ANALYSIS OF CROP-COMPETITION, WEEDS, AND HETERODERA GLYCINES IN WINTER ANNUAL OILSEED ROTATION

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Abstract

Midwest crop production is dominated by two summer annual crops grown in rotation, corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.]. The rotation leaves a productivity gap during the spring and autumn. Winter oilseed crops, such as pennycress (*Thlaspi arvense* L.), and winter camelina [*Camelina sativa* (L.) Crantz], can fill this gap and provide ecosystem and economic benefits. The objectives of this study were to: i) examine the tradeoffs between soybean and winter oilseed yields in the relay-cropping system, ii) determine legacy impacts on corn one year after oilseed-soybean relay-cropping, iii) evaluate the weed suppression abilities of the winter oilseeds. iv) evaluate the host susceptibility of the winter oilseeds to SCN in the greenhouse, and v) evaluate pennycress germplasm collection for major resistance genes to SCN. Three sites were used across Minnesota to evaluate winter oilseeds and commodity crop yields in a relay-cropping production system. Total seed production of the system (winter oilseed crop + soybean) was increased by 20% at one site, while at the other two sites, there was no significant difference in total yield when compared to mono-cropped soybean. Soybean yield was reduced at two-of-three sites by the inclusion of winter oilseeds by 20% and 47%. Soybean yield was unaffected by inclusion of winter oilseeds at the third site. Corn yield, in the subsequent year, was unaffected by the winter oilseed treatments. Weeds were suppressed by the winter oilseeds crops. The pennycress treatment reduced weed biomass by 97% to 100%. Likewise, the camelina treatment reduced weed biomass by 85% to 87%. The inclusion of winter oilseeds in the corn-soybean cropping system can increase overall seed production and suppress early-season weeds. Through greenhouse evaluation it was

determined that pennycress is an alternate host for soybean cyst nematode; while camelina is a poor or non-host. Including pennycress as a winter annual cover crop in rotations with soybean has the potential to increase SCN pest pressure. Genetic screens for SCN resistance found variation in the population. Lines tested ranged in Female Index values from 27-143. Indicating diversity in the germplasm that may be able to be exploited for resistance development. The inclusion of winter oilseeds in the corn-soybean cropping system can increase productivity and decrease weed populations, but also may increase pest pressure of SCN in the cropping system.

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Introduction

The Midwest Region of the United States is dominated by two crop species, corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.). These two crops are commonly rotated with one another, and they account for the first- and second-most hectares planted in the United States (USDA-ERS, 2017). In the Midwest, corn accounted for 29.8 million hectares, while soybean accounted for 29.4 million hectares planted in 2017 (USDA-Farm Service Agency, 2017). As summer-annual crops, corn and soybean are planted in April-May and harvested in October-November in the Midwest. As a result, millions of hectares in the Midwest have exposed soil during the months of heavy rainfall, especially in the spring and autumn. The spring and autumn months are when half of the total annual precipitation of 932 mm falls in the Midwest (Huff and Angel, 1992). Modeled estimates show that during spring months heavy storms detach soil from the bare ground, and soil losses commonly exceed 14 tonnes ha^{-1} yr^{-1} (Gelder et al., 2018). These erosion losses are unsustainable for crop production and result in the additional consequence of nutrient loss from the plant root zone.

Nitrogen fertilizer application and subsequent leaching results in the presence of nitrates in groundwater. The impacts of nitrates in wells and surface waters have been well-documented (McCasland et al., 2012; Van Metre et al., 2016). Several Midwestern watersheds have vulnerable aquifers and high nitrogen loading from agriculture (Nolan and Hitt, 2006). This has resulted in many wells and streams having high levels of nitrates. (Van Metre et al., 2016). In a USGS survey on nitrate contamination, 7% of private wells

tested above the legal limit of 10 ppm-nitrate-N. One-quarter of shallow wells tested in rural farm communities were contaminated above the legal limit (DeSimone et al., 2009). Nitrates at levels above 10 ppm can adversely affect human health, especially the health of infants (De Roos et al., 2003; McCasland et al., 2012; Aschebrook-Kilfoy et al., 2012). In addition to the effects on wells, the excess nutrient accumulation in the Gulf of Mexico has created a hypoxic zone. Agriculture is the largest contributor of nitrates and phosphates into the Gulf of Mexico, making up 70% of overall deposition (Alexander et al., 2008). Cover crops have been investigated to address some of these environmental concerns. However, cover crop adoption has remained low. Non-cover crop users have identified timing challenges and lack of economic return as the primary barriers for cover crop adoption (SARE- North Central, 2015). Cover crop solutions that address these barriers while continuing ecosystem services are needed to increase adoption.

Winter annual oilseeds and inter-cropping

Pennycress (*Thlaspi arvense* L.) and winter camelina [*Camelina sativa* (L.) Crantz] are winter annual oilseed crops being developed for use as cash cover crops in corn and soybean rotations. Enhancing production and providing ecosystem services are the primary focus of incorporating cash cover crops into these rotations (Heaton et al., 2013). The winter oilseed crops potentially provide an additional income (hence, cash cover crop) opportunity for growers without losing the value of their annual grain crops. The relay cropping system begins by seeding the winter oilseeds in autumn into standing corn/soybeans or seeding after the harvest of shorter-season crops (e.g., spring wheat, sweet corn, or silage corn). In the corn-soybean relay cropping rotation, the winter oilseeds

emerge and grow in the autumn, overwinter as rosettes, and then quickly bolt and reach maturity in the spring. Soybeans are interseeded into the winter oilseeds before flowering in May. When the winter oilseeds reach maturity, they are harvested (i.e., direct combined) over the top of the newly emerged soybean plants (Johnson et al., 2017). The soybeans then grow to maturity as in a typical Upper Midwest rotational system. Preliminary research indicates that adding the winter oilseeds to the rotation can produce a total seed oil yield (winter oilseeds + commodity crop) that can be up to 50% greater than the mono-cultured soybeans (Gesch et al., 2014). This increase in overall production might be enough incentive for growers to adopt this winter oilseed crop system. Pennycress and winter camelina were chosen for this system because of their favorable agronomic traits and marketable seed product. Both winter oilseed crops are extremely cold tolerant and reach maturity quickly (by mid-June) making them ideal in a corn and soybean system (Warwick et al., 2002). In addition to the winter oilseeds being favorable crops in cold climates, their oils also have properties that make them good feedstocks for biofuel production (Moser et al., 2009). Previous evidence suggests that the benefits of adding oilseeds into the rotation may include overall greater production in the system as well as other potential benefits such as and noxious weed suppression, and soybean cyst nematode reduction.

One issue remains when evaluating the use of pennycress and camelina in a corn-soybean rotation. This issue is that soybean yields are put at risk when pennycress and camelina are added into the rotation. Although there is evidence that the overall yield of the system is greater, taking a yield reduction in the commodity crop is not ideal. It has been indicated that the critical weed free period in corn is between V3 and V14. (Hall et al., 1992). In soybean the weed free period is between R1 and R5 (Van Acker et al., 1993).

Based on critical weed period alone the presence of camelina and pennycress will result in a yield penalty. Light competition between the relay-cropped soybeans and the winter oilseeds could lead to soybean plant etiolation and subsequent yield loss, as has been observed for soybean interseeded into wheat (Wallace et al., 1996). Reducing light competition and the amount of time the plants are growing together may be paramount to solving this issue.

Weed management and resistance

Resistance weed biotypes are on the rise in agriculture, as cropping system diversity continues to decrease, resulting in an overall increase in the amount of herbicides used to control weeds (Aguilar et al., 2015). Herbicide use has increased drastically since 1960 in United States agriculture from 89 million kg of active ingredient to 234 million kg in 2008. This is largely due to herbicide's inexpensive weed control costs relative to manual or mechanical weed control. In addition, this increase in herbicide usage is due to a massive switch from crop diversity in farming to a landscape dominated by corn and soybeans. Of the total pesticide application in the United States, 39.5% is used on corn, and 21.7% is used on soybean, respectively (Fernandez-Cornejo et al., 2014). Weed resistance to herbicide mode of actions has begun to affect chemical weed control especially in corn and soybean systems which have experienced heavy herbicide use. There are three clear examples of the rise of herbicide resistance in the United States.

First registered for use in 1959, Atrazine [2-chloro-4-(ethylamino)-6-isopropylamino-s-triazine] quickly became a prominent herbicide to control weeds in corn production. Atrazine was a great fit for annual corn production as it had broad spectrum

weed control, it was low cost, there was low risk of crop injury, and great application flexibility with the long residual activity. Atrazine is a PSII limiting herbicide that inhibits the light reactions of photosynthesis by competitively binding to the Q_b binding site of the D1 protein (Fuerst and Norman, 1991). This binding blocks the flow of electrons to cytochrome b_6f, which interrupts the synthesis of ATP and NADPH in the chloroplast as well as forms free radical oxygens. These free radical oxygens damage the cellular components of the plant (Triantaphylidès and Havaux, 2009). Atrazine is used on crops such as corn, due to its ability to perform metabolic detoxification by means of glutathione S-transferase (GST). GST, termed a protective tripeptide, uses atrazine as a substrate and transfers the molecule to the cells vacuole, thereby rendering it inactive (Timmerman, 1989). Within a few years after the release and widespread use of atrazine, resistant weeds were discovered. Resistance in dicot weeds has been found to be due to a target site, point mutation in the D1 protein which prevents the binding of the atrazine molecule. Resistance in monocot weeds has been found to be largely due to elevated GST levels, the same mechanism that provides the selection in corn (Hirschberg and Mcintosh, 1983; Svyantek et al., 2016). Over 50 species of atrazine resistance weeds now exist (Heap, 2014). The herbicide is largely ineffective for the broad spectrum weed control it was intended to provide.

Another class of herbicide with historic weed resistance issues are the ALS (acetohydroxyacid synthase) inhibitors. The ALS inhibitors were first commercialized in 1982, and resistance developed rapidly. The ALS herbicides have a primary mode of action that involves binding and inhibition of the ALS enzyme. The ALS enzyme is the first enzyme involved in the biosynthesis of branched chain amino acids such as isoleucine. The

inhibition of this enzyme starves the plant of these vital amino acids, causing plant death (Umbarger, 1978). ALS inhibitors were widely used in small grain production, namely in wheat, barley, and rice, but selectivity existed for use in other crops as well. Selectivity in crop species was largely due to metabolic deactivation of the herbicide with conjugation to a glucose molecule (Brown, 1990). Resistance in weeds to ALS inhibitors occurs by one of two mechanisms, first is a reduced sensitivity of the target ALS enzyme, and the second is metabolism that results in detoxification of the herbicide (Tranel and Wright, 2002). Over 170 weed species have resistance to ALS herbicide rendering less effective in control of weeds in many of the cropping systems it was designed to protect (Heap, 2018).

