Evaluation of soybean cyst nematode development on the winter oilseeds pennycress and camelina

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Abstract

Soybean cyst nematode (SCN, *Heterodera glycines*) is the most damaging pathogen in soybean *Glycine max* (L.) production within the Midwest region of the US. SCN causes $1.24-1.69 billion USD in yield loss annually in this region. Management practices such as using resistant cultivars, rotation with non-host crops, and seed treatments can effectively control SCN population density. Pennycress (*Thlaspi arvense* L.) and camelina [*Camelina sativa* (L.) Crantz] are being developed as winter oilseed crops that can be implemented in corn-soybean cropping rotations to serve the function of cover crops. Pennycress and camelina can increase yields of Midwest cropping rotations and provide the ecosystem services of cover crops. Pennycress has been reported as an alternative host to SCN. Camelina is classified as a poor or non-host and has been implicated as a potential trap crop as it has been shown to reduce SCN population density in naturally-infested soil. These experiments aimed to i.) examine the impacts of pennycress and camelina crops on SCN population under field conditions, ii.) examine hatch, infection, and development of SCN in pennycress and camelina, and iii.) and screen for SCN resistance in pennycress accessions. Experiments were completed in the field, microplot, and controlled-environment settings. The multi-location field experiment did not detect an effect of including pennycress or camelina on SCN population density in an oilseed-corn-soybean rotation. New females were observed in the spring and early summer on pennycress root samples in microplots, but females were not observed in the autumn. When the SCN life cycle was examined, egg hatch was affected by pennycress root exudates but not by camelina root exudates. Both winter-annual oilseeds had evidence of juvenile penetration into the roots, and the number of juveniles observed

infecting roots was not different between the pennycress and camelina treatments. However, after 35 days of continued development, pennycress produced more adult females than camelina. Camelina samples had near-zero averages of adult female recovery. Eggs produced on pennycress roots were collected and reinoculated, and new females were recovered from pennycress and soybean roots following the reinoculation. This result demonstrates that SCN can complete a life cycle and reproduce on pennycress roots. The SCN screening of pennycress accessions did not reveal any accessions exhibiting a major resistance phenotype. However, some accessions were classified as moderately resistant when compared to the susceptible soybean 'Williams 82.' The susceptible soybean variety 'Williams 82' was used for comparison because it is used as the standard check for soybean resistance screening and classification. Overall, new female development can occur on pennycress in a controlled environment setting, but field experiments failed to show a detectable effect of pennycress on SCN population density.

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Chapter 1 - Literature Review

Introduction

Corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) are summer-annual crops grown in the Midwest region of the United States. These two crops are commonly rotated with one another, and they account for the first- and second-most hectares of U.S. crops planted. Corn accounted for 37.2 million hectares, while soybean accounted for 33.9 million hectares planted in 2020 (USDA- National Agricultural Statistics Service, 2021). As summer-annual crops, corn and soybean are planted April through May and harvested October through November in the Midwest production regions. The summer-annual crop rotation has 5-7 months of a fallow period in which the soil is uncovered and vulnerable to erosion. Modeled estimates predict that heavy storms detach soil from the bare ground during spring months, and soil losses commonly exceed 14 tonnes ha^{-1} yr^{-1} (Gelder et al., 2018). In addition, nearly 75% of the nitrate loss from production fields occurs during the spring months between April and June, when there is a lack of vegetative ground cover in summer-annual rotations (Randall & Vetsch, 2005). The soil and nutrient losses from production fields are unsustainable for crop production and negatively impact wells and recreational waterways.

Nitrogen fertilizer application and subsequent leaching lead to nitrate presence in groundwater. Row-cropping in areas with vulnerable aquifers results in the deposition of nitrates in wells and waterways (McCasland et al., 2012; Van Metre et al., 2016). Several Midwestern watersheds have vulnerable aquifers and high nitrogen loading from

agriculture (Nolan & Hitt, 2006). Subsequently, current cropping practices have resulted in many wells and streams with high levels of nitrates. In a USGS survey on nitrate contamination, seven percent of private wells tested above the recommended safe level of 10 ppm-nitrate-N. In addition, one-quarter of shallow wells tested in rural communities had nitrate concentrations above the recommended safe level (DeSimone et al., 2009). Levels above 10 ppm-nitrate-N can adversely affect human health, especially the health of infants (Aschebrook-Kilfoy et al., 2012; De Roos et al., 2003; McCasland et al., 2012).

In addition to the effects on wells, excess nutrient accumulation in bodies of water has created hypoxic zones that negatively impact aquatic species. Phosphorus is a leading contributor to these oxygen-depleted zones. Phosphorus is bound tightly to soil particles. Therefore, the movement of soil particles off-field by wind or water results in phosphorus deposition in waterways (King et al., 2015). This deposition of phosphorus creates algae growth through a process known as eutrophication. When the algae die and decompose, the bacteria feed on the algae. The bacteria, in turn, consume the dissolved oxygen in the water. The lack of dissolved oxygen is detrimental to marine life in these waterways and can create depleted oxygen zones, such as the one in the Gulf of Mexico (Correll, 1998). Agriculture is the most significant contributor of nitrates and phosphates to the Gulf of Mexico, making up 70% of overall deposition (Alexander et al., 2008).

The implementation of crops that provide continuous living cover addresses these environmental concerns (Kaspar et al., 2001; Randall & Vetsch, 2005). However, implementation of continuous living cover through cover crop adoption has remained low in the United States, covering approximately 15 million acres in the United States in 2017 (Wallander et al., 2021). The annual Sustainable Agriculture Research and Education–

Conservation Technology Information Center (SARE–CTIC) survey showed that growers identified timing challenges and lack of economic return as primary barriers to cover crop adoption. Nevertheless, the survey showed that most grower respondents have a positive outlook on cover crops and are willing to adopt the practice if it is beneficial and economically feasible (CTIC, 2020). However, despite the positive survey, most growers experienced an overall net economic loss when adopting cover crop practices (Plastina et al., 2020). Therefore, cover crop solutions addressing these barriers and adding profitability to the cropping rotation are needed to increase adoption.

Winter-annual oilseed cropping system

Researchers are developing new winter oilseed crops, pennycress (*Thlaspi arvense* L.) and camelina (*Camelina sativa* L.), for implementation in corn and soybean rotations. The winter oilseeds function as cover crops to cover the ground during fallow periods from autumn to spring. Winter oilseeds also have harvestable seeds with value in end-use markets. Enhancing production and providing ecosystem services are the primary focus of incorporating winter oilseeds into these rotations (Heaton et al., 2013; Weyers et al., 2021). Winter oilseed crops can increase productivity and profitability in the corn-soybean rotation (Dose et al., 2017; Gesch et al., 2018). The relay-cropping system is best suited for the northern Midwest, where heat units are limited for oilseed crop establishment in the autumn (G. A. Johnson et al., 2017; Jones, 1994). The winter oilseeds are seeded into standing corn, emerge and grow in the autumn, overwinter as rosettes, and quickly bolt to maturity in late spring. Soybeans are planted green into the winter oilseeds before flowering in May to maximize soybean growing degree days (Hoerning et al., 2020; Reed et al., 2019). Upon physiological maturity, the winter oilseeds are harvested (i.e., direct combined) over the top of the young soybean plants,

and the soybeans grow to maturity like a typical Upper Midwest rotation (Johnson et al., 2017; Walia et al., 2018). Alternatively, the winter oilseed crops can be grown in double-crop rotation, where the oilseeds are planted after corn harvest, and soybean is planted after oilseed harvest (Berti et al., 2017). The double-crop system, without crop overlap, is easier to implement in Midwestern states at lower latitudes, with more heat units (Gesch et al., 2014; Heggenstaller et al., 2009). The winter annual oilseeds pennycress and camelina were selected for this system because of their oil profile, extreme cold tolerance, and ability to reach physiological maturity quickly in spring (Warwick et al., 2002).

Pennycress as a winter-annual oilseed crop

Pennycress is in the *Brassicaceae* family and is also known as field pennycress, fanweed, and stinkweed. Native to Eurasia, pennycress has become widespread in temperate regions of the world. It is commonly found in roadside ditches or agricultural fields as a winter-annual weed in the United States. Field crops such as barley, oats, rape, sunflowers, wheat, and forage crops are affected by weedy pennycress in the system, as yield, seed, or forage quality are reduced. (Holm et al., 1997; Warwick et al., 2002). The weed risk assessment completed by the United States Department of Agriculture in 2015 determined pennycress was “High Risk.” The determination was due to the wide range of environmental conditions pennycress can tolerate, its potential for harm to livestock health, and its importance as an agricultural weed in many cropping systems (USDA-APHIS, 2015). Pennycress has both a spring and winter biotype. The winter biotype is the focus of current research and implementation efforts (Wortman, 2020). Pennycress was first identified as a potential crop for agricultural production in the United States to replace rapeseed oil. The seeds of pennycress contain 33-35% oil. The composition and

properties are similar to rapeseed oil, as it has high erucic acid content and similar viscosity properties (Clopton & Triebold, 1944). Extraction and further testing of the oil found it has a high cetane number of 59.8 and good low-temperature properties, making pennycress oil a good candidate oilseed for biofuel production (Moser et al., 2009a). One of the biofuels under development is renewable jet fuel. In 2017, the United States aviation industry consumed 98 billion liters of fossil fuel. Aviation companies have set goals to offset their fossil fuel usage with renewable fuels. A techno-economic analysis showed that 40-60 thousand ha of pennycress production could produce 19 million liters of renewable aviation fuel annually (Mousavi-Avval & Shah, 2021). In addition to pennycress biofuel potential, seed oil-derived fatty acids from pennycress can be processed to produce wax esters for end-use in cosmetics, lubricants, and plastic manufacturing (Volkova et al., 2016).

The University of Minnesota and other regional partners have worked on pennycress domestication for over 10 years. In 2015 the draft genome of pennycress was published and revealed that pennycress shares approximately 86% nucleotide sequence identity with the genome of the model organism *Arabidopsis thaliana* (Dorn et al., 2015). The genetic similarity between the laboratory model *Arabidopsis* and pennycress facilitated the transfer of genomic information from *Arabidopsis* to pennycress (Dorn et al., 2013). The ease of technology transfer has resulted in rapid pennycress improvement using mutagenesis and CRISPR/Cas9 gene editing (Jarvis et al., 2021). This work has improved agronomic and oil traits necessary for the success of pennycress as a commercial crop. Improved traits include early maturity, reduced pod shatter, reduced seed glucosinolate levels, and an improved oil fatty acid profile (Chopra et al., 2020).

Pennycress is also highly desirable as a new crop to fill the gap of living cover on the landscape when summer-annual crops are not growing. The cover gap leaves an opportunity for soil loss and nitrate leaching, primarily in the spring following heavy rainfall events when the soil is exposed (Randall & Vetsch, 2005). Interseeded pennycress has been shown to provide 37-57% soil cover in the autumn and produce between 0.4–2.5 Mg ha^{-1} of plant biomass in the spring (Wortman, 2020). The plant ground cover during critical months can reduce soil nitrogen loss. In a study evaluating a pennycress and sweet corn rotation, pennycress reduced residual soil nitrate by 27-42% compared to fallow treatments (Moore et al., 2020).

Pennycress has an additional ecosystem benefit of providing early season forage to insect pollinators, as it flowers in early spring, before many plants on the Midwest landscape. Forcella et al. (2021) recorded pollinator visits on pennycress and identified pollinators. The observations included bumblebees, honeybees, solitary bees, butterflies/moths, and beetles. An average of 1.6-5.3 total insects min^{-1} were observed in pennycress fields at peak flowering (Forcella et al., 2021).

A survey of row crop farmers completed by Zhou et al. (2021) encompassed seven Midwest agriculture states and found that 58% of farmers were interested in growing pennycress if profitable. The survey found that a price of \$0.28 USD kg^{-1} at an estimated ~1800 kg ha^{-1} yield was the level at which most growers would consider adoption. Farmers with less debt, more land, higher education levels, and previous knowledge about pennycress were more likely to consider adoption, while farmers who used no-till practices or had no knowledge about pennycress were less likely to want to adopt the crop (Zhou et al., 2021).

Camelina as a winter-annual oilseed crop

Camelina is another oilseed member of the *Brassicaceae* family and is similar to pennycress with its winter-annual life cycle. Camelina is also known as “False flax” and “gold of pleasure” in popular nomenclature (Zubr, 1997). Camelina is native to southeast Europe and southwest Asia (Berti et al., 2016). Camelina has spring and winter biotypes (Mirek & Mirek, 1980). Winter biotypes are extremely winter-hardy and can survive in the Midwest’s northern region (Gesch & Cermak, 2011). Winter camelina has been grown in double-crop and relay-crop rotations (Hoerning et al., 2020; Walia et al., 2021).

Camelina is a good candidate for oil end-use markets. In a review of camelina oil characteristics, Obour et al. (2015) found that camelina seeds have an oil content averaging 35%. The camelina oil profile contains 40% of an omega-3 fatty acid known as α-linolenic acid (18:3n-6), a healthy oil in human and animal feed diets. Camelina meal contains approximately 45% protein with a favorable composition of amino acids similar to soybean meal (Obour et al., 2015). Given this favorable oil and protein profile, camelina has demonstrated end uses in the food, feed, biofuel, lubricant, and cosmetic industries (Zanetti et al., 2021). Numerous strategies were used to improve camelina’s oil characteristics and other properties. Pure line breeding, pedigree breeding, marker-assisted selection, transformation, and gene editing were used by breeders to improve camelina traits (Jiang et al., 2017; Vollmann & Eynck, 2015). Winter camelina provides many of the same ecosystem and productivity benefits as pennycress when planted as a crop in the summer-annual rotation (Gesch et al., 2018). Like pennycress, planting camelina during fallow periods can reduce soil nitrate and soil erosion (Wittenberg et al., 2020). It also has other benefits, such as early-season weed suppression and pollinator forage resources (Forcella et al., 2021; Hoerning et al., 2020).

Prevalence and Economic Importance of SCN

Soybean cyst nematode (SCN, *Heterodera glycines*) is the most damaging pest affecting soybean yield in the United States, resulting in twice the magnitude of yield loss than any other pathogen (Bandara et al., 2020). In a survey conducted from 2015 to 2019, the average soybean yield loss per year was 94 million bushels, valued at $1.24-1.69 billion USD (Bradley et al., 2021) SCN is widespread in soybean growing regions of the United States and the geographic distribution of SCN has increased over time (Tylka & Marett, 2021; Workneh et al., 1999). For example, a statewide survey completed in Missouri in 2005 revealed that 50% of samples tested were positive for SCN. The number increased to 88% in a statewide survey completed in 2016, demonstrating that SCN has become more prevalent in the state over time (Howland et al., 2018).

SCN reduces soybean yield by greater than 30% without aboveground symptoms (Niblack, 2005). The soybean yield is estimated to be reduced at an SCN population density of 470 eggs kg^{-1} soil (Francl, 1986). Wang et al. (2003) investigated the aboveground and belowground symptoms of soybean that result from infection by SCN. The aboveground symptoms include stunting, leaf yellowing, reduced seed pod size, and seed pod density Belowground, SCN infection decreases nodules formed by beneficial nitrogen-fixing bacteria and reduces soybean root development (J. Wang et al., 2003). Plants infected by SCN become susceptible to other soybean pathogens because the damaged root systems create favorable growth and development opportunities. Other soil-borne pathogens associated with SCN infection are *Phytophthora*, *Pythium*, *Fusarium*, and *Rhizoctonia* (Niblack & Tylka, 2008).

In Minnesota, SCN was first detected in 1978 and has since spread to 71 of 87 counties (MacDonald, 1980; Gregory L. Tylka & Marett, 2021). The highest prevalence of SCN is in the southcentral and southwestern regions of the state, where summer-annual rotations with soybean are typical (S. Chen & Grabau, 2018). Since its discovery in the state, SCN has been a major limiting factor in soybean production (Chen et al., 2001).

Biology and Life Cycle of SCN

Nematodes are multicellular, triploblastic, unsegmented aquatic animals. Nematodes are in the phylum *Nematoda* under the kingdom *Animalia* (De Ley & Blaxter, 2002). The soybean cyst nematode is part of the family *Heteroderidae*, which consists of 18 genera. All members of the 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 reproductive system. Of special importance to the SCN life cycle as a plant pathogen 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 to increase genetic diversity.

The life cycle of SCN consists of an egg, four juvenile, four molt, and an adult stage. The molt stages occur between the developmental phases. First-stage juvenile (J1)

develops within the egg and then molts to the second-stage juvenile (J2) prior to egg hatch. There are three defined egg-hatching behaviors for SCN: constitutive, inducible, and dormancy (Niblack et al., 2006). Constitutive hatching occurs when soil temperatures are adequate. Most constitutive hatching occurs the first year after fertilization. A high proportion of eggs in the gelatinous matrix, those eggs not inside the female body, hatch constitutively. When a host plant is still actively growing, these constitutively hatching eggs allow for immediate reinfection (Thompson & Tylka, 1997). Inducible hatching only occurs when a host plant is present. The host plants release root exudates received by the nematode eggs and stimulate the eggs to hatch (Schmitt & Riggs, 1991). Inducible hatching will not occur when a non-host crop is in rotation. The final phase, dormancy, is initiated by dropping soil temperatures in the autumn. (Edward P. Masler & Rogers, 2011). Unlike the other two types of hatching, dormant eggs will not hatch despite favorable environmental stimuli or signals from host plants. Niblack et al. (2006) reported that eggs could stay dormant for many years.

The characterization of the hatching factors involved in SCN egg hatch is ongoing. One advancement discovered hatching factors associated with kidney beans (*Phaseolus vulgaris*). The molecule was named glycinoeclepin A (bis-[p-bromophenacyl] ester). SCN eggs treated with glycinoeclepin A had a higher egg hatch percentage than the DI water control (Masamune et al., 1982). Hatching begins when larvae inside the egg use their stylet to thrust against the side of the egg wall. The action creates a series of perforations in the eggshell, and a slit forms allowing the juvenile to leave the egg. SCN leaves the egg in the J2 stage (Doncaster & Shepherd, 1967).

SCN develops from a single cell egg to a fully formed J2 in approximately 172 hours under optimized development temperatures. The primary motor nervous system and the stylet are fully formed in late pre-hatch J2 development. Pre-hatch egg development follows the same timeline if the eggs are free in an egg sac or retained within the mother. The J2 nematodes hatch from the egg and move across the film of water present on the soil particles toward the host plant. The nematodes use chemotaxis to move toward and find a host plant to infect. Chemotaxis is regulated by the nematode's nervous system and guides the nematode toward the source of stimulation. In the case of SCN, the source of stimulation is thought to be root secretions from host plants (Y. Hu et al., 2017; Rasmann et al., 2012).

After locating a target plant, the J2 nematode penetrates the root and causes necrosis at the penetration site. The J2 uses cell-wall-degrading enzymes secreted through its stylet to migrate intracellularly through the root cortex. Once at the vascular stele, the J2 establishes a feeding site (Smant et al., 1998). This feeding site is called a syncytium. The syncytium is a multinucleate cell with one enlarged nucleus resulting from adjacent cell coalescence. The syncytium functions as a feeding cell providing the nematode with the nutrients it needs for development. The nematode acts as a metabolic sink. Nutrients are transferred from the plant to the syncytium and then to the nematode. 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 males revert to a vermiform shape and leave the root as an adult. The females remain inside the root but rupture the root cortex. This root rupturing and formation of

the vulva allows sexual reproduction between the male in the soil matrix and the female attached to the root. Adult males are attracted to females by sex pheromones, such as vanillic acid. The pheromones allow the males to find and fertilize the females that have protruded from the roots (Jaffe et al., 1989). After fertilization, the female releases a gelatinous matrix of eggs into the soil but most of the fertilized eggs are retained within the female body. Finally, the female dies, and her cuticle tans and forms a tough protective cyst, encasing the eggs. Cysts are the overwintering and survival structure of the soybean cyst nematode (Bonner & Schmitt, 1985).

