EVALUATION OF TRAP CROPS FOR THE MANAGEMENT OF WIREWORMS IN
SPRING WHEAT IN MONTANA

by

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DEDICATION

My work is dedicated to my father, mother, wife, brother and my adviser who were always there for me and supported me every day.

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ABSTRACT

Wireworms, the larval stage of click beetles, have slender, shiny, hard bodies with three pairs of legs. They are polyphagous and have cryptic habitats. In recent years, increasing wireworm numbers and damage have become a major problem for growers in the Golden Triangle Region of Montana. The control of this pest is very difficult because after the removal of Lindane by US Environmental Protection Agency (USEPA) in 2009, the use of the second generation insecticides have not been able to provide effective control of wireworms. Thus, using insecticides to control wireworms has not been effective at high population levels. Also, little is known about the biology and feeding habit of wireworms. The goal of this study is to evaluate trap crops that can protect the spring wheat by attracting wireworms. First, field trials were conducted at two locations to investigate on the use of peas *Pisum sativum* L. (Fabaceae), lentils *Lens culinaris* L. (Fabaceae), canola/rapeseed *Brassica napus* L. (Brassicaceae), corn/maize *Zea mays* L. (Poaceae), durum *Triticum durum* Desf. (Poaceae) and barley *Hordeum vulgare* L. (Poaceae) as traps intercropped with spring wheat *Triticum aestivum* L. (Poaceae). The efficacy of trap cropping was determined by damage assessment in wheat plants along 1m transects and by counting wireworm populations on wheat and trap crop rows of a plot. Secondly, the effect of intercrop spacing on plant damage and the number of wireworms was determined for pea and lentil trap crops. To support field trials potted experiments were set up in the shade house to determine wireworm distributions in two-choice assays. Collectively the results from this study showed that pea and lentil trap crops resulted in significantly lower damage in wheat plants and they attracted more numbers of wireworms compared to other crops. Results from shade house trails supported observations from the field trials. An intercropping spacing of 0.5m between pea or lentil and the spring wheat crop produced highest density of spring wheat plants. The results from these experiments will add to the basic understanding of trap cropping for wireworms control.

CHAPTER ONE

GENERAL INTRODUCTION

Wireworms, larvae of click beetles are the common pest of the grassland and are reported of being more common in no tillage farming. They are polyphagous pest and can feed on seeds, seedling, plant residues, tubers, and roots of the crops. They have of the long list of host range like wheat, corn, barley, strawberry, potatoes, and others. After the banned of the longer persistent insecticides, currently permitted insecticides (neonicotinoids) are not able to kill wireworms. Biological controls are not effective to provide control so in such scenario there is increased interest in cultural methods especially trap crops to control the wireworms population. The goals of my project is to examine the concept of trap cropping to manage wireworms and protect the spring and find out the best trap crop for spring wheat. To start with, a field trial was conducted at the two locations to investigate on the use of peas *Pisum sativum* L. (Fabaceae), lentils *Lens culinaris* L. (Fabaceae), canola/rapeseed *Brassica napus* L. (Brassicaceae), corn/maize *Zea mays* L. (Poaceae), durum *Triticum durum* Desf. (Poaceae), barley *Hordeum vulgare* L. (Poaceae) as trap crops intercropped with spring wheat *Triticum aestivum* L. (Poaceae). The efficacy of trap cropping was determined by damage percentage of wheat within a one meter wheat plant counts and wireworms populations on wheat and trap crop rows of an experimental plot.

Secondly, based on the first trial, the effect of row spacings between the trap crops (pea and lentil) and intercropped spring wheat on wireworms infestation in field

was determined. The laboratory experiments was also carried out in the indoor/shade house to assess the effect of trap crops and wheat sowing together on distribution of wireworms. The results from these experiments can contribute on the knowledge of trap cropping in spring wheat and feeding habit of wireworms. This will give a potential information on the management of wireworm and idea to deviate larvae from main/cash crop. The results from these experiments will add to the basic understanding of trap cropping for wireworm control unknown.

CHAPTER TWO

REVIEW OF WIREWORMS IN NORTH AMERICA

Wireworms in North America

Wireworm, the soil dwelling larva of click beetle (Coleoptera: Elateridae) is one of the major pest of spring and winter wheat in Montana, North Dakota, South Dakota and Minnesota (Hermann et al. 2012). Larvae are yellowish, slender, shiny, hard bodies having three pairs of legs. They can have a long period in the soil as larvae (3-7 years) before completing development to the adult beetle. Comstock and Slingerland in 1891 recognized wireworms as serious pest of US agriculture. Larvae of many species are destructive, very common in grassland, feeding in grass, forage, fruits and vegetables (Vernon et al. 2001). Wireworms are also serious pests of corns, small grains, potatoes and strawberry crops feeding on the roots and tubers (Gratwick 1989, Johnson 2002, Parker and Howard 2001, Wilkinson 1963). According to field surveys in 2011 and 2012, *Limonius californicus* (Mannerheim) and *Hyponoidus bicolor* (Eschscholtz) (Coleoptera: Elateridae) are two predominant species of wireworms causing damage to spring wheat in Western Triangle Area of Montana (Morales Rodriguez et al. 2014, Reddy et al. 2014).

Elateridae is the ninth most species rich family of Coleoptera with 10,000 identified species in 400 genera universally (Johnson 2002). In Nearctic region, nearly 1,000 species are described and in North America, Elaterids have been ranked as the seventh most species rich family with 965 species in 91 genera (Marske and Ivie 2003). Johnson in 2009 reported 921 species in 99 genera of Family Elateridae in North

America. In Montana, 168 species of click beetles in 43 genera were identified (Seibert 1993). About 100 potential species of Elaterids are reported for the Holarctic includes Europe, North Africa, Middle East, Northern Asia (North China, Japan, etc.), and North America (Vernon and van Herk 2013). In Canada, 369 different species of click beetles are reported (Bousquet 1991). Bousquet et al. (2013) lists 133 Elateridae species for Alberta, 102 for Saskatchewan, and 100 for Manitoba. Click beetles are characterized for their clicking mechanism which is due to the flexible union of the mesothorax and prothorax. If a click beetle is placed on its back, the prosternal spine fits into a groove on the mesosternum that produces a click sound and propels the beetle into the air, allowing it to right itself. The adult beetle is elongate, 15 to 30 mm long, parallel sided, having posterior corners of the pronotum prolonged backward into the spines (Triplehorn and Johnson 2005).

According to a survey done by Burghause and Schmitt (2011), *Agriotes lineatus* (Linnaeus 1767), *Agriotes obscurus* (Linnaeus 1758) and *Agriotes sputator* (Linnaeus 1758) are the common wireworm species causing crop damage in Europe. These three species are considered to be introduced and now established in the Pacific Northwest. Nowadays they are common in North America, Canada, Mongolia, Kazakhstan, Israel, Iran and Korea (Ritter and Richter 2013). *Agriotes lineatus* and *A. obscurus* were first reported in the State of Washington in 1997 (Vernon and Pats 1997). In 2005, these species were also discovered in Southern Washington (LaGasa et al. 2006) and Oregon (Andrews et al. 2008). These wireworms have been reported as major pest of corn,

potatoes, strawberries, small fruits and organic vegetables produced in Canada and Europe (LaGasa et al. 2006).

In the Pacific North Coast, *Limonius canus* (LeConte) commonly known as the Pacific coast wireworm, *L. californicus* (sugar beet wireworm), *L. infuscatus* (Motschulsky) known as the western field wireworm and *L. subauratus* (LeConte) is known as the Columbia Basin wireworm are the most common economically important species of irrigated land (Andrews et al. 2000, 2008, Mail 1932). Three major economically important wireworms species of Idaho, Washington and Oregon were the inflated wireworm, *Ludilts inflatus* (Say), the dry-land wireworm, *Ludius noxius* (Hyslop), and the wet-land wireworm, *Pheletes occidentalis* (Candeze) (Lane 1925), but after two decades these species were no longer considered important (Glen 1950).

Aeolus, *Agriotes*, *Ctenicera*, *Dalopius*, *Limonius*, and *Melanotus* are the most recognized economic important species in the Pacific Northwest (Lane 1925). *Ctenicera pruinina* (Horn) followed by *L. californicus*, *L. infuscatus* and *M. longulus oregonensis* (LeConte) were the dominant species in Oregon (Toba 1992). *Melanotus* spp. are usually found in heavy clay soil (Glen et al. 1943). According to Hasting and Cowen (1954), *Athous* species were the most dominant in Central Montana and *L. canus* in irrigated areas (Mail 1932) but not on dry land. *Ctenicera glauca* (Germar), *C. destructor* (Brown) and *A. mellilus* (Saylor) were found in wheat fields in central Montana (Morrill 1983). *Aeolus. mellilus* prefers drier soil but are also found in irrigated soil (Glen et al. 1943). *Limonius californicus*, *L. infuscatus*, *H. bicolor* and *A. mellillus* were identified as the

most common species of Montana from a three year survey since 2011 to 2013 (Morales Rodriguez et al. 2014). *Hypnoidus bicolor* is common in grassland having heavy soil (Glen et al. 1943). Table 1.1. shows the distribution of some common wireworms in North America along with list of crops they damage.

Life Cycle

Female click beetle lays creamy white to grey eggs, singly or in clusters of 100 to 300 eggs during May and June. These beetles have long life cycles of about 3 to 8 years depending on the species. Eggs are small, spherical having a diameter of 0.5 mm and are deposited near the soil surface (Parker and Howard 2001). In dry soil, the eggs can suffer desiccation that may result in egg mortality (Doane 1969). Number of eggs laid varies depending on soil moisture and grassland as it affect the egg cover for protection and food preference by female (Gough et al. 1942). It takes 4 to 6 weeks to hatch egg depending on temperature. At 24.5 °C, it takes 13 to 14 days for the eggs of *H. bicolor*, *C. aeripennis* and *C. destructor* to hatch (Doane 1966). At 15, 25 and 29°C, embryonic development of *A. ustulatus* (Schall) occurs within 45, 14 and 13 days respectively (Furlan 1996). However, *A. sordidus* (Illiger) takes 11 to 17 and 9 to 11 days for development at 25 and 29 °C respectively. An incubation period of 10 to 11 days, 9 to 15 days and 15 to 20 days were recorded by Rabb (1963) for *C. vespertinus* (Fabricius) at 30°C, 26.5°C and 24°C temperature, respectively.

Newly immersed larvae are whitish in color and around 1.5mm in length. They can grow around 25mm and turn shiny yellowish brown color (Roberts 1919). Well

grown larvae are long slender, yellow, hard bodies having three pairs of legs with biting and chewing mouthparts (Anonymous 1983). *Hypnoidus bicolor* have eyes, a single or triple-pointed nasale, caudal notch on last abdominal segment, the triangular plate between the head and the first pair of legs is partially divided and a pair of hairs is present on the upper side of the last body segment; which are the characterized features (Etzler 2013). In *A. mellilus*, caudal notch is present on last abdominal segment, have dark head and eyes with anal hooks and frons tapering to blunt point are some of the distinguishing characters (Etzler 2013). Similarly, single nasale is triple pointed, no eyespots, solid triangular segment appearing right before the first pair of legs, claw-shaped appendices on last abdominal segment form a keyhole shape and pointing away from the body are the distinguishing characters of *L. californicus* (Etzler 2013).

Larvae may pass through 8 to 14 instars depending on genera and soil conditions. Under laboratory conditions, *L. californicus* larvae have 10 to 13 instars for female and male, respectively (Stone 1941). *Limonius californicus* requires 7 to 11 years (EPPO 2005b), *M. communis* (Gyllenhal) and *M. pilosus* (Blatchley) takes 6 years (Fenton 1926) and *Agriotes* spp. spends about 2 to 5 years (have 14 larvae instars) to pass larval stage (Parker and Howard 2001). A well-developed larva of *L. californicus* and *H. bicolor* were reported to be 25-30mm and 10-15mm in length, respectively (Seal 2011). The rate of development of egg to adult beetle may differ within the same clutch of eggs (Andrews et al. 2008). The first molting depends on soil moisture and temperature, however, the second and third moltings depends only on soil temperature (Parker and Howard 2001). *Agriotes* have 8 to 11 larval instars in third year with sufficient food and moisture in

laboratory conditions, whereas under semi-natural conditions *A. obscurus* and *A. lineatus* have 13 and 12 larval instars (Sufian 2012). With molting, wireworms are supposed to increase the extent of feeding on plant materials. Food type does affect the development of the larvae. *Agriotes obscurus* larvae feeding on wheat were reported to double their weight in 25 weeks, whereas those feeding on carrot increased 10% of body weight and on potato do not gain weight (Evans and Gough 1942).