A final, large-scale, example of weed resistance to common herbicides is glyphosate. Glyphosate [2-(phosphonomethylamino)acetic acid] works by inhibiting the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme in plants. This enzyme catalyzes a step in the shikimate pathway of amino acid biosynthesis. The inhibition of this enzyme stops aromatic amino acid formation. Glyphosate came into large scale use in agriculture following the introduction of RoundUp Ready crops. RoundUp Ready crops contained a transgene that encoded for a glyphosate-tolerant EPSP synthase (Padgette et al., 1995). Growers readily adopted glyphosate, as it produced broad spectrum weed control without crop damage. In 2014, worldwide glyphosate use was 825,804 Mega grams compared with just 56,296 Mega grams in 1994. This is nearly a 15-fold worldwide increase in glyphosate use. This widespread use has largely been driven by the introduction of RoundUp Ready technology (Benbrook, 2016). Weed resistance in glyphosate is due to three known mechanisms. First, is limited translocation, in which the glyphosate is not effectively translocated to active meristems by way of inhibited phloem loading. Second,

is mutation within the EPSP target site, binding to the target enzyme is inhibited by this mechanism. The third mechanism of weed resistance is gene amplification, in which there are multiple copies of the target site present and the glyphosate molecules cannot bind to all sites. These mechanisms of resistance in weeds render glyphosate ineffective (Bromilow and Chamberlain, 2000; Gaines et al., 2010). 38 weed species have developed resistance to glyphosate, and despite its continued heavy use, the herbicide is limited in broad spectrum control (Heap, 2018).

Resistant weeds are a large management issue for growers in the Midwest. Species of common ragweed, giant ragweed, and waterhemp have all been identified as prominent weeds having herbicide resistance (Heap, 2014). Specifically it has been found that the decline in glyphosate effectiveness, and rise of glyphosate resistant weeds cost soybean growers an average of $8.00/acre in 2012, and reduced returns by $22.00/acre that same year (Livingston et al., 2015). If herbicides became completely ineffective due to weed resistance, economic losses on corn and soybean acres would total $43 billion annually in the U.S. and Canada. Yield losses in soybeans would exceed 49%, while yield losses in corn would exceed 52%. This cost is an unacceptable burden for agriculture, and other weed management techniques must be investigated.

Soybean Cyst Nematode

Soybean cyst nematode (SCN, *Heterodera glycines*) is the most prevalent pest affecting soybean yield in the United States. The estimated yield reduction reported in 2009 was 120,048,000 bushels (Koenning and Wrather, 2010), at an annual cost of $1.2 billion dollars. In heavily infested fields SCN has been shown to reduce soybean yield by greater than 30% (University of Minnesota-Extension, 2011). SCN was first detected in

Minnesota in 1978 (MacDonald, 1980), and has since spread to 67 counties in Minnesota (Tylka and Marett, 2017). Since its discovery in the state, SCN has been reported to be a major limiting factor in soybean production (Chen et al., 2001).

Nematodes are classified as multicellular, triploblastic, unsegmented aquatic animals. Currently nematodes are regarded in a separate phylum, Nematoda under the kingdom Animalia (De Ley and Blaxter, 2002). The soybean cyst nematode is part of the family Heteroderidae which consists of 18 genera. All members of family Heteroderidae are sedentary parasites. They are characterized by the tanning and drying, otherwise known as cutinization, of the body wall of the sedentary adult female following egg production. The adult female cyst of the soybean cyst nematode can be identified in the soil with the naked eye as they are typically over 1mm in length. The cyst is lemon-shaped with a small terminal cone, setting it apart from other nematodes of the family Heteroderidae. (Robinson et al., 1996). Nematode morphology consists of numerous organ systems typical of the kingdom Animalia, including the body wall, digestive system, nervous system, secretory-excretory system, and the reproductive system. Of special importance to the soybean cyst nematode life cycle is the digestive system, which facilitates the formation of the feeding cell, the nervous system, which allows the nematode to sense stimuli, and the reproductive system, which allows for sexual reproduction and increases genetic diversity.

The life cycle of the soybean cyst nematode consists of an egg, four juvenile stages, four molts, and an adult stage. In general, the nematode life cycle is as follows: Egg_J1_M1_J2_M2_ J3_M3_J4_M4_adult nematode (Figure 1.1). In SCN the first-stage juvenile (J1) develops within the egg, and then molts to the second-stage juvenile (J2) prior to egg hatch. Egg hatch from encysted eggs occurs in three different phases. The first phase

is constitutive hatching. Constitutive hatching occurs whether or not a suitable host plant is present. The large majority of constitutive hatching occurs the first year after fertilization. The second phase of hatching is inducible hatching. These inducible eggs only hatch when a host plant is present. The host plant sends out root exudates or chemical signals that are received by the nematode eggs, and hatching is induced. Inducible hatching will not occur when a non-host crop is in the rotation. The third phase of hatching is dormancy. Dormancy in SCN is induced when temperatures cool in the fall; for constitutive and inducible eggs the dormancy is broken in the spring if other conditions are met (Alston & Schmitt, 1988; Masamune, Anetai, Takasugi, & Katsui, 1982). These conditions are related to environmental stimuli such as temperature and moisture availability (Masler & Rogers, 2011). Unlike the other two types of hatching, despite favorable environmental stimuli, dormant eggs will not hatch. Dormant eggs have been reported to survive up to eight years in the soil. The J2 nematode hatches from the egg and as an aquatic animal, it moves across the film of water present on the soil particles toward the host plant. The nematodes use a process called chemotaxis to move toward and find a host plant. Chemotaxis is believed to be regulated by the nervous system of the nematode and provide guidance for the nematode toward the source of stimulation. In the case of SCN, the source of stimulation is root secretions from host plants (Rasmann et al., 2012; Hu et al., 2017). After locating a target plant, the J2 nematode penetrates the root of this host plant within a few minutes and causes necrosis at the penetration site. The J2 migrates to the feeding site by cutting a slit in the walls of cells with their stylet. The J2 juvenile establishes a feed site near the vascular system. This feeding site is called a syncytium. The syncytium is a multinucleate cell with one enlarged nuclei. This multinucleate cells arises from the

coalescing of adjacent cells. The syncytium functions as a feeding cell providing the nematode with the nutrients it needs from the plant vascular tissue. The female nematode acts as a metabolic sink, in which the nutrients are transferred from the plant to the syncytium, and then to the nematode. The living nematodes are required for the maintenance and development of the syncytium. If the nematode dies, the syncytium will degrade (Gipson et al., 1970). The nematodes develop into the J3 juvenile inside of the root, at the fourth molt stage the male nematodes leave the root, while the female nematodes remain inside the root but rupture the root cortex. This root rupturing as well as the subsequent formation of the vulva, allows for sexual reproduction between the male in the soil matrix and the female still inside of the root. The J4 male juveniles are attracted to the females by sex pheromones, namely vanillic acid, and are able to find and fertilize the J4 females still inside the roots (Jaffe et al., 1989). After fertilization, the female releases a gelatinous matrix of eggs into the soil, although a large number of eggs are retained within the female body (400-600). Subsequently, after the female dies, her cuticle tans and forms a tough protective cyst, encasing eggs. This is the overwintering and survival structure of the soybean cyst nematode. The typical life cycle of SCN is completed within 21-30 days under the optimal temperature of 25°C (Lauritis et al., 1983). In the Midwest 3-4 full SCN life cycles can occur in a single season. Eggs from a single female range from 40-600, but the average is 200 per female (Sipes et al., 1992).

SCN consists of different variants that are able to infect and reproduce on certain lines of soybean. Although not morphologically distinct, the SCN variants are able to infect different types of genetically distinct soybean varieties. These SCN variants are termed HG types. HG types are determined by testing the SCN populations on soybean cultivars

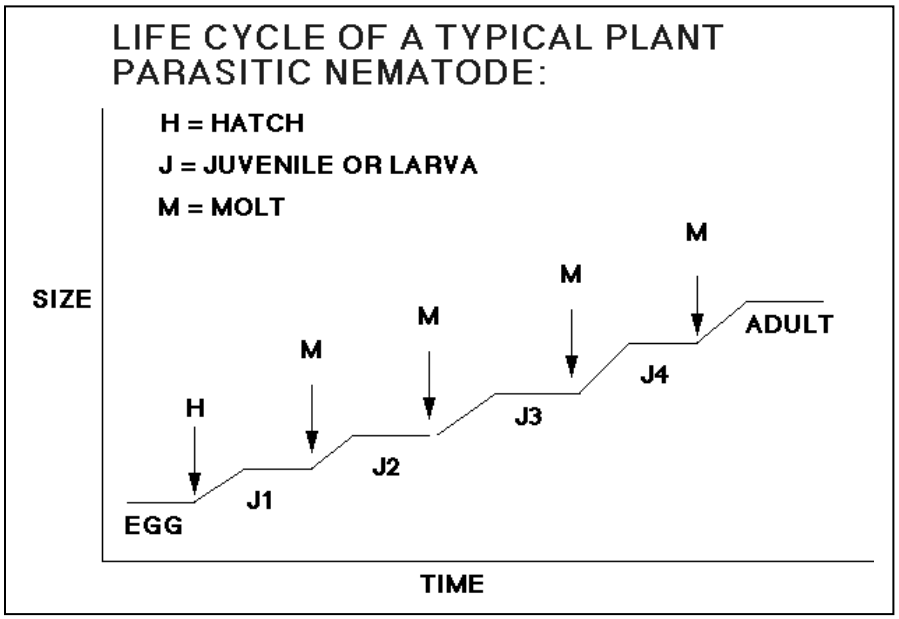
known as differentials. There are seven differentials used to determine HG Types. Lee 74 is used as the standard susceptible for calculation of the Female Index (FI). Female index values less than 10 are considered resistant (Niblack et al., 2002). Currently in soybean SCN is controlled through the deployment of resistant soybean cultivars. Today, in the United States, 95% of the soybeans available on the market contain the PI 88788 type resistance locus. The remaining 5% of soybeans on the market contain Peking and/or PI 437654 type resistance (Joos et al., 2013; Tylka and Mullaney, 2015). Resistance to SCN due to these sources of resistance in soybean cultivars is not a function of the lack of penetration into the root but rather the lack of full female development once inside of the root (Li and Chen, 2005). In resistant soybeans, the nematode is unable to establish or maintain a feeding cell. In PI-88788 type resistance, the Rhg1 gene is required in high copy number. The Peking type resistance has a low copy number of Rhg 1, but also requires Rhg4 to confer resistance to SCN (Concibido et al., 2004). PI-88788 resistance is breaking down due to its overuse and lack of rotation with other sources of resistance. About 70% of SCN populations have begun to overcome host resistance due to PI 88788, thus reducing its effectiveness (Niblack et al., 2007). With the breakdown of the major resistance deployed on the landscape, other options may be needed to control SCN.