Research involving potato cyst nematode (*Globodera rostochiensis*) revealed that the tanning process is induced by an enzyme (phenoloxidase) located in the cuticle of the female cyst. The enzyme is partitioned when the female is living, and tanning does not occur. However, the partitioning process breaks down when the female dies and the cuticle tans (Awan & Hominick, 1982). The presence of a phenolic compound and its role in cyst tanning was confirmed in SCN under an electron microscope. As the tanning process of the cyst progressed, the phenolic compound became deposited at higher concentrations in the cyst wall (Kondo & Ishibashi, 1975). Eggs from a single female typically range from 40 to 600, but the average is 200 per female (Sipes et al., 1992).

Winter-annual oilseeds and SCN

Pennycress and camelina contain glucosinolates, compounds that have pesticidal (and nematicidal) properties. The degradation of these glucosinolates leads to biologically active products such as isothiocyanates and oxazolidinethiones, which act as natural pesticides (Fenwick & Heaney, 1983; Gimsing & Kirkegaard, 2008). Soil-applied pennycress seed powder has been shown to reduce SCN population levels (Grabau & Chen, 2014). These results indicate that the biologically active derivatives of the

glucosinolates present in the seed powder may negatively affect SCN populations in the soil. Contrary to the seed powder experiment results, pennycress has been reported as an alternative host to SCN (facilitates development) in weed surveys performed in the greenhouse (Poromarto et al., 2015; Venkatesh et al., 2000). However, as a winter-annual, pennycress endures cold winter temperatures. The effect of cold temperatures on SCN population development on pennycress in the field is unknown. Camelina was tested in SCN greenhouse surveys and was reported as a poor or non-host of SCN (does not facilitate development) (Acharya et al., 2019; Poromarto & Nelson, 2010). A poor or non-host can act as a trap crop of SCN and reduce population numbers if infection occurs, but development does not proceed to the adult female stage. (Chen et al., 2001; Warnke et al., 2006). Like pennycress, camelina has not been tested on the field scale.

Soil and environmental effects on SCN development

Soil properties are a major determining factor in SCN population density and associated soybean damage. Generally, higher pH levels have a positive correlation with SCN population density. Soybean yield decreased as pH increased in fields with SCN (Pedersen et al., 2010). pH is also highly correlated with another soybean limiting phenomenon known as Iron Deficiency Chlorosis (IDC). In IDC, alkaline soils (pH > 7) convert iron into a plant unavailable form resulting in soybean plant deficiency (Merry et al., 2021). The combination of IDC and high SCN population density causes additional soybean yield loss (Chen et al., 2007). Soil textural classes (sand, silt, clay) are correlated with SCN, and more severe damage to soybean yield is also associated with higher sand content soils (Koenning & Barker, 1995; Perez-Hernandez et al., 2019).

Temperature plays a crucial role in SCN development as poikilotherm organisms cannot regulate their body temperature (Barker et al., 1992). Higher temperatures are

positively correlated with higher rates of embryogenesis and hatching, and the window for the most rapid SCN development is between 20 to 28℃, with maximum rates of development observed at 24℃ (Alston & Schmitt, 1988; Melton et al., 1986). The estimated thermal minimum of development is 5℃, and the J1-J2 nematode molt does not occur at temperatures below 10℃. However, development is known to proceed to the J2 stage at 15℃ (Alston & Schmitt, 1988; Ross, 1964). Low temperatures act directly on the nematode by restricting enzyme activity, muscle movement, or phase transition of membrane lipids (Hill & Schmitt, 1989). While development does not proceed at low temperatures, SCN in roots can enter a quasi-hibernation state where development ceases, but the nematode maintains a viable feeding site as survival for a period of 20d at a temperature of 0℃ was observed in roots (Creech, Santini, et al., 2007). Larvae (J2 nematodes in the absence of a host) can survive in the soil for 630 days at 0℃ (Slack et al., 1972). SCN egg hatch is also affected by cold temperatures as a dormancy phenomenon is observed following the onset of cold temperatures in the autumn (Edward P. Masler et al., 2008). Egg hatching and juvenile infectivity peak in midsummer and are reduced drastically from October through May in Missouri, United States (Yen et al., 1995). In the overwintering stage of the SCN, eggs can survive in cysts exposed to -40℃ for seven months (Ichinohe, 1955).

Host range of SCN

The host range of SCN is not limited to soybean, as Edible beans (*Phaseolus vulgaris*), mung bean (*Phaseolus aureus*), and green pea (*Pisum sativum*) are alternative crop hosts of SCN grown in the United States (Niblack & Chen, 2004). In addition, over 150 genera of weeds found in crop production systems of the United States can serve as hosts to SCN (Rocha, Gage, et al., 2021). Notable winter annual weeds commonly found

in Midwest agriculture fields include purple deadnettle (*Lamium purpureum* L.), henbit (*Lamium amplexicaule* L.), pennycress, Shepherd's purse (*Capsella bursa-pastoris*), common chickweed (*Stellaria media*), bittercress (*Cardamine hirsuta*), common mallow (*Malva sylvestris*), Canada thistle (*Cirsium arvense*), and common cocklebur (*Xanthium strumarium*) (Johnson et al., 2008; Riggs, 1987; Rocha et al., 2021). Most weeds were identified as alternative hosts in controlled temperature settings such as a greenhouse or growth room. However, there have been field confirmations of weeds hosting SCN. For example, new cysts containing viable SCN eggs were recovered from purple deadnettle plants and henbit plants sampled in the autumn from fields in Indiana (Creech et al., 2005). These results from henbit and purple deadnettle indicate that cysts and eggs can be produced on winter-annual weeds before temperatures decrease too low for development in the autumn. However, it is unclear if cover crops facilitate enough development to affect overall SCN field population densities. The presence of purple deadnettle, henbit, common chickweed, shepherd's purse, and bittercress resulted in no detectable change in SCN egg population density under field conditions (Mock et al., 2012).

Cover crops can also facilitate the development of SCN as cover crop species, including cabbage (*Brassica oleracea*), red clover (*Trifolium pratense*), alfalfa (*Medicago sativa*), hairy vetch (*Vicia villosa*), pea, tillage radish (*Raphanus raphanistrum*), ryegrass (*Lolium* spp.), and cereal rye (*Secale cereale*) are alternative hosts (Schmitt & Riggs, 1991). Many of these cover crops facilitated development to the adult female stage. However, the number of SCN females recovered from the cover crops was less than 1% of the number of females produced on the susceptible soybean 'Williams 82,' which may indicate that development is too low to significantly increase

SCN population densities in the field (Kobayashi-Leonel et al., 2017; Schmitt & Riggs, 1991).

Control of SCN

Rotation with non-host crops is a primary strategy for the control of SCN. Many row crops grown in the United States are non-hosts to SCN, including corn, alfalfa, barley (*Hordeum vulgare*), cereal rye, oats (*Avena sativa*), wheat (*Triticum spp*.), and canola (*Brassica napus*), which are all commonly grown in rotation with soybean (Strunk, 2020). Nematode juveniles that hatch due to constitutive hatching when the non-host crop is in the rotation will die due to starvation or predation; however, many SCN eggs will remain dormant and not hatch (Niblack et al., 2006). One year of non-host corn rotation is insufficient to drastically reduce SCN population density, as demonstrated in a 7-year study, but annual rotation with corn prevented long-term increases (Noel & Wax, 2003). Therefore, several years of non-host crop rotation is recommended to reduce SCN populations to non-damaging levels if significant yield loss has occurred on soybean (Tylka, 2021).

A study performed by Rocha et al. (2021) demonstrated that growing wheat (non-host) in double-crop rotation with soybean reduces SCN population density. The results showed that wheat double-crop treatments reduced SCN population density in soybean production at the soybean R1 stage by 32% and, after soybean harvest, by 33% compared to the fallow control (Rocha et al., 2021).

Non-host cover crop species may facilitate infection but not allow complete development and therefore function as a trap crop (Trivedi & Barker, 1986). Brown mustard (*Brassica juncea*), camelina, and crambe (*Crambe cordifolia*) were evaluated for their ability to reduce SCN population density in the field. Brown mustard and camelina

significantly reduced SCN population density by 51 and 48%, respectively, while crambe did not exhibit a significant change (Acharya et al., 2019).

Another control strategy is seed treatments or biocontrol agents applied to seeds to reduce SCN population density growth. A review by Munkvold et al. (2014) found that chemical seed treatments have widespread use in various crop species to achieve goals such as protection from seed-borne and soil-borne pathogens and protection from fungi and insects. The seed treatments have contact, local systemic, and whole plant systemic activity, and these treatments help improve seedling emergence and vigor and minimize plant loss. In addition, the advantage of using seed treatments over soil-applied products include a reduced amount of product used per hectare and reduced off-target effects on other organisms (Munkvold et al., 2014). Seed treatments for SCN have had varying results. Beeman & Tylka (2018) showed that a product containing the active ingredient fluopyram reduced reproduction in the greenhouse by 35 to 97%. The seed treatment also significantly reduced J2 hatching, motility, and root penetration compared to the control (Beeman & Tylka, 2018). In a three-year field experiment, fluopyram treatments increased soybean yield and significantly reduced SCN egg and juvenile counts compared to the control (Roth et al., 2020). However, Bissonnette et al. (2020) showed that fluopyram effects on SCN population density were field location-specific. The study results indicate that fluopyram reduced SCN reproductive factor in a few small plot experiments by 36 to 60% and one of the strip trial experiments by 50%. However, in 22 other small plot experiments and 11 other strip trial experiments, there was no effect of including fluopyram seed treatment on SCN reproductive factor (Bissonnette et al., 2020).

Seed treatments using bacteria have also been developed to reduce SCN population density. A product containing a gram-positive, spore-forming bacterial strain called *Bacillus firmus* is proposed to have toxic effects on nematodes by creating a living barrier around the root system, preventing nematodes from entering the plant (Wilson & Jackson, 2013). However, when juvenile hatching, motility, and root penetration were measured, the *Bacillus firmus* seed treatment did not affect these traits (Beeman & Tylka, 2018).

Fungal isolates have also demonstrated nematicidal activity—two fungal isolates from *Purpureocillium* spp. and a fungal isolate from *Ilyonectria* sp. significantly reduced SCN reproduction on a susceptible soybean variety at both high and low initial SCN population densities (Haarith et al., 2021). Another seed-based biological control method is the arbuscular mycorrhiza *Glomus etunicatum*, and in the greenhouse, the mycorrhiza treatment reduced the number of nematode females on the roots by 28% (Benedetti et al., 2021). Another effective fungal biological control is *Aspergillus niger*, a fungus that produces organic acids, heterocyclic compounds, and biological enzymes thought to suppress plant-parasitic nematodes. In vitro experiments showed that *Aspergillus niger* NBC001 culture filtrate caused 89% mortality of 2nd stage juveniles and inhibited 98% of egg hatching (N. Jin et al., 2021).

Genetic resistance is the most common control strategy used for SCN today, and soybean cultivars resistant to SCN were first used in the United States starting in 1978 (Blok et al., 2018). The widespread deployment and use of resistant commercial cultivars have protected soybean yields and prevented significant increases in SCN population density (McCarville et al., 2017). Starting in the 1990's soybean cultivars were developed

using the breeding line PI 88788. Today, 95% of the soybeans available on the market contain the PI 88788 – derived resistance gene, while the remaining 5% contain Peking or PI 437654 type resistance genes (Joos et al., 2013; Tylka & Mullaney, 2015). Resistance in soybean prevents most nematodes from reaching full maturity in the plant root as the nematode penetrates the root but cannot maintain the syncytium feeding cell through development (Li & Chen, 2005).

Research on SCN resistance began with the first discovery of *rhg* loci (resistant to *Heterodera glycines*) in 1960 (Caldwell et al., 1960). Resistance to SCN involves multiple pathways with copy number variation at the *rhg1* and *Rhg4* loci (Cook et al., 2012). A high copy number (7 to 10 copies) of three genes at *rhg1-b* in a 31-kb segment is involved in PI 88788 type resistance. These genes include an amino acid transporter, an N-ethylmaleimide-sensitive factor attachment protein (α-SNAP), and a wound-inducible protein (WI12). There is no resistant-type gene expression at *Rhg4* in PI 88788 type resistance (Cook et al., 2012, 2014). The Peking-type resistance has a low copy number of *rhg1* (1 to 3 copies) but also requires *Rhg4* to confer resistance to SCN (Concibido et al., 2004). The genes involved in Peking resistance are a low-copy α-SNAP gene at *rhg1-a* paired with a resistant-type SHMT (serine hydroxymethyltransferase) gene. SHMT is encoded by *Rhg4* (S. Liu et al., 2017). The developmental stage the nematode reaches prior to syncytial degradation is different depending on the type of derived resistance. In PI-88788 type resistance, SCN development can proceed to the J3 or J4 juvenile stage prior to mortality, but in Peking-type resistance, SCN development does not proceed past the J2 infection stage before mortality occurs (Klink et al., 2009, 2010). Biotypes of SCN that can overcome PI-88788–derived resistance are emerging due to its overuse and

lack of rotation with other sources of resistance. Approximately 70% of SCN populations surveyed have begun to overcome host resistance derived from PI 88788 (Niblack et al., 2007).

Rotating resistance sources derived from Peking and PI88788 effectively reduces SCN population density in the field compared to continuous cropping with one resistance source (Chen, 2020). A shift in SCN virulence has also been observed when two resistance sources are deployed as rotation from PI 88788 derived resistance to Peking resistance significantly reduced SCN population density but selected for broader SCN virulence (Meinhardt et al., 2021; Thapa et al., 2021). As the effect of major resistance genes is diminished, other resistance strategies may be needed to control SCN. One strategy is gene pyramiding which involves the presence of multiple known resistance alleles in one cultivar (Joshi & Nayak, 2010). Resistance pyramids have effectively reduced SCN population density as it has been demonstrated that multiple sources of resistance in one cultivar result in greater resistance than any single resistance source (Meinhardt et al., 2021).

Another novel resistance strategy is RNA interference (RNAi). RNA interference involves gene silencing of genes involved in plant physiological and developmental processes (Kaur et al., 2021). Researchers have used RNAi in transgenic soybean to down-regulate genes encoding for the major sperm protein (MSP), which has inhibited SCN life cycle development (Steeves et al., 2006). Down-regulation of other soybean genes related to SCN reproduction and fitness successfully reduced SCN development. For example, the downregulation of *Cpn-1*, *Y25*, and *Prp-17* genes resulted in a 79-95%

reduction in eggs g root^{-1}, and this host-derived resistance was comparable with levels of control available through commercial soybean resistance (Tian et al., 2016)

Chapter 2 - Impact of Pennycress and Camelina as Winter Oilseed Crops on the Soybean Cyst Nematode Population in Minnesota Corn-Soybean Rotation System

Synopsis

Pennycress (*Thlaspi arvense* L.) and camelina (*Camelina sativa* (L.) Crantz) are winter oilseed crops that can be implemented in cropping systems of the U.S. Midwest. Incorporating winter oilseed crops into the cropping system offers ecosystem and productivity benefits when the ground is otherwise fallow. However, adding a new crop into an established cropping system may increase pest or pathogen risk. Pennycress and camelina have been identified as a host and poor/non-host, respectively, of the soybean cyst nematode (SCN, *Heterodera glycines*), a devastating soybean pathogen. The objective of this experiment was to investigate whether adding pennycress or winter camelina to a soybean-corn rotation affected SCN population density. The experiment was a two-level factorial with a split-plot design which included low and high SCN initial population densities as main plots and oilseed crops (pennycress, camelina, and fallow) as subplots conducted at three field sites in Minnesota. Throughout the study, the higher initial SCN population density treatment generally resulted in significantly higher SCN population density when compared to the low initial population density treatment. There was no effect on SCN population density when camelina or pennycress was included as a treatment. The results indicate that winter oilseeds can be added to soybean-corn rotations without inducing a detectable effect on SCN population density in Minnesota.

Introduction

Midwest crop production in the United States primarily consists of corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) grown in annual rotation with one another (S. Wang et al., 2020). Fallow periods in corn and soybean production are several months long, extending from harvest to the following year's plant (Tonitto et al., 2006). The winter oilseed species pennycress (*Thlaspi arvense* L.) and camelina (*Camelina sativa* [L.] Crantz) are being developed to provide living groundcover during fallow periods in Midwest agriculture (Mohammed et al., 2020; Sindelar et al., 2015). Enhancing production and providing ecosystem services are the primary focus of incorporating the winter annuals into these rotations (Heaton et al., 2013). Research indicates that adding winter oilseeds to a soybean rotation can produce a total seed oil yield larger than soybeans in mono-culture (Gesch et al., 2014). The oil extracted from the winter oilseeds has end-use potential as a biofuel in diesel and jet fuel marketplaces (Fan et al., 2013; Mohammad et al., 2018). The winter oilseeds function as cover crops to provide ecosystem services vital to Midwest agriculture through continuous living cover in the autumn, winter, and spring months (Weyers et al., 2021). The winter oilseeds have been shown to reduce nitrate concentrations by over 80% compared to fallow-tilled systems, reduce soil erosion and loss of total suspended solids during heavy spring rainfall, and provide forage to pollinators in the early spring when pollinator resources are scarce (Forcella et al., 2021; Weyers et al., 2019).

SCN is the most prevalent pest affecting soybean in the United States. It is estimated that soybean yield losses from SCN have an economic impact ranging from

$1.24 to 1.69 billion USD in the United States annually (Bandara et al., 2020). Soybean is protected from SCN-related yield loss through resistance derived from the breeding line PI 88788, used in approximately 95% of commercial soybean cultivars (Kandel et al., 2017; Tylka et al., 2019). However, widespread reliance and deployment of PI 88788 resistance have resulted in a breakdown of the effectiveness of the resistance, as evident through SCN population density increase and yield decrease in fields using PI 88788 derived resistance (McCarville et al., 2017).

Cover crops as part of a crop rotation solution to SCN management have been proposed, as cover cropping is an increasingly adopted practice by growers in the United States (Wallander et al., 2021). Cover crops may affect SCN population density by serving as a trap crop, releasing inhibitory allelochemicals, or stimulating hatching (Niblack & Chen, 2004). Recent laboratory and greenhouse studies indicate that cover crops such as annual ryegrass (*Lolium multiflorum*), daikon radish (*Raphanus sativus var. Longipinnatus*), and crimson clover (*Trifolium incarnatum*) may act to reduce SCN population density through one or more of these mechanisms (Acharya et al., 2019; Harbach et al., 2021).