Mature larvae usually pupate during July to September (Gratwick 1989). Larvae burrow deep into the soil and hollow out small pupation cells in 5 to 30 cm below ground (Comstock et al. 1891). The pupal stage passes for about 3 to 4 weeks. Once they turn adult they can usually remain inside the cell and hibernate (Parker and Howard 2001) or come out from the cell and overwinter in soil (Gratwick 1989).

Factors Influencing Growth, Development and Movement of Wireworms

Wireworms growth, development, and movement in soil highly depend up on soil moisture and temperature (Campbell 1937). Wireworms are highly active from April to May and September to October (Parker and Howard 2001). *Hypnoidus bicolor* remains active from April to July having the sex ratio (m:f) of 1:2 (Doane 1961). In *S. aeripennis* (Kirby), males become active two to three weeks earlier than females starting from mid-April to mid-July. The soil moisture range of 9 to 12% is mostly preferred by wireworms. Wireworms are affected highly by the process of muscular inhibition so their movement increases in dry soil condition whereas reduce in moist and dry soil conditions. Exposure in saturated soil condition, larvae suffer desiccation and die (Lefko et al. 1998a,

Campbell 1937). Food is the source of moisture thus, wireworms are less active when they are nearby food source (Campbell 1937). Soil temperature of 10 to 25°C is highly preferred by wireworms (Fisher et al. 1975). The biannual vertical migration of wireworms in soil is governed by soil temperature. As the upper surface soil temperature increases during mid-summer, wireworms burrow down the soil and move downwards (McColloch and Hayes 1923). As temperature drops in fall, larvae move upward toward soil surface. With the decreasing soil temperature in late October, wireworms return to deeper soil depth for overwintering (Fisher et al. 1975). At 10 to 25 °C soil temperature, wireworms stay within the soil depth of 8-10cm but as the soil temperature rises above 25°C or fall down below 10°C, they move vertically down below 15cm in soil (Fisher et al. 1975). Older larvae are more active and can show better movement than younger ones which mainly result in aggregation which may be a reason for the patchy damage in the field condition (Doane 1977a).

Feeding, molting of larvae, cultivation practices and soil type affect wireworms movement in soil (Vernon et al. 2014). Later instar larvae of *S. aeripennis* stay within 10cm of soil regardless of temperature but as the temperature falls down (in early August), the younger larvae drive themselves deep below 15 cm in soil (King et al. 1933). Feeding behavior of wireworms is affected by the prior soil temperature and moisture (Zacharuk 1962) so while conducting any study on field collected wireworms they should be kept in handle well (van Herk and Vernon 2013).

In early summer or mid-June as the temperature of soil rises, *H. bicolor* larvae move down in the soil. They are highly sensitive to heat and desiccation than

Selatosomus destructor (Brown) which mostly co-occurs with them (King et al. 1933). In the presence of food source, wireworms are able to tolerate high temperature. *Limonius californicus* can survive in dry soil at low temperature but at high temperature they move down in soil (Zacharuk 1962). Hence, it is extremely hard to predict wireworms population by sampling as movement of wireworms is highly dependent on food source, soil moisture and temperature. Sampling for larvae should be conducted at peak activity period that is during June or August for *Hypnoidus* spp. and *Selatosomus* spp. at two week time interval (Doane 1981).

Wireworms Species Found in Field Trials

Hypnoidus bicolor

They are very common in wheat fields of Western Triangle Areas in Montana. Adults have black pronotum and reddish brown elytra. Larvae are yellowish, slender, 8-10 mm in length with eye spot and caudal notch on last abdominal segment. The triangular plate between the head and the first pair of legs is partially divided and a pair of hairs is present on the upper side of the last body segment (Etzler 2013). Adults are active during April to July with sex ratio (m: f) of 1:2 (Doane 1961). They have 2-3 years of larval stages but under adverse soil conditions can have longer larval period (King et al. 1933). The larval stage showed clumpy distribution in the field (King et al. 1933). Female becomes active earlier than male and flight starts sooner in females. They are widely distributed in Montana, Idaho, North and South Dakota, Washington, New York, New Hampshire, Minnesota, Michigan, Ohio, Nevada, and Arizona states of North

America (Morales Rodriguez et al. 2014). Similarly, they are common in Canadian provinces such as Alberta, Manitoba, Saskatchewan, British Columbia, and Yukon (Bousquet 1991, Bousquet et al. 2013, Johnson 2009). Sometimes they show the characteristics of parthenogenesis. The adult emerging from parthenogenetic eggs are all females.

Hypnoidus bicolor is polyphagous and a serious pest of cereals (Campbell et al. 1989), potato, garden crops, and sugar beets. This species is reported to co-occur with *S. aeripennis* causing higher damage to potatoes and wheat.

Aeolus mellillus

These beetles are reddish orange in color, small sized (8- 12 mm) with black patch on pronotum with black on elytra. Larvae are flat, 8 to 18mm in length, yellowish having dark head and eyes; anal hooks and horns are tapering to blunt point which are the distinguishing characters (Etzler 2013). It has been reported that this species has flexible life history as both adult and larvae can undergo overwintering (Jewett 1940). The completion of entire life cycle varies based on overwintering stages. For overwintering adult, lifecycle is completed in 53 to 105 days. Adults oviposit from May to September, eggs are hatched within 1-2 weeks, larval period of 5-10 weeks and pupate in 1- 3 weeks. In case of overwintering larvae, 276 to 365 days are required to complete the life cycle where larval period lasts for about 35- 50 weeks (Jewett 1940).

Aeolus mellillus is well known for its parthenogenetic form laying 15- 18 eggs per day over two weeks periods (Stirrett 1936). Species are well distributed in Montana, Oregon, New Hampshire, Idaho states of US and Alberta, Manitoba, Saskatchewan,

Ontario and British Colombia provinces of Canada (Morales Rodriguez et al. 2014). They are mostly active in mid-June to mid-July (Doane 1977b). Larvae of *A. mellillus* mostly occurs in irrigated land. They are highly active and predaceous in nature, attacking cereals crops, cutting off the wheat stem (Glen et al. 1943).

Limonious californicus

According to Etzler (2013), adults are black, slender, 8-12 mm long, with distinct backward pointed projections in middle of narrow tapering body. Larvae are long, cylindrical, yellowish, 25-40mm having bulbous parameres with lateral margin narrowing toward back, a solid triangular segment appears right before the first pair of legs, claw-shaped appendices on last abdominal segment form a keyhole shape and are pointed away from the body (Etzler 2013). Larval stage lasts up to 3 to 7 years passing through 10 to 13 instars. About 72 % development occur in first two years and 19% in the third year (Stone 1941). Males are active a week earlier than females. Oviposition occurs in early summer, female lays about 500 to 1400 eggs on the upper surface of soil within a week of mating. Males can survive for 31 days and females can live up to 46 days after mating. A well-developed larva start to pupate from June to October around 15cm below soil. Pupation lasts for about 3 weeks and a new adult beetle comes out. In cold conditions, adult life duration can get extended more than 7 years (Stone 1941).

Larvae are commonly known as sugar beet wireworms are very common in the Pacific North West of North America mostly on irrigated soil conditions receiving annual rainfall around 450mm. California, Idaho, Montana, Oregon, Washington in USA and Alberta, British Colombia, Manitoba, Saskatchewan in Canada have this species (Al

Dhafer 2009, Bousquet 1991, Bousquet et al. 2013, Johnson 2009, Morales Rodriguez et al. 2014). Larvae are polyphagous causing severe damage to cereals, sugar beets, potatoes, maize/corn and beans (EPPO 2005b).

Wireworms as a Crop Pest

Wireworms are polyphagous in nature having biting and chewing mouth parts (Triplehorn and Johnson 2005). Larvae are reported to be attracted by carbon dioxide gas released from the germinating seeds (Doane 1961). Wireworms are one of the major pests of spring wheat in Montana, North Dakota, South Dakota and Minnesota (Hermann et al. 2012). They were identified as agriculture pest in 19th century in USA (Comstock et al. 1891). Because of their high species diversity, long larval stage, poorly known taxonomy and life history, cryptic habitat, they are very difficult pest to deal with (Hyslop 1916). They cause serious damage to forage, fruits and vegetables (Vernon et al. 2001). Wireworms are also pest of corns, small grains, potato, sugar beets, strawberries and vegetables feeding on roots and tubers (Gratwick 1989, Parker and Howard 2001, Reddy et al. 2014) also presented in Table 1. In North America, wireworms are increasing problem causing bare spots in fields and damage range of 5-25% (Parker and Howard 2001).

In Montana, 164 species of click beetles have been identified out of which 23 species of potential economic importance (Seibert 1993). Wireworms damage to crop depends on factors like plant density, growth stages, vigor, plant species and wireworms density in soil (Anonymous 1983). Larvae mostly feed on underground parts causing

crop thinning and serious yield loss (Anonymous 1983, Miles 1942). In wireworms damaged crops/plants, the central leaf dies and other leaves show signs of wilting or yellowing causing thin crop stand resulting in bare spots in field (Vernon et al. 2009).

Cropping history of field plays a vital role in wireworms damage. Field with 1 to 5 years of fallow, crop rotation with cereals or alfalfa and in soil having high manure content; wireworms infestation is higher (Schepl and Paffrath 2005). Wheat is the mostly grown cereal crop in North America employing >15000 people in the state and wheat accounts for about 25% of agriculture revenue in Montana (Montana Wheat and Barley Committee 2005). Montana is third state in barley and wheat production with 1,000,000 acres of barley and 5,770,000 acres of wheat in 2012 that was valued at 0.25 and 1.1 billion U.S. dollars respectively (USDA National Agricultural Statistics Service, <http://www.nass.usda.gov>). Larvae feed on the roots of wheat, bore into the stem causing wilting and dying resulting in thin plants stand (Kulash et al. 1955, Simmons et al. 1998). About 12 genera of wireworms are reported as serious pest of potatoes worldwide (Jansson and Seal 1994). In USA, potato is planted in spring and harvested in fall which make them susceptible to wireworms damage. Larvae make tunnels and small holes on surface of potato causing cosmetic damage and reducing crop quality. Low wireworms population (<100,000 larvae/ha) is capable of causing economic loss in potato field (Parker and Howard 2001, Parker and Seeney 1997). Damage from wireworms facilitate slug damage and dry core symptoms. Tunnels and holes made by wireworms provide access for nematodes, slugs, millipedes and grubs to cause further damage. Moreover, it was found that dry core symptoms was two times higher in wireworms damaged potatoes

(Keiser 2007). Wireworms are serious pest of corns, the third important agriculture crop worldwide (FAO 2005) causing up to 35% crop damage (Apablaza et al. 1977). In small grains, wireworms damage can reach 65% on standing crops which may require replanting of crops (Smith et al. 1981). Larvae attack beans, carrots, tomatoes, *Brassicas* in seedling and later stages (Miles 1942). Spring and winter wheat is more preferred over barley and rye (Glogoza 2001). Smaller wireworms (<5 mm length) are not considered capable of causing significant crop damage, however, as the size increases (>15mm), they are more capable to cause destructive crop damage (Parker and Howard 2001).

Sampling of Wireworms

Wireworms are very difficult to sample because of their ambiguous habitat. In every 2-3 years, there is one generation of wireworms (Parker and Howard 2001). They may go up to 1.5m deep in soil making very difficult to predict its population (Andrews et al. 2008). Sampling of wireworms depend on objectives like survey, species monitoring, population estimate, threshold level monitoring, and spatial and temporal distribution.