Pennycress and camelina, as members of the Brassicacaceae family, have been shown to contain compounds known to have pesticidal (and nematicidal) properties. The brassica species contain glucosinolates. The degradation of these glucosinolates leads to biologically active products such as isothiocyanates and oxazolidinethiones which act as natural pesticides (Fenwick and Heaney, 1983; Gimsing and Kirkegaard, 2008). A greenhouse study that assessed organic soil amendments and their effects on SCN levels,

showed that pennycress seed powder reduced SCN population levels.(Grabau and Chen, 2014). These results indicate that the biologically active derivatives of the glucosinolates present in the seed powder, exhibit a negative effect against SCN populations in the soil. Contrary to the results of the seed powder experiment, pennycress has been shown to be a host to SCN (facilitates reproduction) in SCN weed surveys performed in the greenhouse (Venkatesh et al., 2000; Poromarto et al., 2015). The effect of pennycress on SCN in the field has not been tested. Camelina has also been tested in similar SCN weed surveys and it has been found to be a poor host of SCN (does not facilitate reproduction) (Poromarto and Nelson, 2010). A poor plant host can act as a trap crop of SCN and reduce reproduction ability and population numbers (Chen et al., 2001; Warnke et al., 2006). Like pennycress, camelina has not been tested on the field scale.

Tables and Figures

Figure 1.1 The general life cycle of a nematode.



M denotes the four molt stages of a parasitic nematode life cycle.

Chapter 2 : Light competition and weed suppression in winter annual oilseed relay-cropping system

Materials and methods

Experimental Description

Field experiments were conducted in 2015, 2016, and 2017 at the University of Minnesota Rosemount Research and Outreach Center in Rosemount, MN (44°43'04"N 93°05'53"W), the Southwest Research and Outreach Center in Lamberton, MN (44°14'22"N 95°18'43"W), and the United States Department of Agriculture Swan Lake Research Farm near Morris, MN (45°41'14"N 95°47'59"W). The soil at the Rosemount site was a well-drained Waukegan silt loam (fine-silty, over-sandy mixed, mesic Typic Hapudoll) with a pH of 6.5 and an organic matter content of 3.8%. The soil at the Lamberton site was a well-drained Normania loam (fine-loamy, mixed, mesic Aquic Hapludoll) silt loam with a pH of 6.1 and an organic matter content of 3.8%. The soil at the Swan Lake site was a well-drained Barnes loam soil (fine-loamy, mixed, superactive, frigid Calcic Hapludoll) with a pH of 7.0 and an organic matter content of 3.9%. All field sites tested high for soil K and P.

The experimental design was a factorial design with four replicates. Two soybean cultivars were used: one cultivar containing soybean cyst nematode (*Heterodera glycines*, SCN) resistance ('Peking'), and a second cultivar not containing SCN resistance. There two oilseed treatments plus the no-oilseed control. The 4.6 m wide by 9.1 m long plots were planted with either pennycress ('MN106'), or winter camelina ('Joelle'). The control plots did not contain winter oilseeds.

Field pennycress and winter camelina were planted at a rate of 16.8 kg ha^{-1}, both with 95% live seed germination. The pennycress was treated with gibberellic acid (GA3)

for 24 hr to achieve the 95% seed germination rate (Metzger, 1983; Karimmojeni et al., 2014). ProGibb® T&O Plant Growth Regulator Solution was used for the gibberellic acid treatment at a concentration of 0.08% (w/w) of active ingredient. The winter oilseeds were planted with a high-clearance interseeder with 2.1 m of clearance, equipped with a Gandy® Orbit-Air Cover Crop Seeder (Gandy Company, Owatonna, MN). The seed was direct-broadcasted and incorporated between standing corn rows at stage R5, from 20-28 August 2015. The corn was harvested for silage from 5-10 September 2015. The oilseed stands at Lamberton and Rosemount were damaged with the single-row silage harvesters that were used. The single row harvesters resulted in substantial wheel traffic on the growing winter annuals, prompting a need for reseeding. The Morris site was harvested with commercial silage harvest equipment, thereby avoiding tire damage to the oilseeds. Since two of the three sites suffered significant tire damage, the winter annual oilseeds at all locations were reseeded at 16.8 kg ha^{-1}. The winter oilseeds were re-planted 14 to 18 of September 2015 using a Brillion® seeder with 10 cm spacing. A fertilizer application of N-P-K at 78-34-34 kg ha^{-1} was broadcast over each plot from 15 to 20 April 2016. Well-adapted full season soybean maturity groups were selected for each location. For the Rosemount and Morris sites, Nutech 7186, Peking source resistance to SCN, 1.7 maturity group, with glyphosate [N-(phosphonomethyl) glycine] resistance, and Pioneer P16T04R, susceptible to SCN, 1.6 maturity group with glyphosate resistance were planted. At Lamberton, Pioneer P22T69R, Peking source resistance to SCN, 2.2 maturity, with glyphosate resistance, and Pioneer P91Y90 susceptible to SCN, 1.9 maturity, with glyphosate resistance were planted. The soybeans were no-till planted at Lamberton, Rosemount, and Morris on 20 May, 6 May, and 30 April 2016, respectively, at a rate of 320,000 seeds ha^{-1} and 76 cm row-spacing.

The control "fallow" plots received a glyphosate application at a rate of 0.84 kg a.e. ha^{-1} 20 to 23 May 2016 to control emerging weeds, whereas weed control was not required in the winter annual plots. All plots received a glyphosate treatment at the 0.84 kg a.e. ha^{-1} rate 20 to 25 June 2016 after oilseed harvest.

Aboveground biomass of the winter oilseeds was hand-harvested in 0.25 m^2 quadrats, samples were collected in each plot 14 to 17 June for pennycress and 16 to 24 June for winter camelina. The remainder of the plots were harvested with a plot combine keeping the cutting bar 5 cm above the growing soybeans. The harvest samples were dried for 3 d at 40°C to obtain dry matter weight. Overall winter oilseed biomass, prior to seed threshing was recorded. Threshed oilseeds were cleaned and weighed. The soybeans were harvested 10 to 13 October 2016 using a plot combine. The middle two rows of every plot were harvested as the representative sample for the plot. The harvested samples from the combine were dried, weighed, and weights adjusted to 13% moisture.

Corn was planted 5 to 11 May 2017. In conjunction with corn planting, N-P-K at 168-34-34 kg ha^{-1} was broadcast over the experimental site. The cultivar planted was Dekalb® DKC49-72RIB, 99 d relative maturity with glyphosate resistance. The corn was planted on a 76 cm row spacing with 89,000 seeds ha^{-1}. On all plots, a tank mix of clopyralid (0.59 kg a.e. ha^{-1}) and glyphosate at a rate (0.84 kg a.e. ha^{-1}) was applied 12 to 15 June 2017, to control emerging weeds. The corn was harvested 2 to 8 November 2017 using a plot combine. The middle two rows of every plot were harvested as a representative sample. The harvest samples were dried and adjusted to 15.5% moisture.

Experimental Measurements

Photosynthetically active radiation (PAR) interception was measured after the soybeans were interseeded into the growing winter oilseed crops. Five above- and below-canopy measurements were recorded in each plot using a line quantum sensor (Model 191 SB, Li-Cor, Lincoln, NE). The bi-weekly measurements were made between 1200-1400 h on sunny days until winter oilseed harvest. For below canopy measurements, the sensor was placed at the average height of the emerging soybeans (1-10cm) under the winter oilseed crop canopy. For above canopy measurements the sensor was placed horizontally 1 m from the ground. Tau (τ) was calculated as the ratio of below canopy to above canopy PAR measurements ($\tau = \frac{I_A}{I_0}$, where I_A was the above canopy interception, and I_0 was the below canopy interception). Soybean populations, height, and relative maturities were assessed prior to oilseed harvest, 14-24 June, 2016. The two middle rows of soybeans in each plot were assessed for height, number of plants in a 1 m length, and relative maturity using visual scoring. Two measurements of soybean height, soybean populations, and soybean relative maturities were taken in each plot. The natural weed populations in the plots were assessed for dry weight 15-19 May 2016, prior to glyphosate application control plots. Weed biomass in two randomly placed 0.25 m^2 quadrats was collected, dried for 3d at 40°C, and weighed.

Statistical Analysis

The experiment was a factorial design with each treatment occurring independently. Linear mixed effect models were used to estimate the effects of soybean cultivar and oilseed crop treatment on seed yield, crop biomass, and weed biomass (RCoreTeam, 2016). Analytical assumptions for an ANOVA were examined by graphical

inspection of the residual plots. Block was treated as a random effect in the model. Soybean cultivar and oilseed crop treatment were treated as fixed effects. Soybean cultivar was included as a fixed effect in the full model because the soybean cultivars were from unique genetic backgrounds and varied in relative maturity. These physiological differences in the cultivar affected the response variables tested. A generalized analysis across all locations for the parameters was prevented due to treatment interactions ($P< 0.05$), thus the three locations were analyzed separately. At Morris, soybean stand counts in one of the blocks were 40% lower than the stand counts in the other three blocks. This block was in a low-lying area. For this reason this block and its respective plots were excluded from analysis. At Rosemount, Thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*) infested nine corn plots during 2017; these plots were excluded from analysis. The mean separation procedure used to investigate the significant differences between treatments was Fisher's LSD with an associated $P< 0.05$.

Results

Precipitation and Temperature

At all sites, the maximum and minimum air temperatures were slightly higher than the 30-yr (1984-2013) averages in the three years of the study. The minimum air temperatures were notably higher than the 30-yr average in the winter months of November- February at all sites. The deviation from the mean was 3-4°C in these winter months at all sites. The maximum air temperatures were highest in July at all three sites and was consistently between 28-30 °C in all years recorded. This was consistent with the 30-year average. Yearly precipitation amounts were higher than the 30-yr average at all sites in all years (Figure 2.1), but the timing of precipitation varied from normal patterns.