In greenhouse experiments and weed surveys, pennycress has been reported as an alternative host to soybean cyst nematode (SCN, *Heterodera glycines*), and the number of observed SCN cysts on pennycress plants has represented 16-34% of the number of cysts observed on the susceptible soybean (Hoerning et al., 2022; Poromarto et al., 2015; Venkatesh et al., 2000). Camelina was recorded as a poor or non-host of SCN in similarly designed experiments, and camelina reduced SCN population density when tested in naturally-infested soil (Acharya et al., 2019; Poromarto & Nelson, 2010). It is unknown if

adding the oilseed crops to the corn-soybean field rotation will affect the population density of SCN as it did in controlled temperature greenhouse studies. Low soil temperatures during the autumn, winter, and spring, when the oilseeds undergo vernalization, may affect SCN development. SCN, as a poikilothermic organism, cannot regulate its body temperature (Barker et al., 1992). The body temperature fluctuates with the environment, and soil temperature influences embryogenesis and SCN development (Barker et al., 1992). The SCN life cycle is interrupted at temperatures below 10°C (Alston & Schmitt, 1988; Ross, 1964). If juveniles infect the roots in the autumn before the soil reaches 10°C and are killed by freezing winter temperatures, the oilseeds may function as a trap crop (Trivedi & Barker, 1986). However, SCN could also stop development and remain dormant in the roots over the winter and then restart development when soil temperatures rise, as observed in the winter-annual weed purple deadnettle (*Lamium purpureum*) (Creech, Santini, et al., 2007). It is unknown how the oilseeds will function at the field level outside of the controlled temperature conditions of the greenhouse. The objective of this study was to investigate whether adding pennycress and camelina as winter oilseed cover crops to a soybean-oilseed-corn-soybean rotation affected SCN field population density.

Materials and Methods

Location of the field experiment

A three-year experiment consisting of a rotation of soybean in year one, winter oilseed/corn in year two, and soybean in year three, was established in 2016 at three sites in Minnesota. The sites were selected to represent the corn-soybean growing regions in Minnesota. The three sites were the Rosemount Research and Outreach Center in Rosemount, MN (44.70638°, -93.07398°), the Southern Research and Outreach Center in

Waseca, MN (44.09151°, -93.54650°), and West Central Research and Outreach Center in Morris, MN (45.59098°, -95.87537°). 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 4.4%. The soil at the Waseca site was a well-drained Glencoe clay loam (Fine-loamy, mixed, mesic Cumulic Endoaquolls) silt loam with a pH of 8.0 and an organic matter content of 7.0%. The soil at the Morris site was a well-drained McIntosh silt loam (Fine-silty, mixed, frigid Aquic Calciudolls) with a pH of 8.0 and an organic matter content of 5.1%. All field sites tested greater than 30 mg kg^{-1} P and more than 100 mg kg^{-1} K.

Experimental design and treatments

The experiment was a split-plot design with six replicates. The main plot factor, initial population density, consisted of two levels. A low level (~3500 eggs/100 cm^3 soil) was achieved by planting a soybean cultivar containing SCN resistance ('Peking'), and a high level (~6200 eggs/100 cm^3 soil) was achieved by planting a cultivar that did not contain SCN resistance in year 1. The sub-plot factor, oilseed, consisted of three levels (pennycress, winter camelina, and no oilseed control). The treatment factors were assigned to plots which were the experimental unit. Plots were 4.6-m wide and 9.1m long.

Field management

Glyphosate [N-(phosphonomethyl) glycine] was applied as a burndown herbicide treatment (0.84 kg a.e. ha^{-1}) from 21 to 30 April 2016 to prepare for no-till planting. 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 resistance, and ‘Prairie Brand PB-1611R2’, susceptible to SCN, 1.6 maturity group with glyphosate resistance were planted. At Waseca, ‘Pioneer P22T69R’, Peking-source resistance to SCN, 2.2 maturity group, with glyphosate resistance, and ‘Prairie Brand PB-2419R2’ susceptible to SCN, 2.3 maturity group, with glyphosate resistance were planted. The soybeans were no-till planted at Rosemount, Waseca, and Morris on 6 May, 24 May, and 20 May 2016, respectively, at a rate of 320,000 seeds ha^{-1} and 76 cm row-spacing. To control emerging weeds, glyphosate was applied (0.84 kg a.e. ha^{-1}) from 25 to 30 June 2016. The soybeans were harvested from 10 to 19 October 2016 using a plot combine. Field pennycress and winter camelina were planted at soybean harvest at a rate of 13.5 kg ha^{-1}, both with 90% live seed germination. The planting density was approximately 2,600,000 seeds ha^{-1} for pennycress and 2,100,000 seeds ha^{-1} for camelina. The pennycress was treated with gibberellic acid (GA3) for 24 hr to achieve a 90% seed germination rate (Karimmojeni et al., 2014; Metzger, 1983). ProGibb® T&O Plant Growth Regulator Solution was used for the gibberellic acid treatment at an active ingredient concentration of 0.08% (w/w). The oilseeds were planted using a Truax Flex II Grain Drill (Truax Company Inc., New Hope, MN) seeder with a 20 cm row spacing. Before the winter oilseed main stem elongation stage, a fertilizer application of $N–P_2O_5–K_2O$ at 78–34–34 kg ha^{-1} was broadcast over each plot from 20 to 30 April 2017. The oilseed plots were harvested at physiological maturity with a plot combine from 20 June to 5 July 2017. The cutting bar of the combine was set at 10 cm above the soil surface. After the oilseed harvest, glyphosate was applied as a burndown herbicide treatment (0.84 kg a.e. ha-1) from 27 June to 12 July 2017 to prepare for no-till corn planting. Croplan CP2330VT2P/RIB corn with 83 d relative

maturity and glyphosate resistance was planted. The corn was planted on a 76 cm row spacing with 90,000 seeds ha^{-1} from 1 July to 14 July 2017. It was harvested from 1 to 8 November 2017 using a plot combine. The plots received a glyphosate application at 0.84 kg, a.e. $ha^{-1,}$ and S-metolachlor application at a rate of 0.56 kg a.e. ha^{-1} from 15 to 25 May 2018 to control emerging weeds. Soybean 'Prairie Brand PB-2419R2' susceptible to SCN, 2.3 maturity group, with glyphosate resistance, was no-till planted at Waseca on 17 May 2018. 'Prairie Brand PB-1611R2,' susceptible to SCN, 1.6 maturity group, with glyphosate resistance, were planted at Rosemount and Morris on 30 May and 25 May 2018, respectively. The seeding rate was 320,000 seeds ha^{-1} with 76 cm row spacing. An additional application of glyphosate at a rate of 0.84 kg, a.e. ha^{-1}, and Sodium salt of fomesafen at a rate of 1.68 kg a.e. ha^{-1}, was applied from 18 to 23 June 2018 to all plots. The plots were harvested from 18 to 24 October 2018 using a plot combine.

Plant data collection

Aboveground biomass of the winter oilseeds was hand-harvested in two 0.25 m^2 quadrats per plot; samples were collected from 14 to 20 June 2017. The two samples were averaged per plot. Stand counts for the oilseeds were also collected from 14 to 20 June 2017. Two 1-meter row samples per plot were assessed from the middle plot rows, and the two samples were averaged per plot. The harvest samples were dried for 72 h at 60°C to obtain dry matter weight. Dry matter was achieved when the weight of the samples was constant for two consecutive readings. Total winter oilseed biomass prior to seed threshing was recorded. Threshed oilseeds were cleaned and weighed. Corn was harvested with a plot combine to obtain yield. The middle two rows of every plot were harvested as a representative sample. The harvest samples were dried, weighed, and adjusted to 15.5% moisture. For soybean, the middle two rows of every plot were

harvested as the representative sample to obtain yield for the plot using a plot combine. The harvested samples from the combine were dried, weighed, and weights adjusted to 13% moisture.

Sampling for SCN

Nematode egg densities were initially determined at oilseed planting (Pi) in the autumn of 2016. In 2017 egg density was sampled at four periods: one month prior to oilseed harvest (P1), oilseed harvest (P2), two months after corn planting (P3), and corn harvest (P4). In 2018 egg density was sampled at four periods: soybean planting (P5), one month after soybean planting (P6), two months after soybean planting (P7), and soybean harvest (Pf). A soil sample composed of 20 soil cores was collected across an area of 6.1 m × 3.0 m from the center of each plot. Each soil core was taken to a depth of 20 cm with a 2-cm-diameter soil probe. Percent increase or decrease was calculated using overall treatment means and the percent change equation. The experiment's SCN population change factor (PCF) was determined by dividing SCN population density at a given sampling period by initial SCN population density (Pi = egg density at oilseed planting). PCF was calculated on a per plot basis and analyzed as described in the linear mixed model procedure used for SCN population density

Lab processing of soil samples

Before processing, the soil samples were transferred to and stored at 4°C in a refrigerator. Each soil sample was mixed with the cores from the same plot, and the cysts were extracted from a subsample of 100 cm^3 soil with a semiautomatic elutriator (Byrd et al., 1976). After extraction, cysts were separated from debris with centrifugation in 76% (w/v) sucrose solution (Liu & Chen, 2000). Cysts were crushed to release the eggs on a 150-µm-aperture sieve with a rubber stopper mounted on a motor (Faghihi & Ferris,

2000). The eggs were collected on a 25-μm-aperture sieve and separated from debris by centrifugation in a 35% (w/v) sucrose solution (Liu & Chen, 2000). An aliquot of egg suspension was used to count eggs. The number of eggs per 100 cm^3 of soil was determined.

Data Analysis

Linear mixed-effect models were used to estimate the effects of the factors on the response variables. All analyses were conducted with R, version 4.0.5. For SCN population density, a count data parameter, a Poisson error structure was initially attempted, but overdispersion was present as the conditional variance was larger than the conditional mean of the dependent variables. As a result, the final model was a random intercept generalized linear mixed-effect model with a negative binomial response (packages lme4, MASS; Bates et al., 2015; Venables & Ripley, 2002). A random intercept generalized linear mixed-effect model with a normal distribution error structure was used for biomass, yield, and stand count data. Finally, the DHARMa package was used to examine residuals and test functions for misspecification issues such as overdispersion, zero inflation, and residual autocorrelation (Hartig, 2020).

Analytical assumptions for the mixed analysis of variance were examined using DHARMa and visual inspection of the residual plots. Block and location were treated as random effects in all models. Location was initially designed as a random effect in the experiment, and the three locations chosen represented the most prevalent soybean growing regions in Minnesota (Bolker et al., 2009). Soybean cultivar and oilseed crop treatment were treated as fixed effects. Sampling periods were analyzed independently. One block at the Morris site was damaged by herbicide drift from an adjacent field operation. This block and its respective plots were excluded from the analysis. Planter

skips were also present in some plots. If the stand count was 50% lower than the site average and plant skips were noted in field notes, these plots were excluded from the analysis. At Morris, a hard freeze damaged corn in 2017. The corn was unable to be combine harvested at this location. Wald χ^2-tests were used to assess the significance of the fixed effects (Fox & Weisberg, 2019). When the significance of fixed effects exceeded the predetermined significance threshold of $P < 0.05$, the differences between means were examined using least-square means (package: lsmeans; Lenth, 2016). For figures and tables, means were reported on the original scale.

Results

Population Density of SCN

SCN population densities were collected and reported at nine sampling intervals to assess the effect of initial population density and oilseed crop on SCN egg densities in the field. The effects of initial SCN population densities (I), oilseed crop (O), and the interaction between these two factors (I x O) on egg densities are presented in Table 2-1. At pennycress establishment in the autumn of 2016, an initial SCN population was determined to create the baseline population for the study. Plots with susceptible soybeans in the previous year were denoted as a "high" initial population (6,215 eggs/ 100 cm^3 soil average of the three sites), and plots with resistant soybeans in the previous year were denoted as a "low" initial population (3,572 eggs/ 100 cm^3 soil).

At the sampling period P1 in spring 2017, after winter and soil thaw, and at sampling period P2, following pennycress harvest, the only significant factor driving SCN egg density was the initial field population density treatments. The pattern continued for the next two sampling periods in 2017 as initial field population density was the only significant factor through sampling period P3, mid-season double-crop corn,

and P4 at corn harvest. The overall mean SCN population density decreased over the first year from Pi to P4 when the oilseed and corn crops were present in the cropping system by 44%. For the significant factor of initial field population density, the “high” treatment decreased by 40%, while the “low” treatment decreased by 52% in the first year of the experiment.

In 2018, SCN susceptible soybean was planted in all plots. Pre-plant soybean sampling period (P5) and early season vegetative soybean (P6) showed the same pattern as observed in the corn year, in which initial field population density was the only factor that was significant (Table 2-2). At sample period P7 in August, when soybean development was at R1, there was no detectable difference in the initial field population factor as both treatments experienced growth in SCN population density with susceptible soybean in the rotation. From P6 to P7, the “low” initial field population density increased by 262%, while the “high” initial treatment increased by 127%. The higher growth in SCN population density of the “low” treatment continued throughout the susceptible soybean rotation. At the final sampling period (Pf), the initial field population density factor was again significant. The difference between treatments was driven by a substantial increase in SCN population density on the “low” initial treatment during the susceptible soybean cropping treatment. From Pi to Pf, the “low” treatment increased by 138%. The “high” treatment increased 10% from baseline levels in the same two-year period. There was no difference between the three-oilseed crop (O) treatments during any sampling period from 2016 to 2018 (Table 2-1). The presence or absence of the oilseed crops did not have a detectable effect on SCN population density. There was no

interaction between the initial population density and oilseed crop treatment (I x O) at any sampling period (Table 2-1).

Population Change Factor

A population change factor (PCF) was determined at four time periods, pennycress harvest (PCF1), corn harvest after year one (PCF2), SCN susceptible soybean planting (PCF3), and soybean harvest (PCF4). The initial sample period (Pi) was used as the baseline for the PCF calculation. SCN population density and PCF followed the same generalized trend, as the only significant factor was the initial field population density. The oilseed factor or (I x O) interaction was not significant at any PCF. Both the "low" and "high" treatments had PCF values below 1.0 following pennycress harvest and corn harvest (Table 2-5), indicating attrition of the SCN population density during this period. At oilseed and corn harvest, the PCF was significantly higher in the "high" initial population density treatment, indicating a lower rate of population change than in the "low" treatment. In 2018 at PCF3 following soybean planting, the "high" treatment was significantly higher than the "low" treatment, with 1.16 and 0.75, respectively. Following the 2018 growing season, susceptible soybean was grown in all plots; the "low" initial field density had higher reproduction (PCF=4.91) than the "high" initial field density treatment (PCF = 1.44). PCF values for all treatments are in Table 2-6.

Plant density, yield, and biomass

The oilseed crop (O) treatment effect impacted winter-oilseed seed yields, stand density, and biomass (Table 2-3). Overall, camelina had better growth than pennycress. Camelina seed yield (0.76 Mg ha^{-1}) was 48% greater than the pennycress seed yield (0.40 Mg ha^{-1}). Camelina stand density was 40% greater than pennycress stand density (Table

2-4). Camelina biomass (10.87 Mg ha^{-1}) was 56% greater than pennycress biomass (4.79 Mg ha^{-1}). The treatment effect of initial population density or the interaction of treatment effect (I x O) did not impact oilseed stand density, seed yield, or biomass. In 2017, corn stand count and yield were not influenced by treatment factors or the interaction (Table 2-3). Corn stand counts averaged 87,548 plants ha^{-1}, and corn yield averaged 5.97 Mg ha^{-1} across treatments. In 2018, neither treatment factors nor their interaction was influenced by soybean stand count and seed yield (Table 2-3). Soybean stand counts averaged 315,467 plants ha^{-1}, and soybean yield averaged 3.01 Mg ha^{-1}.

Discussion

Field trials were conducted using a double-cropping system for Midwest winter oilseed production (Gesch et al., 2014). The cropping system includes the winter oilseed planted into a double-crop rotation with corn and soybean. The field locations chosen for this study were in south, southwest, and western Minnesota. These state regions have historic soybean-corn rotational production representing millions of hectares of production in the state, and reflect the corn-soybean management practices used across the Midwest (Galzki et al., 2018). Growers manage SCN in Midwest soybean production regions by deploying resistant soybean varieties and rotation with non-host crops. These strategies have controlled SCN population density in production fields for decades (Joos et al., 2013; Noel & Wax, 2003; Tylka, 2020).

SCN management disruptions may occur when adding a new crop to the corn-soybean rotation, especially if the added crop is an alternative host to SCN. In previous weed surveys and greenhouse assessments, pennycress has been reported as an alternative host to SCN with 16-34% of females compared to susceptible soybean (Hoerning et al., 2022; Poromarto et al., 2015; Venkatesh et al., 2000). In screening for SCN-resistance,

when the comparative number of females to susceptible soybean is less than 10 (Female Index <10), the soybean varieties are considered resistant to the population of SCN tested. This relative number of female development (FI =10) was chosen as SCN population density at or below 10% of the numbers observed on susceptible soybean could not sustain themselves over a single growing season (Golden et al., 1970; Niblack et al., 2002). Camelina also has been reported as an alternative host of SCN but has been described as a poor host as females collected from camelina roots were less than 1% of females collected on susceptible soybean (Poromarto & Nelson, 2010). In another study, no females were observed on camelina, and including camelina in naturally SCN-infested soil reduced SCN population density compared to the control treatment, implicating it as a potential trap crop (Acharya et al., 2019).

Pennycress and camelina included in field rotation in this study did not impact SCN population density compared with the control treatment. The low/high initial population density, established by planting either resistant or susceptible soybeans prior to planting the oilseeds in autumn 2016, impacted SCN population density in the field. This effect was consistent through the oilseed and corn rotation in 2017 and during susceptible soybean plant and early season sampling in 2018. Sampling periods P5 through Pf included actively growing SCN susceptible soybean in all plots. In 2018, the increase in overall population density across plots resulted from the susceptible soybeans in the rotation. The effect of the initial population density treatment factor was no longer significant ($P > 0.05$) at sample period 7 (P7), but by the end of the season, the factor again impacted SCN population density at the final sample period (Pf). In a reversal of the trend prior to sample period Pf, the plots with the low initial population density

treatment had a higher final population density than the plots with the high initial population density treatment. The reason for the trend reversal is unknown, but two explanations are proposed. First, it is known that infection with SCN has a density-dependent effect on root growth and biomass (Endo & Veech, 1970; Wang et al., 2007); therefore, it is possible that the high initial population density treatment which had a higher SCN population density at the start of 2018, had lower root growth. This lower root growth may have resulted in fewer root infection sites for the later generations of SCN when the temperatures warmed in late summer and the development rate increased. However, there were no yield differences between initial population density treatments at the end of the season, which would be expected if root biomass accumulation was causal to observed differences present by the end of 2018. The second explanation involves the presence of natural biocontrol agents and their role in SCN suppressive soils. The rebound of SCN population density in the low initial population density treatment may be explained because the resistant cultivar not only lowers SCN population density but also lowers biological antagonists, which results in the soil being less suppressive (Chen, 2020; Hu et al., 2019; Strom et al., 2020). Subsequently, in 2018, when the susceptible soybean was added to the rotation, the less suppressive soil allowed a greater SCN development and reproduction, resulting in a higher SCN population density at soybean harvest.

In a previous study evaluating winter-annual weeds in a field rotation, sparse weed density was hypothesized to explain the lack of effect on the SCN population density of known alternative winter weed hosts. However, winter-annual weeds were observed at a density of 36 and 75 plants m^{-2}, lower than the density of winter oilseed

crops in this study (Creech et al., 2008). At harvest, the stand density was 76 plants m^{-2} for pennycress and 127 plants m^{-2} for camelina. The hypothesis that these plant densities were insufficient to facilitate J2 navigation and infection is still possible but unlikely, given that susceptible soybean had a lower stand density of 31 plants m^{-2} but increased overall SCN population density in 2018. In addition, the oilseed rows were drilled every 20 cm, whereas the soybean rows were 76 cm apart. Therefore, it seems unlikely that J2 could navigate and infect susceptible soybean at wider row spacing and lower plant density but could not navigate to the more prevalent and narrower row-spaced oilseed crops.