It is important to have reliable estimate of the number of wireworms in any field to know degree of crop damage and how well the controlling techniques are relatively effective. Different sampling techniques can be used for this purpose. Taking soil core sample is absolute sampling of wireworms with major objectives of estimating temporal and spatial larval distribution and population size. King (1928) sampled 0.1 square foot of soil for estimation of larvae population. Hawkins (1934) used 1 square foot surface

area having the depth of 6 to 8 inches to estimate population size. The larval population size was determined by excavating two feet square and depth of two feet from two spots in a plot (Lacroix 1935). The comparative study on sampling techniques of wireworms showed that one square foot unit gives more precise estimate of wireworms population than smaller units of 1/4 and 1/16 square foot and at least 50 samples are need to estimate the low population level whereas about 25 samples are sufficient for estimating the intermediate and higher level of larval population in field (Jones 1937).

Use of traps or baits in soil give an estimate of wireworms population in a relative plot or field is one of the relative sampling techniques that is being used recently. All these baiting sampling use crop seeds that is based on the principle that germinating seeds release carbon dioxide to which wireworms are attracted (Doane 1961, Doane et al. 1975). Simmons et al. (1998) compared seven baiting sampling techniques to determine field wireworms population and found that corn-wheat baits gave the highest precision and accuracy in intensive sampling and extensive sampling program. It is also a cost effective method of sampling wireworms in soil. Cereal trap crops are reported to be more reliable in detecting wireworms than soil core sample (Parker 1996). Baits having cereals seeds (barley and wheat), corns and sorghum have been more effective to trap wireworms (Furlan et al. 2010). Wireworms can detect the germinating seed or respiring crops (CO₂ source) from 20cm in greenhouse condition (Doane et al. 1975). Black plastics were used to cover baits on soil surface which increases the soil temperature resulting in rapid the seed germination process resulting in greater number of larvae per

baits (Ward and Keaster 1977). It also increased the cost and time of sampling that may not be affordable in some cases.

Management Practices

The main reason for using different management practices is to keep the insect pest population below economic injury level. There is always a certain threshold level set for a particular insect which is a base line whether or not to employ control practices. Different threshold levels have been established for the wireworms based on practitioner experience (Pedigo et al. 1986). According to Rice and Simmons (1999), one larvae per bait found at interval of a week has potential of causing damage to crop. In case of potatoes, 0.4 larvae per square foot of soil (screen method) is established as economic threshold level (Cranshaw 2002). This estimate is approximately 17,000 to 20,000 larvae per acres. Capturing wireworms by baits is affected by soil moisture and temperature, baiting seeds, temporal and spatial use of baits (Parker 1996).

Wireworms management practices can be categorized into three different heading:

- A. Chemical control method
- B. Biological control method
- C. Non-chemical or Cultural control method

Chemical Control Method

The use of chemical insecticides to control wireworms population. In past, organochlorine and organophosphate insecticides were used to control wireworms

(Vernon et al. 2001). Cyclodiene insecticides since 1960 to 80s, gave effective control over wireworms, however, because of their long persistence characteristics in soil they were banned. Since then, Organophosphate (broad spectrum insecticides) were proven to keep larvae under control and reduce damage (Horne and Horne 1991).

Organophosphates, pyrethroids, neonicotinoids have been used to control larvae in corn and potato (Kuhar et al. 2003). However, the results obtained from various organophosphate insecticides are inconsistent and contradictory due to variation in wireworms distribution, soil type and application methods. Type of insecticides use and their effectiveness varies with soil type, climatic conditions and wireworms pressure (Munson et al. 1986). Newer insecticides like neonicotinoids and fipronil are been tested but only moderate control on wireworms have been reported (Kuhar et al. 2003).

Lindane (Benzene hexachloride insecticide) seed treatment (Lange et al. 1949) was able to keep wireworms population low in past 30-40 years. These insecticides were so effective to control wireworms population below economic injury level which reduced the number of research in wireworms between 1970 to 2000 (Vernon et al. 2009, Vernon and van Herk 2013). Due to the long lasting nature in soil and toxicity to human, non-target species and environment; Lindane (main weapon) was limited in 2002 and banned in 2009 by Enviromental Protection Agency. Imidacloprid, thiamethoxam and clothianidin are currently available neonicotinoids seed treating chemicals to control wireworms. van Herk and Vernon (2007) reported that wireworms feeding on neonicotinoid treated seeds/seedling get toxicated and retreat but later on recover resulting in increased population and resistance against the insecticides and repeated

attack on crops. However, they are able to reduce the damage in wheat by inducing morbidity for short time about one to two after application (Vernon et al. 2009).

Insecticides are used in liquid or granular forms, fumigants and mostly as seed treatment. Chemical application as pre-plant treatment or in furrow has been proven to give most effective control (Toba 1987). Carbofuran, has been effective in in-furrow and ethoprop and terbufos were effective in row band application (Munson et al. 1986).

If other integrated pest management (IPM) tools are not able to keep the larvae down then only we should use chemicals. Since chemicals have negative effect on human health, soil environment and natural pollinators there is increasing demands of alternative methods for the control and management of wireworms (Vernon et al. 2009).

Biological Control Method

The use of biological agents like predators, parasitoids, pathogens to suppress wireworms population fall under the biological control methods. They do not provide immediate control but once the agents are established, it is inexpensive and provide long term management of wireworms keeping them below threshold level. There are more than 700 species of entomopathogenic fungi (Robert et al. 1991) which are natural enemies of arthropods (Coleoptera, Diptera, Hymenoptera, Hemiptera, and Lepidoptera). Two species of fungi *Beauveria* and *Metarhizium* were able to reduce wireworm damage in the field (Jansson and Seal 1994). However, due to the cryptic habit and soil dwelling nature of wireworms, biological control can be difficult (Arnold 1981). Spores of pathogenic fungi grow directly on the inner body of insects (wireworms) as they come in contact with cuticle of larvae (Bateman et al. 1996). It may take 5 days to kill insect and

after dying the larvae serve as the source for secondary spread of fungus spores. Spores or conidia can long live in soil than on plant surface. Fungal effectiveness is highly dependent on temperature and relative humidity. Optimal temperature range of 20-30°C and high relative humidity above 90% for 8-10 hours/day is required for fungal infection (McCoy et al. 1988).

Fungi have been studied several times to explore their potential to control insect pest (wireworms) (Kabaluk et al. 2001, Kabaluk and Ericsson 2007). *Metarhizium brunneum* F52, (Ascomycota: Hypocreales), *Beauveria bassiana* (Hypocreales: Clavicipitaceae) and entomopathogenic nematodes (family: Steinernematidae and Heterorhabditidae) have proven their effectiveness against soil borne pests (Ansari et al. 2009). Entomo-pathogenic fungi have been successful to control wireworms in Canada (Kabaluk et al. 2001), Italy, UK (Parker and Howard 2001) and Switzerland (Keller and Schweizer 2001). In corn fields of Canada, *M. brunneum* has been identified to be pathogenic on wireworms (Ericsson et al. 2007). V1002 and LRC181A strains of *M. brunneum* have been successful to cause 90-100% mortality of *A. lineatus* under laboratory condition whereas *B. bassiana* and *Paecilomyces fumosoroseus* (Wize) had no effect on *A. lineatus*. Fungus *Zoophthora elateridiphaga* (Kenneth) (Entomophthorales: Zygomycetes) has been successful to cause 75 – 100% mortality of *A. sputator* in grassland ecosystem in Switzerland (Keller 1994). *Metarhizium brunneum* produces toxin that paralysis the host and eventually kill it within two weeks. Seed treated with *M. brunneum* has proven its efficacy to increase yield by reducing the wireworms damage in potatoes and corn (Tharp et al. 2007). Researchers have used these fungi in combination

with the insecticides to increase the efficacy to control wireworms and reduce insecticidal resistance. High mortality of *A. lineatus* and *A. obscurus* was noticed in Canada with combined application of *M. brunneum* and Spinosad (Erisson et al. 2007). A field study conducted in Europe presented that commercial strain of *B. bassiana* was able to reduce wireworm population compared to untreated check plot (Ladurner et al. 2008). Similarly, *B. bassiana* under laboratory conditions showed a significant mortality in high number of wireworms (50%) compared to low number of wireworms (17%) and untreated boxes (13%) respectively (Sufian 2012). Although nematodes and bacteria are been used as biological agent to reduce soil borne pests, yet they are not been successful to control wireworms (Toba and Turner 1983). *Steinernema carpocapsae* (Weiser) has shown some promising result to control wireworms when used with resistant varieties or some insecticides (Schalk et al. 1993). Reddy et al. (2014) reported significant differences among the application methods of entomopathogenic fungi rather than the species of fungi. *M. brunneum* F52, *B. bassiana* GHA and *M. robertsii* DWR 346 applied as formulated granules or soil drenches, resulted in high wheat plant counts and yields than the fungus-coated seed treatments or the untreated control. Imidacloprid seed treatment showed the similar result as these two fungi.

Cultural Control Method

Cultural control methods refer to those cultivation or seeding techniques which modify the soil environment and hinder the wireworms population to increase. Portable trench barrier was useful to avoid adult *A. obscurus* entering field (Vernon and van Herk 2013). In Florida, black light traps was effective in concentrating adult *M. communis* in

sugarcane field (Cherry and Hall 1986) but could not collect *C. vespertinus* in North Carolina (Baumler 2008). These practices are mediated through crop which affect the pest reducing their damage and pressure in the field conditions. Crop rotation, soil drying, flooding, early or late planting, ploughing/cultivation, using resistant cultivars, trap cropping are some of the common practices employed to control wireworm.

Cultivation. Cultivation causes larvae and pupa expose to predators and pathogens, low moisture and high temperature resulting in reduction of large percentage of wireworms density (Salt and Hollick 1949). Cultivation of field in which crops are grown for more than a year is highly effective to reduce wireworms numbers and the rate of reduction is directly proportional to number of wireworms in field (Anonymous 1948). In moderately infested field (100,000/acre), cultivation over two years have been proven to reduce 37% of initial population and similarly, reduction of 87% in initial population of 1.8 million/acre respectively (Anonymous 1948). Wireworms pupate during July and August at the depth of 15 to 30 cm. Ploughing field at 23 cm depth was able to kill 90% of pupa (Andrews et al. 2008). Three times ploughing in summer reduced larvae significantly in ploughed plots compared to un-ploughed check from 1.75 to 0.2 per bait (Seal et al. 1922). Due to relatively high moisture content and low temperature of clay soil, ploughing seems to be promising in sandy soil than clay. Hot and dry soil condition drive larvae to form pupa deeper in soil, thus ploughing such soil may not be effective to control wireworms (Shirck 1945). Long term cultivation has been effective to reduce wireworms pressure in Czechoslovakia, Central Bohemia and Florida (Jansson and Lecrone 1991). After five years of cultivation, click beetle population was noticeably

reduced to 90% (Dirlbek et al. 1973). Fields in Catalonia (Spain) those had extensive tillage, needed none of the insecticidal treatment to control wireworm (Pique et al. 1998). During early spring when wireworms are most active and concentrated on upper soil surface, cultivation is the highly successful to control wireworms (Parker and Howard 2001).

Soil Drying and Flooding. Soil moisture manipulation is one of the non-chemical tools to control wireworms. *Limonius californicus* and *L. canus* prefer irrigated soil, *H. bicolor* favors heavy soil and *C. pruinina* likes dry soil condition. In sandy loam soil, drying of soil surface up to 40 cm thoroughly for few weeks during summer facilitated in death of *L. californicus* eggs and larvae (Landis and Onsager 1966). *Limonius canus* and *L. californicus* do not survive well in dry soil (moisture < 8%) (Landis and Onsager 1966) and even saturated soil resulted in death of *L. californicus* (Shirck 1945). Flooding is mostly practiced in areas having nearby water source. It not only kills larvae and pupa but also prevents adult female beetles from laying eggs. Flooding at higher temperature during summer is more appropriate to reduce the wireworms population (van Herk and Vernon 2006). Flooding soil at 30°C killed all *L. californicus* and *L. canus* within 4 days but as the temperature is lowered to 10-15°C, mortality percentage reduces to 26 % (Lane and Jones 1936). According to van Herk and Vernon (2006), flooding for 55 days at 5 to 10°C killed 90% larvae. Flooding and drying alternatingly in Florida at 4-2-4 weekly cycle provided complete reduction of *Cryptocephalus falli* (Lane) and *M. communis* (Genung 1970).