At Morris and Rosemount, lower than average precipitation occurred during the months of April, May and June of 2016, a time of critical crop-crop interaction in the oilseed relay cropping system.

Yield

Across all locations, the soybean cultivar (S) and the interaction between soybean cultivar (S) and oilseed crop (O) treatment (i.e., pennycress, and winter camelina) did not impact oilseed biomass (Table 2.1). Oilseed crop (O) treatment as a main effect, did impact the oilseed biomass. Pennycress and winter camelina differed in biomass production at all three sites ($P< 0.05$; Table 2.1). Winter oilseed biomass was greater for camelina and exceeded the biomass of pennycress at all locations. Camelina biomass was greatest at Morris, followed by Rosemount, and Lamberton. Pennycress biomass yield followed the same trend with the highest oilseed biomass at Morris, followed by Rosemount, and Lamberton (Table 2.2). Winter oilseed seed yields were impacted by winter oilseed crop (O) treatment, but not soybean cultivar (S) or the interaction soybean cultivar (S) and oilseed crop (O) treatment ($P< 0.05$; Table 2.1). At Morris, camelina seed yield was 30% greater than the pennycress yield. At Rosemount and Lamberton there was no significant difference between the camelina and pennycress seed yields ($P< 0.05$; Table 2.2). Soybean yields were not impacted by the soybean cultivar (S), the oilseed crop (O) treatment, or the interaction soybean cultivar (S) and oilseed crop (O) treatment ($P< 0.05$; Table 2.1) at the Lamberton location. At the Morris and Rosemount locations soybean yields were not impacted by the soybean cultivar (S), or the interaction soybean cultivar (S) and oilseed crop (O) treatment but were impacted by the winter oilseed crop (O) treatment ($P < 0.05$; Table 2.1). The soybean yields were reduced in the winter oilseed treatments by

approximately 20% compared to the mono-cropped soybean treatment at Rosemount. At Morris, the soybean yields in the oilseed treatments were reduced by 41% compared to the mono-cropped soybean treatments. Total combined yields (winter oilseed crop + soybean) were not affected by soybean cultivar (S), the winter oilseed crop (O) treatment, nor the interaction soybean cultivar (S) and oilseed crop (O) treatment *(P< 0.05:* Table 2.2*)* at Morris or Rosemount. At Lamberton, total combined yields were affected by the winter oilseed crop (O) treatment, but not by soybean cultivar (S) nor the interaction soybean cultivar (S) and oilseed crop (O) treatment *(P< 0.05:* Table 2.2). The Rosemount and Morris locations had combined yields that did not differ from the mono-cropped soybean yield at those sites. At Lamberton the combined yields produced 20% greater total seed yield than the mono-crop soybeans. At all locations, corn yields taken in 2017, following the relay-cropping in 2016, were not affected by soybean cultivar (S), the winter oilseed crop (O) treatment, nor the interaction soybean cultivar (S) and oilseed crop (O) treatment *(P< 0.05:* Table 2.2*)*.

Light and Crop-Crop Competition

For both Lamberton and Rosemount the below canopy light interception for the growing soybean plants was consistently between 40-50% of the total light reaching the top of the canopy for both the pennycress and camelina cropping systems (Figure 2.2). At Morris, the TAU values in both cropping treatments were lower than that of the other two locations. The TAU values were below 0.3 for the pennycress treatments and below 0.2 for the camelina treatments indicating only 20-30% of available light was reaching the below-canopy soybeans (Figure 2.2).

At Lamberton, the soybean populations and soybean heights were not impacted by the soybean cultivar (S) or the winter annual crop (O) treatment, nor the interaction soybean cultivar (S) and oilseed crop (O) treatment ($P < 0.05$; Table 2.1 and Table 2.3). Soybean development, measured at oilseed harvest was hindered by the winter oilseeds. The soybean growth stage in winter oilseed plots averaged R4 compared to the R5-R6 in the fallow treatments (data not shown). At Rosemount soybean populations were not impacted by the soybean cultivar (S), the winter oilseed crop (O) treatment, nor the interaction soybean cultivar (S) and oilseed crop (O) treatments ($P< 0.05$; Table 2.1 and Table 2.3). Soybean height was impacted by oilseed crop (O) treatment, but not impacted by the soybean cultivar (S), or the interaction of the soybean cultivar (S) and oilseed crop (O) treatments ($P< 0.05$; Table 2.1 and Table 2.3). The soybean growing under pennycress canopy were 23% taller than the mono-cropped treatment, and those under camelina were 45% taller than the soybeans in the mono-cropped treatment (Table 2.3). The soybean development in the winter annual plots at Rosemount averaged R3, while the development in the fallow plots averaged R4 at the time of winter oilseed harvest. At Morris, the soybean crop was impacted by winter oilseed crop (O) treatment ($P< 0.05$; Table 2.1 and Table 2.3) for both the height of the soybeans and the populations, but these attributes were not impacted by the soybean cultivar (S), or the interaction of the soybean cultivar (S) and oilseed crop (O) treatments ($P< 0.05$; Table 2.1). The soybeans in the pennycress plots were 46% shorter than the mono-cropped soybeans, and the soybeans in the camelina plots were 35% shorter than the mono-cropped soybeans at the time of winter oilseed harvest. Soybean establishment and development were reduced in the winter oilseed plots at Morris. The camelina and pennycress plots had 45 and 50% lower soybean population densities

than the mono-cropped soybean treatment, respectively (Table 2.3). At Morris, the growth stage of soybean in the winter oilseed plots ranged VE-V1, while the range of development in the fallow plots was V3-V4.

Weed Suppression

At both Rosemount and Lamberton, soybean cultivar (S) and the interaction between soybean cultivar (S) and oilseed crop (O) treatment (e.g. pennycress, and winter camelina) did not impact weed biomass (Table 2.1). However, the weed biomass was impacted by winter oilseed crop (O) treatment ($P < 0.05$; Table 2.1) at both Rosemount and Lamberton. At Morris, the weed seedbank was low due to years of weed-free management, and weeds did not emerge in high enough quantities for assessment. All treatments at Morris exhibited weed biomass less than 2.2 g m^{-2}, and thus, were not analyzed for this site. The mono-cropped soybean treatment was used as the "weedy control," for comparison. The most common weeds at both locations were common lambsquarters (*Chenopodium album* L.) and dandelion (*Taraxacum officinale* Weber ex Wiggers).

Early season weed control was observed at Rosemount for both winter oilseed treatments. The winter camelina treatment exhibited weed biomass of 24 g m^{-2}, while the pennycress treatment had 6 g m^{-2}, as compared to 161 g m^{-2} for the mono-cropped soybean treatment (Figure 2.3). The winter annual crop treatments resulted in 97% and 85% early season weed control for pennycress and camelina, respectively. At Lamberton, early season weed suppression was also observed, the camelina treatment had 6 g m^{-2} of weed biomass, while the pennycress had 0 g m^{-2}. In comparison, the mono-cropped soybean treatment had

49 g m^{-2} of weed biomass (Figure 2.3). The winter oilseed crop treatments suppressed weeds by 88% and 100%, respectively.

Discussion

Yield

The Morris and Lamberton sites deviated significantly from what has been reported in previous literature on this relay-cropping system. At Morris, the inclusion of pennycress and camelina resulted in a substantial soybean yield penalty. Pennycress reduced soybean yield by 41% and camelina reduced soybean yield by 53% compared to the mono-cropped soybean treatment (Table 2.2). The probable cause for this substantial reduction in soybean yield was the abnormally low precipitation in 2016 during May and June (Figure 2.1). The competition for available soil moisture during this time was greater at Morris than the other sites. Soybean yields can be drastically affected by water stress. Studies have indicated soybean yields decreased as much as 55% when severe water stress has occurred in the soybean growing season. Soybeans under stress, including water stress, have been shown to abort pods, have delayed seed set, and mature earlier causing this yield loss (Doss et al., 1974; Foroud et al., 1993). Maximum water use for the winter oilseeds, like many cover crops species that grow in the spring, occurs around late May through June. This time period coincides with late-development and seed fill when soil water content is drawn down by the growing cover crops (Clark et al., 1997; Nielsen et al., 2015). Gesch and Johnson, 2015 found that the inclusion of camelina in the soybean cropping system resulted in 26-50 mm less water in that rotation compared to mono-crop soybean. The additional water use of the winter annuals, along with the low precipitation amounts was likely causal in the soybean yield loss. In Rosemount, lower than average precipitation also occurred

during the period of May and June. This likely penalized soybean yields for the same reasons as those at Morris. Previous studies investigating the winter oilseed-soybean relay-cropping system reported a 20-35% reduction in soybean yield caused by the inclusion of pennycress and camelina (Gesch et al., 2014; Johnson et al., 2017). Similar results were recorded at Rosemount where soybean yields were reduced by pennycress by 18%, and camelina by 19% compared to the mono-cropped soybean treatment (Table 2.2). At Lamberton soybean yields were not affected by the inclusion of the pennycress or camelina crops in the system, while the winter oilseeds still yielded 0.8 Mg ha^{-1} and 1.2 Mg ha^{-1}, respectively. The camelina-soybean and pennycress-soybean cropping system had a total yield (camelina and soybean combined) that exceeded the yield of the mono-cropped soybean by approximately 20% (Table 2.2). Lamberton received notably high precipitation during May 2016, and combined May plus June precipitation exceeded the 30-year average. Water was a limiting factor for soybean development in the months of May and June at both the Morris and Rosemount locations. Water limitation was likely a contributing factor to soybean yield loss at these two sites when comparing the winter annual crop treatments to the mono-cropped soybean treatment.

Another factor related to water use that likely contributed to potential water stress and subsequently lower soybean yields at Morris compared to the other sites, was the extensive productivity of the winter oilseeds. Biomass productivity in Morris was as much as two-fold greater than the other sites (Table 2.2). It is well known that there is a direct positive correlation between biomass accumulation and evapotranspiration (Nielsen et al., 2006), which also likely impacted soil moisture availability for soybeans in the relay treatments in the present study. Water limitation may have not been the only yield-limiting

factor, however. Light competition for the soybeans under the growing winter oilseed canopy likely also played a role.

Despite the soybean yields being decreased at two-of-three sites tested, the overall seed production of the winter oilseed treatments (oilseed + soybean) was the same as, or exceeded, the seed production of the mono-cropped soybean treatment. Total yield is an important indicator of the success of this system. The fact that soybean yield was decreased at two sites should not degrade the prolific production ability of this system. However, the mechanisms by which soybean yield was affected should be examined.