Another explanation for the lack of effect observed with the winter oilseed treatments is the period of time that the crops are in the system. Pennycress and camelina are planted in autumn, overwinter, and are harvested in early summer (Cubins et al., 2019; Hoerning et al., 2020). At the southernmost location in this study, Waseca, the 10cm soil temperature dropped below 10°C in late October and generally did not rise above this temperature until late April during the study period (UMN-SROC, 2019). SCN fails to complete a life cycle at soil temperatures below 10°C (Alston & Schmitt, 1988; Ross, 1964). For six of the nine months that the oilseed crop is in rotation, temperatures are below the temperature threshold needed for life cycle completion. The rate of SCN development is known to increase linearly at temperatures between 15 and 30°C, meaning development is slower at lower temperatures (Alston & Schmitt, 1988). The discrepancy in SCN development between temperature-controlled greenhouse settings and the field could be due to this temperature affect, as development occurs much slower under field conditions. The SCN egg hatch and juvenile emergence rate declines with

decreasing temperatures in the autumn then increases as dormancy is broken and soil temperatures rise in the spring (Bonner & Schmitt, 1985; Hill & Schmitt, 1989). However, the spring development rate might be too slow prior to winter oilseed harvest for an entire SCN life cycle to be completed.

A final factor that may impact SCN life cycle completion in winter oilseeds is seasonal diapause. In climates with cold winters, SCN enters seasonal diapause beginning in early autumn (Yen et al., 1995). This diapause response drastically reduces egg hatch and may indicate that SCN does not infect and develop in the oilseeds in the autumn after planting.

Adding winter oilseed crops to Midwest cropping rotations enhances ecosystem services and profitability for growers by adding a third crop in a rotation and providing continuous living cover on the landscape during typically fallow months. This study indicates that winter pennycress and camelina can be added to the cropping system without affecting SCN field population density in Minnesota's corn and soybean production regions. However, as the soil temperature is likely correlated with the lack of effect observed in the cropping rotation, study locations farther south with warmer soil temperatures and differently adapted populations of SCN should be examined for changes in SCN population density when pennycress or camelina are added to the rotation.

Tables

Table 2-1: Mixed-model analysis of variance for SCN population density (eggs 100 cm^{-3} soil) following different main season and winter oilseed crops.

Crop	2016 Oilseed	2017 Oilseed	2017 Oilseed	2017 Corn	2017 Corn	2018 Soybean	2018 Soybean	2018 Soybean	2018 Soybean
	Pi	P1	P2	P3	P4	P5	P6	P7	Pf
Initial Population (I)	36.28***	50.00 ***	36.86 ***	45.22 ***	46.41***	26.74***	31.17***	0.58	8.01**
Oilseed (O)	1.05	1.33	1.21	0.17	0.35	2.38	1.28	1.04	1.88
I x O	1.76	2.26	3.36	1.39	0.67	0.42	0.62	4.68	0.36

The fixed effects of initial SCN population [(I) low and high], oilseed [(O) camelina, none, and pennycress], and their interactions were tested using the Wald chi-square method. All models included location and block as random effects. A separate analysis was performed for each sample period (Pi-Pf). *, **, and *** represent significance of Wald chi-square tests at a $P < 0.05$, 0.01, and 0.001, respectively.

Table 2-2: Population densities (eggs 100 cm^{-3} soil) of SCN in field plots in Minnesota from 2016-2018 following different main season and winter oilseed crops.

Crop	2016 Oilseed	2017 Oilseed	2017 Oilseed	2017 Corn	2017 Corn	2018 Soybean	2018 Soybean	2018 Soybean	2018 Soybean
	Pi	P1	P2	P3	P4	P5	P6	P7	Pf
Initial Population Factor									
Low	3572a	3058a	2609a	2129a	1724a	2163a	1776a	6433	8499b
High	6215b	6287b	4581b	4335b	3709b	3057b	2715b	6150	6866a
Oilseed Factor									
Camelina	4883	5537	3994	3161	2871	3094	2529	6521	7957
None	5601	4801	4240	3927	2998	2694	2132	6526	7522
Pennycress	4725	4309	2985	3057	2662	2238	2245	5834	7051

Different letters within columns indicate a difference between means examined using least-square means with a significance value of $P < 0.05$. A separate analysis was performed for each sampling period (Pi – Pf)

Table 2-3**:** Mixed-model analysis of variance for biomass, stand count, and yield.

Crop	2016 Oilseed Biomass (Mg ha^{-1})	2017 Oilseed Grain Yield (Mg ha^{-1})	2017 Oilseed Stand Count (plant ha^{-1})	2017 Corn Grain Yield (Mg ha^{-1})	2017 Corn Stand Count (plant ha^{-1})	2018 Soybean Grain Yield (Mg ha^{-1})	2018 Soybean Stand Count (plant ha^{-1})
Initial Population (I)	0.28	0.45	0.88	1.03	1.93	1.61	0.09
Oilseed (O)	62.22 ***	93.84***	66.18***	3.71	0.09	0.67	0.60
I x O	0.59	0.59	0.01	0.10	1.13	2.54	2.67

The fixed effects of initial SCN population [(I) low and high], oilseed [(O) camelina, none, and pennycress], and their interactions were tested using the Wald chi-square method. All models included location and block as random effects. *, **, and *** represent significance of Wald chi-square tests at a $P < 0.05$, 0.01, and 0.001, respectively.

Table 2-4: Agronomic measurements collected from field plots in Minnesota from 2016-2018 for biomass, stand count, and yield.

Crop	2017 Oilseed Biomass (Mg ha^{-1})	2017 Oilseed Grain Yield (Mg ha^{-1})	2017 Oilseed Stand Count (plant ha^{-1})	2017 Corn Grain Yield (Mg ha^{-1})	2017 Corn Stand Count (plant ha^{-1})	2018 Soybean Grain Yield (Mg ha^{-1})	2018 Soybean Stand Count (plant ha^{-1})
Initial Population Factor							
Low	7.34	0.57	984,738	5.74	88,580	2.94	314,952
High	7.82	0.58	1,043,054	6.21	86,515	3.09	315,981
Oilseed Factor							
Camelina	10.87a	0.76a	1,266,584a	5.37	87,669	3.02	320,163
None	-	-	-	6.07	86,758	3.07	313,022
Pennycress	4.79b	0.40b	761,208b	6.47	88,216	2.95	313,215

Different letters within columns indicate a difference between means examined using least-square means with a significance value of $P < 0.05$.

Table 2-5: Mixed-model analysis of variance for population change factor (PCF)

Population Change Factor	2017 PCF Winter Oilseed Harvest	2017 PCF Corn Harvest	2018 PCF Soybean Plant	2018 PCF Soybean Harvest
Initial Population (I)	6.25*	7.13 **	20.17***	44.33 ***
Oilseed (O)	0.04	2.90	3.77	2.00
I x O	4.80	1.10	0.54	0.86

The fixed effects of initial SCN population [(I) low and high], oilseed [(O) camelina, none, and pennycress], and their interactions were tested using the Wald chi-square method. All models included location and block as random effects. *, **, and *** represent significance of Wald chi-square tests at a $P < 0.05$, 0.01, and 0.001, respectively.

Table 2-6: Population Change Factor (PCF) on a per plot basis from field plots in Minnesota from 2016-2018.

Population Change Factor (PCF)	2017 PCF Winter Oilseed Harvest	2017 PCF Corn Harvest	2018 PCF Soybean Plant	2018 PCF Soybean Harvest
Initial Population Factor				
Low	0.70a	0.50a	0.75a	4.91b
High	0.93b	0.78b	1.16b	1.44a
Oilseed Factor				
Camelina	1.04	0.87	1.18	3.35
None	0.70	0.51	0.78	2.29
Pennycress	0.74	0.59	0.97	3.21

Different letters within columns indicate a difference between means examined using least-square means with a significance value of $P < 0.05$.

Chapter 3 Microplot evaluation of SCN development in pennycress as affected by planting date

Synopsis

Pennycress (*Thlaspi arvense* L.) is a winter-annual crop that can be incorporated into Midwest corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) rotations to provide ecosystem services and increased productivity. Under greenhouse conditions, pennycress is an alternative host to soybean cyst nematode (SCN, *Heterodera glycines*), a devastating pathogen in soybean. Cold temperatures hinder SCN development, and it is unknown if development is facilitated during the pennycress winter-annual life cycle. This study examined SCN juvenile and female development in microplots following five different autumn planting date treatments. Juveniles were present in pennycress roots in the autumn and spring. However, mature females were not observed in the autumn on pennycress plants. Mature female development on pennycress plants was observed in the spring before plant senescence and harvest. The latter two autumn planting dates, September 26 and October 10, had the highest cumulative female count. The earliest autumn plant date (August 15) had the lowest cumulative female count. Spring senescence date appears to impact female development, as the August 15 date was the first treatment to senesce, and later senescing treatments had higher cumulative females recovered. Early pennycress planting dates or earlier maturing pennycress varieties may reduce overall SCN female development.

Introduction

Pennycress (*Thlaspi arvense* L.) is under development as a winter-annual crop that can provide ecosystem services and produce seedpods with harvestable value, and it is being introduced into U.S. Midwest corn (*Zea mays* L.) and soybean (*Glycine max* [L.]

Merr.) rotations (Zanetti et al., 2019). These rotations cover ~175 million acres of the region (Jez et al., 2021). Using the fallow period in corn-soybean rotations to add an additional crop can capture radiation, rainfall, and nutrients otherwise unused or lost in the summer-annual rotation during this period (Heggenstaller et al., 2009). Pennycress has been implemented in double-crop or relay-crop rotations and can add 2000 kg/ha of oilseed yield to the corn-soybean rotation (Hoerning et al., 2020; Johnson et al., 2017). In addition, pennycress oilseeds contain 30-36% oil and 20% protein making the crop viable for end-use in sustainable aviation fuel and feed markets (Hojilla-Evangelista et al., 2015; Moser et al., 2009). Pennycress also provides various ecosystem services to the corn-soybean rotation when the ground is typically fallow. These ecosystem services include preventing soil erosion, reducing nitrate-N leaving the field through leaching, and providing early season forage to pollinators (Cecchin et al., 2021; Forcella et al., 2021; Moore et al., 2020; Weyers et al., 2019).

Soybean cyst nematode (SCN, *Heterodera glycines*) is the most damaging pest affecting soybean in the United States and is widespread across the soybean production regions (Tylka & Marett, 2021). Annual yield losses caused by SCN on soybean in the United States is estimated at $1.24-1.69 billion USD (Bandara et al., 2020; Bradley et al., 2021). SCN-related soybean yield losses of up to 60% have been recorded in susceptible cultivars, and yield losses of 30% can occur in soybean without noticeable aboveground symptoms (Mueller et al., 2016; J. Wang et al., 2003). Growers currently manage SCN primarily using resistance incorporated from plant introduction PI 88788, present in 95% of all commercial cultivars (Joos et al., 2013; Gregory L Tylka & Mullaney, 2015). In addition to the use of resistance, SCN management recommendations include crop

rotation with non-host crops, seed-applied nematicides, biological controls, and weed management (Beeman & Tylka, 2018; Bissonnette et al., 2020; Mueller et al., 2016; Rocha et al., 2021; Wight et al., 2018).

Adding a plant species to an existing rotation can affect SCN population density (Creech et al., 2007). Pennycress has been reported as an alternative host to SCN in greenhouse experiments, and the number of cysts recovered from pennycress plants has represented 16-34% of the number of cysts observed on the susceptible soybean control (Hoerning et al., 2022; Poromarto et al., 2015; Venkatesh et al., 2000). Pennycress is an overwintering crop that requires a vernalization period to induce spring flowering, and it can survive temperatures below -20℃ (Warwick et al., 2002). This cold tolerance is ideal for plant survival in Minnesota, where winter temperatures in January and February [30-year normal temperature means (1991-2020)] for Saint Paul, MN, are -9.2℃ and -6.8℃, respectively, and daily average minimum temperatures are -13.3℃ for January and -11.5℃ for February (National Centers for Environmental Information, 2021). Unlike greenhouse studies, where temperatures were maintained from 24-27℃ to facilitate SCN development, field weather conditions are much colder (Hoerning et al., 2022; Venkatesh et al., 2000). SCN are poikilothermic organisms that cannot regulate their body temperature (Barker et al., 1992). Therefore, low soil temperatures influence SCN development rates, and juveniles do not proceed to the J2 stage below temperatures of 10℃ (Alston & Schmitt, 1988; Ross, 1964). SCN development is hypothesized to occur within two periods of the pennycress winter-annual life cycle based on minimum thermal temperatures. Development period one occurs in the autumn from pennycress planting until the soil temperature falls below 10℃. Development period two occurs in the spring

after the soil temperature warms above 10℃ until pennycress plant senescence. The objective of this experiment was to i.) quantify new female development on pennycress under field conditions. and ii.) examine juveniles in the roots of pennycress in autumn and spring.

Materials and Methods

Plot preparation and nematode population classification

The microplot experiments were conducted in a research field location at the University of Minnesota-Twin Cities Saint Paul Research Center from 1 August 2018 to 31 September 2019 and repeated once in the same period in 2019-2020. The field site had no previous detection of SCN and was planted into perennial ryegrass in May of 2018. Microplots were established by removing the top layer of ryegrass sod from a 1 m^2 area. The field soil in the microplots was removed from the plots to a depth of 25 cm. The microplots were filled with naturally infested field soil from the Southern Research and Outreach Center in Waseca, MN (44.09151°, -93.54650°). A total of 3000 kg of soil was collected from the top 10 cm of the soil profile in a field planted with a susceptible soybean variety in late July 2018 and collected from the same site in July 2019. The susceptible soybean variety was Prairie Brand 2419, with 2.4 RM planted in mid-May. The soil at the Waseca site was a well-drained Glencoe clay loam (Fine-loamy, mixed, mesic Cumulic Endoaquolls) silt loam with a pH of 8.0 and an organic matter content of 7.0%. The soil was thoroughly mixed with a hand shovel and roto-tiller to obtain equal levels of SCN in each microplot. The soil was then moved into the microplots. The average SCN density of the mixed, naturally infested soil in the microplots was 7,380 eggs/100 cm^3 soil at the first pennycress planting in 2018 and 6,840 eggs/100 cm^3 in 2019. The SCN population present at the site was tested and confirmed as HG Type 2.5.7

using the standard HG typing procedure (Niblack et al., 2002). The full results of the HG test are presented in Table 3-1.

Experimental design and planting

The experiment used a randomized design with two plot replicates. The winter pennycress accession 'MN106' was planted at five predetermined dates in the autumn: August 15, August 29, September 12, September 26, and October 10. Pennycress seeds were planted in a 10 x 10 grid within a microplot equidistant from each other. 2-3 seeds were planted in each grid location to ensure germination. After pennycress planting, the soil was hand-watered to field holding capacity. Three days after germination, the seedlings were thinned to one plant per grid location. Plants were approximately 8 cm apart and at least 10 cm from the microplot edge. In the same grid pattern as the pennycress plants, a susceptible soybean variety, 'Sturdy,' was planted in microplots on two planting dates in the spring, May 15 and May 29. Two soybean seeds were planted per grid location, and the seedlings were thinned three days following germination. An application of N at 76 kg ha^{-1} was broadcast over each plot on 12 April 2019 and 14 April 2020. Maturity dates for each planting date treatment for pennycress and soybean are presented in Table 3-2. For soybean and pennycress, full maturity was when 95% of the pods reached their mature color. Environmental temperature and precipitation data were collected at the University of Minnesota, St. Paul Campus Climate Observatory, located adjacent to the microplot field.

Sampling procedure and timing

The pennycress microplot sampling began 14 days after plant emergence and continued through November 15 in the autumn. Sampling restarted in the spring after the snow melted and the soil thawed on April 15. The pennycress microplots were sampled at

7-day intervals. There were five sampling periods spanning from June 4 to July 2 (hereafter PC1-5) when white SCN females were present in the samples collected. Zero values for white SCN females were present at other intervals in the pennycress microplots.

Soybean was included as a comparison tool for the sampling methodology. Direct comparison was not possible between the two botanical species because they have different life cycles. Pennycress is a winter-annual, and soybean is a summer-annual. Soybean microplot sampling occurred at 14-day intervals. The sampling began two weeks after soybean emergence and continued until November 15. White SCN females were recovered in eight sampling periods from June 18 through September 24 (SB1-8).

SCN root sample processing for cysts

Before removing the soil and root sample, the aboveground plant material was removed. The soil around the plant root was dug up using a spade hand shovel to a 10cm diameter and a depth of 15 cm. The samples were processed within 24 hours after sampling. The volume of soil removed with each plant root was approximately 1200cm^3. The sample was placed into a 3000 ml pitcher and filled with 2000 ml of water. The roots were manually rubbed in the water to remove cysts from the roots. The water in the pitcher was stirred to get the soil suspended in the water and decanted five times over an 850 μm-aperture sieve (#20) nested over a 250-μm-aperture sieve (#60). The debris remaining on the 850-μm-aperture sieve was sprayed with high-pressure water for 30 seconds, and the remaining material was discarded from the sieve (Krusberg et al., 1994). The cysts on the 250-μm-aperture sieve were rinsed into a 50 ml centrifuge tube with 76% (w/v) sucrose solution (Liu & Chen, 2000). The tubes were centrifuged for 5 minutes at 2500 rpm. Following centrifugation, the top pellet portion of the tube

containing cysts was poured onto a 250-µm-aperture sieve. Tap water was used to rinse the cysts into another 50ml tube to await counting. Counting occurred under a stereoscope within 48 hours after sample processing. Only new white females were counted on a per plant basis, as old cysts were present in the soil. New white females indicated that SCN female development occurred within a few weeks of sampling on the roots.

SCN root staining procedure for juveniles

At two sample periods in pennycress microplots, roots were stained to determine the amount of J2 and J3/J4 stage juveniles present in the roots. The two periods were: the end of the autumn for pennycress (October 30 hereafter R1) and the mid-season of pennycress spring growth (June 16 hereafter R2). The staining procedure was modified from the soybean root staining procedure described in Byrd et al., 1983. The stock solutions of acid fuschin consisted of 3.5 g acid fuchsin, 250 ml acetic acid, and 750 ml distilled water. First, the roots were washed, cut into approximately 2 cm segments, and placed into a 250ml beaker containing 50 ml of 1.5% NaOC1 solution mixed in DI water. The segmented roots were kept in the bleach solution for 4 minutes. Agitation and stirring occurred twice during this 4-minute soak. Next, the roots were removed from the bleach solution, placed on a 250-µm-aperture sieve (#60), and rinsed three times with DI water. Then the roots were put into a 250 ml beaker with 50 ml of DI water and soaked for 15 minutes. Next, the roots were removed from the DI water and placed into a 250 ml beaker with diluted acid fuschin stain (3.33% stock). The roots were stained in the solution for 5 minutes. In the acid fuschin stain, the roots were heated with a 1000-watt microwave for 1 minute. After allowing the sample to cool to room temperature (approximately 10 minutes), the roots were removed and again placed on a 250-µm-

aperture sieve (#60) and rinsed three times with DI water. The soybean roots were then returned to a 250 ml beaker with DI water, soaked for 10 minutes, and destained in acidified glycerin. The roots were then spread across a petri dish, and acidified glycerin was pipetted over the roots for preservation. Next, roots were pressed between glass plates and observed under a dissecting microscope. Destaining in acidified glycerin did not occur for the pennycress roots, as the procedure damaged the pennycress roots.