Crop Rotation. Wireworms population is highly affected by cropping history and crop rotation. Crop rotation to alfalfa, buckwheat, lettuce, and sunflowers reduced wireworms population dominated by *L. canus* and *L. californicus* (Gibson et al. 1958). In Idaho, continuous alfalfa for 4 years has consistently reduced the wireworms population in heavily infested field (6-10 larvae/sq ft) whereas rotation to red cover and continuous vegetable showed dramatic increase in population (Shirck 1945). Rotation with one winter cereal has successfully reduced the wireworms compared with two winter cereals rotation cycle (Schepl and Paffrath 2005). Mustard and cabbage are rich in glucosinolate and rotation to these crops have provided effective control over soil borne pest (Lichtenstein et al. 1964). Incorporation of *Brassica* meal in potato potted experiment resulted in reduced wireworms damage (Furlan 2007). In highly infested field, pea, beans and linseed crop can be planted as they are tolerant to wireworm damage (Anonymous 1948). Robertson (1993) reported that sorghum, mung beans, soybeans, if grown in preceding season increase the infestation of wireworms damage whereas winter wheat resulted in lower infestation. Few weeks delay in spring planting of susceptible crops helped to escape from active feeding period of larvae. Corn planted in May were found to be less damaged then planted in April (Bryson 1930). In Florida, wireworms damage have been influenced by the management of preceding summer cover crops (Jansson and Lecrone 1991).

Resistant Cultivars. Host plant manipulation is one of the important tools of IPM. Several resistant cultivars have been developed that have reduced wireworms incidence and damage. Rawlins (1943) observed that potato cultivars Warba, Heavyweight and

Rural Russet showed less tuber damage (35 to 40%) compared to damage in Burbank Russet, Irish Cobbler and Bliss Triumph (50 to 55%) in New York. Out of 50 potato cultivars tested for wireworms resistant, Anco and Irish Cobbler were the only two potato cultivars which showed less than 16% tuber damage (Kwon et al. 1999). Slight difference in tuber damage by wireworms was reported in seven commonly grown potato cultivars in UK (Parker and Howard 2000). The concentration of total glycol-alkaloid and reducing sugar respectively are positively and negatively correlated with the wireworms resistant (Johnson et al. 2008). Somatic potato hybrid cultivars are reported to have same or better resistant level as compared to insecticidal control (Novy et al. 2006).

Trap Cropping. Agriculture Canada in collaboration with van Herk et al. (2008) have clearly shown repellence mechanism can be an effective measure of crop protection rather than by mortality of wireworms. Trap cropping has been one of the best way of controlling pest in absence of insecticidal use. It is an environmental friendly approach with no risk of pesticide resistance in which trap crops are planted either in row intercropping or along border to protect the main crop from the certain pest. Trap crops attract insects or other organisms to protect main or target crop (Hokkanen 1991). Shelton and Badenes-Perez (2006) defined trap crop as plant stands that are, per se or vis manipulation, deployed to attract, divert, intercept, or retain targeted insect or pathogens they vector in order to reduce damage to main crop. Trap crops can serves as sink in which pest offspring cannot survive and thus, preventing pest movement from trap crops to main crop (Shelton and Badenes-Perez 2006). Trap cropping focuses on plant and pest interaction that is habitat manipulation for biological control of pest (Landis et al. 2000).

Insecticides and biological control can be supplemented to increase the effectiveness of trap crops. Plant breeders have developed many cultivars that are able to attract insect pest and natural enemies, and low larval survival (Cook et al. 2002). Only 10% area can be utilized for trap crop to protect the main crop (Hokkanen 1991). About forty successful cases of trap crops are described by Hokkanen (1991). Trap crops have shown its effectiveness in trapping and assembling wireworms in numbers of crops such as strawberry (Miles and Petherbridge 1927, Vernon et al. 2001) and sugar beets (Petherbridge 1938). Larvae are found to be attracted toward CO₂ within distance of 15 to 20 cm (Doane et al. 1975). In Canada, it has been reported that wireworms are found to be attracted to and cause higher damage to alfalfa than mustard, barley and buckwheat (Mackenzie and Hammermeister 2010). The germinating cereals like wheat and oat sown in between rows of strawberry attract wireworms (Miles and Petherbridge 1927). The mortality of strawberry reduced by three times when wheat was intercropped during autumn (Petherbridge 1938). Wheat corn bait traps set up in sweat potato fields were helpful to reduce tuber damage from wireworms (Seal et al. 1992). Planting wheat rows one week prior to strawberry plantation as trap crop reduced the mortality of strawberry by reducing wireworms feeding on strawberry roots and has proven to an inexpensive pest management method (Vernon et al. 2000). Peas were more attractive to wireworms than potatoes, wheat and oilseed crops, and was recommended as an effective trap crop for potato in Austria (Landl and Glauningner 2013). In potato row which was 0.75 m away from the pea row, 2.4 times more numbers of wireworms were caught then in potato row that is 1.5m away from the pea row (Landl and Glauningner 2013). This suggests

wireworms are able to cover distance of 0.75m under the field condition. Crop preferences at closed row spacing by wireworms depend on specific plant chemical (Horton 2007). Wireworms used the volatile chemicals to locate the host plant. In a bioassay, they were reported to be attracted towards synthetic blend of four chemicals (hexanal, (*E*)-hex-2-enal, (*E*)-non-2-enal and (*E,Z*)-nona-2,6-dienal) produced by barley roots (Barsics et al. 2016). Carrot and beans are successful as attractive crops in organic potato field reducing the infestation in tubers (Schepl and Paffrath 2003).

Table 1.1. Distribution of wireworms and crops affected by them in North America.

Species	Distribution	Crop affected	Citations
<i>Limonius californicus</i>	United States: CA, ID, MT, OR, WA. Canada: AB, BC, MB, SK	Cereals, corn, potato, sugar beet, onion, beans, field-grown vegetable	1,2,3,4,5,11,15,16,20,22,23
<i>L. canus</i>	United States: CA, ID, MT, OR, WA. Canada: AB, BC, MB, SK	Cereals, potato, beans, corn, field-grown vegetable	1,2,3,4,5,11,16,20,22,23
<i>L. infuscatus</i>	United States: CA, FL, IA, ID, IL, IN, MA, MT, MO, NE, NH, OR, PA, RI, SD, UT, WA Canada: AB, BC	Cereals, potato, field crops	1,2,4,5,11,16,20,22,23
<i>Hypnoidus bicolor</i>	United States: ID, MN, MT, OR, WA Canada: AB, AK, BC, LB, MB, NB, NS, NT, ON, PQ, SK, YK	Cereals, potato, most field and garden crops	2,4,10,15,20
<i>Aeolus mellillus</i>	United States: CT, ID, MT, NH, OR, PA, RI, WA Canada: AB, BC, MB, NT, ON, PQ, QC, SK	Cereals, corn, Brassicaceae, potato	4,7,8,15,20
<i>Agriotes lineatus</i>	United States: OR, WA, Canada: BC, NF, NS, PE	Cereals, potato, carrot, beans, garden vegetables	2,4,5,13,14,17,18,20
<i>A. obscurus</i>	United States: OR, WA Canada: BC, NF, NS, PE	cereals, carrot, potato, vegetables, beans	2,4,5,6,14,17,19,18,20

Table 1.1. Continued

<i>A. mancus</i>	United States: ME, MI, NJ, NY, PA Canada: AB, LB, MB, NB, NS, ON, PE, SK	cereals, carrot, potato, vegetables, beans	4,5,6,13,20
<i>Melanotus communis</i>	United States: AL, AR, AZ, CO, FL, GA, IA, IL, IN, LA, MO, NC, NE, NY, OH, PA, SC, SD, TX, VA Canada: ON, PQ	cereals, potato, beans, carrot, garden vegetables	4,5,6,9,12,19,20,21
<i>Selatosomus aeripennis</i>	United States: in all states Canada: AK, BC, MB, NT, SK, YK	Cereals, corn, sugar beet, field-grown vegetables, ornamental plants	10,15,20
<i>S. destructor</i>	United States: in all states Canada: AB, AK, BC, MB, NT, SK, YK	Cereals, corn, potato, sugar beet, field-grown vegetables, ornamental plants	10,15,20

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CHAPTER THREE

EVALUATION OF TRAP CROPS FOR THE MANAGEMENT OF WIREWORM IN SPRING WHEAT IN MONTANA

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Ashish Adhikari

Contributions: Conducted field trials and bioassays, design study, the data collection and analysis. Wrote the manuscript.

Co-Author: Gadi VP Reddy

Contributions: Provided supervision and feedback in the study design and comments to the manuscript.

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Abstract

The polyphagous larvae of click beetles (Elateridae), which undergo a long larval period in the soil, are major pests of spring wheat in Montana, USA. Presently available insecticides are unable to provide control of wireworms populations and the use of natural enemies has not been successful under field conditions. In this study, we examined the effect of seven trap crops, pea, lentil, canola, corn, durum, barley and wheat, for their attractiveness to wireworms compared to control spring wheat. Experimental plots were located in two commercial grain fields near Valier and Ledger, Montana, USA, from May to August in 2015 and 2016. Wheat damaged by wireworms was recorded and their relative locations in wheat rows and adjacent trap crops rows within a plot was determined using soil samples. In 2016, variable row spacing (0.25, 0.5, 0.75 and 1 m) between the trap crops (pea and lentil) and wheat was assessed. Shade house bioassays were conducted using potted pea, lentil, and wheat plants to support field trials results. *Limonius californicus* larvae, released at the center of each pot were sampled on 4 and 10 days after sowing. Wheat intercropped with pea and lentil had significantly fewer damaged plants and fewer wireworms compared to the control, for both locations and years. Shade house results corresponded with field results, more wireworms were collected near pea and lentil regions compared to wheat in potted bioassays. In the spacing trials, wheat plant counts were also significantly higher when paired with pea and lentil, particularly at the 0.5 m spacing. However, consistent trends associated with inter row spacing and the number of damaged wheat plants was not

noted. Regardless of inter-row spacing, significantly fewer wireworms were associated with wheat when intercropped with pea and lentil trap crops.

Key words: wireworm, *Limonius californicus*, *Hypnoidus bicolor*, intercropping, spacing, crop damage

Introduction

Wireworms, the soil dwelling larvae of various species of click beetles (Elateridae) are one of the major pest groups attacking spring wheat in Montana, North Dakota, South Dakota, and Minnesota (Hermann et al. 2012). Wheat is the dominant cereal crop grown in North America. Montana is the third state for wheat and barley production in the US with 5,265,000 harvested acres of wheat and 850,000 harvested acres of barley in 2015, valued at 0.25 and 0.93 billion U.S. dollars respectively (2015 State Agriculture Overview). Wireworms populations are found in a wide range of habitats including grasslands, cultivated land, and forests (Trogott et al. 2015). In soil, larvae move in search of food and feed on roots, seeds, seedlings of various plants, decaying organic matter, insects (Vernon et al. 2009). Larval feeding can facilitate secondary damage to crops from pathogens and snails (Keiser et al. 2012). Because of high species diversity, long larval lifespan in the soil, poorly known taxonomy and life history, and cryptic habitats of wireworms, they are difficult to control (Trogott et al. 2015) and often result in stand thinning, poor growth, cosmetic damage, and reduced yield (Barsics et al. 2013, Parker and Howard 2001).

Johnson (2009) reported 921 species of click beetles in 99 genera in North America. In Montana, 164 species in 43 genera were identified, among these 23 wireworms species were of potential economic importance (Seibert 1993). According to field surveys in 2011 and 2012, *Limonius californicus* (Mannerheim) and *Hyponoidus bicolor* (Eschscholtz) are the most important wireworms damaging spring wheat in the Western Triangle Region of Montana (Morales-Rodriguez et al. 2014).