Competition and Crop-Crop Interaction

Stem lengthening, or etiolation, is a well-documented soybean response to light stress (Green-Tracewicz et al., 2012; Liu et al., 2015). The low light levels being intercepted by the soybeans at Rosemount, along with the overall light depreciation under the winter annual canopy, resulted in architectural changes in soybean. The soybeans developed a vine-like architecture with thinner and taller stems, and underwent other etiolation physiological responses that resulted in reduced plant vigor and yield (Van Acker et al., 1993; Page et al., 2010; Green-Tracewicz et al., 2012; Ruberti et al., 2012; Wu et al., 2016). The 19-21 day light competition, along with less than 50% of the photosynthetically active radiation penetrating the winter oilseed canopy during the critical light period resulting in the etiolation response, gives an indication of why the soybean yields were significantly reduced in the winter oilseed plots at the Rosemount site (Figure 2.2).

At Morris the winter oilseed yields were far higher than seen in previous literature (Gesch et al., 2014; Johnson et al., 2017). This was likely due to the high seeding rate at Morris, where the tire traffic from the silage harvester was lower than the traffic at the other

two sites. At Rosemount and Lamberton the tire traffic killed the growing oilseeds, at Morris this did not occur. All sites were replanted regardless of surviving oilseed populations, which resulted in denser winter oilseed stands at Morris. Higher seeding rates have been correlated with higher plant densities and higher oilseed yields in camelina (Urbaniak et al., 2008). Thus, at the Morris location the crop-crop interaction was greater than that at the other sites. The accumulation of oilseed biomass severely stressed the growing soybeans under the canopy. The soybeans still elicited an etiolation stress response at Morris as can be seen by the images in Figure 2.4. The soybeans portrayed the classic vine-like structure seen at Rosemount (Green-Tracewicz et al., 2012; Liu et al., 2015). However, the etiolation response did little to improve the light condition for the soybeans, but did reduce soybean fitness (Tateno and Taneda, 2007; Ruberti et al., 2012). The reduced soybean fitness and substantial light stress, along with moisture stress under the canopy, likely resulted in the short phenotype and soybean death response. The substantial biomass of the winter oilseeds, competition, low light interception levels below the winter oilseed canopy, and abnormally low precipitation greatly affected soybean growth in the winter oilseed treatments. This is reflected in the soybean yield data in which the winter oilseeds reduced the soybean yields by 40-50% at the Morris (Table 2.2).

Light is vitally important to growth and yield of soybean in relay-cropping systems. This was apparent when looking at the effects of varying stands of the winter annual crop had on soybean yield. When overlap of soybean and cover crop in the system was minimized as at the Lamberton site, soybean yields were unaffected. When light competition was worsened for the soybeans as at Rosemount, and more apparently at the Morris, the soybean yields were greatly reduced. Lack of precipitation during May and

June also appeared to exacerbate the problem. The soybean stand populations were reduced by the cover crop treatments in Morris. Likely a function of the elicted etiolation response which reduced soybean fitness, and the long duration of light and precipitation stress. The soybean stress response, light and precipitation interactions, and agronomic practices for soybean planting into winter oilseed cover crops need to be further explored.

One agronomic practice of interest that may result in reduced light and water resource competition for the soybeans is a skip-row planting technique (Duncan and Schapaugh, 1997). This technique was used by Gesch et al. (2014), who blocked every third opener (61 cm) on a no-till drill with 19-cm wide row spacing to plant winter camelina in autumn. The soybeans were then relay-planted in 61-cm spaced rows the following spring. This allows for reduced resource competition for the germinating and growing soybeans (Gesch et al., 2014). Moreover, this technique helped optimize the growing environment for the relayed soybean, resulting in lower yield penalties. This type of skip-row technique would not have been possible with the planting strategy used in this study (broadcast seeding in-between standing corn rows). However, this technique could have been simulated in a broadcast planting system by band-killing the winter oilseeds with herbicide at row spacing intervals conducive to the soybean planter used, or band-tilling at the same width with a field implement. An agronomic technique in which the soybean plants endure less light and water resource competition must be investigated to optimize yields of both the winter annual and the soybean.

Weed Suppression

Resistance weed biotypes are on the rise in agriculture, as cropping system diversity continues to decrease, there has been an overall increase in the number of herbicides used

to control weeds (Aguilar et al., 2015). Weed resistance to herbicide mode of actions has begun to affect chemical weed control. This is especially true in corn and soybean systems which have experienced heavy herbicide use and herbicide selection pressure. Resistant weeds are a management issue for corn-soybean producers. The inclusion of the cover crops that overwinter and provide spring weed competition in corn-soybean rotations have been shown to reduce weed biomass of both summer and winter annual weeds by up to 95 and 98%, respectively when compared to the no-oilseed crop control (Yenish et al., 1996; Fisk et al., 2001; Hayden et al., 2012). Likewise, in the present study it was found that winter oilseed crops resulted in lower weed biomass when the oilseeds were present in the system. In this study, camelina reduced weed biomass by up to 88%, while pennycress reduced weed biomass by up to 100%. The winter oilseeds reduce weed biomass in the spring and early-summer when they are present in the system. Cover crops and these winter oilseed crops may be an integral part of a pest management program for early emerging and herbicide resistant weeds such as ragweed, and common lambsquarters. However, it is likely the same competition mechanisms that result in soybean yield loss in the relay system also hinders weed emergence and development. The trade-offs between weed suppression and soybean yield by the winter oilseed crops requires further investigation.

Lastly, one agronomic practice of interest that may result in reduced light and water resource competition for the soybeans is a skip-row planting technique (Duncan and Schapaugh, 1997). This technique was used by Gesch et al. (2014), who blocked every third opener (61 cm) on a no-till drill with 19-cm wide row spacing to plant winter camelina in autumn. The soybeans were then relay-planted in 61-cm spaced rows the following spring. This allows for reduced resource competition for the germinating and growing

soybeans (Gesch et al., 2014). Moreover, this technique helped optimize the growing environment for the relayed soybean, resulting in lower yield penalties. This type of skip-row technique would not have been possible with the planting strategy used in this study (broadcast seeding in-between standing corn rows). However, this technique could have been simulated in a broadcast planting system by band-killing the winter oilseeds with herbicide at row spacing intervals conducive to the soybean planter used, or band-tilling at the same width with a field implement. An agronomic technique in which the soybean plants endure less light and water resource competition must be investigated to optimize yields of both the winter annual and the soybean.

Conclusions

The markets for camelina and pennycress are still developing. The markets for the oils and proteins produced by these winter oilseeds are being investigated for food-use, human protein supplement, animal feed, biodiesel, and other bio-based products. With new markets emerging it is difficult to quantify the price that the winter oilseeds will garner. The prioritization of oilseed crop versus soybean crop in this relay-cropping system will likely be affected by market conditions. The results of this experiment shed light on agronomic techniques to maximize profit of the winter oilseeds and/or the soybeans grown in the system.

Overall, the winter oilseed relay system has the potential to out-yield mono-cropped soybean by 20%, when examining total seed yield of the system. Light, timing, and precipitation play key roles in the establishment and development of relay-cropped soybeans. The longer the soybeans spend in the system with the winter annual crops, the greater the yield penalty. This was likely due to light competition, as was quantified by the

light quantity under the canopy of winter oilseeds, as well as oilseed water use and low precipitation; however, temperature and other micro-environmental effects cannot be dismissed. Early season weeds are suppressed by the inclusion of winter oilseeds in the relay-cropping system. Early season weed suppression up to 100% was recorded for pennycress, and suppression up to 88% for camelina. Light competition was, again, likely the causal factor for decreased germination and development of natural weed populations, but other environmental factors possibly played a role as well. Further research must be initiated to determine the causal effects of the soybean yield penalty and weed suppression in the relay-cropping system. Moreover, light quantity and water availability play key roles in the success of this system, and agronomic techniques should be employed (e.g., skip-row planting) to reduce early season competition between the winter oilseeds and growing soybeans.

Tables and Figures

Fixed Effects (by location)	Winter oilseed biomass	Winter oilseed yield	Soybean yield	Combined yield	Soybean Population	Soybean Height	Weed Biomass
Lamberton							
Soybean (S)	0.47 $_{(1,34)}$	0.89 $_{(1,34)}$	0.65 $_{(1,34)}$	0.01 $_{(1,34)}$	0.65 $_{(1,34)}$	0.13 $_{(1,34)}$	0.01 $_{(1,34)}$
Oilseed (O)	31.66 $_{(2,34)}$***	19.32 $_{(2,34)}$***	0.49 $_{(2,34)}$	11.87 $_{(2,34)}$***	2.58 $_{(2,34)}$	2.74 $_{(2,34)}$	89.85 $_{(2,34)}$ ***
S x O	0.26 $_{(2,34)}$	0.50 $_{(2,34)}$	0.74 $_{(2,34)}$	1.04 $_{(2,34)}$	1.12 $_{(2,34)}$	1.05 $_{(2,34)}$	1.36 $_{(2,34)}$
Rosemount							
Soybean (S)	0.01 $_{(1,36)}$	0.49 $_{(1,36)}$	3.98 $_{(1,36)}$	2.32 $_{(1,36)}$	2.36$_{(1,36)}$	3.00$_{(1,36)}$	1.75$_{(1,36)}$
Oilseed (O)	24.66 $_{(2,36)}$***	18.91 $_{(2,36)}$***	13.65 $_{(2,36)}$***	0.49 $_{(2,36)}$	1.53$_{(2,36)}$	46.18$_{(2,36)}$***	9.05$_{(2,36)}$***
S x O	0.01 $_{(2,36)}$	0.23 $_{(2,36)}$	0.54 $_{(2,36)}$	0.35 $_{(2,36)}$	0.14$_{(2,36)}$	1.10$_{(2,36)}$	1.73$_{(2,36)}$
Morris							
Soybean (S)	0.77 $_{(1,26)}$	2.05 $_{(1,26)}$	0.11 $_{(1,26)}$	1.36 $_{(1,26)}$	0.67$_{(1,26)}$	0.13$_{(1,26)}$	NA
Oilseed (O)	90.64 $_{(2,26)}$***	37.84 $_{(2,26)}$***	76.93 $_{(2,26)}$***	2.48 $_{(2,26)}$	37.50$_{(2,26)}$***	33.41$_{(2,26)}$***	NA
S x O	0.56 $_{(2,26)}$	0.26 $_{(2,26)}$	0.15 $_{(2,26)}$	1.48 $_{(2,26)}$	0.62$_{(2,26)}$	0.94$_{(2,26)}$	NA

Table 2.1. Mixed model analysis of variance for the fixed effects in the full mixed effects model. Each location was analyzed separately. *, **, and *** represent significance of F tests at α = 0.05, 0.01, and 0.001, respectively.