Data Analysis

The experiment was a completely randomized design with two replications that occurred in 2019 and 2020. A microplot was the experimental unit. All analyses were conducted with R, version 4.0.5. Linear mixed-effect models were used to estimate the effects of the factors on the response variables. A negative binomial error structure was used for SCN females and juveniles, count data parameters. The final model was a random intercept generalized linear mixed-effect model with a negative binomial response (packages lme4, MASS; Bates et al., 2015; Venables & Ripley, 2002). Analytical assumptions for the mixed analysis of variance were examined by visual inspection of the residual plots. Data from the two years of the experiment were combined for analysis. Year was treated as a random effect in the model. Sampling periods, as defined by Julian Date, were analyzed independently. For the cumulative total female count for the pennycress treatments, the five sampling period observations were combined for each treatment replicate and tested for the effect of planting date treatment. Likelihood Ratio χ^2-tests were used to assess the significance of the fixed effects (Fox & Weisberg, 2019). When the significance of fixed effects exceeded the predetermined significance threshold of $P < 0.05$, the differences between means were examined using

least-square means (package: lsmeans; Lenth, 2016). For figures and tables, means were reported on the original scale.

Results

Precipitation and temperature

Total precipitation amounts on a calendar basis were above the 30-year historical average (1991-2020) of 831mm in 2018 (877mm) and 2019 (1091mm) and below the historical average in 2020 (656mm). Pennycress stand establishment occurs from August through October, and sufficient rainfall is critical for proper establishment. During the pennycress establishment period in 2018, the total precipitation amount of 343mm was higher than the 30-year average (272mm); in 2019, the total precipitation amount for the establishment period was also higher than the 30-year average (424mm). Cumulative precipitation was also higher than the 30-year average (301mm) during the pennycress spring growth and seed set period (April through June) in 2019 (322mm) but lower in the same period in 2020 (260mm). The maximum and minimum air temperatures were consistent with the 30-year averages throughout the three years of the study as deviations from the normal range of 1-5℃ in the study period. Notably, in the autumn of 2018, the observed maximum averages were 3.1℃ below normal in October and 4.4℃ below normal in November. Observed minimum averages in the same months were 1.2℃ and 3.3℃ below normal, respectively. During the period of pennycress spring development in May of 2019, the maximum average was 3.1℃ below normal, and the minimum average was 2.3℃ below normal. The months of October and November were also below normal in 2019. The maximum average was 2.4℃ below normal, and the minimum average was 1.3℃ below normal for October. For November, the maximum average was 3.4℃ below normal, and the minimum average was 1.4℃ below normal.

SCN female counts in pennycress and soybean root samples

In the autumn of 2018 and 2019, SCN females were not observed in any sample period beginning after pennycress planting until the end of autumn sampling on November 15. SCN females were collected and reported at five sampling periods in the spring. These five sampling periods had new female observations in both years of the experiment. The autumn planting date treatment effect was significant in all five sampling periods (Table 3-3). New SCN females were recorded in all five treatments at each sampling period (Table 3-4). At PC1, the planting date treatment of September 26 was the only treatment that was higher than the other treatments, with 7.4 females. At PC2, the treatments for the September 12 and September 26 were higher than the other three. September 12 was the second-highest, with 9.1 females, and September 26 was the highest, with 23.1 females. For PC3, the September 26 treatment was the second highest with 16.5 females, and the October 10 planting date treatment was the highest with 28.6 females. In the sample period, PC4, August 29, and September 26 planting date treatments were the highest observations at 23.0 and 28.9 females, respectively. The final sampling period, PC5, showed the September 26 planting date as the second-highest treatment at 12.6 females and October 10 as the highest mean observation at 23.6 females. The highest recorded mean observation was 28.9 at sampling period PC4 for the planting date treatment on September 26. The lowest mean value occurred for the planting date treatment on August 15 on PC5, with an average of 1.5 females. The highest cumulative observations were recorded in the planting date treatments on September 26 and October 10, with 76.8 and 88.5 females, respectively. The August 29 and September 12 autumn planting dates were lower than the latest two autumn plantings but higher than the first planting date, August 15. The August 15 planting date had the

lowest recorded cumulative observation at 13.6 females. White females observed on pennycress, and a broken cyst containing eggs, are presented in Figure 3-3.

Susceptible soybean planted in spring at two different planting date treatments (May 15 and May 29) were sampled every 14 days using the same methodology for sampling pennycress. New SCN females were not observed on the treatments for soybean until Julian Day 169 (SB1). The soybeans treatments were used as a point of comparison for SCN female development, as the main objective was to quantify female development on pennycress. The soybean samples assist in understanding the magnitude of development across the two plant species, given the same volume of soil collected. The soybean planting date treatment effect was significant in four sampling periods (Table 3-5). For the May 15 planting date treatment, the means ranged from 5.8-to 169.3 females. For the May 29 planting date treatment, the means ranged from 3.0 to 132.8 females (Table 3-6). When comparing the magnitude of female development across species, the highest observed mean value across the pennycress treatments was 28.9 females. The highest female average on pennycress represents 17% of the observed highest female average on the susceptible soybean in the May 15 treatment and 22% in the May 29 treatment.

SCN juveniles in pennycress root samples

Plant roots were collected during two sample periods on October 31 in autumn and June 18 in spring (R1-2). For the autumn pennycress sampling period R1, both the J2 and J3/J4 juvenile response variables were significant ($P < 0.05$) (Table 3-7). The October 10 planting date treatment (planted 21 days before sampling) had no observed juveniles in the roots. For the J2, the August 15, August 29, and September 26 planting date treatments were not different from each other and ranged from 8.6-9.6 J2 juveniles

per gram root. For the J3 and J4 juvenile stage counts, the September 12 planting date had the highest observed mean at 10.4 J3/J4 juveniles per gram root. There were no significant differences among planting date treatments for the mid-pennycress sampling period R2 (June 18) (Table 3-7). For the J2 juveniles, the average across pennycress planting date treatments was 30.4, and for the J3/J4 juveniles, the average across pennycress treatments was 27.5 (Table 3-8).

Discussion

In previous greenhouse assessments, pennycress was reported as an alternative host to SCN at greenhouse growing temperatures of 24-27°C, in which pennycress female counts were 16-34% of the susceptible soybean check (Hoerning et al., 2022; Poromarto & Nelson, 2010; Venkatesh et al., 2000). Winter pennycress is an overwintering crop that requires a vernalization period to initiate flowering in the spring. In Minnesota, it is planted between August and October, overwinters, and is harvested in late June when using accession'MN106' (Cubins et al., 2019; Hoerning et al., 2020). SCN development rates are affected by low temperatures that occur in winter (Alston & Schmitt, 1988; Barker et al., 1992; Ross, 1964). Previously, it was unknown if SCN could complete the life cycle in the autumn prior to soil temperatures decreasing too low for development. Likewise, it was unknown if the life cycle could be completed in spring and early summer after the soil warmed but before natural pennycress senescence. This microplot experiment showed that SCN did not develop to new females in the autumn at the microplot location in Saint Paul, MN, as no new females were observed in the samples during the two years of the experiment. However, observed temperatures were lower than the 30-year normal in October and November in both years of the experiment.

Temperature is correlated with SCN development, and a more normal autumn may facilitate development to the mature female stage prior to winter.

In the spring of the year, new females were observed on the pennycress plants over the five sampling periods that stretched 35 days. The September 26 and October 10 pennycress planting dates had the highest cumulative means and the highest average female observations. The lower cumulative mean observed on the first planting date (August 15) may be caused by earlier senescence dates recorded for the earliest autumn planted treatments (Table 3-2). The number of days that the August 15 treatment senesced prior to the October 10 treatment was 18 days in 2019 and 17 days in 2020. The effect of maturity group and date of senescence on SCN development has been demonstrated in soybean as later maturity dates have higher SCN egg numbers than plants with earlier maturity dates (Hill & Schmitt, 1989). For the closely related cereal cyst nematode (*Heterodera Avenae*), which infects barley plants, there was a clear relationship between the rate of maturation of the host and the number of eggs produced. When the host matured earlier, the number of eggs produced was lower (Banyer & Fisher, 1976). Therefore, SCN spring development on pennycress may be mitigated with earlier autumn planting dates or faster-maturing varieties.

Soybean is the primary host for SCN and was included in this study as a basis of comparison. It would be expected that SCN female counts would be higher on soybean as the plants are present in the field when temperatures are more suitable for SCN egg hatch and development. The percentage of egg hatch increases with increasing soil temperature, and SCN can mature in 2-3 weeks between the temperatures of 24-28°C (Bonner & Schmitt, 1985; Schmitt, 1991). The highest observed mean of females on pennycress was

28.9 during PC4 (May 19). On soybean, the highest observed mean of females was 169.3 during SB3 (July 16). The observations were 21 days apart, but soybean had 586% of the number of females observed on pennycress.

Juveniles inside the roots were observed at all treatment dates in all planting date treatments except the October 10 planting date treatment in the autumn for pennycress. The autumn sampling period had substantially lower observed juveniles than the mid-pennycress sampling period. The lower juvenile count can likely be explained by lower egg hatch rates resulting from falling temperatures and the induction of seasonal diapause. In climates with cold winters, SCN enters seasonal diapause, typically beginning in early autumn (Yen et al., 1995). Diapause drastically reduces egg hatch and subsequent juvenile root penetration. It is unknown if juveniles can survive extended periods of sub-zero temperatures inside the root. If the juveniles are killed by freezing winter temperatures, pennycress may function as a trap crop, potentially reducing the SCN population density (Trivedi & Barker, 1986). It was reported that -4°C was lethal to SCN juveniles in water, and the formation of ice crystals was the apparent cause (Slack et al., 1972). Ice crystal formation is likely to be prevented in pennycress roots because pennycress protects its root cells from damage through cold-induced freezing tolerance (Ouellet & Charron, 2013). Previous research with purple deadnettle, another SCN alternative host and winter-annual, showed that juveniles could survive for 20 days at 0°C inside the roots and continue development once temperatures warmed (Creech et al., 2007). More research is needed to understand which temperatures are fatal to juvenile SCN inside of pennycress roots.

In conclusion, this study showed that juveniles infect pennycress roots in autumn and spring. New females were not observed in the autumn on pennycress plants in the two years of the study, meaning SCN development was stopped before reaching the mature female stage. It is unknown if cold winter temperatures kill the juveniles or if the juveniles can survive in the root. If the juveniles are killed, pennycress may be a trap crop and reduce SCN population density. Mature female development on pennycress plants can occur in the spring before plant senescence and harvest. The latter two autumn planting dates, September 26 and October 10 had the highest cumulative females. The earliest autumn plant date (August 15) had the lowest cumulative females and earliest senescence date. Therefore, early pennycress planting dates or improved pennycress varieties that reach maturity earlier may reduce overall SCN female development.

Tables

Table 3-1: *Heterodera glycines* (HG) Type Test for the population of SCN found in soil collected from Waseca, MN

Differential #	Differential Name	Replications	Female/plant	Female Index (%)	HG Type
1	PI 548402 (PEKING)	5	2.4	0.4	
2	PI88788	5	102.4	15.0	2
3	PI90763	5	0	0.0	
4	PI437654	5	0	0.0	
5	PI209332	5	108.0	15.8	5
6	PI89772	5	0	0.0	
7	PI548316 (CLOUD)	5	248.8	36.4	7
	Pickett	4	39.0	5.7	
Wm 82	Williams 82	5	684.4		

Table 3-2: Maturity dates for the two years of the experiment in 2019 and 2020 for pennycress and soybean plants.

Crop	Planting Date Treatment	Maturity Date 2019 (Julian Day)	Maturity Date 2020 (Julian Day)
PC	August 15	171	168
PC	August 29	174	170
PC	September 12	181	176
PC	September 26	188	185
PC	October 10	189	185
Soybean	May 15	281	277
Soybean	May 29	289	288

Table 3-3: Likelihood-ratio $\chi 2$-test for planting date treatment on the amount of SCN live females collected in pennycress microplots on 7 -day intervals from June 2- July 4 represented as sampling periods PC1-5.

Sampling Period	$\chi 2$	Num Df	P-value
PC1	13.9	4	0.01**
PC2	78.4	4	<0.001***
PC3	75.7	4	<0.001***
PC4	34.3	4	<0.001***
PC5	163.1	4	<0.001***
Cumulative	184.7	4	<0.001***

The fixed effects of planting date [August 15, August 29, September 12, September 26, October 10] were tested with the likelihood-ratio $\chi 2$ method. All models included year as a random effect. Each sample period was analyzed separately. Num Df = numerator degree of freedom. *, **, and *** represent significance of likelihood-ratio $\chi 2$ tests at a $P < 0.05$, 0.01, and 0.001.

Table 3-4: Mean SCN live females by pennycress planting date in microplots on 7 -day intervals from June 2- July 4 represented as sampling periods PC1-5.

	Sampling Period					
Planting Date	PC1 (6/4)	PC2 (6/11)	PC3 (6/18)	PC4 (6/25)	PC5 (7/2)	Cumulative
August 15	2.3a	2.0a	3.3a	4.5a	1.5a	13.6a
August 29	2.1a	2.9a	6.5ab	23.0b	2.3a	36.6b
September 12	3.6ab	9.1b	7.8bc	9.4a	4.5a	34.4b
September 26	7.4b	23.1c	16.5c	28.9b	12.6b	76.8c
October 10	2.5ab	5.6ab	28.6d	16.4ab	23.6c	88.5c

†The values in the same column followed by different letters denote significant difference at the $P \leq 0.05$ level using Tukey–Kramer least-square means

Table 3-5: Likelihood-ratio $\chi 2$-test for planting date treatment on the amount of SCN live females collected in soybean microplots on 14-day intervals from June 18 through September 24 represented as sampling periods SB1-8

Sampling Period	$\chi 2$	Num Df	P-value
SB1	36.6	1	<0.001***
SB2	0.5	1	0.49
SB3	75.8	1	<0.001***
SB4	1.4	1	0.23
SB5	18.5	1	<0.001***
SB6	0.1	1	0.86
SB7	20.8	1	<0.001***
SB8	1.0	1	0.31

The fixed effects of planting date [May 15 and May 29] were tested with the likelihood-ratio $\chi 2$ method. All models included year as a random effect. Each sample period was analyzed separately. Num Df = numerator degree of freedom. *, **, and *** represent significance of likelihood-ratio $\chi 2$ tests at a $P < 0.05$, 0.01, and 0.001.

Table 3-6: Mean SCN live female count by soybean planting date treatment in microplots on 14 -day intervals from June 18 through September 24 as sampling periods SB1-8.

	Sampling Period							
Planting Date	SB1 (6/18)	SB2 (7/2)	SB3 (7/16)	SB4 (7/30)	SB5 (8/13)	SB6 (8/27)	SB7 (9/10)	SB8 (9/24)
May 15	18.5	71.8	169.3b	111.3	77.5a	51.8	35.5b	6.8
May 29	3.0a	66.0	67.5a	94.8	132.8b	50.5	18.8a	5.0

† The values in the same column followed by different letters denote significant difference at the $P \leq 0.05$ level using Tukey–Kramer least-square means

Table 3-7: Likelihood-ratio $\chi 2$-test to examine the pennycress planting dates on SCN juveniles collected in pennycress microplots during two sampling periods 10/31 (R1) and 6/18 (R2).

Sampling Period	Juvenile Stage	$\chi 2$	Num Df	P-value
R1	J2	20.5	4	< 0.001***
R1	J3/J4	12.7	4	0.01*
R2	J2	2.4	4	0.67
R2	J3/J4	2.1	4	0.71

The fixed effects of planting date [August 15, August 29, September 12, September 26, October 10] were tested with the likelihood-ratio $\chi 2$ method. All models included year as a random effect. Each sample period was analyzed separately. Num Df = numerator degree of freedom. *, **, and *** represent significance of likelihood-ratio $\chi 2$ tests at a $P < 0.05$, 0.01, and 0.001.

Table 3-8: Mean SCN juvenile count by life stage collected in pennycress microplots during two sampling periods 10/31 (R1) and 6/18 (R2) compared among pennycress planting dates.

	J2 / g root	J3+J4 / g root	J2 / g root	J3+J4 / g root
Planting Date	R1 (10/31)	R1 (10/31)	R2 (6/18)	R2 (6/18)
August 15	8.6b	8.1ab	34.3	26.6
August 29	8.8b	9.3ab	26.3	28.8
September 12	6.5ab	10.4b	33.8	33.0
September 26	9.6b	4.9ab	30.5	24.9
October 10	0.00a	0.00a	27.3	24.1

† The values in the same column followed by different letters denote significant difference at the $P \leq 0.05$ level using Tukey–Kramer least-square means

Figures

Figure 3-1: Monthly average minimum and maximum air temperatures (°C) and monthly total precipitation in 2018, 2019, and 2020 compared with the 30 yr average (1991–2020) collected at the University of Minnesota, St. Paul Campus Climate Observatory located adjacent to the microplot field.