Wireworms can live long and show biannual vertical movement in soil. In early May, temperature is around 13-15°C, wireworms are within 10cm soil depth from the surface. As the temperature reaches 22-25 °C during June and July, the larvae move vertically down and again in August to mid-October come towards the surface to feed near surface (Lafrance 1968) which make difficult to sample them or predict changes in their density. Poor species-specific knowledge due to difficulty in identifying larvae to species is one factor that impedes the development of IPM tactics for wireworms. Highly persistent organochlorine and organophosphates, formerly available for use, provided effective control of wireworms (Vernon et al. 2013) when used as a seed treatment (Lange et al. 1949). Consequently, during this historical period, very little research was conducted on wireworm biology and species diversity. Currently, neonicotinoid seed treatments are the most common management approach, but provide only partial control of wireworms (Barsics et al. 2013). Van Herk and Vernon (2007) reported wireworms feeding on neonicotinoid-treated seeds/seedlings get intoxicated but often survive, the time of recovery depends on concentration of insecticides and wireworms weight, however, increasing both population and resistance against the insecticides.

As an alternative to chemical, consideration has been given to the use of entomopathogenic fungi for control wireworms in Canada (Kabaluk et al. 2001), Italy, the UK (Parker and Howard 2001) and Switzerland (Keller and Schweizer 2001). The principal species examined have been *Metarhizium brunneum* F52 (formerly *M. anisopliae* F52), (Ascomycota: Hypocreales) and *Beauveria bassiana* (Hypocreales: Clavicipitaceae). However, various factors such as changes in relative humidity and temperature, high cost

of production, and the need for high application rates have hindered the practical use of these biological agents to control wireworms as compared to insecticides (Bourassa et al. 2001, Jaronski 2010).

Wireworms populations are becoming more common with the wider use of no-till farming practices and disappearance of residues of older pesticides from wheat land soils (Jedlicka and Frouz 2007). Currently, neither chemicals nor biocontrol agents (fungi, nematodes) provide effective control of wireworms at high population densities. As a consequence, there is interest in other control measures, including the use of trap crops. Trap crops are proposed as a better method to control wireworms (Staudacher et al. 2013). Trap crop is defined as plant stands that are, per se or vis manipulation, deployed to attract, divert, intercept, or retain targeted insect or pathogens they vector in order to reduce damage to main crop (Shelton and Badenes-Perez 2006). Trap crops have been shown to be effective in trapping and concentrating wireworms in a number of crops such as strawberry (Miles and Petherbridge 1927, Vernon et al. 2001) and sugar beets (Petherbridge 1938). The mortality of strawberry plants was reduced to about 30% of that in monocultures (strawberry only) when wheat was intercropped in strawberry fields in autumn (Petherbridge 1938). Wheats and corns planted as trap crops in sweet potato fields was found to reduce tuber damage from wireworms (Seal et al. 1992). Peas were more attractive to wireworms than potatoes, wheat, or oilseed crops, and were recommended as a potential trap crop for potato in Austria (Landl and Glauning 2011). Carrot and beans were also successful as trap crops in organic potato fields reducing wireworm infestation in tubers (Schepl and Paffrath 2003). Crop preferences of

wireworms at close row spacing depends on the specific plant chemicals (Horton 2007) used by wireworms to locate their host plants.

There have been no studies of trap crops to control wireworms in spring wheat. Based on previous findings, we chose to study pea, lentil, canola, durum, barley, and corn intercropped with spring wheat. The objectives of this study were to (1) examine the concept of trap crops to control wireworms in spring wheat, and find the best trap crop, (2) examine the effect of trap crops and row spacing on wireworms infected in spring wheat, and (3) determine the effects of trap crops on distribution of wireworms.

Materials and Methods

Trials Sites

The field trials were conducted at two sites: Ledger (48.2583° N, 111.8257° W) and Valier (48.3078° N, 112.2498° W) in the Golden Triangle region of Montana, from May to August in both 2015 and 2016. Both fields have a history of wireworm infestation. Soil at the Valier site is a sandy loam while that of Ledger is silt and clay, rich in humus. Both fields have central pivot system for irrigation and spring wheat is the main crop grown in both.

Effectiveness of Trap Crops in Field Trials

Experimental Design.

The study area measuring 40×12.6 m was established and divided into 42 experimental units each measuring 1.2×4.8 m. A complete randomized block design

with seven treatments and six replications was used. The blocks were separated by 1 m buffers and the two plots within each block were separated by 0.45 m. Each experimental plot had four rows spaced 0.3 m apart. The main crop (Duclair spring wheat) was planted in the first and third rows and the trap crop was planted in the second and fourth rows. The six trap crops treatments were Montech pea (T1), Hyeless 955 canola (T2), sweet corn (T3), Montrail durum (T4), Metealfa barley (T5) and Green Land lentil (T6). All these seeds are commercially available in market. Four rows of Duclair wheat served as a no-choice control (T0). The plots were maintained under minimum tillage and planted with a four-row experimental drill (Fabro in Swiftcurrent, Saskatchewan). Seeding rates were 22, 26, 26 and 24 seeds per 0.3 m for wheat, lentil, barley, and durum, respectively. Similarly, 7 and 53 seeds/m were planted for corn and pea, respectively. There was no irrigation at the time of sowing. The herbicide ammonium sulfate (AMS) was broadcast at the time of planting at 2.24 kg /ha as were fertilizers at an N, P and K ratio of 224.2, 0, and 22.4 kg /ha.

Sampling for Plant Damage and Wireworm Density.

To determine the level of crop damage from wireworms, the number of plants or seedlings in each plot was counted along a randomly selected 1 m line intercept (Canfield 1941; Jonasson 1988). From two rows of spring wheat in an experimental plot, three 1 m wheat plant counts were collected. Within this 1m, wheat plants were categorized as damaged or not damaged. Wireworms are reported to attract towards the root system (Barsics et al. 2016) of wheat and they cause injury to root during feeding process. The

larvae feed on germinating seeds or young seedlings, seldom cut them off. It has been reported that in wheat plant damaged by wireworms the central leaves die and the damaged plants exhibited symptoms of wilting or yellowing in appearance or were stunted in growth (Vernon et al. 2009). Wireworms do the most damage in early spring when they are near the soil surface (Vernon et al. 2009). In both fields, the first recording was taken two weeks after planting. The first five recording was taken at weekly intervals but the last three recording was done at two week intervals. From each site, a total of eight sampling were recorded.

To determine the density of wireworms larvae, destructive soil sampling method was employed. A square metal tools measuring 15 cm on each side (0.15 cubic meters) was used for sampling. Within an experimental plot, two random samples from the wheat rows and two random samples from the adjacent trap crop rows were collected. The soil samples were taken after plant counts recordings. Total eight soil samples were collected from both locations during both years. Samples were placed in plastic bags, labeled and brought to the lab. In lab, samples were manually processed, a bag of soil sample was put into the plastic tray (30cmX15cmX6cm) and the numbers of wireworms in each sample were recorded. Wireworms from samples were kept in small plastic cups of size 8cm diameter and 5 cm depth filled with sphagnum peat moss and soil (from the soil samples) and stored in a refrigerator at 8 °C. Wireworms were retained in the refrigerator for the later use in the shade house experiments. Wireworms were identified using the keys of wireworms described by Etzler (2013).

Determination of Optimal Spacing for Trap Crops

Location and Experimental Design.

These trials were conducted in 2016 at the Valier and Ledger locations. A complete randomized block design was again used, with row spacing treatments of 0.25, 0.5, 0.75, and 1 m, between the spring wheat crops intercropped with the pea and lentil trap crops and control (wheat only), each replicated four times. Spring wheat alone served as the no-choice control. Row spacing treatments was used as blocking and all trap crops treatments were randomly assigned to plots within each block. There were 48 plots in total, each measuring 4 m². The blocks and the plots were separated from each other by 1.5 m buffer distance. The crop varieties used were Montech pea, Green Land lentil and Duclair wheat, respectively. Crops were manually planted at the same rate used for the first field trials. Due to spring weather conditions plots were planted in late; in Ledger, on the third week of May and in Valier, on last week of May.

Sampling for Plant Counts and Wireworm Densities.

Wheat plant counts was taken using the 1 m line intercept method from randomly selected wheat rows intercropped with trap crops. For each counting event, the same randomly 1m row of wheat was used and count was taken. The first count was taken two weeks after planting. The following three counts were taken at weekly intervals and the last two at two week intervals. A total of six plant counts from wheat rows were taken from both locations.

In each experimental plot, two soil samples were taken at random, one each from a randomly selected wheat row and trap crop row immediately after plant counts were completed. Wireworm larvae were sampled, identified and stored as described previously.

Shade House Bioassay

Shade house bioassays were conducted at the Western Triangle Agriculture Research Centre in Conrad, Montana during August 2016. An average room temperature of 18 to 22 °C was maintained throughout the experiment. Assay units were square plastic flower pots (10.5 cm square and 9.2 cm deep) filled with a mixture of soil from the soil sample collected from Valier after wireworm processing, peat moss (Alaska Peat Moss Inc.) and filtered sand for construction in ratio 4:2:1. Two trap crops, Montech pea and Green Land lentil, were tested in two-choice assays with Duclair spring wheat. Five grams of wheat seeds was planted in two opposite sides in middle quadrant and five grams of pea or lentil seed was planted in the remaining two sides similarly in middle quadrant. We put the seeds together at the center of these four opposite quadrants at the depth of 4 to 5 cm. *Limonius californicus* larvae those collected from the field trails were used for the experiments. Larvae of size not less 1.5 cm were kept in plastic pot (12 cm diameter by 8 cm deep) filled with peat moss (Alaska Peat Moss Inc.) for a week before the experiments were setup to starve them. Nine larvae of *L. californicus* were then released in the center of the middle quadrant of the pot, eight pots for each comparison. On days 4 and 10 of the respective assays four pots each for trap crops and control were

destructively sampled by divided the pot and soil into nine equal quadrants, comprised of the four corners, the four sides and the center. The numbers of wireworms in each quadrant were recorded.

Statistical Analysis

Analysis of variance (ANOVA) was used to analyze data in r-software version 3.1.3. For both field trials: trap cropping, and spacing and trap crops, we used date of sampling as a fixed variable and performed ANOVA. Tukey HSD post-hoc at 95% confidence interval was used for pair-wise comparison among treatments for plant counts and damage. Values of $P < 0.05$ were considered significant. Paired t-test at 95% confidence interval was used to analyze the difference for wireworms counts between treatments for the first experiment to find the best trap crops attracting more wireworms. In spacing trials, we use ANOVA to analysis the date of numbers of wireworms recorded from the wheat rows intercropped to the treatment trap crops. If the results were significance, Tukey HSD post hoc test was used for the pair wise comparison. Chi-square tests of fitness were performed for the greenhouse bioassays and P -values < 0.05 were considered significant.

Results

Effect of Trap Crops on Spring Wheat Damage in Field Trials

In both years, at the Valier field location, significance difference in damage (%) to the spring wheat crop was found between the different trap crop treatments (in 2015, F

= 9.01, df = 6, and $P < 0.01$ and in 2016, $F = 15.54$, df = 6, and $P < 0.01$). In 2015, except for the barley ($P > 0.05$) and canola ($P > 0.05$) treatments, significance differences were found between tested trap crops and the spring wheat only control (Figure.3.1.).

Likewise, in 2016, damage levels in wheat plants intercropped with pea ($P < 0.01$), lentil ($P < 0.01$), and corn ($P < 0.05$) were significantly lower than in the wheat alone control (Figure.3.2.). In Valier, in both years, pea ($P < 0.01$) and lentil ($P < 0.01$) treatments showed the lowest damage to wheat plants.