Corn Yield (Mg/ha)	No winter oilseed crop	13.3ns	9.9ns	11.2ns
	Pennycress	12.8ns	9.4ns	11.9ns
	Camelina	13.1ns	9.4ns	12.3ns
Combined yield (winter oilseed + soybean) (Mg/ha)	No winter oilseed crop	4.0ns	4.8a	4.3a
	Pennycress	3.8ns	4.0b	2.5b
	Camelina	3.9ns	3.9b	2.0c
Soybean yield (Mg/ha)	No winter oilseed crop	4.0b	4.8ns	4.3ns
	Pennycress	4.6ab	5.1ns	4.4ns
	Camelina	5.0a	5.1ns	4.8ns
Winter oilseed yield (Mg/ha)	Pennycress	0.8a	1.1a	1.9b
	Camelina	1.2a	1.1a	2.7a
Winter oilseed biomass (Mg/ha)	Pennycress	3.3b	3.4b	4.6b
	Camelina	4.3a	4.7a	9.0a
		Lamberton	Rosemount	Morris

Table 2.2. The oilseed crop treatment effects on winter oilseed seed and biomass, soybean yield, and corn yield. The within row means for a given attribute with different letters denote significant difference at the $P < 0.05$ level using LSD mean separation procedure. ns denotes there was no significant difference within column means at the $P < 0.05$ level.

	Soybean stand count (value/m)			*Soybean height (cm)*		
	Camelina	Pennycress	No winter oilseed crop	Camelina	Pennycress	No winter oilseed crop
Lamberton	26.3 ns	25.4 ns	28.1 ns	10.3 ns	11.3 ns	9.6 ns
Rosemount	22.3 ns	21.8 ns	24.3 ns	30.5 a	25.9 b	21.0 c
Morris	14.6 a	13.2 a	26.4 b	13.0 a	10.8 b	20.0 c

Table 2.3. Oilseed crop treatment effects on soybean population and height.The within row means with different letters denote significant difference at the $P < 0.05$ level using LSD mean separation procedure. ns denotes there was no significant difference.

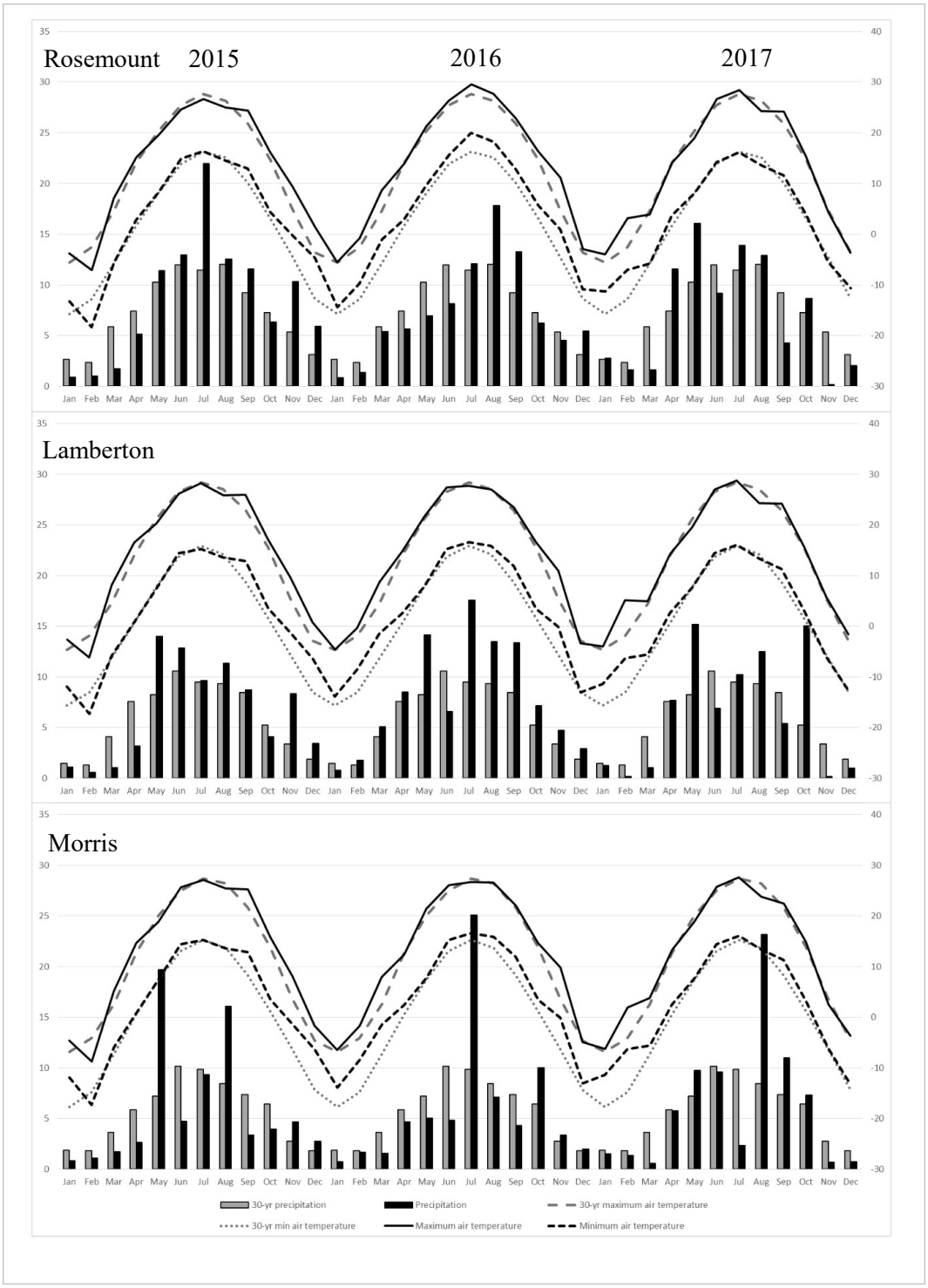


Figure 2.1. Monthly average minimum and maximum air temperatures (°C) and monthly total precipitation in 2015, 2016, and 2017 compared with the 30 year average (1984-2013) at the three sites.

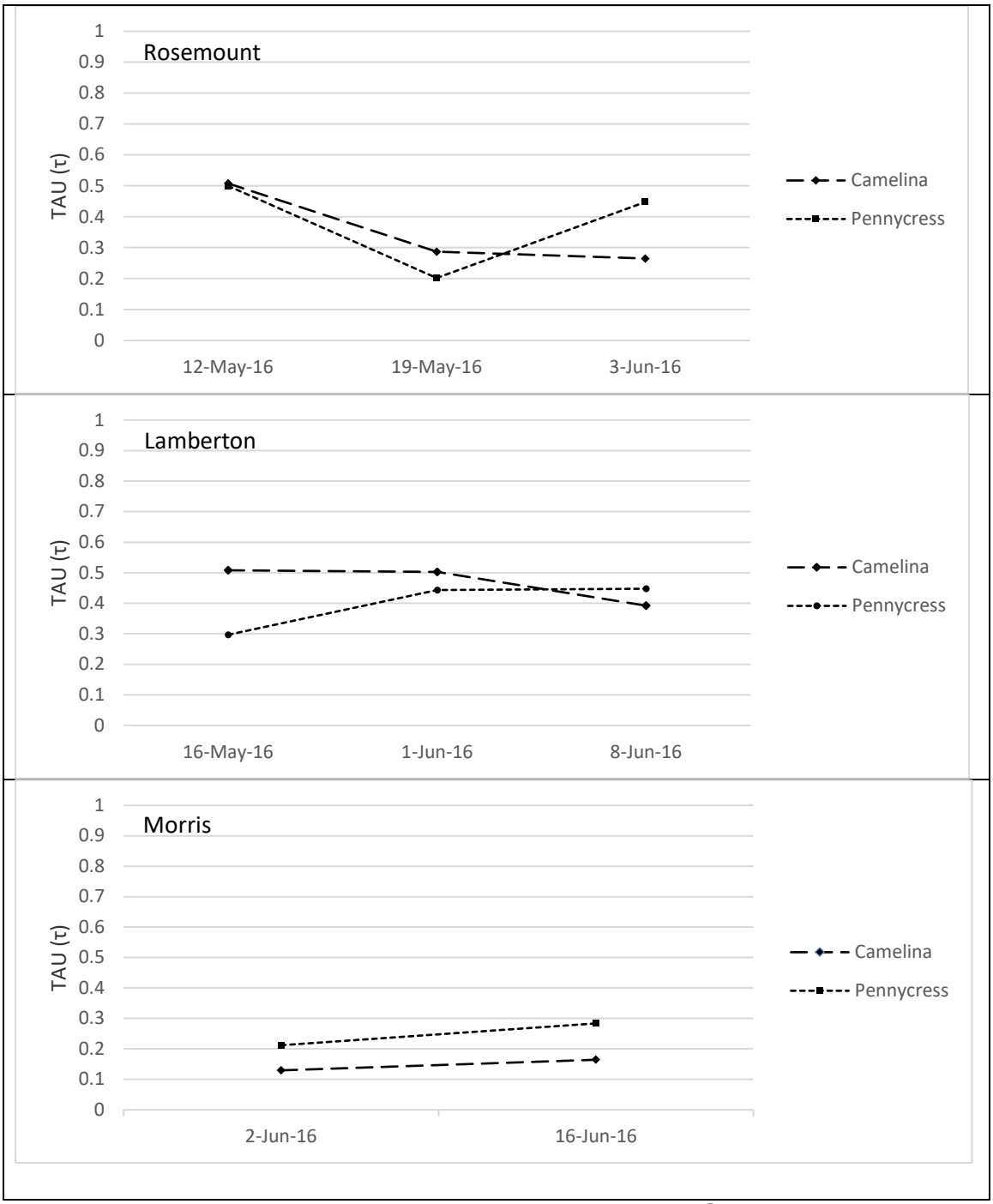


Figure 2.2 Photosynthetically active radiation measured as TAU. $\tau = \frac{I_A}{I_0}$, where I_A was the above canopy interception, and I_0 was the below canopy interception. Error bars represent Standard Error.

