 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological research 111:947-966.

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

Rashed, A., Rogers, C. W., Rashidi, M., and Marshall, J. M. 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. Arthropod-Plant Interactions 11:147-154.

Reddy, G. V., Tangtrakulwanich, K., Wu, S., Miller, J. H., Ophus, V. L., Prewett, J., and Jaronski, S. T. 2014. Evaluation of the effectiveness of entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat. Journal of Invertebrate Pathology 120:43-49.

Rehner, S. A., and Kepler, R. M. 2017. Species limits, phylogeography and reproductive mode in the *Metarhizium anisopliae* complex. Journal of Invertebrate Pathology 148:60-66.

San-Blas, E., Pembroke, B., and Gowen, S. R. 2012. Scavenging and infection of different hosts by *Steinernema carpocapsae*. Nematropica 42:123-130.

Schulte, M. J., Martin, K., Büchse, A., and Sauerborn, J. 2009. Entomopathogens (*Beauveria bassiana* and *Steinernema carpocapsae*) for biological control of bark-feeding moth Indarbela dea on field-infested litchi trees. Pest Management Science 65:105-112.

Sufyan, M., Abbasi, A., Gogi, M. D., Arshad, M., Nawaz, A., and Neuhoff, D. 2017. Efficacy of Beauveria Bassiana for the Management of Economically Important Wireworm Species (Coleoptera: Elateridae) in Organic FarmingWirksamkeit von Beauveria bassiana für das Management wirtschaftlich wichtiger Drahtwurmarten (Coleoptera: Elateridae) im ökologischen Landbau. Gesunde Pflanzen 69:197-202.

Toba, H. H., Lindegren, J. E., Turner, J. E., and Vail, P. V. 1983. Susceptibility of the Colorado potato beetle and the sugarbeet wireworm to *Steinernema feltiae* and *S. glaseri*. Journal of Nematology 15:597-601.

Traugott, M., Benefer, C. M., Blackshaw, R. P., van Herk, W. G., and Vernon, R. S. 2015. Biology, ecology, and control of elaterid beetles in agricultural land. Annual Review of Entomology 60:313-334.

Van Herk, W., and Vernon, R. 2006. Effect of temperature and soil on the control of a wireworm, *Agriotes obscurus* L.(Coleoptera: Elateridae) by flooding. Crop Protection 25:1057-1061.

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

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

Vernon, R. S., Herk, W. G. V., Clodius, M., and Harding, C. 2009. Wireworm management I: stand protection versus wireworm mortality with wheat seed treatments. Journal of Economic Entomology 102:2126-2136.

Appendix-1

OXFORD UNIVERSITY PRESS LICENSE TERMS AND CONDITIONS

Nov 28, 2018

This Agreement between University of Idaho, Aberdeen Research and Extension Center -- Pooria Ensafi ("You") and Oxford University Press ("Oxford University Press") consists of your license details and the terms and conditions provided by Oxford University Press and Copyright Clearance Center.

License Number	4477541486899
License date	Nov 28, 2018
Licensed content publisher	Oxford University Press
Licensed content publication	Journal of Economic Entomology
Licensed content title	Soil Type Mediates the Effectiveness of Biological Control Against *Limonius californicus* (Coleoptera: Elateridae)
Licensed content author	Ensafi, Pooria; Crowder, David W
Licensed content date	Jul 6, 2018
Type of Use	Thesis/Dissertation
Institution name	
Title of your work	Ph.D. Student
Publisher of your work	University of Idaho
Expected publication date	Nov 2018
Permissions cost	0.00 USD
Value added tax	0.00 USD
Total	0.00 USD
Title	Ph.D. Student
Institution name	University of Idaho
Expected presentation date	Nov 2018
Portions	I need to put a PDF copy of this article as an appendix in my Ph.D. dissertation,
Requestor Location	University of Idaho, Aberdeen Research and Extension Center 1693 S 2700 W ABERDEEN, ID 83210 United States Attn: Pooria Ensafi
Publisher Tax ID	GB125506730
Billing Type	Invoice
Billing Address	University of Idaho, Aberdeen Research and Extension Center 1693 S 2700 W