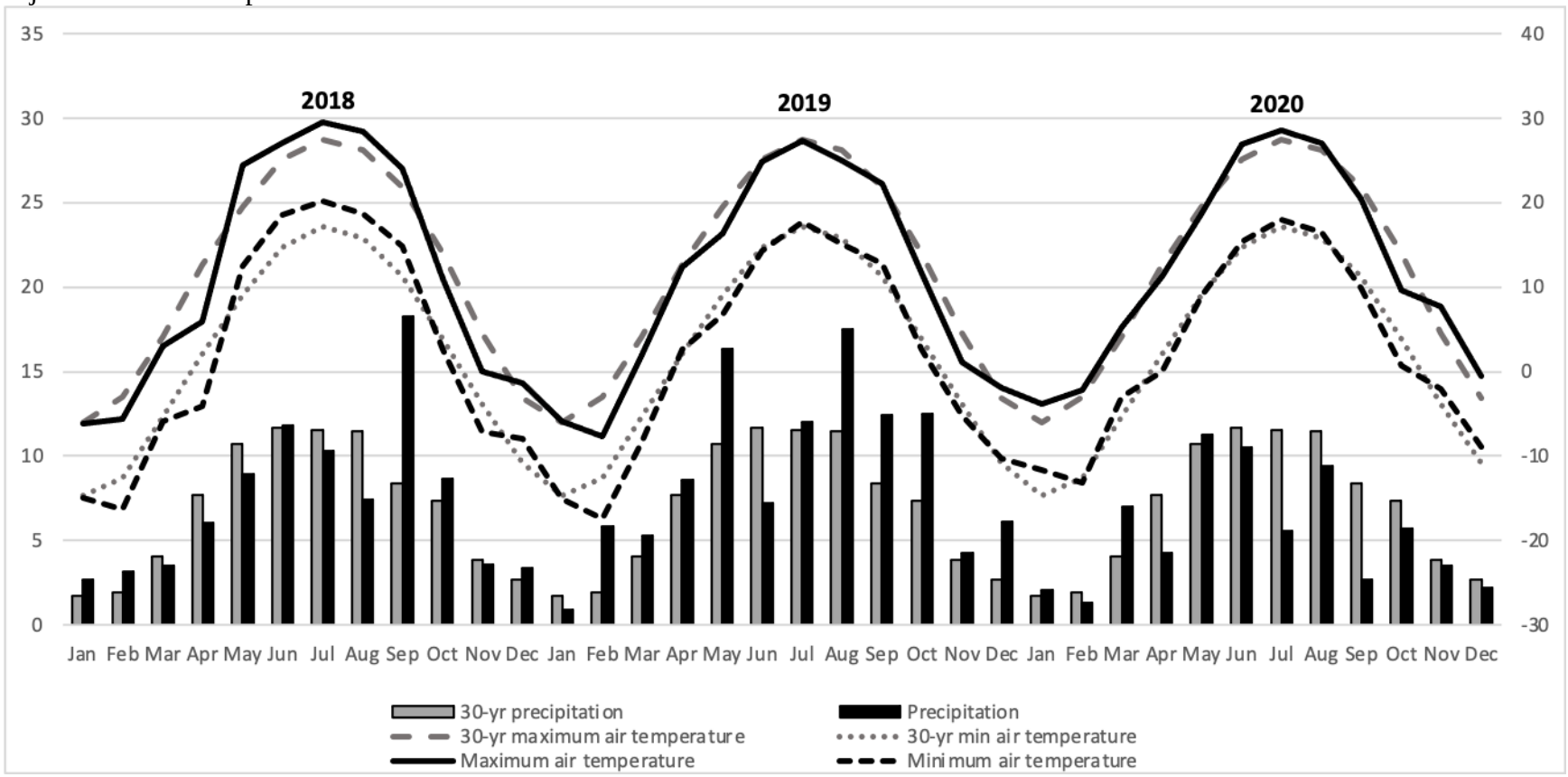
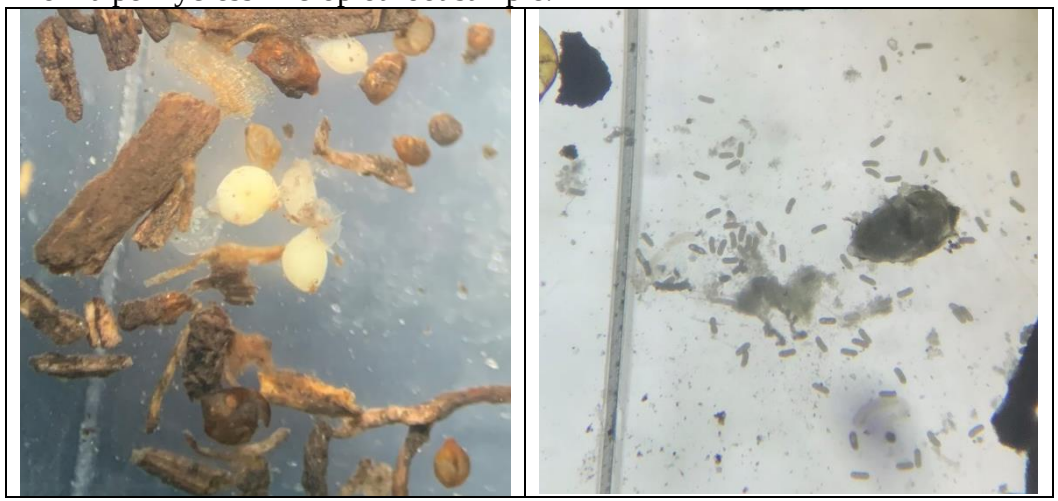


Figure 3-2: Pennycress and soybean microplots photographed in early June of 2019.

Figure 3-3: SCN white females (left) and eggs from a ruptured white female (right) when examined under microscope after collection from a pennycress microplot root sample.



Chapter 4 - Assessing hatch, penetration, and female development of SCN in winter annual oilseed crops.

Synopsis

Soybean cyst nematode (SCN, *Heterodera glycines*) is the most damaging pathogen in soybean *Glycine max* (L.) production in the US Midwest. SCN causes significant yield loss annually in this region. Pennycress (*Thlaspi arvense* L.) and camelina [*Camelina sativa* (L.) Crantz] are being developed as winter oilseed crops that can be implemented in corn-soybean cropping rotations to serve the function of cover crops. Pennycress and camelina can increase the overall yields of Midwest cropping rotations and provide the ecosystem services of cover crops. Pennycress has been reported as an alternative host to SCN. Camelina is classified as a poor or non-host. Camelina has been implicated as a potential trap crop as it has been shown to reduce SCN population density in naturally-infested soil. This experiment aimed to evaluate the impact of pennycress and camelina on the hatch and development of SCN. The results of the egg hatch experiment showed that pennycress significantly increases hatch more than the water control. Pennycress and camelina do not differ in the number of juveniles observed in the root samples, but more SCN females were recovered from pennycress roots. Camelina had a near-zero average of SCN female observations. SCN was observed to complete its life cycle on pennycress plants as the eggs harvested from pennycress roots were reinoculated and developed into new females on soybean and pennycress roots. Given the results, camelina largely does not facilitate nematode development to the mature female stage inside roots, while pennycress supports the development and reproduction of SCN.

Introduction

Soybean cyst nematode (SCN, *Heterodera glycines*) is the most damaging yield-suppressing pathogen in soybean (*Glycine max* L.) production in the United States (Bandara et al., 2020; Mueller et al., 2016; Tylka & Marett, 2021). Growers currently manage SCN in soybean production fields through the deployment of resistance, crop rotation with non-host crops, seed-applied nematicides, biological controls, and weed management (Beeman & Tylka, 2018; Bissonnette et al., 2020; Rocha, Pimentel, et al., 2021; Wight et al., 2018) Nearly all of the available commercial cultivars in the United States have resistance derived from plant introduction PI 88788 (Joos et al., 2013; McCarville et al., 2017; Tylka et al., 2019). Widespread deployment and continued use of PI 88788 derived resistance have resulted in the increased ability of SCN to reproduce on this derived resistance which has made SCN management more difficult (Howland et al., 2018; Niblack et al., 2007)

Growers are increasingly adopting cover cropping in the United States, and the use of cover crops increased by 50% from 2012 to 2017 (Wallander et al., 2021). Cover crops are planted after cash crop harvest and produce autumn and spring ground cover. They provide vital ecosystem services such as reducing soil erosion and nutrient loss and providing early season forage to pollinators (Forcella et al., 2021; Kaspar & Singer, 2011; Meisinger & Ricigliano, 2017; Weyers et al., 2019). Cover crops can affect SCN population density on a field scale by serving as alternative hosts, releasing allelochemicals, and acting as trap crops (Acharya et al., 2019; Harbach et al., 2021; Niblack & Chen, 2004). SCN has a wide host range that includes soybean, dry bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), other leguminous crops, and weed species (Poromarto & Nelson, 2010; Venkatesh et al., 2000). Plants that serve as alternative hosts

have been shown to lengthen the development period for SCN beyond the period in which the primary host is in rotation (Creech et al., 2007). Allelochemicals released by plants are known to affect SCN egg hatch and infection as crops such as Crimson clover (*Trifolium incarnatum*), rapeseed (*Brassica napus*), and other mustard species have been shown to significantly increase SCN root penetration (Harbach et al., 2021; Warnke et al., 2008). Cover crops that increase egg hatch and infection can function as trap crops if nematodes penetrate roots, but feeding does not occur, resulting in nematode death (Trivedi & Barker, 1986). Including trap crops in rotation can reduce the population density more than a no-cover control, as was demonstrated with sugar beet cyst nematode (*Heterodera schachtii*) and oilseed radish (*Raphanus sativus*) (Wight et al., 2018).

Pennycress (*Thlaspi arvense* L.) and camelina [*Camelina sativa* (L.) Crantz] are winter oilseed crops that can increase overall yields of Midwest cropping rotations and provide the ecosystem services of cover crops due to their soil coverage in the autumn and spring (Berti et al., 2017; Hoerning et al., 2020; Patel et al., 2021). In greenhouse experiments and weed surveys, pennycress has been reported as an alternative host to SCN, as SCN females and cysts were recovered from pennycress roots in controlled environment experiments (Hoerning et al., 2022; Poromarto et al., 2015; Venkatesh et al., 2000). Camelina was recorded as a poor or non-host of SCN in similar experiments, and camelina reduced SCN population density greater than the control treatment when tested in naturally infested soil (Acharya et al., 2019; Poromarto & Nelson, 2010). Many unknowns remain about how the life cycle of SCN is facilitated and influenced by the winter oilseed species pennycress and camelina. The objectives of this experiment were to i.) determine whether root exudates from the winter oilseeds affect the hatching of

SCN ii.) examine SCN development in the winter oilseeds iii.) and quantify reproduction of SCN on pennycress.

Materials and Methods

Soil medium preparation

For all experiments, the same soil medium was used. The soil medium was a 1:1 mix by volume of field soil (not infested with SCN) and construction sand. The field soil was a Nicollet clay loam (fine-loamy, mixed, superactive, mesic Aquic Hapludoll) with a particle size distribution of 40% sand, 35% silt, and 25% clay. Once mixed, the soil was autoclaved at 121°C and 110 kPa for 180 minutes.

Egg collection procedure

Eggs of SCN for the hatching experiments were obtained from the roots of the susceptible soybean accession 'Sturdy' grown in SCN-infested field soil of population SY-97 (HG Type 7) obtained from the University of Minnesota Southern Research and Outreach Center in Waseca, Minnesota. SCN females and cysts were collected from the roots of 5 to 8-week-old soybean plants. Females and cysts were washed off soybean roots with a vigorous water stream through an 850-µm-aperture sieve onto a 250-µm-aperture sieve and extracted by centrifugation in 76% (w/v) sucrose solution (Liu & Chen, 2000). The eggs were released by crushing the cysts on a 150-µm-aperture sieve with a rubber stopper mounted on a motor and collected on a 25-µm-aperture sieve (Faghihi & Ferris, 2000). The eggs were separated from debris by centrifugation in a 35% (w/v) sucrose solution for 5 min at 1500g (Liu & Chen, 2000).

Collection of root exudates and soil leachate

Experiments were conducted under greenhouse and laboratory conditions in August 2021 and October 2021. Root exudates were collected from four different plant treatments 1) pennycress accession 'MN106', 2) susceptible soybean 'Sturdy,' 3) wheat

variety ‘LANG-MN,’ and 4) camelina variety ‘Joelle.’ Seeds of the four cropping treatments were planted in 10 cm diameter Terra Cotta pots (Gertens, Inver Grove Heights, MN) filled with 500 cubic centimeters of soil medium. Per pot seedling density was four for the winter oilseeds, three for the wheat, and two for the soybeans. The seedling per pot density was normalized by species root biomass at 35 days post-planting. The plants were watered daily to water-holding capacity. Fertilizer was applied at a rate of 15 ml of Peters Hydrop-Sol 5-11-26 (ICL Specialty Fertilizers, Summerville, SC). on day 7 and day 21 post-planting. Soil leachate was collected as a control treatment from pots without any plants containing only the soil medium. Thirty-five days after planting, the pots were ready for root exudate collection.

The pots were not watered 24 hours prior to collection taking place. Soil leachate (SL) and root exudate (RE) collection methods from Sikora & Noel (1997) were adapted and used for this experiment. Briefly, 100-ml of double-distilled water was poured into each pot. After 1 hour, an additional 300-ml of double-distilled water was poured into each pot, and leachate was collected in 600-ml beakers positioned beneath each pot. Leachates from the three replications of the pots of the same treatment were combined. The collections were sterilized using a 150-ml bottle-top filter with 0.22-um pores (Celltreat Scientific Products, Pepperell, MA) on 50-ml centrifuge tubes connected to a vacuum. The tubes were labeled, stored at 4 °C, and used within 24 hours for the hatching experiment. Soil leachate obtained from pots containing only the sand-soil mix served as the control.

Egg hatching experiments

Eggs were collected in water and quantified by examining a subsample of egg suspension with an inverted microscope. The hatching experiments were conducted in

sterile six-well tissue culture plates (Corning Costar, Tewksbury, MA), with one plate serving as a single experimental unit. The hatching methods of Harbach et al. (2021) were adapted and used for this experiment. Throughout the experiment, the top three wells of the tissue culture plates were used for one treatment of REs and SLs, and the bottom three wells were left unused to avoid possible volatile contamination. The treatment solutions were as follows: 1) exudate from pennycress accession‘MN106,’ 2) exudate from susceptible soybean ‘Sturdy,’ 3) exudate from wheat variety ‘LANG-MN,’ 4) exudate from camelina variety ‘Joelle’ 5) soil leachate from soil control without any plants, and 6) a solution of 4 mM ZnCl2 (known hatching stimulant). On the first day of the experiment, 4 ml of the treatment solution was deposited into the top left well of each plate. A sieve with 30-um-pore nylon mesh (Elko Filtering Company, Miami, Florida) was placed into the well, and 200-300 SCN eggs were added to each sieve.

The plates were incubated in the dark at 25°C for 7 days. After 7 days, the sieves with the unhatched eggs were moved to the top right well, and a new solution was added to the well. The plates were then returned to the incubation chamber at 25℃ for another 7 days. When juveniles hatched in the sieves, they moved down through the mesh and remained in the liquid within the well. On day 14, at the end of the experiment, the number of hatched juveniles in the wells was counted. The unhatched eggs remaining on the sieve were also counted. Cumulative percent hatch was calculated by dividing the total number of juveniles hatched in the experiment by the total number of hatched juveniles plus the remaining unhatched eggs. The experiment was repeated once, and each experiment was organized using a completely randomized design with four replications of each treatment per run of the experiment.

Juvenile development and root staining experiments

Cone-tainers (Stuewe and Sons Inc., Tangent, OR) with a 106-cm^3- capacity (SC7U) were filled with the soil medium and planted with one seed of the following plant treatments 1) winter pennycress accession 'MN106,' 2) susceptible soybean 'Sturdy,' and 3) winter camelina variety 'Joelle.' Eggs were transferred to a 500 ml beaker, and a suspension of 250 eggs/ ml of sterile water was prepared. After 14 days of plant growth, two holes of 3 cm depth (halfway between the cone wall and the plant stem) were made with a 5-ml pipette tip (Fisher-Scientific, Waltham, MA). Two milliliters of the 500 eggs/ml sterile water was pipetted into each hole for a total of 2000 eggs/cone. The holes were covered with the soil medium, and the plants were completely randomized on trays and placed in the greenhouse. After 14 days, the plants were removed from the greenhouse, and the roots were washed and prepared for staining. The staining procedure described in Byrd et al. (1983) was used and adapted for this experiment. The stock solution of acid fuschin was composed of 3.5 g acid fuchsin, 250 ml acetic acid, and 750 ml distilled water. The roots of the plants were washed and cut into approximately 2 cm segments. The roots were placed into a 250ml beaker, and 50 ml of 1.5% NaOC1 solution mixed in DI water was added to the beaker. The roots were kept in the bleach solution for 4 minutes; agitation and stirring occurred twice during this 4-minute soak. The roots were removed from the bleach solution, placed on a 250-μm-aperture sieve (#60), and rinsed three times with DI water. The roots were put into a 250 ml beaker with 50 ml of DI water and soaked for 15 minutes. The roots were removed from the DI water and placed into a 250 ml beaker with diluted acid fuschin stain (3.33% stock solution). The roots were stained in the solution for 5 minutes. In the acid fuschin stain, the roots were heated in the microwave for 1 minute. After allowing the sample to cool to room temperature

(approximately 10 minutes), the roots were removed and again placed on a 250-µm-aperture sieve (#60) and rinsed three times with DI water. The roots were then returned to a 250 ml beaker with DI water and soaked for 10 minutes. Destaining in acidified glycerin did not occur for the pennycress or camelina roots, as this portion of the procedure damaged the roots. Destaining did occur for the wheat and soybean plants, and the plants were placed in 20 ml of acidified glycerine and heated to boiling (approximately 30 seconds in the microwave). The roots were spread across a petri dish, and acidified glycerin was pipetted over the roots for preservation. The roots were pressed between glass plates and observed under a dissecting scope. The number of SCN juveniles visible in the roots was counted and classified by visual life stage as either J2 or J3/J4.

Fourteen days after inoculation, four replications of each plant treatment were moved to the growth room in Waseca for an additional 21 days to facilitate continued SCN development to the mature female stage. Throughout the growing period, fertilizer was applied every two weeks at a rate of 5 ml of Peters Hydrop-Sol 5-11-26 (ICL Specialty Fertilizers, Summerville, SC) per cone-tainer. After 35 days post-inoculation, the plants were removed from the growth room. Aboveground biomass was cut at the soil level and removed. The soil from the cone was emptied into a 1000 ml pitcher and decanted five times to remove the cysts from the soil. Newly formed females and cysts were washed off roots with a vigorous water stream through an 850-µm-aperture sieve onto a 250-µm-aperture sieve and extracted by centrifugation in 76% (w/v) sucrose solution (Liu & Chen, 2000). Females were counted under dissecting scope.

Reinoculation experiments

Winter pennycress seedlings 'MN106' were germinated in cone-tainers (Stuewe and Sons Inc., Tangent, OR) with a 106-cm^3- capacity (SC7U) and filled with soil medium. The tray containing the cone-tainers were maintained in the greenhouse with temperature ranging from 25-28°C and a daylight period of 16-h. The cone-tainers were watered daily to water-holding capacity. After 21 days in the greenhouse, the seedlings were moved to a growth chamber set to 12-h light day and 4 °C for a 21-day vernalization treatment. After vernalization, a suspension of 250 eggs/ ml of sterile water was prepared from SCN population SY97 collected from 'Sturdy.' Two holes of 3 cm depth (halfway between the cone wall and the plant stem) were made with a 5-ml pipette tip (Fisher-Scientific, Waltham, MA). Two milliliters of the 500 eggs/ml sterile water were pipetted into each hole for a total of 2000 eggs/cone. Pennycress plants were then moved to the SCN growth room in Waseca, MN, for 35 days. The growth room is maintained at 28°C and 16-h light day. Throughout the growing period, fertilizer was applied every 14 d at a rate of 5 ml of Peters Hydrop-Sol 5-11-26 (ICL Specialty Fertilizers, Summerville, SC) per cone-tainer. Aboveground biomass was cut at the soil level and removed. The soil from the cone was emptied into a 1000 ml pitcher and decanted five times to remove the cysts from the soil. Newly formed females and cysts were washed off pennycress roots with a vigorous water stream through an 850-µm-aperture sieve onto a 250-µm-aperture sieve and extracted by centrifugation in 76% (w/v) sucrose solution (Liu & Chen, 2000).

The eggs were released by crushing the cysts on a 150-µm-aperture sieve with a rubber stopper mounted on a motor and collected on a 25-µm-aperture sieve (Faghihi and Ferris, 2000). The eggs were separated from debris by centrifugation in a 35% (w/v)

sucrose solution for 5 min at 1500g (Liu & Chen, 2000). The pellet portion of the 50-ml centrifuge tubes was poured over a 25-µm-aperture sieve and rinsed twice with water. The eggs were returned to 50-ml tubes and filled to the 20-ml level. The eggs were counted under an inverted microscope. The eggs were diluted with sterilized water to 500 eggs/ml. Pennycress plants 'MN106' and susceptible soybean plants 'Sturdy' were germinated and grown in cone-tainers in the greenhouse as described previously for 14 days. The 14-day-old plants were moved to the growth room to prepare for inoculation with the eggs developed from pennycress plants. Two holes of 3 cm depth (halfway between the cone wall and the plant stem) were made with a 5-ml pipette tip (Fisher-Scientific, Waltham, MA). Two ml of the 500 eggs/ml sterile water was pipetted into each hole for a total of 2,000 eggs/cone. The holes were covered with the soil medium, and the plants were completely randomized on trays and placed in the greenhouse. After 35 days, the plants were processed for eggs using the same procedure described above. However, before cysts were crushed for eggs, the total cyst count per cone was determined under a dissecting scope.