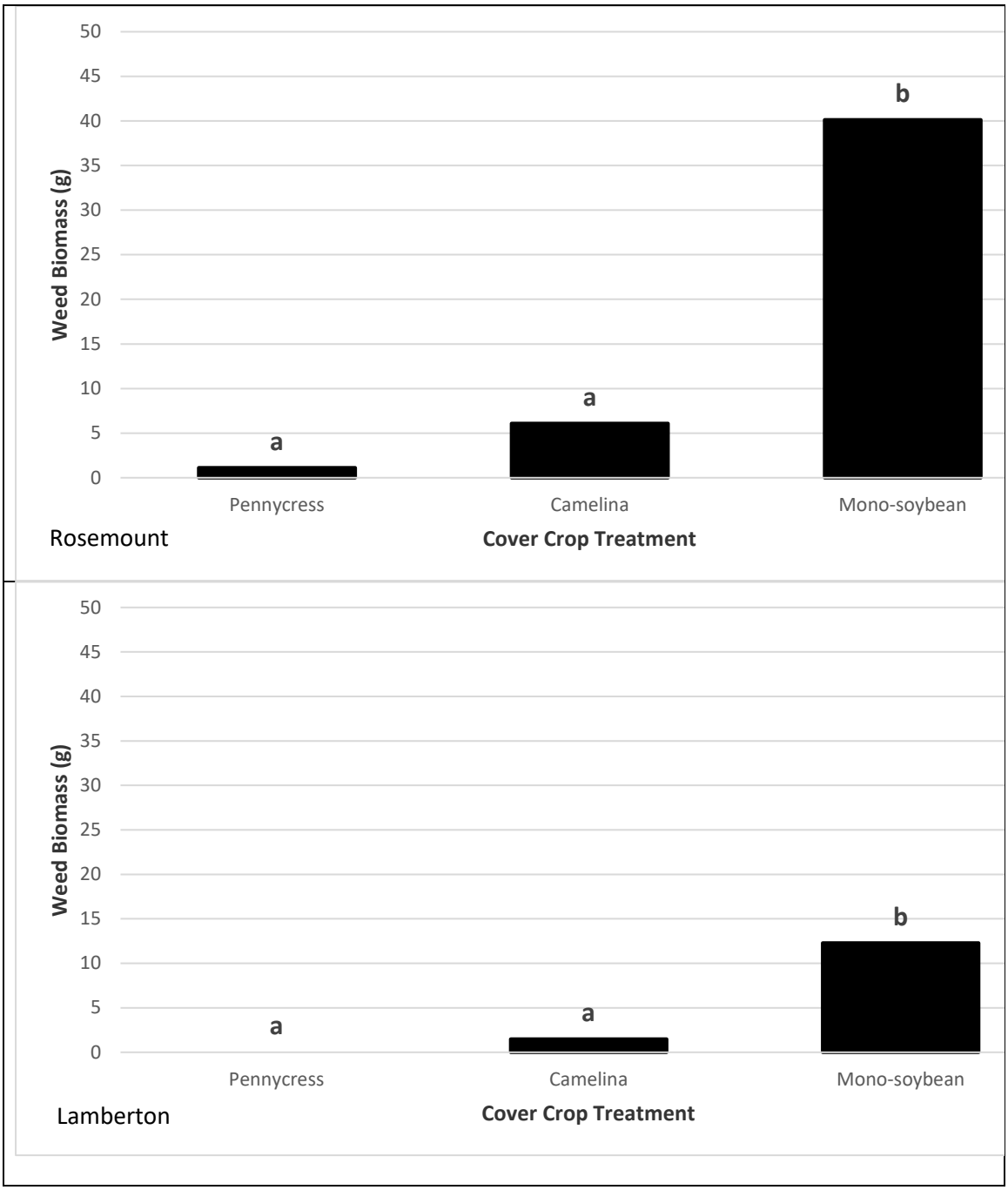


Figure 2.3. Weed control effected by winter oilseed cover cop at the Rosemount and Lamberton experiment sites. Different letters denote significant difference at the $P < 0.05$ level using LSD mean separation procedure.

Soybeans in camelina oilseed treatment	Soybeans in non-oilseed treatment

Figure 2.4. Pictures taken after oilseed harvest in late-June 2016 at Morris showing soybeans with oilseed crop treatment versus non-oilseed crop treatment.

Chapter 3 : Host Suitability of Winter Oilseed Crops Pennycress and Camelina for Heterodera glycines.

Materials and methods

Experiment 1

The experiment was designed as a randomized complete block design with three factors, replicated four times in the greenhouse. The main plot factor was seven crop treatments. The crop treatments were three lines of pennycress and three lines of camelina present within the UMN breeding programs, and a susceptible soybean cultivar. The three lines of pennycress used in the experiment were: MN103, MN106, MN108. The three lines of camelina were: HPX-WG1-35 (WG1), Joelle, and BISON. The susceptible soybean cultivar was 'Sturdy'. The sub-plot factor was HG-Type. The three SCN races used were race 1 (HG Type 2.5.7), race 3 (HG Type 0), and race 14 (HG Type 1.3.6). The sub-sub-plot factor were the inoculation levels. The inoculation levels used were 0, 2,000, and 20,000 eggs 100 cm^{-3} of soil.

A soil without SCN infestation was collected from a field in Waseca. The soil collected was a Nicollet clay loam (fine-loamy, mixed, superactive, mesic Aquic Hapludoll) with a pH of 6.1 and an organic matter content of 3.8%. The soil was screened, mixed, and distributed to four lots that were used in the four blocks (replicates). The soil was heat-treated in a commercial oven for 24 hours at the temperature of 48°C. This temperature has been shown to kill nematodes, and potentially insects and mites while keeping other soil microbes alive (Liu et al., 2016). The soil was mixed again after heating. The soil was divided to 1-kg lots and placed in 1-gallon plastic bags. To each bag 500 grams of sand

was added to facilitate drainage. The bag was then thoroughly mixed to ensure even distribution of sand and soil. SCN was cultured on SCN-susceptible soybean 'Sturdy' in the greenhouse. Females and cysts were collected from roots of 45-d old soybean plants for uniform ages of SCN eggs among the three populations. The eggs were diluted in 10 ml of water and added into each bag. The eggs were mixed into the soil thoroughly. The soil of each bag was used for one 16-cm-diameter plastic pot.

Seedlings of pennycress and camelina were first grown in the greenhouse in cones, with one plant in each cone. 21 d after planting, the seedlings were moved to the growth chamber set to 12-h light d and 4 °C for vernalization treatment. After 21 d, the plants were transplanted to 16-cm-diameter pots in the greenhouse that contained SCN inoculated soil. To transfer the pennycress and camelina plants to a pot, 80% of the previously inoculated soil was applied to each pot. The pennycress and camelina plants were transplanted from the cones to the pots. Four plants were transplanted to each pot and the remaining soil was added.

For the soybean treatments, 10 soybean seeds were planted per pot using the same planting technique as for pennycress and camelina. The pots were maintained in the greenhouse with the temperature set at 28 °C and daylight of 16 h. The pots were watered daily to water-holding capacity. After emergence, the soybean seedlings were thinned to provide five plants per pot. Fertilizer was applied every two weeks at a rate of 25 ml of a solution of Peters Hydrop-Sol 5-11-26 (Water Wise And Organic Gardening Supplies) per pot.

Sixty days after inoculation, the experiment was terminated. The shoots were cut at the soil level, and the samples were dried for 3 d at 40°C to obtain dry matter. The soil in each pot was thoroughly mixed, and a soil subsample of 100 cm^3 was used to extract cysts with a semiautomatic elutriator (Byrd et al., 1976) and then the cysts were separated from debris with centrifugation in 63% (w/v) sucrose solution. The eggs were released from the cysts by crushing the cysts on a 150-µm-aperture sieve with a rubber stopper mounted on a motor and collected on 25-µm-aperture sieve (Faghihi and Ferris, 2000). The eggs were separated from debris by centrifugation in a 35% (w/v) sucrose solution. An aliquot of egg suspension was used to count eggs. The number of eggs per 100 cm^3 of soil was determined.

Experiment 2

This experiment was conducted to determine resistance of germplasm to SCN. The experiment was a randomized complete block design with one factor, replicated three times. The main factor was crop treatment of 119 lines of pennycress from the UMN Germplasm collection. Forty-one spring-type lines, 78 winter-type lines, as well as two susceptible soybean cultivars (‘Sturdy’ and ‘Williams 82’) were included. the same soil without SCN infestation that was used in Experiment 1 was also used for Experiment 2. The soil was screened, mixed, and distributed to six lots. The soil was autoclaved prior to the experiment in a steam autoclave at 121 °C. The soil was mixed again after heating. Cone-tainers (4 cm diameter and 13.5 cm height) were filled with a soil mixture of 50% field soil and 50% clean sand. Pennycress seedlings were treated in gibberellic acid (GA3) for 24h prior to plating to ensure germination. Pennycress seedlings were sown in the

greenhouse. The pots were maintained in the greenhouse with the temperature set at 28°C, and daylight of 16 h. The pots were watered daily to water-holding capacity. After emergence the seedlings were thinned to one plant per cone. Fertilizer was applied every two weeks at a rate of 4 ml of a solution of Peters Hydrop-Sol 5-11-26 (Water Wise And Organic Gardening Supplies) per cone. 21 d after planting, the seedlings were moved to a growth chamber set to 12-h light d and 4°C for vernalization treatment. After 21 d in the growth chamber the cones were inoculated.

Eggs of SCN race 3 were obtained from fresh culture in the greenhouse (about 45 d). An egg suspension of 800 eggs ml^{-1} were prepared. Two holes of about 3 cm depth along two sides of the plant (close to the cone wall) were made with a 1-ml pipette tip. Two thousand (2,000) eggs in 2.5 ml of water were added in one hole, and another 2,000 eggs were added in the other hole, with a total of 4,000 eggs per plant (or cone). Additional autoclaved sand-soil mixture about 0.5 cm of the tube height were added to cover the hole and nematodes. The cone-tainers were arranged in randomized blocks on the benches in the growth room with the temperature set at 28°C and artificial light of 16 hours per day. Water was supplied with a sprinkler irrigation system. Fertilizer was at the same rate and frequency throughout the experiment. After 35 d (one SCN generation), the experiment was terminated. The shoots were cut at the soil level the samples were dried for 3 d at 40°C to obtain dry matter. The roots were rinsed over a #60 (250 um aperature) sieve then collected and dried for 3 d at 40°C to obtain dry matter. The soil from the cone was decanted five times to remove the cysts from the soil. The soil suspension containing cysts was then poured over #20 and #60 sieves. The cysts and debris from the #60 sieve was rinsed into a centrifuge tube with

76% sucrose, which separated the cysts from the debris. The cysts were then counted under a microscope.