Similarly, in both years at the Ledger field location, significance differences were observed in damage (%) to spring wheat intercropped with trap crops ($F = 59.49$, df = 6, and $P < 0.01$ in 2015; and $F = 13.68$, df = 6, and $P < 0.01$ in 2016). In 2015, except for the barley ($P > 0.05$) trap crop treatment, significant differences were detected in damage (%) to spring wheat plants intercropped with other trap crops ($P < 0.05$) compared to the wheat alone control. Damage to wheat plants was significantly lower in the pea ($P < 0.01$) and lentil ($P < 0.01$) treatments than in the other treatments ($P < 0.05$) (Figure. 3.1). In 2016, except for the durum ($P > 0.05$), significant differences were found between trap crops and the control ($P < 0.05$). Moreover, significantly lower damage (%) in wheat plants intercropped with pea ($P < 0.01$) and lentil ($P < 0.01$) trap crop treatments than other ($P < 0.05$) was observed (Figure. 3.2). In both years, regardless of location, wheat plant damage % by wireworms was higher in first four weeks of sampling, then decreased, and leveled off.

Effect of Trap Crops on Wireworm Densities in Field Spring Wheat

In 2015 and 2016, at the Valier field location, significance differences in wireworm numbers were found between the soil samples taken from wheat rows intercropped with different trap crops and the control spring wheat alone (in 2015, $df = 6$, $P < 0.01$ and in 2016, $df = 6$, $P < 0.05$). However, significantly lower numbers of wireworms in the spring wheat were observed only when intercropped with pea ($t = 3.41$, $P < 0.05$ in 2015, and $t = 3.24$, $P < 0.05$ in 2016). While comparing the wireworm numbers recorded from the different trap crops rows with control wheat rows we found significant lower number of wireworms in corn rows ($P < 0.05$ in both years) and canola ($P < 0.05$ in 2016 compared to trap wheat rows).

In both years, at the Ledger field location, significant differences in wireworm numbers were found between soil samples collected from wheat rows intercropped with trap crops and the wheat only control (in 2015, $df = 6$, $P < 0.01$ and in 2016, $df = 6$, $P < 0.05$). However, significantly different wireworm numbers were only recorded from the soil samples from wheat rows intercropped with treatment pea ($t = 3.993$, $P < 0.05$ in 2015, and $t = 3.16$, $P < 0.05$ in 2016). Moreover, while comparing the wireworm numbers recorded from the different trap crops rows with control wheat rows we found significantly lower numbers of wireworms in corn rows ($P < 0.05$ in both years) than trap crop pea.

In both years, wireworm numbers in soil core samples were high until the fifth sampling date after which wireworm numbers declined. In 2015, we collected a total of 693 and 380 wireworms at the Valier and Ledger respectively (Figure. 3.3 and 3.4). In

2016, we collected 301 wireworms in Valier among which 25 were *Aeolus mellillus*, 117 *Hypnoidus bicolor*, and 159 *Limonius californicus*. In Ledger, 262 wireworms were collected from soil samples among which 15 were *Aeolus mellillus*, 125 *Hypnoidus bicolor* and 124 *Limonius californicus*.

Effect of Trap Crops and Row Spacing on Wheat Plant Density

At Valier, wheat plant counts showed significant differences between trap crops ($df= 2$, $F= 158.8$, $P<0.01$) and between row spacing levels ($df= 3$, $F= 58$, $P<0.01$). Significantly higher numbers of wheat plants per meter were recorded in plots intercropped with pea ($P<0.01$) and lentil ($P<0.01$) compared to control wheat plots (Figure. 3.5). At intercropping spacing of 0.25 and 0.5 m between the trap crops and spring wheat significantly more numbers of wheat plants were found than at 0.75 and 1m spacing (Figure. 3.6). Moreover, a significant interaction was observed between trap crops and spacing levels ($df= 6$, $F= 131.61$, $P<0.01$) which is presented in Table 3.1.

At the Ledger field location, significant differences in wheat plant density was found among trap crops ($df= 2$, $F=33.66$, $P<0.01$) and row spacing levels ($df=3$, $F= 4.36$, $P<0.01$). While wheat plant counts intercropped with pea and lentil treatments did not differ significantly from each other. Wheat plant counts in the plots intercropped with pea and lentil were significantly higher than that in the wheat only control ($P<0.01$) which is shown in Figure. 2.5. Wheat plant counts were significantly higher at 0.75 m spacing compared to 0.25 m ($P<0.01$), but wheat plant numbers were not significantly different among the 0.75, 0.5 and 1 m row spacing levels (Figure.3.6). Moreover, there was

significant interaction between spacing and trap crop ($df=6$, $F=3.53$, $P<0.01$) presented in Table 3.2.

Effect of Trap Crops and Row Spacing on Wireworm Density

At the Valier field location, the number of wireworms collected from wheat rows intercropped with different trap crops showed significant differences ($df= 5$, $F= 5.87$, $P<0.01$), but no differences were observed for the different row spacing levels used. There was no significant interaction between trap crops and row spacing levels. Wireworms numbers in wheat rows intercropped with pea ($P=0.05$) or lentil ($P<0.01$) were significantly lower than in control wheat rows. From the soil samples, we collected 209 wireworms of which 11 were *Aeolus mellillus*, 70 *Hypnoidus bicolor* and 128 *Limonius californicus*.

At the Ledger field location, there were no significant differences among trap crops and row spacing levels for the numbers of wireworms recorded from the soil samples in wheat rows intercropped with different trap crops at different row spacing levels, $P > 0.05$ for both factors. There was not any significant interactions between trap crops and row spacing levels. We found 178 wireworms from the soil samples among which 15 were *Aeolus mellillus*, 73 *Hypnoidus bicolor* and 90 *Limonius californicus*.

Effect of Trap Crops on Wireworms distribution in Shade-House Bioassay

In a shade-house bioassay, the distribution of wireworms within pots on the 4th day after planting/sowing showed significant differences when wheat was planted with

pea ($P < 0.01$) or lentil ($P < 0.01$) compared to the no-choice control (wheat alone). Pea and lentil when sowing with wheat have more numbers of wireworms associated within their quadrants compared to the wheat sowing together. Moreover, pea and lentil quadrants were likely to have more numbers of wireworms compared wheat quadrants in control pots. Similar results were obtained on the 10th day after planting, with both pea ($P < 0.01$) and lentil ($P < 0.01$). More wireworms were observed in pea and lentil quadrants compared to wheat. On both 4 and 10 days of sampling, the distribution of wireworms were not uniform (one larva in each nine quadrants) when wheat was sown with trap crops pea and lentil having significant P-value in both sampling dates, however, in control the distribution was uniform ($P > 0.05$) presented in Table. 3.3 and Table 3.4. We never found wireworms evenly distributed across all nine sections of the pot, confirming that wireworms have either random or aggregated distribution but not uniform distribution in the soil.

Discussion

This study found pea and lentil to be the most effective trap crops for management of wireworms in spring wheat in Montana. When spring wheat was intercropped with either pea or lentil, damage to wheat from wireworms was reduced significantly and two to three fold more wireworms were collected from the soil taken from pea or lentil rows than intercropped wheat rows. Both pea and lentil are legumes and can fix nitrogen in soil increasing the soil fertility. Pea is also resilient to wireworms damage and drought (Griffiths 1974, Miles and Petherbridge 1927). The Ledger field

location has silt clay soil. The lower numbers of wireworms were collected compared to the Valier location which has sandy loam soil, suggesting that soil composition might affect wireworms distribution. *Hypnoidus bicolor* was the predominant wireworms species in Ledger while *Limonius californicus* was the predominant species in Valier but the ratios were almost close to equal.

Wireworms have been found to follow carbon dioxide gradients associated with plant roots (Doane et al. 1975). According to Johnson and Nielsen (2012), soil-borne pests rely primarily such CO₂ gradients to find hosts, and secondarily use as clues the more specific volatile organic compounds (Hiltpold et al. 2013) and semio-chemicals found on the surface of roots (Barsics et al. 2012) to determine host suitability. Durum, barley, and wheat all are from the same family Poaceae. Interestingly, the percentage of damaged wheat plants by wireworms within treatments in our study was almost same across these three crops, and the numbers of wireworms associated with these trap plants were not significantly different. Vernon et al. (2000) reported wheat to be an effective trap crop reducing wireworms damage in strawberries when used as an intercrop in strawberry rows. A number of studies have shown wireworms populations to be attracted to wheat (Miles and Petherbridge 1927, Kabanov 1975, Parker, 1994). Corn (also Poaceae) intercropped with spring wheat in this study, was able to reduce the initial wireworms damage for the two to three weeks after sowing, however, more numbers of wireworms were collected from the adjacent wheat rows, resulting in increased damaged to wheat then. Furlan et al. (2010) found wireworms to be attracted towards corn and sorghum. The temperature (<) was not so warm at the time of seed sowing during field

trials, however, corn needs warm temperature () at the time of germination, corn seeds failed to germinate which may be a reason that caused wireworms to move towards adjacent wheat row few weeks after sowing. Canola, which belongs to the Brassicaceae was able to reduce damage in wheat plants only in the low-density wireworms population location (Ledger) in our study. However, at Valier, the higher density the results were not significance. It has been reported that ionic thiocyanate (SCN-) present in Brassica did not have any effect on wireworm feeding (McCaffrey et al. 1995).

Regardless of years or locations, wheat intercropped with lentil or pea showed significantly lower damage wheat plants compared to other tested crops in our study. Possible explanations for this may be that besides carbon dioxide, both these crops provide easily accessible sugar (Miles and Petherbridge 1927, Griffiths 1974). Pea and lentil have nitrogen fixing bacteria which is may be a major factors involve in wireworms attraction towards them as nodules are the source of nutrition. In addition, wireworms are polyphagous and legumes (both pea and lentil) are a good source of protein, so it is possible that wireworms might like to feed on legumes even though other food sources are available. Wireworms may be also feeding on the nodule bacteria which are the source of protein. However, we never found wireworms to be totally absent in wheat rows intercropped with pea or lentil, and furthermore it seems that the attractiveness of pea diminishes over time (Miles and Petherbridge 1927, Griffiths 1974). Thus, we hypothesized that wireworms move back and forth between wheat and pea or lentil but spend a greater amount of time on pea and lentil, which was the main reason why more wireworms were caught on these trap crops and there was a reduced level of damage to

adjacent wheat plants. Pea has also been reported to be an effective trap crop in reducing the wireworms damage and numbers in potato field (Landl and Glauning 2011).

In the spacing levels trial, wheat plant counts were significantly higher when intercropped with pea or lentil and at a spacing level of 0.25 and 0.5 m in sandy soil (Valier) and at 1m, 0.75 m, and 0.5m in heavy clay (Ledger). It has been suggested that wireworms can move considerable horizontal distances (Langenbuch 1932), and under laboratory conditions, wireworms were found to move up to 20 cm to reach a CO₂ source (Doane et al. 1975). Moreover, Vernon et al. (2000) found wireworms to be attracted towards wheat from 50 cm away. Under field conditions, it was determined that wireworms were able to travel a distance of 0.75 m (Landl and Glauning 2011). Our results are similar to these findings, while Miles and Petherbridge (1927) reported wireworms to be attracted to CO₂ sources from 1.2 m away, we did find wireworms movement towards pea or lentil at spacing of 1 m, however, the results were not significant and consistent.

Wireworms numbers were significantly lower in wheat rows of pea and lentil treatments compared to control wheat rows in sandy soil with a high wireworms population pressure, suggesting that the distance from which wireworms can detect CO₂ and VOCs depends on plant type and soil properties. Aeration and drainage in soil also influence its CO₂ content (Russel 1936).

In the shade-house choice bioassay, we found that wireworms were attracted towards lentil and pea when sown with wheat on both the 4th and 10th days after sampling. We also observed that released wireworms did not follow a uniform

distribution pattern but rather were seen to have random or aggregate when pea and lentil trap crops are present. Surprisingly, in the control we still had no evidence to support an aggregated distribution pattern, perhaps because in monocultures wireworms populations (in this case *L. californicus*) follow a uniform distribution pattern (equal attraction). It is more likely that pea and lentil have some specific compounds in their roots that are highly attractive to wireworms. Barsics et al. (2016) found wireworms to be attracted to a synthetic blend of aldehydes, but live barley roots were more attractive than this synthetic blend of VOCs, confirming the role of CO₂ as a strong attractant for wireworms.