Data Analysis

All analyses were conducted with R, version 4.0.5 (R Core Team, 2020). For each experiment, the main effect of treatment was fixed. Experimental run and replication within run were random effects. Each treatment occurred independently. The culture tissue plate was the experimental unit for the egg hatch experiment. For the penetration and reinoculation experiments, a cone-tainer was the experimental unit. The final model for the experiments was a random intercept generalized linear mixed-effect model (packages lme4, MASS; Bates et al., 2015; Venables & Ripley, 2002). Analytical assumptions for the mixed analysis of variance were examined by visual inspection of the

residual plots. If analytical assumptions were not met for count data, Poisson or negative binomial regression was used. Data from the two runs of the experiment were combined for analysis. Likelihood Ratio $\chi 2$-tests were used to assess the significance of the fixed effects (Fox & Weisberg, 2019). When the significance of fixed effects exceeded the predetermined significance threshold of $P < 0.05$, the differences between means were examined using least-square means (package: lsmeans; Lenth, 2016). If Poisson and negative binomial regression were used, means were reported on the original scale in figures and tables.

Results

Egg hatch experiments

The effect of root exudate and soil leachate treatment on the cumulative percentage of egg hatch was significant ($\chi 2=74.05$, df = 5, $P > \chi 2 = 1.47e\text{-}14$). On average, the number of eggs hatched after 14 days was 34% in the DI water control and 68% in the $ZnCl_2$ hatching stimulant treatment. The camelina and wheat treatments had the lowest percent hatch of the crop treatments and were not significantly different ($\alpha < 0.05$) from the DI water control treatment. The pennycress treatment was significantly higher than the DI water control but not significantly different from the wheat and camelina treatments. The soybean treatment was significantly higher than the control, wheat, and camelina treatments. The soybean root exudate treatment and the pennycress treatment were not significantly different. The $ZnCl_2$ hatching stimulant treatment resulted in the highest proportion of egg hatch and was significantly higher than the other treatments (Table 4-1).

Root Penetration Experiments

The effect of plant treatment on the juvenile count in the roots and cyst count per plant was significant ($\alpha < 0.05$, Table 4-2). The soybean treatment had significantly

higher juveniles per gram root than the winter oilseeds pennycress and camelina. The pennycress and camelina treatments were not significantly different (Table 4-3). The average number of juveniles observed in camelina was 37% of the average number observed in soybean (116.75). For pennycress, the average was 52% of the number observed in soybean. For cyst development, the soybean plant treatment had the highest number of cysts, the pennycress had the second highest, and the camelina had the lowest. All treatments were significantly different (Table 4-4). The number of cysts observed on camelina averaged 0.09% of the number of cysts observed on soybean, and the number of cysts on pennycress averaged 23% of the cysts on soybean.

Reinoculation Experiment

The effect of plant treatment on cyst count, egg count, and eggs/cyst was significant ($\alpha < 0.05$, Table 4-5). Susceptible soybean was significantly higher than the pennycress treatment for cyst count, egg count, and eggs/cyst (Table 4-6). The average number of cysts on the pennycress treatments was 63% of those collected on susceptible soybean (135). The average eggs collected on pennycress was 27% of the average eggs on susceptible soybean (26,023). The average eggs/cyst for pennycress was 42% of the average eggs/cyst collected on susceptible soybean (194).

Discussion

When not feeding on a plant host, neutral lipids are the main food reserve for cyst nematodes. The lipid reserve is finite, and movement from the hatch site is limited for the J2 infective stage of SCN (Storey, 1984). To maximize survival and infection in suitable host plants, the cyst nematodes have synchronized egg hatch with the signals from host root exudates (E P Masler & Perry, 2018). Host-specific compounds referred to as hatching factors have been identified for SCN. The hatching factors include

glycoalkaloids and terpenes such as Glycinoeclepins that induce egg hatch (Masamune et al., 1982). Hatching factors have high specific activities for cyst nematodes and stimulate hatch at concentrations as low as 10^{-10} g ml^{-1} (Devine & Jones, 2000). A percentage of the eggs will hatch without the presence of root exudates when environmental and moisture conditions are favorable through a phenomenon known as constitutive hatching (Thompson & Tylka, 1997). In the absence of root exudates, the highest rate of hatching in free eggs occurs at a temperature of 24 ℃, soil moisture of 25%, and a pH of 6.0 (Tefft et al., 1982). In this experiment, 34% of eggs hatched in the DI water control. The camelina and wheat root exudates did not significantly increase the hatch over the control; this finding is consistent with previous examinations that root exudates from non-host crops do not increase egg hatch (Tsutsumi & Sakurai, 1966). Soybean root exudate exhibited higher egg hatch rates than the DI water control, consistent with previous studies examining soybean root exudates' effect on egg hatch (Okada, 1971; Tefft & Bone, 1984). Pennycress also stimulated a higher egg hatch than the DI water control, consistent with pennycress' status as an alternative host to SCN (Hoerning et al., 2022; Poromarto et al., 2015; Venkatesh et al., 2000).

Root exudates and semiochemical signals from plants also influence juvenile navigation to the root-feeding site. Specific chemical compounds are known attractants in plant species that host SCN, but other attractants released by plant roots, such as carbon dioxide and ethylene, aid in juvenile navigation to root tissue more generally (Hu et al., 2017; Rasmann et al., 2012). Pennycress and camelina treatments were not different in the number of juveniles $gram^{-1}$, but soybean was significantly higher than the two winter oilseeds. The juveniles in pennycress and camelina roots averaged 37% and 52% of the

observed juveniles in soybean, respectively. This finding is consistent with Harbach et al. (2021), who found that two mustards in the Brassicaceae family had approximately 30-50% of the juveniles per fresh root gram compared to soybean. In all treatments, juveniles in both the J2 and the J3/J4 stages were observed (Figure 4-1). For all treatments, mature females were observed. The pennycress treatment represented 23% of the cyst average on soybean. The cyst count on camelina was very low. Non-zero observations were in two of eight samples, and these observations were one and two cysts per sample. The observations on camelina are consistent with the average of two cysts observed on camelina across six camelina accessions found in a study by Poromarto & Nelson 2010. In this same study, an average of 477 cysts were observed on susceptible soybean, meaning camelina cyst count was 0.4% of the cysts on soybean. In another study accessing camelina, zero cysts were observed on roots across the runs of the experiment (Acharya et al., 2019). These results show that most juveniles who penetrated the roots will not develop to the mature female stage in the camelina treatment. The mechanism for this phenomenon of failed development is unclear, but it is possible that the plant recognizes SCN effectors and launches a localized response to kill the syntactical host cell, similar to the known mechanism in resistant soybean varieties (Bayless et al., 2016; Guo et al., 2015). The ability of SCN to infect plant roots but not develop into mature females has been previously documented in brown hemp (*Crotalaria juncea*) and showy rattlebox (*Crotalaria spectabilis*). However, the response differed because hatching in the root exudates of these two plants stimulated hatch greater than the DI water control, a requirement of trap crops (Kushida et al., 2003). In this study,

camelina did not stimulate egg hatch more than the DI water control and therefore did not meet the definition of a trap crop (Trivedi & Barker, 1986)

Pennycress is a known SCN alternative host, although SCN development is lower than susceptible soybean as the cyst or egg count represented 16-34% of the count on the susceptible soybean check-in three experiments (Hoerning et al., 2022; Poromarto et al., 2015; Venkatesh et al., 2000). These studies documented SCN development, but to date, the ability of SCN to complete a reproduction cycle has not been tested. In this context, reproduction is defined as the ability for viable cysts to be produced, leading to further infection (Triantaphyllou, 1975). Cysts from pennycress 'MN106' were crushed, and the eggs from the cysts were reinoculated into the soil with susceptible soybean and pennycress (non-vernalized). The susceptible soybean and non-vernalized pennycress were included to simulate the potential for soybean or volunteer pennycress to follow the pennycress crop. Cysts and eggs were produced on both subsequent treatments, meaning that eggs produced on pennycress are viable and infective. When comparing the reinoculation treatments of pennycress and soybean, the eggs produced on pennycress were 27% of the average eggs produced on susceptible soybean. The cyst and egg/cyst counts were also lower. Pennycress averaged 82 eggs per cyst, while susceptible soybean plants averaged 195 eggs per cyst. Lower cyst, egg, and egg/cyst counts in pennycress, when compared to soybean, indicate that field population density growth of SCN may occur at a smaller magnitude in pennycress than in soybean production.

In conclusion, cumulative egg hatch averages among crop treatments ranged from 42 to 58%. The pennycress and soybean treatments had higher cumulative egg hatch than the DI water control, and both crops are known hosts of SCN. Wheat and camelina, non-

hosts of SCN, did not influence egg hatch greater than the control treatment. The root penetration experiment showed no differences between the juveniles in pennycress and camelina roots, but they were significantly lower than the number of juveniles in soybean roots. Juveniles penetrated camelina roots with a similar magnitude as pennycress roots. However, unlike pennycress, camelina facilitates very little female development. Most SCN juveniles can successfully penetrate the root tissue but cannot mature. Pennycress can facilitate the development and reproduction of SCN, as the eggs produced on pennycress roots developed to maturity when reinoculated on soybean and non-vernalized pennycress plants. Given the same egg inoculation level, the pennycress plants had lower cysts, eggs, and eggs/cyst than the susceptible soybean.

Tables

Table 4-1: Cumulative percent hatch by root exudate and soil leachate treatment for the egg hatch experiment.

Root Exudate Treatment	Cumulative Percent Hatch
Control (DI Water)	34.1a
Camelina	41.8ab
Pennycress	50.1bc
Soybean	57.8c
Wheat	42.5ab
$ZnCl_2$	68.2d

† The values in the same column followed by different letters denote significant difference at the $P \leq 0.05$ level using Tukey–Kramer least-square means

Table 4-2: Likelihood-ratio $\chi 2$-test for SCN juveniles in the root penetration experiment

Response	$\chi 2$	Num Df	Prob > $\chi 2$
Juvenile	33.79	2	4.59e-8***
Cyst	542.93	2	< 2.2e-16 ***

The fixed effects of plant treatment [camelina 'Joelle,' pennycress 'MN106,' and soybean 'Sturdy,'] were tested with the likelihood-ratio $\chi 2$ method. All models included run and replication with run as random effects. Num Df = numerator degree of freedom. *, **, and *** represent significance of likelihood-ratio $\chi 2$ tests at $P < 0.05$, 0.01, and 0.001

Table 4-3: Juveniles by plant treatment for the root penetration experiment

Plant Treatment	Juveniles gram root^{-1}
Camelina	43.0a
Pennycress	60.8a
Soybean	116.8b

† The values in the same column followed by different letters denote significant difference at the $P \leq 0.05$ level using Tukey–Kramer least-square means

Table 4-4: Cyst count by plant treatment for the root inoculation experiment.

Plant Treatment	Cyst
Camelina	0.4a
Pennycress	95.1b
Soybean	410.1c

† The values in the same column followed by different letters denote significant difference at the $P \leq 0.05$ level using Tukey–Kramer least-square means

Table 4-5: Likelihood-ratio $\chi 2$-test for SCN response variables in the reinoculation experiment as influenced by plant treatment

Response	$\chi 2$	Num Df	Prob > $\chi 2$
Cyst	14.55	1	1.37e-4***
Eggs	21.42	1	3.69e-6***
Egg/Cyst	24.94	1	5.91e-7***

The fixed effects of plant treatment [camelina 'Joelle,' pennycress 'MN106,' and soybean 'Sturdy,'] were tested with the likelihood-ratio $\chi 2$ method. All models included run and replication with run as random effects. Num Df = numerator degree of freedom. *, **, and *** represent significance of likelihood-ratio $\chi 2$ tests at $P < 0.05$, 0.01, and 0.001

Table 4-6: Cyst, egg, and egg/cyst by plant treatment for the reinoculation experiment.

	Response		
Plant Treatment	Cyst	Egg	Egg/Cyst
Pennycress	85.17a	7023.33a	81.95a
Soybean	135.00b	26023.33b	194.83b

† The values in the same column followed by different letters denote significant difference at the $P \leq 0.05$ level using Tukey–Kramer least-square means

Figures

Figure 4-1: Image of the 6-cell tissue culture hatching apparatus used for the egg hatching experiment

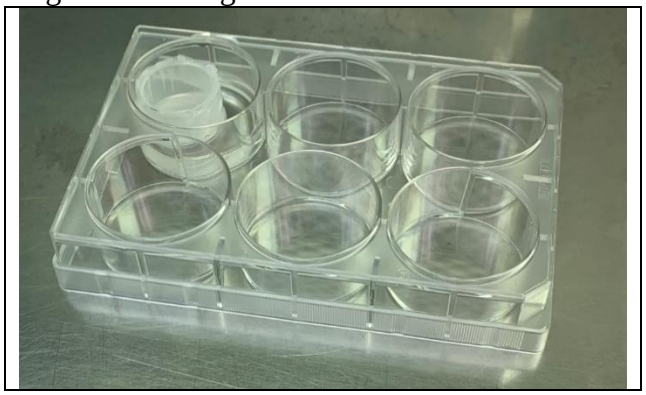
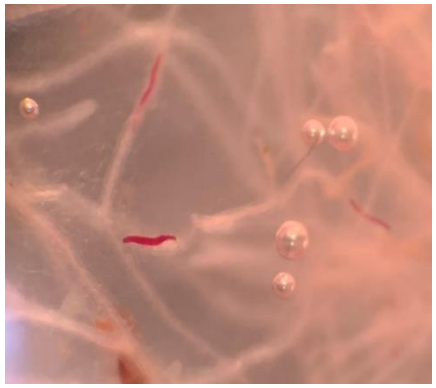


Figure 4-2: Image from dissecting scope of juveniles stained in roots shows that juveniles have developed beyond the infective J2 stage in each of the three crops sampled.

Camelina	Pennycress	Soybean
		

Chapter 5 - Bioassay to evaluate pennycress for resistance to SCN

Synopsis

Soybean cyst nematode (SCN, *Heterodera glycines*) is the most widespread and damaging pest affecting soybean in the United States. Therefore, crops under development for inclusion in soybean rotations must be evaluated for SCN alternative host status. Pennycress (*Thlaspi arvense* L.) is a harvestable winter-annual oilseed crop intended for inclusion in Midwest corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) rotations. Pennycress provides the ecosystem services of a cover crop while also increasing productivity because of its harvestable seed that contains high quantities of oil suitable for various markets. Pennycress is classified as an alternative host to SCN. In SCN management, resistance is a common strategy to control SCN population density and damage. In this study, 51 pennycress accessions were screened via bioassay for SCN resistance. The results from the study show that natural variation exists within the pennycress germplasm, but the resistance is largely classified as moderate when using the "female index" (FI) soybean classification system (FI = 10-30). A pennycress accession with major resistance (FI < 10) was not discovered. Some accessions with specific mutations induced by mutagenesis exhibited lower FI values (FI < 15); these accessions need further evaluation to ensure a robust resistance response.

Introduction

Soybean cyst nematode (SCN, *Heterodera glycines*) is the most damaging pest affecting soybean yield in the United States. Annual yield losses caused by SCN on soybean in the United States is estimated at $1.24-1.69 billion USD (Bandara et al., 2020; Bradley et al., 2021). Growers manage SCN through multiple practices, including

growing resistant soybean cultivars, crop rotation, and nematicidal crop protection products

(Chen et al., 2001; Hassan et al., 2013; McCarville et al., 2017). Additional crops that are known alternative hosts to SCN include beans (*Phaseolus* spp.), pea (*Pisum sativum*), clovers (*Trifolium* spp.), and vetches (*Vicia* spp.) (Schmitt & Riggs, 1991; Smith & Young, 2003). Therefore, growers managing SCN are recommended to avoid these alternative host crops in the rotation when attempting to control SCN population density in the field (Tylka, 2021).

Crops under development for inclusion in soybean rotations must be evaluated for SCN alternative host status to ensure the crop will not increase SCN management challenges. Extending pest life cycles to alternative hosts in a growing season is known as the "green bridge effect" and can result in crop damage and lower yields in rotation (Belder et al., 2008; Mahoney et al., 2016). Pennycress (*Thlaspi arvense* L.) is a harvestable winter-annual oilseed crop intended for inclusion in Midwest corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) rotations (Hoerning et al., 2020). Enhancing production and providing ecosystem services are the primary focus of incorporating pennycress into these rotations (Heaton et al., 2013). Greenhouse SCN surveys classify pennycress as a low-moderate alternative host of SCN, representing 16-34% of the reproduction observed on the susceptible soybean control (Hoerning et al., 2022; Poromarto et al., 2015; Venkatesh et al., 2000). Therefore, a better understanding of the host-pathogen interaction is required before implementing pennycress into a corn-soybean rotation.

Resistance is a common control mechanism in soybean for SCN. Research on SCN resistance began with the first discovery of *rhg* loci (resistant to *Heterodera glycines*) in 1960 (Caldwell et al., 1960). Resistance to SCN in soybean involves multiple pathways with copy number variation at the *rhg1* and *Rhg4* loci. The *rhg1-b* resistance allele from PI-88788 is characterized by its high copy number (Cook et al., 2014). Peking-type resistance has a low copy number of *rhg1* (1 to 3 copies) and requires *Rhg4* to confer resistance to SCN (Concibido et al., 2004). Resistance is quantified in soybean using the "Female Index." The female index is calculated by comparison of the female count on a test accession to a known susceptible soybean accession such as "Lee 74" or "Williams 82" (Sikora & Noel, 1991). Resistance to SCN in soybeans is not HG type-specific; rather, the HG type test measures the virulence or ability of SCN populations to reproduce on the differentials used to develop resistant soybean cultivars (Tylka, 2018). There are seven differentials used to determine HG Types. Female index values less than ten are considered resistant (Niblack et al., 2002). Using best practices developed for soybean SCN screening, the objective of this study was to evaluate breeding and mutation-derived pennycress accessions for resistance to SCN.

Materials and Methods

Cone-tainer Bioassay for SCN Resistance

Soybean cyst nematode bioassays were conducted in a growth room at the University of Minnesota Nematology laboratory in Waseca, MN. The bioassay (Bioassay 1) evaluating pennycress accessions from the UMN breeding program and the US National Plant Germplasm System collection was conducted in 2017 and 2019. The bioassay (Bioassay 2) evaluating advanced UMN breeding program accessions and mutant accessions and breeding accessions submitted by Western Illinois University and

Illinois State University were conducted in 2020 and 2021. The same experimental protocol was used across experiments and experimental runs. The experiments were randomized complete block designs with one treatment factor (Accession ID), replicated six times. Bioassay 1 evaluated six pennycress accession submissions from the UMN breeding program, all of which were winter-type pennycress. Bioassay 1 also evaluated 19 accessions from the US National Plant Germplasm System collection, in which four accessions were spring-type pennycress and 15 accessions were winter-type. Bioassay 2 evaluated 37 pennycress accessions, in which 13 winter-type pennycress accessions were from the UMN breeding program. Three winter-type pennycress accessions were from the Illinois State University breeding program, and 21 were from the Western Illinois University breeding program. The susceptible soybean cultivar 'Williams 82' was included in all experiment runs as a control.

In every experiment, the same soil medium was used. The soil medium was a 1:1 mix by volume of field soil (not infested with SCN) and construction sand. The field soil was a Nicollet clay loam (fine-loamy, mixed, superactive, mesic Aquic Hapludoll) with a particle size distribution of 40% sand, 35% silt, and 25% clay. Once mixed, it was autoclaved at 121°C and 110 kPa for 180 minutes. Cone-tainers (Stuewe and Sons Inc., Tangent, OR) with a 106-cm3- capacity (SC7U) were filled with the soil medium and planted with three seeds of each pennycress accession. The cone-tainers were maintained in the greenhouse at a temperature of 28°C and light day at 16 h. Cone-tainers were watered daily to water-holding capacity.