Statistical Analysis

Experiment 1

Linear mixed effect models were used to estimate the effects of crop treatment, inoculation level, and SCN Race on SCN egg counts (RCoreTeam, 2016). Block was treated as a random effect in the model. Crop treatment, inoculation level, and SCN Race were treated as fixed effects. Biomass was used as the covariate in the ANCOVA analysis. Log (y+1) transformation was used on our response variable to satisfy assumptions of the model, namely homogeneity of variances and normal distribution of the residuals. Analytical assumptions for an ANOVA were examined by graphical inspection of the residual plots. The mean separation procedure used to investigate the significant differences between treatments was Fisher's LSD with an associated $P < 0.05$. The means were back-transformed for presentation, but all analysis and mean separation was performed on the Log (y+1) transformed scale.

Experiment 2

Linear mixed effect models were used to estimate the effects of crop treatment on SCN cyst counts (RCoreTeam, 2016). Block was treated as a random effect in the model and pennycress line was treated as a fixed effect. Two cones of each treatment were averaged in each replication for one observation in each replication. Root biomass was used as a covariate in analysis. Analytical assumptions for an ANOVA were examined by graphical

inspection of the residual plots. The mean separation procedure used to investigate the significant differences between treatments was Fisher's LSD with an associated $P < 0.05$.

Results

Experiment 1

The crop treatment was significant at the $P < 0.05$ level (Table 3.1). Significant SCN reproduction occurred on the three pennycress lines tested. The SCN reproduction on the pennycress lines was not statistically different from the susceptible soybean check treatment. Very little SCN reproduction occurred in the three camelina lines tested. The camelina lines tested were significantly less than the pennycress and soybean crop treatments (Table 3.2). The average egg value across camelina lines was 39.9 eggs $100cm^{-3}$ of soil. The average egg value across pennycress lines tested was 4076.0 eggs $100cm^{-3}$, and the average value for the susceptible soybean variety sturdy was 6437.2 eggs 100 cm^{-3}.

Experiment 2

In this evaluation, all the pennycress lines resulted in SCN reproduction, and the completion of the life cycle, as the plants were inoculated with eggs and cysts were counted. The 119 lines tested ranged in FI values from 27-143, indicating 27% to 143% reproduction as compared to the susceptible soybean cultivar "Sturdy". The definition of SCN resistance has not be established. However, in soybean it is considered resistant if a line can exhibit a FI value less than 10, or 10% of the reproduction on the susceptible soybean cultivar. Moderate resistance is defined as a FI value between 10-30. One

pennycress line, a winter line identified as #39 in the UMN Breeding program had an FI value of 27 (Figure 3.1), a value within the moderate resistance range when using the soybean resistance definition. Eleven of the lines tested could be considered highly susceptible, as they had more cysts than the SCN-susceptible soybean control "Sturdy" that was used to calculate the FI index values were (Figure 3.1).

Discussion

This study confirmed the findings of Venkatesh et al. 2000 and Poromarto et al. 2015, that pennycress serves as an alternate host to SCN. Within the context of this study camelina appears to be a poor or non-host. Camelina does not pose a potential issue in the relay cropping system with corn and soybeans, as it relates to SCN pest pressures. Alternatively, pennycress could pose a potential issue if incorporated into a system where the main commodity crop is a host to SCN, such as the corn-soybean relay cropping system that is being investigated.

The germplasm evaluation showed varying levels of SCN reproduction on the 119 lines tested. Major gene resistance, like that seen in soybean (Bayless et al., 2016) was not discovered in the pennycress populations tested. One line exhibited "moderate resistance" with an FI value of 27, however significant reproduction still occurred on this line, indicating this resistance could be qualitative versus the resistance in soybean which relies on multiple copies of one resistance gene Rhg1 (Cook et al., 2012). The 11 lines that facilitated more reproduction than the susceptible soybean variety are of interest as they may indicate diversity in the pennycress germplasm that can be exploited to develop

resistance. A genome wide association study using the 119 lines screened may give QTL regions that affect the SCN reproduction in pennycress. Using the draft genome (Dorn et al., 2015) in combination with SNPs in a genome wide association study may give areas of the genome to target for pennycress breeders interested in incorporating SCN resistance.

Soil temperature plays a key role in the emergence and development of SCN. In both experiments temperature was maintained near 28°C, a temperature that facilitates SCN reproduction. The optimal temperature for SCN development is 25°C (Alston and Schmitt, 1988). At this temperature it takes 21-30 d for a full life cycle to be completed (Lauritis et al., 1983). SCN development is known to cease when soil temperatures drop below 10°C, and development to the first-stage juvenile will not occur until the soil increases to 10°C in the Spring of the year (Alston and Schmitt, 1988). 10-cm-depth soil temperatures in the Southern Minnesota corn-soybean production region drop below 10°C near October 15th, and do not warm up above 10°C until around May 1st. Pennycress yields are optimized when planted 24 August through 18 September (Dose et al., 2017). This optimum planting time may allow 1-2 life cycles to occur during the Fall, in this rotation. SCN reproduction can also take place during the spring of the year, as the snow melts and the pennycress plants begin to develop and bolt to maturity. Pennycress is harvested on or near 1 July (Dose et al., 2017), and the soil temperatures rise above 10°C at or near 1 May. This results in about 60 d in which pennycress is in the rotation at temperatures that facilitate SCN reproduction. Therefore, during spring and early summer it is possible one or more life cycles of SCN reproduction may take place before pennycress senescence and harvest. In the case of relay-cropping, the soybean will already be in the system prior to pennycress

harvest. This “green bridge effect” could result in continuous host plants in the system, resulting in greater pest pressure for the soybean crop. However, further studies are needed to determine SCN population development in pennycress under field conditions and their effect on soybean production.

Conclusions

This study indicates that pennycress may be an alternate host for soybean cyst nematode; while camelina is a poor or non-host. Including pennycress as a winter annual cover crop in rotations with soybean has the potential to increase SCN pest pressure, thereby putting the soybean crop at risk. Temperature and other factors likely play a key role in SCN development and reproduction on pennycress, and they must be further explored, especially under field conditions. A potential solution to this issue is to search for genetic resistance to SCN within pennycress populations. Results from this experiment show that natural variation does exist within the pennycress germplasm, but this resistance appears to be qualitative. A major resistance phenotype was not discovered. Additional screening, association mapping, and QTL discovery may be the options to find genetic resistance in pennycress that breeders can target. A solution involving agronomic or genetic resistance must be discovered so pennycress can be included in the relay-cropping system on millions of Midwest acres.

Tables and Figures

Source of Fixed Variation	
SCN Eggs	
Biomass	$2.32_{(1,103)}$
Crop (C)	$20.01_{(6,103)}$***
Inoculation Level (I)	$1.15_{(1,103)}$
Race (R)	$0.32_{(2,103)}$
C x I	$1.51_{(6,103)}$
C x R	$1.16_{(12,103)}$
I x R	$0.92_{(2,103)}$
C x I x R	$0.73_{(12,103)}$

Table 3.1. F values for Experiment 1 (numerator df, denominator df) for the fixed effects in the full mixed effects model.*, **, and *** represent significance of *F* tests at α = 0.05, 0.01, and 0.001, respectively. Each year was analyzed separately.

Cover Crop Treatments	2016
Eggs (count/100cm^{3}soil)	
Camelina-Joelle	32.8a
Camelina-Bison	41.5a
Camelina-WG-1	45.5a
Pennycress-MN103	4187.1b
Pennycress-MN106	4401.8b
Pennycress-MN108	3640.0b
Soybean-Sturdy	6437.2b

Table 3.2. The cover crop treatment effects on SCN egg population density from Experiment 1. The within column means with different letters denote significant difference at the P<0.05 level using LSD mean separation procedure. ns denotes there was no significant difference within column means at the P<0.05 level.

Source of Fixed Variation	
SCN Eggs	
Biomass	N/A
Crop Treatment	$222.0_{(119,121)}$

Table 3.3. F values for Experiment 2 (numerator df, denominator df) for the fixed effects in the full mixed effects model.Each year was analyzed separately.

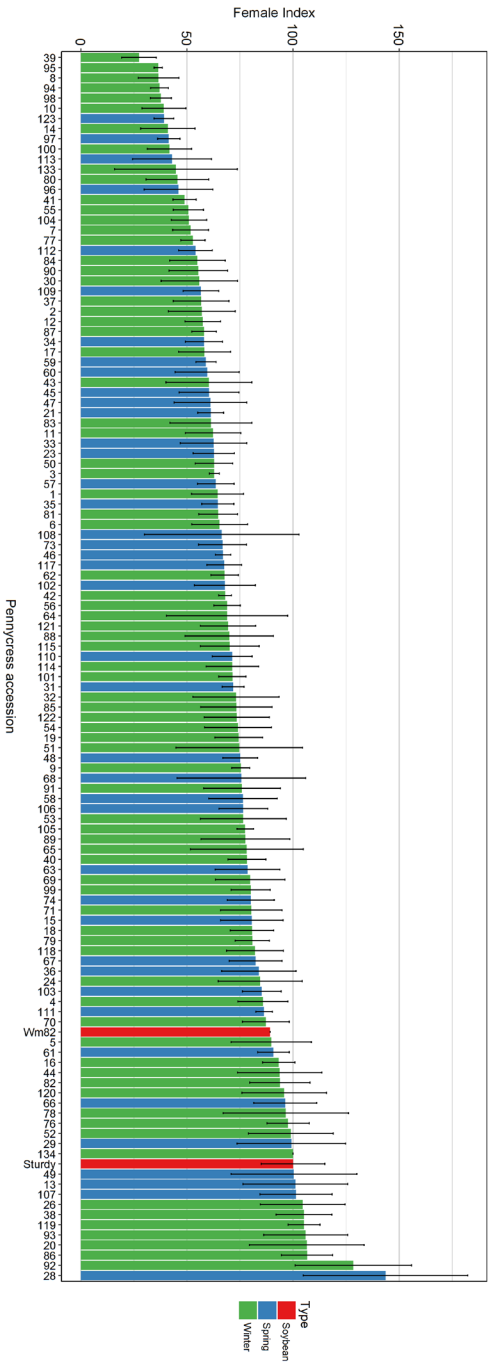


Figure 3.1. Female Index Values of breeding lines tested in Experiment 2 Error bars denote standard error.

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