Wireworm larvae are polyphagous (Parker and Howard 2001) and can persist in soils for long periods, resulting in increased damage in seeds and seedlings every year (Vernon et al. 2009). The cryptic habitat (Hyslop 1916) of wireworms also makes them more difficult to control. Neonicotinoids are available for wireworm control, but are not consistently effective at controlling wireworms (Van Herk and Vernon 2007). Under such conditions, trap crops may be a better method to control wireworms (Staudacher et al. 2013). Trap crops confine wireworms movement, concentrating them in their root zones (Schallhart et al. 2011) and helping to protect the main crop. The VOCs compounds to which wireworms are attracted vary widely with cropping conditions. Diffusion properties in the air and water phases of a soil can play significant roles in wireworms detection of CO₂ and VOCs through olfaction or taste. Moreover, the chirality of the compounds affect wireworms behavior (D'Alessandro et al. 2013). Further work is needed to determine the specific compounds in the roots of peas and lentil that attract wireworms. These compounds could be used as baits to trap and kill wireworms

populations in the field. Studies on agronomic practices such as irrigation and fertilization should also be carried out to examine the role of cropping conditions on trap crops and wireworms densities. Cost-benefit ratio analyses of trap cropping to control wireworms are needed to persuade growers to adopt these cultural practices.

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Table 2.1. Interaction between intercropping spacing and trap crops in Valier, Montana in 2016.

Treatments Interaction	0.25 P ^z	0.25 L	0.25 W	0.5 P	0.5 L	0.5 W	0.75 P	0.75 L	0.75 W	1 P	1 L	1 W
0.25 P	X	X	X	X	X	X	X	X	X	X	X	X
0.25 L	Ns ^y	X	X	X	X	X	X	X	X	X	X	X
0.25 W	**	**	X	X	X	X	X	X	X	X	X	X
0.5 P	Ns	Ns	**	X	X	X	X	X	X	X	X	X
0.5 L	Ns	Ns	**	Ns	X	X	X	X	X	X	X	X
0.5 W	**	**	Ns	**	**	X	X	X	X	X	X	X
0.75 P	Ns	Ns	**	Ns	Ns	**	X	X	X	X	X	X
0.75 L	Ns	Ns	**	Ns	Ns	**	Ns	X	X	X	X	X
0.75 W	**	**	**	**	**	**	**	**	X	X	X	X
1 P	**	**	Ns	**	**	Ns	**	**	**	X	X	X
1 L	**	**	Ns	**	**	Ns	**	**	**	Ns	X	X
1 W	**	**	**	**	**	**	**	**	Ns	**	**	X

^y Ns = not significant, * and ** indicate significant interactions at $P < 0.05$ and 0.01 , respectively according to three way ANOVA, Tukey HSD.

X = already compared between the treatments.

^z P = Pea, L = Lentil, and W = Wheat.

Table 2.2. Interaction between intercropping spacing and trap crops in Ledger, Montana in 2016.

Treatments Interaction	0.25 P ^z	0.25 L	0.25 W	0.5 P	0.5 L	0.5 W	0.75 P	0.75 L	0.75 W	1 P	1 L	1 W
0.25 P	X	X	X	X	X	X	X	X	X	X	X	X
0.25 L	Ns ^y	X	X	X	X	X	X	X	X	X	X	X
0.25 W	*	Ns	X	X	X	X	X	X	X	X	X	X
0.5 P	Ns	Ns	**	X	X	X	X	X	X	X	X	X
0.5 L	Ns	Ns	**	Ns	X	X	X	X	X	X	X	X
0.5 W	Ns	Ns	Ns	**	**	X	X	X	X	X	X	X
0.75 P	Ns	Ns	**	Ns	Ns	*	X	X	X	X	X	X
0.75 L	**	**	**	*	*	**	**	X	X	X	X	X
0.75 W	Ns	Ns	Ns	*	*	Ns	Ns	**	X	X	X	X
1 P	Ns	Ns	**	Ns	Ns	*	Ns	**	Ns	X	X	X
1 L	Ns	Ns	*	Ns	Ns	*	Ns	**	Ns	Ns	X	X
1 W	Ns	Ns	Ns	Ns	Ns	Ns	Ns	**	Ns	Ns	Ns	X

^y Ns = not significant, * and ** indicate significant interactions at $P < 0.05$ and 0.01 , respectively according to three way ANOVA, Tukey HSD.

X = already compared between the treatments.

^z P = Pea, L = Lentil, and W = Wheat.

Table 2.3. Number of wireworms found in different regions of soil from plastic pots sown with wheat and trap crops in the bioassay on four days after sowing. Chi-square values are at α 0.05.

Treatment	Pea vs Wheat			Lentil vs Wheat			Wheat vs Wheat		
Sampling on 4 days after sowing	Pea	Wheat	Nth	Lentil	Wheat	Nth	Wheat (T)	Wheat	Nth
	15	13	8	16	11	9	12	10	14
P-value	0.0002			0.0006			0.1223		

Table 2.4. Numbers of wireworms found in different regions of soil from plastic pots sown with wheat and trap crops in the bioassay on ten days of sowing. Chi-square values are at α 0.05.

Treatment	Pea vs Wheat			Lentil vs Wheat			Wheat vs Wheat		
	Pea	Wheat	Nth	Lentil	Wheat	Nth	Wheat (T)	Wheat	Nth
Sampling on 10 days after sowing	16	8	12	17	10	9	11	12	13
P-value	0.007			0.0003			0.06		

Table 2.5. Wireworms numbers recorded from the wheat rows (W) and trap crops (Pea, Canola, Corn, Durum, Barley, Lentil and Control) rows from location Valier in 2015.

Date	Pea		Canola		Corn		Durum		Barley		Lentil		Wheat	
	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap
1	6	9	3	7	5	13	9	9	4	5	5	3	4	8
2	7	18	10	7	22	5	4	12	19	8	9	14	14	14
3	3	5	3	10	8	3	2	13	9	3	5	7	14	10
4	5	12	10	7	13	8	10	12	13	15	4	8	9	13
5	3	9	8	5	5	2	2	5	6	10	8	5	8	7
6	2	6	2	6	11	3	6	5	5	6	3	6	4	6
7	2	4	3	5	1	2	2	1	4	5	2	3	1	2
8	0	2	1	0	1	0	1	1	1	2	0	0	4	2

Table 2.6. Wireworms numbers recorded from the wheat rows (W) and trap crops (Pea, Canola, Corn, Durum, Barley, Lentil and Control) rows from location Ledger in 2015.

Date	Pea		Canola		Corn		Durum		Barley		Lentil		Wheat	
	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap
1	3	6	4	5	5	3	6	8	10	6	3	4	8	10
2	2	6	1	4	4	4	2	4	8	9	5	3	2	3
3	3	11	7	1	6	5	3	4	6	9	8	7	6	9
4	1	6	7	4	5	2	4	6	5	2	4	6	11	8
5	2	2	6	7	2	2	5	1	1	5	0	5	5	3
6	0	2	4	1	2	1	2	1	0	1	0	0	2	0
7	0	2	0	0	3	0	0	1	0	2	2	3	1	0
8	0	2	0	0	1	0	0	0	2	0	1	2	1	1

Table 2.7. Wireworms numbers recorded from the wheat rows (W) and trap crops (Pea, Canola, Corn, Durum, Barley, Lentil and Control) rows from location Valier in 2016.

Date	Pea		Canola		Corn		Durum		Barley		Lentil		Wheat	
	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap
1	2	5	4	3	1	2	3	4	4	1	1	3	6	4
2	2	7	2	2	6	4	7	8	3	5	2	4	7	6
3	3	7	2	3	6	2	5	3	3	6	3	8	3	6
4	2	6	3	2	5	3	4	4	3	4	3	6	4	5
5	1	4	3	1	4	1	2	4	2	3	2	3	5	3
6	0	2	2	0	2	1	2	3	2	3	1	1	2	1
7	0	2	0	1	1	0	0	1	0	1	1	2	1	2
8	0	1	0	1	0	0	1	0	0	0	1	0	2	1

Table 2.8. Wireworms numbers recorded from the wheat rows (W) and trap crops (Pea, Canola, Corn, Durum, Barley, Lentil and Control) rows from location Ledger in 2016.

Date	Pea		Canola		Corn		Durum		Barley		Lentil		Wheat	
	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap	W	Trap
1	1	7	4	3	4	5	3	4	2	3	2	6	6	4
2	2	8	5	7	3	5	5	6	4	6	3	6	6	7
3	2	5	5	4	4	1	3	5	4	3	2	4	6	7
4	2	4	4	1	4	2	5	2	2	2	3	4	4	2
5	1	3	2	1	1	0	2	1	0	1	1	3	2	4
6	0	1	1	0	2	0	1	0	0	1	1	1	0	2
7	0	2	1	0	0	0	0	0	0	1	0	1	1	0
8	0	2	0	0	0	0	0	1	0	0	0	0	0	0

Table 2.9. Mean of wheat plant counts recorded from randomly selected 1m line intercept in wheat rows at different sampling dates when intercropped with trap crops (Pea, Canola, Corn, Durum, Barley, Lentil and Control) from location Valier in 2015. Undamaged is the numbers of wheat plant counts which were not damaged from the wireworms.

Dates	Wheat vs Pea		Wheat vs Canola		Wheat vs Corn		Wheat vs Durum		Wheat vs Barley		Wheat vs Lentil		Wheat vs Wheat	
	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total
1	31.00	36.00	31.83	38.88	26.83	30.83	30.00	35.00	31.28	37.33	31.28	35.27	26.00	35.00
2	27.27	33.00	26.56	36.22	22.78	27.44	26.56	32.44	25.72	33.28	28.22	34.00	22.94	31.61
3	24.33	26.53	22.78	27.00	16.56	21.28	20.50	25.06	19.44	24.83	25.33	28.05	18.22	22.17
4	20.07	23.33	16.28	19.83	14.06	16.78	15.28	18.66	15.00	18.22	22.61	26.11	14.33	17.33
5	20.93	22.93	18.06	20.89	14.56	17.06	16.60	19.50	15.66	18.38	21.17	23.11	14.61	17.17
6	20.27	23.20	18.17	20.22	14.61	15.83	16.67	18.61	16.33	18.05	20.56	23.33	14.39	16.22
7	21.00	23.00	18.50	21.11	14.90	16.01	16.80	18.88	16.50	18.56	21.10	23.11	14.56	16.56
8	21.21	23.11	18.50	20.88	15.00	16.22	17.00	19.02	16.30	18.66	20.78	22.33	14.33	16.89

Table 2.10. Mean of wheat plant counts recorded from randomly selected 1m line intercept in wheat rows at different sampling dates when intercropped with trap crops (Pea, Canola, Corn, Durum, Barley, Lentil and Control) from location Ledger in 2015. Undamaged is the numbers of wheat plant counts which were not damaged from the wireworms.

Dates	Wheat vs Pea		Wheat vs Canola		Wheat vs Corn		Wheat vs Durum		Wheat vs Barley		Wheat vs Lentil		Wheat vs Wheat	
	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total
1	33.22	37.22	32.17	38.17	31.06	38.06	31.33	37.33	30.94	39.94	34.00	36.17	29.66	35.66
2	31.33	35.55	29.39	34.61	29.78	36.00	28.39	34.11	28.72	37.88	31.11	32.17	26.78	34.61
3	27.90	29.83	22.33	27.61	24.22	28.00	26.61	31.94	24.56	30.61	26.50	28.55	22.17	27.72
4	27.50	29.80	22.00	25.61	24.67	27.77	25.33	29.72	24.78	29.77	27.11	28.17	22.11	26.50
5	27.50	29.88	23.00	23.88	24.83	25.83	25.55	27.55	25.55	29.77	27.17	28.22	22.11	26.50
6	27.22	28.38	22.11	23.17	25.17	26.28	25.11	27.66	25.11	29.22	27.11	28.00	22.88	26.11
7	27.50	28.44	23.00	24.11	25.00	26.88	25.11	27.33	25.00	29.00	26.11	27.78	23.00	26.33
8	28.60	29.22	22.56	23.81	24.89	25.81	25.33	27.11	25.78	29.11	27.00	28.11	23.10	26.11

Table 2.11. Mean of wheat plant counts recorded from randomly selected 1m line intercept in wheat rows at different sampling dates when intercropped with trap crops (Pea, Canola, Corn, Durum, Barley, Lentil and Control) from location Valier in 2016. Undamaged is the numbers of wheat plant counts which were not damaged from the wireworms.