After emergence, the seedlings were thinned to one plant per cone. Twenty-eight days after planting, the seedlings were moved to a growth chamber set to 12-h light d and

4°C for vernalization treatment. Winter and spring pennycress lines were subjected to the same vernalization treatment for consistency in experimental conditions among ecotypes. The cones were prepared for inoculation after 21 days in the growth chamber. Eggs of SCN were obtained from the roots of the susceptible soybean accession ‘Sturdy’ grown in SCN-infested field soil of population SY-97 (HG Type 7) obtained from the University of Minnesota Southern Research and Outreach Center in Waseca, Minnesota. SCN females and cysts were collected from the roots of 5 to 8-week-old soybean plants. Females and cysts were washed off soybean roots with a vigorous water stream through an 850-µm-aperture sieve onto a 250-µm-aperture sieve and extracted by centrifugation in 76% (w/v) sucrose solution (Liu & Chen, 2000). The eggs were released by crushing the cysts on a 150-µm-aperture sieve with a rubber stopper mounted on a motor and collected on a 25-µm-aperture sieve (Faghihi & Ferris, 2000). The eggs were separated from debris by centrifugation in a 35% (w/v) sucrose solution for 5 min at 1500g (Liu & Chen, 2000).

An egg suspension of 800 eggs ml-1 was prepared. Two holes of 3 cm depth along two sides of the plant were made with a 5-ml pipette tip. Two thousand (2,000) eggs in 2.5 ml of water were added to each hole for 4,000 eggs per plant. Additional autoclaved sand-soil medium was added to cover the hole and nematodes. The cones were arranged in randomized blocks on the benches in the growth room with the temperature set at 28°C and artificial light for 16 hours per day. Water was supplied with a sprinkler irrigation system twice daily to water-holding capacity. Throughout all stages of the growing process, fertilizer was applied every 14 d at a rate of 4 ml of a solution of Peters Hydrop-Sol 5-11-26 (ICL Specialty Fertilizers, Summerville, SC) per cone. After

35 d (one SCN generation), the experiment was terminated. Images of the plants at the pre-inoculation stage and the end of the experiment are in Figure 5-3. Biomass was cut at the soil level and removed. The soil from the cone was emptied into a 1000 ml pitcher and decanted five times to remove the cysts from the soil. Newly formed females and cysts were washed off pennycress roots with a vigorous water stream through an 850-µm-aperture sieve onto a 250-µm-aperture sieve and extracted by centrifugation in 76% (w/v) sucrose solution (Liu & Chen, 2000). The newly formed females and cysts were counted under a dissecting microscope.

Data Analysis

All analyses were conducted with R, version 4.0.5 (R Core Team, 2021). For each experiment, the main effect of Accession ID was fixed. Experimental run and replication within run were random effects. Each treatment occurred independently. The two runs of the experiments were combined for analysis. The final model for the experiments was a random intercept generalized linear mixed effect model (packages lme4, MASS; Venables & Ripley, 2002; Bates et al., 2015). Analytical assumptions for the mixed analysis of variance were examined by visual inspection of the residual plots. Likelihood ratio $\chi 2$-tests was used to assess the significance of the fixed effects (Fox & Weisberg, 2019). Accessions noted with poor root growth or dead plants were excluded from the analysis. In soybean evaluations, it is recommended to exclude accessions from the analysis if the individual accessions have Coefficient of Variation (CV) values above 30% (Niblack et al., 2009). Many of the pennycress accessions in this analysis had a CV value over 30%; however, screening for SCN resistance in pennycress does not have an established evaluation method. Therefore, no accessions were excluded from the analysis, and to quantify the female index, the value was calculated on an accession by replication

basis to calculate a standard error. The variation in response could be quantified and represented with this calculation method rather than averaging across all accessions and replications of the susceptible soybean check. The Female index for each pennycress accession was calculated as:

Equation 5.1: Female Index (FI) = $\frac{Number\ of\ females\ found\ on\ a\ test\ accession}{Number\ of\ females\ found\ on\ a\ susceptible\ soybean\ check}$

The classification scheme for soybean resistance response in SCN screening of soybean is as follows: Female Index <10 is resistant, Female Index 10-30 is moderately resistant, Female Index ≥30-60 is moderately susceptible, and ≥60 is considered susceptible (Bayless et al., 2016; Schmitt & Shannon, 1992). As there is no established resistance classification scheme for pennycress, this classification scheme was used.

Results

Bioassay 1

The fixed effect pennycress accession on Female Index was significant (Prob > $\chi 2$ = 1.28e-13) (Table 5-1). Pennycress accession #23 ('Custer Trail') had the lowest FI average value at 17.3. Pennycress accession #22 (Ames32873) had the highest FI value of 77.2. The range in standard error values was 2.4-13.7. Using the soybean resistance classification scheme, no accessions were considered resistant with an FI value < 10. Three accessions were considered "moderately resistant," with an FI value of 10-30. These were accession #23 (FI=17.3, Custer Trail), accession #11 (FI= 23.1, Ames31008), and accession #9 (FI = 27.4, PI633414). Accessions #23 and #11 were winter-type pennycress accessions. Accession #9 was a spring-type pennycress accession. All accessions screened in bioassay one are presented in Figure 5-1.

Bioassay 2

The fixed effect pennycress accession on Female Index was significant (Prob > $\chi 2$ = 3.31e-8) (Table 5-1). The range in FI average values was 11.8 (MI-1) to 32.4 (tt8). The range in standard error values was 0.9 to 6.8. According to the soybean classification scheme, no pennycress accessions were considered resistant with an FI less than 10. All the accessions tested except two accessions were considered "moderately resistant." Five accessions had FI values less than 15. These accessions were #20, #26, #5, #37, #13 with average FI values of 11.8, 14.0, 14.1, 14.6, and 14.9, respectively. All accessions screened in bioassay two are presented in Figure 5-2.

Discussion

The response of 51 pennycress accessions to inoculation with SCN population SY97 (HG Type 7) was evaluated in this study. The pennycress accessions have not been previously screened against SCN population SY97. In these evaluations, the pennycress accessions were inoculated with eggs produced on soybean 'Williams 82.' The eggs hatched and successfully developed to the adult female stage on the pennycress accessions. As was noted in the Materials and Methods, a defined level of SCN resistance for pennycress has not been established. Therefore, the soybean resistance classification scheme and protocol to compare female counts on test accessions to a susceptible soybean were used (Bayless et al., 2016; Niblack et al., 2002; Schmitt & Shannon, 1992). There are obvious issues with comparing across botanical species in quantifying resistance. The families of soybean (*Fabaceae*) and pennycress (*Brassicaceae*) are estimated to have diverged 125-136 million years ago and have undergone a diverse scenario of evolutionary events in this period (Hyung et al., 2014). As a result, pennycress and soybean have different life cycles, seed size, seed composition, shoot

morphology, root morphology, nutrient requirements, and numerous other physiological differences (Best & Mcintyre, 1975; Cubins et al., 2019; Hicks, 1978). Despite obvious differences, pennycress, as a newly developed crop, does not have a consistently susceptible accession that can be used as a point of comparison. Williams 82 is a well-established control that has been used in SCN screenings for decades, and for this reason, it was used as a comparative tool in these experiments (Sikora & Noel, 1997). A consistently susceptible pennycress accession that can be used for comparative analysis and resistance discovery is needed for future evaluations. Pennycress accession Ames32873, with its average FI value of 77.2, representing ~ 77% of the female development observed on 'Williams 82', may be a suitable candidate. However, additional studies are needed to ensure that Ames32873 is consistently susceptible and could be used reliably as SCN pennycress control for resistance screening.

Three accessions in bioassay 2 are of interest, as they originated from the same genetic backgrounds. These pennycress accessions had induced mutations by mutagens such as EMS and fast neutrons (FA), which caused a generation of distinct sets of mutations in every cell in the seed (Chopra et al., 2018). Three mutagens of interest are 'tt2' and 'Waxy,' which had FI values below 15, and 'tt8,' which had the highest FI value. Gene *tt2* encodes for MYB repeat proteins. MYB transcription factors are involved in plant development, secondary metabolism, hormone signal transduction, disease resistance, and abiotic stress tolerance (Katiyar et al., 2012). MYB transcription factors have been implicated in disease resistance to tobacco mosaic virus (TMV; *Tobamovirus, Virgaviridae*) in cultivated tobacco (*Nicotiana tabacum*) (Jin & Martin, 1999), and the regulation of these transcription factors may be a target for further testing. The 'Waxy'

accession has a mutation that targets wax biosynthesis. A mutation in wax biosynthesis production has been associated with disease resistance to powdery mildew (*Golovinomyces orontii*), anthracnose (*Colletotrichum lindemuthianum*), and rust (*Pucciniales* sp.) pathogens (Uppalapati et al., 2012; Weidenbach et al., 2014). Additional testing is needed to evaluate if the resistance response from 'tt2' and 'Waxy' is robust. Accession 'tt8' had the highest FI value in bioassay 2. The 'tt8' accession has a mutation at *tt8*, which encodes a transcription factor shown in *Arabidopsis* to regulate fatty acid biosynthesis (Chen et al., 2014). *TT8* affects stress response and brassinosteroid and jasmonic acid biosynthesis by binding to promoters of the genes involved. When *Arabidopsis* plants had loss-of-function mutations to *TT8*, they exhibited higher *Pseudomonas syringae* infection and disease symptoms and reduced growth in the presence of other abiotic and biotic stressors (Rai et al., 2016). *TT8* loss-of-function in pennycress may facilitate greater development by SCN, but further testing is needed to confirm this response.

The information from these bioassays can also characterize the genetic mechanisms facilitating SCN infection using the technique employed in Arabidopsis and root-knot nematode (Meloidogyne sp.) by looking at genetic differences between genetic differences the susceptible and resistant accessions (Gleason et al., 2016; Hamamouch et al., 2011). Another resistance strategy could be to use the information from these and other pennycress SCN screenings to perform a genome-wide association study to identify QTL regions that affect the SCN development in pennycress and provide information for pennycress breeders interested in incorporating SCN resistance (Vuong et al., 2015; Zhang et al., 2017).

In conclusion, the results from this experiment show that natural variation exists within the pennycress germplasm, but resistance exhibited in pennycress lines screened is classified as moderate when using the FI soybean classification system. A pennycress accession with major resistance (FI < 10) was not discovered. Additional screening of accessions exhibiting unique mutations, association mapping, and QTL discovery may be the options to find genetic resistance in pennycress that breeders can target.

Tables

Table 5-1: Likelihood-ratio $\chi 2$-test for Female Index (FI) as influenced by pennycress accession

Experiment	Response	$\chi 2$	Num Df	Prob > $\chi 2$
Bioassay 1	Female Index	111.7	23	1.28e-13***
Bioassay 2	Female Index	101.9	36	3.31e-8***

The fixed effect of pennycress accession was tested with the likelihood-ratio $\chi 2$ method. All models included run and replication with run as a random effect. Num Df = numerator degree of freedom. *, **, and *** represent significance of likelihood-ratio $\chi 2$ tests at $P < 0.05$, 0.01, and 0.001

Table 5-2: Pennycress accession source and identification information for bioassay 1

Accession ID	Name	Source	Growth Habit	Origin (City, State)
1	MN107	UMN	Winter	Coates, Minnesota
2	MN108	UMN	Winter	Sherburne, Minnesota
3	MN110	UMN	Winter	Rosemount, Minnesota
4	MN114	UMN	Winter	St. Louis, Missouri
5	Ames29531	GRIN	Winter	North Dakota
6	Ames30985	GRIN	Winter	Yankton, South Dakota
7	Ames29118	GRIN	Winter	Beecher, Illinois
8	PI633415	GRIN	Spring	Belgern, Germany
9	PI633414	GRIN	Spring	Wachstedt, Germany
10	Ames31000	GRIN	Winter	Fort Collins, Colorado
11	Ames31008	GRIN	Winter	Nunn, Colorado
12	Ames31013	GRIN	Winter	La Porte, Colorado
13	Ames31014	GRIN	Winter	Longmont, Colorado
14	Ames31015	GRIN	Winter	Lyons, Colorado
15	Ames31016	GRIN	Spring	Colorado
16	Ames31019	GRIN	Spring	Aspen Park, Colorado
17	Ames31020	GRIN	Winter	Aspen Park, Colorado
18	Ames31022	GRIN	Winter	Leadville, Colorado
19	Ames31026	GRIN	Winter	Parlin, Colorado
20	PI672505	GRIN	Winter	Beecher, Illinois
21	Ames32867	GRIN	Winter	Armenia
22	Ames32873	GRIN	Winter	Armenia
23	CusterTrail	UMN	Winter	Custer Trail, South Dakota
24	BlackHills	UMN	Winter	Black Hills, South Dakota

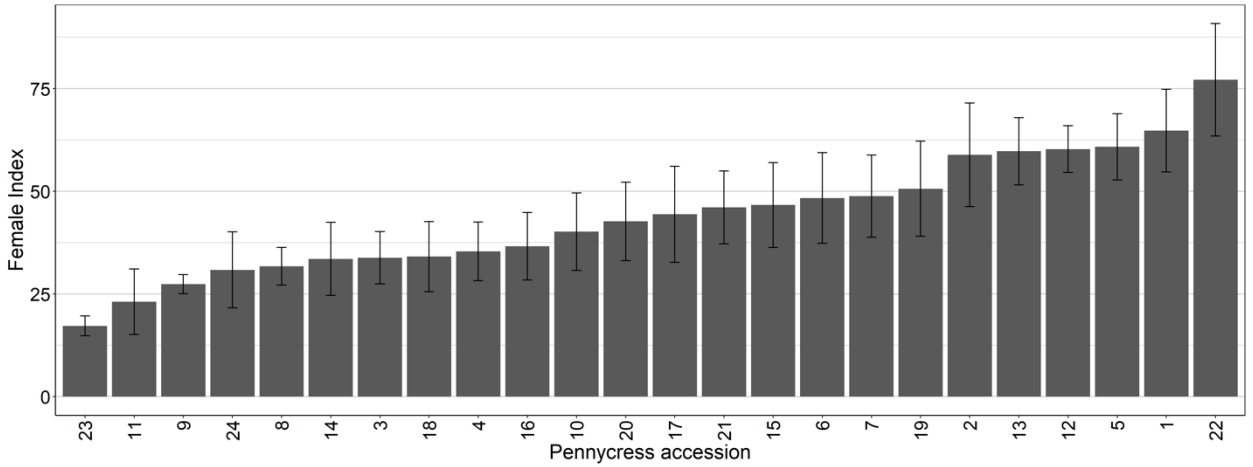
Table 5-3: Pennycress accession source and identification information for bioassay 2

Assession ID #	Name	Source	Growth Habit	Origin (City, State)	Note
1	aop2	UMN	Winter	Coates, MN	low glucosinolate level
2	myc3	UMN	Winter	Coates, MN	low glucosinolate level
3	D2-247-4	UMN	Winter	Coates, MN	low glucosinolate level
4	tt8	UMN	Winter	Coates, MN	yellow seedcoat and low fiber level
5	tt2	UMN	Winter	Coates, MN	yellow seedcoat and low fiber level
6	ttg1	UMN	Winter	Coates, MN	yellow seedcoat and low fiber level
7	Bloom	ISU	Winter	Bloomington, IL	NA
8	ElizabethS2	ISU	Winter	Unknown	NA
9	Norm	ISU	Winter	Normal, IL	NA
10	TAMN137	UMN	Winter	Guthrie Center, IA	NA
11	15-1	WIU	Winter	Wall,SD	NA
12	15-2	WIU	Winter	Moorcroft,WY	NA
13	15-3	WIU	Winter	Sheridan,WY	NA
14	15-4	WIU	Winter	Broadview,MT	NA
15	15-5	WIU	Winter	Vaugh,MT	NA
16	EF1	WIU	Winter	Peoria,IL	NA
17	W12	WIU	Winter	Macomb,IL	NA
18	NY10	WIU	Winter	Ithaca,NY	NA
19	OH-20	WIU	Winter	Columbus,OH	NA
20	MI-1	WIU	Winter	Applegate,MI	NA
21	WY-01	WIU	Winter	Buffalo,WY	NA
22	13-2	WIU	Winter	St. Clair,MO	NA
23	13-11	WIU	Winter	Lawrenceburg,IN	NA
24	13-19	WIU	Winter	Newcastle,IN	NA
25	13-25	WIU	Winter	San Jose,IL	NA
26	13-34	WIU	Winter	Geneva,IL	NA
27	13-39	WIU	Winter	Rolling Meadows,IL	NA

28	KS-1	WIU	Winter	Oakley,KS	NA
29	NE-1	WIU	Winter	Bruning,NE	NA
30	T-1	WIU	Winter	Unknown	Large flowers
31	MT1-2	WIU	Winter	Unknown	NA
32	MN16172	UMN	Winter	Unknown	NA
33	MN16167	UMN	Winter	Unknown	NA
34	MN16089	UMN	Winter	Unknown	NA
5	MN16188	UMN	Winter	Unknown	NA
36	Aneuploid	UMN	Winter	Coates, MN	Extra chromosome
37	Waxy	UMN	Winter	Coates, MN	High wax ester level

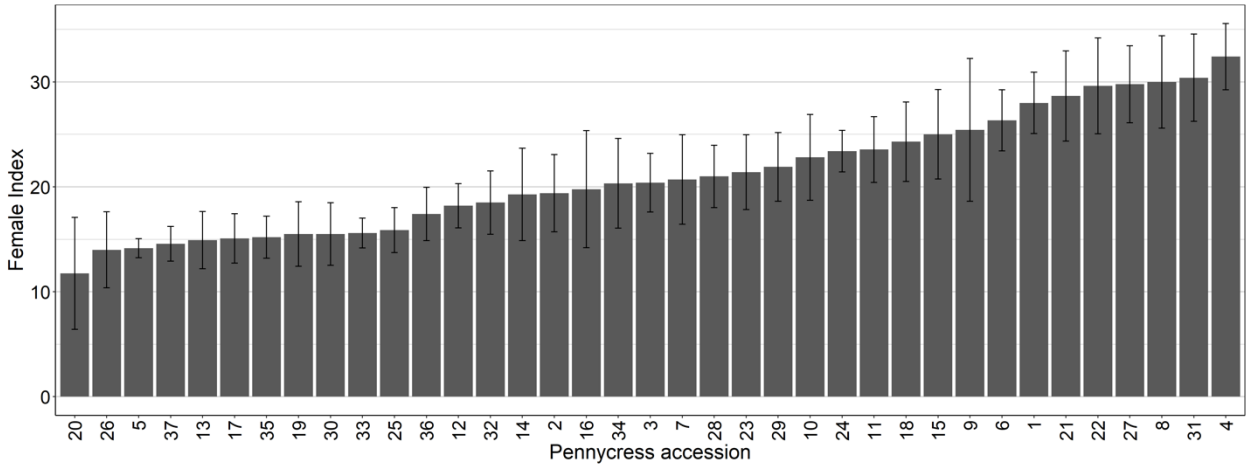
Figures

Figure 5-1: Female Index values of pennycress bioassay 1 tested in growth room evaluation



Error bars denote standard error. For accession identification, see table 5-2.

Figure 5-2: Female Index values of Bioassay 2 tested in growth room evaluation.



Error bars denote standard error. For accession identification, see table 5-3

Figure 5-3: Photos of Pennycress a) after vernalization; and b) at the end of the evaluation from Bioassay 2, Run 2.

a.

b.

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