Dates	Wheat vs Pea		Wheat vs Canola		Wheat vs Corn		Wheat vs Durum		Wheat vs Barley		Wheat vs Lentil		Wheat vs Wheat	
	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total
1	22.33	24.83	18	21.22	17.38	20.05	14.88	17.22	15.88	18.44	20.61	23.05	16.16	20.22
2	19.5	22.44	16.5	20.38	15.5	18.61	15.44	18.44	14.66	18	18.66	21.61	14.55	18.27
3	18.77	20.88	15.83	18.66	14.05	17.16	13.94	16.41	13.66	16.66	18.5	20.33	13.94	17
4	17.16	19.28	14	16.66	13.72	16.05	12.61	14.88	13.22	15.33	17.05	19.11	12.27	15.16
5	17.22	18.94	13.83	16.05	13.72	15.66	12.88	15.05	13.22	15.66	17.22	18.83	12.33	14.66
6	17.22	18.61	13.94	15.77	14.44	15.88	13.33	15.38	13.22	15.05	16.83	18.44	12.16	13.94
7	17.11	18.66	14.16	15.61	14.66	16	13.66	15.27	13.77	15.22	17	18.22	12.38	14
8	17.88	19.16	14.22	15.77	14.55	16.05	13.72	15.22	14.27	15.5	17.44	18.44	12.44	13.8

Table 2.12. Mean of wheat plant counts recorded from randomly selected 1m line intercept in wheat rows at different sampling dates when intercropped with trap crops (Pea, Canola, Corn, Durum, Barley, Lentil and Control) from location Ledger in 2016. Undamaged is the numbers of wheat plant counts which were not damaged from the wireworms.

Dates	Wheat vs Pea		Wheat vs Canola		Wheat vs Corn		Wheat vs Durum		Wheat vs Barley		Wheat vs Lentil		Wheat vs Wheat	
	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total	Undamaged	Total
1	40.33	36.722	36.94	31.44	39	32.77	38.38	32.05	37.77	32.27	42.88	38.5	36.22	29.05
2	38.61	32.88	34	28.33	36.11	30.05	34.16	28.88	35.11	29.16	39.38	34.5	32	25.83
3	34.55	31.33	31.44	26.44	32.38	27.38	32.11	27.83	31.27	27.11	37.16	34	28.11	22.83
4	31.94	28.66	28	23.7	29.38	25.38	28.16	23.83	28.72	25.16	32.66	29.83	25.22	21.11
5	30.27	27.55	27.27	24.38	28.94	25.44	27.22	24.27	27.88	25.5	31.77	28.88	24.66	21.72
6	30.83	27.88	27.55	25.05	29.38	26.77	27.88	24.66	28.83	25.77	32	29.27	24.94	21.88
7	30.94	28.33	26.44	24.61	29.11	27.11	28.55	25.55	28.38	26.11	31.55	29.83	25.55	23.05
8	30.44	28.66	27.38	25	28.55	26.11	27.88	24.55	28.38	25.66	31.22	29.83	25.16	22.22

Table 2.13. Wireworms numbers recorded from the wheat rows (WP = wheat rows in pea, WL = wheat rows in lentil, and Wheat = wheat rows in control) and trap crops (Pea, Lentil and Control (Trap W)) rows, when spring wheat was intercropped with trap crops at different row spacing levels 1m, 0.75m, 0.5m and 0.25m, from location Valier in 2016.

Spacing	1m						0.75m						0.5m						0.25m					
Trap crop	Pea		Lentil		Control		Pea		Lentil		Control		Pea		Lentil		Control		Pea		Lentil		Control	
	WP	Pea	WL	Lentil	Wheat	Trap W	WP	Pea	WL	Lentil	Wheat	Trap W	WP	Pea	WL	Lentil	Wheat	Trap W	WP	Pea	WL	Lentil	Wheat	Trap W
Date																								
1	0	3	1	3	2	2	1	2	2	3	2	1	1	2	2	3	2	2	2	4	1	3	3	3
2	1	2	1	0	1	1	0	2	1	3	2	2	1	2	1	2	2	2	2	3	1	2	4	3
3	2	3	1	2	1	2	1	1	1	3	2	2	1	2	0	3	3	2	1	3	1	2	2	3
4	1	2	0	2	2	2	0	2	0	2	2	1	2	3	1	2	1	1	1	2	1	2	1	1
5	0	2	0	0	0	1	2	1	1	2	1	1	1	1	0	1	2	1	1	2	0	2	2	2
6	1	0	0	1	0	1	0	1	0	1	0	0	0	1	0	2	0	1	1	2	0	2	1	0

Table 2.14. Wireworms numbers recorded from the wheat rows (WP = wheat rows in pea, WL = wheat rows in lentil, and Wheat = wheat rows in control) and trap crops (Pea, Lentil and Control (Trap W)) rows, when spring wheat was intercropped with trap crops at different row spacing levels 1m, 0.75m, 0.5m and 0.25m, from location Ledger in 2016.

Spacing	1m						0.75m						0.5m						0.25m					
Trap crops	Pea		Lentil		Control		Pea		Lentil		Control		Pea		Lentil		Control		Pea		Lentil		Control	
	WP	Pea	WL	Lentil	Wheat	Trap W	WP	Pea	WL	Lentil	Wheat	Trap W	WP	Pea	WL	Lentil	Wheat	Trap W	WP	Pea	WL	Lentil	Wheat	Trap W
Date																								
1	0	2	1	2	1	2	1	3	6	1	1	2	1	2	0	2	3	2	2	3	1	2	1	2
2	1	0	0	0	1	1	0	1	1	3	1	1	2	3	0	1	4	0	2	3	1	2	6	2
3	2	2	2	2	1	1	0	2	1	3	1	2	1	1	0	2	2	1	1	5	0	2	2	2
4	2	2	1	2	1	1	1	2	0	3	2	1	1	4	1	2	0	1	0	3	1	2	0	0
5	0	1	1	1	0	1	1	1	1	2	1	1	0	2	0	1	1	1	1	2	0	1	1	1
6	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	2	0	1	0	0

Table 2.15. Mean of wheat plant counts (Pea W = wheat intercropped with pea, Len W = wheat intercropped with lentil and Wheat = wheat in control) recorded from randomly selected 1m line intercept in wheat rows at different sampling dates when intercropped with trap crops (Pea, Lentil and Control) at the row spacing level of 1m, 0.75m, 0.5m and 0.25m between spring wheat and trap crops from location Valier in 2016.

Spacing	1m			0.75m			0.5m			0.25m		
Trap crop	Pea W	Len W	Wheat	Pea W	Len W	Wheat	Pea W	Len W	Wheat	Pea W	Len W	Wheat
1	17.5	19.25	14.75	25.25	28.25	12.75	27.25	26	20.5	27	26.5	18.75
2	16.5	17.75	12.5	23.5	27	10.75	25.5	24.25	18.25	24.75	24	16.75
3	14	15.5	9.75	21.25	24	8.75	22.25	22.5	15.75	22.5	21.5	14.75
4	12.75	14.75	8.5	19.25	22.75	7.25	21	20.75	14.25	21.5	19.5	13.5
5	13	14.5	8	19.75	23	8.5	20.5	21.25	15	22.25	21	14.25
6	13.75	15	9	20.25	22.5	9.75	20.75	21.25	15.75	20.75	21.75	15

Table 2.16. Mean of wheat plant counts (Pea W = wheat intercropped with pea, Len W = wheat intercropped with lentil and Wheat = wheat in control) recorded from randomly selected 1m line intercept in wheat rows at different sampling dates when intercropped with trap crops (Pea, Lentil and Control) at the row spacing level of 1m, 0.75m, 0.5m and 0.25m between spring wheat and trap crops from location Valier in 2016.

Spacing	1m			0.75m			0.5m			0.25m		
Trap crop	Pea W	Len W	Wheat	Pea W	Len W	Wheat	Pea W	Len W	Wheat	Pea W	Len W	Wheat
1	21.25	31	38.75	22	25.75	36.5	20.5	25	38.25	23.25	26.25	38.25
2	19	25.75	35	20.5	23	32.5	19.25	23.5	34.25	22.25	25	36
3	18.75	27	32.5	19.5	22	28.5	18.75	22.25	29.75	21.25	23.5	33.25
4	18	26.75	31	20	20.5	27	18.25	21	25.75	21.5	23.75	29
5	17.75	25.5	30.75	20	21.5	28	18	20.5	26.75	21	23.25	29.5
6	17.75	25	29	19	22.25	28.25	17.75	19	26.5	20	24.5	28.25

Figure. 2. 1. Figure. 2.1. Effect of trap crops on wheat plants damaged (%) by wireworms in Valier and Ledger locations in 2015. The control was wheat paired with wheat. Different letters above bars indicate significant differences by two way ANOVA, Tukey HSD $\alpha=0.05$.

Figure. 2. 2. Effect of trap crops on wheat plants damaged (%) by wireworms in Valier and Ledger locations in 2016. Different letters above bars indicate significant differences by two way ANOVA, Tukey HSD $\alpha=0.05$.

Figure. 2. 3. Total number of wireworms collected from wheat rows and trap crops rows during soil sampling in Valier and Ledger locations in 2015.

Figure. 2. 4. Total number of wireworms collected from wheat rows and trap crops rows during soil sampling in Valier and Ledger locations in 2016.

Figure. 2. 5. Mean number of wheat plants/m when intercropped with different trap crops at spacing level of 0.25, 0.5, 0.75 and 1 m between wheat and different trap crops. Different letters over bars represent significant differences according to three way ANOVA, Tukey HSD $\alpha = 0.05$.

Figure. 2. 6. Mean number of wheat plants/m, when spring wheat is intercropped with pea, lentil and wheat at spacing levels of 0.25, 0.5, 0.75 and 1 m. Different letters over bars represent significant differences according to three way ANOVA, Tukey HSD $\alpha = 0.05$.

CHAPTER FOUR

GENERAL CONCLUSIONS AND SOME SUGGESTED FUTURE STUDIES

Wireworms are the economic pest of spring wheat in Golden Triangle Areas of Montana. Recently available neonicotinoids insecticides are not able to kill wireworms and provide protective role for only one or two weeks. Since wireworms can live from 3 to 11 years depending upon species so if the wireworms population is not control by chemicals, wireworms population increases every year causing repeated attack on crops and damage. Biological controls have not been successful to control wireworms. From our study, it has been proven that trap crops like pea and lentil are effective in reducing wireworms damage in spring wheat. Relatively, more numbers of wireworms are recorded in pea and lentil trap crops rows compared to the main crops spring wheat when intercropped together. From potted bioassay, it is clear that when wheat is sown with pea or lentil, wireworms are more likely to be found in region of pea and lentil. In field trials of row spacing and trap crops, significantly more wheat plant counts was recorded when intercropped with trap crops pea or lentil. For the row spacing of 0.5m between trap crops and spring wheat more wheat plants counts were recorded in both locations. Thus, based on our studies, pea and lentil are the effective trap crops for the management of wireworms in spring wheat in Golden Triangle Regions of Montana.

Past studies have also shown that trap crops are highly attractive to wireworms. Thus, identifying the possible compounds presents in roots of trap crops may help on better understanding of the attraction phenomenon. The cost benefit ratio of the trap

cropping can be investigated to convince the farmers for the adaptation of this technique. We can also use trap crops as a bait to concentrate the wireworms and use insecticides to kill them. Last but not the least, to optimize agronomical practices for the trap crops should be develop which can play an important role for the success of this study.

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