Globodera pallida Control Using *Brassica juncea* Seed Meal Extract and the Trap Crop *Solanum sisymbriifolium*

A Thesis

Presented in Partial Fulfillment of the Requirements for the

Degree of Master of Science

with a

Major in Plant Pathology

in the

College of Graduate Studies

University of Idaho

by

Bhupendra Bhatta

Major Professor: Louise-Marie Dandurand, Ph.D.

Committee Members: Matthew J. Morra, Ph.D.; Edwin Lewis, Ph.D.

Department Administrator: Edwin Lewis, Ph.D.

August 2021

Authorization to Submit Thesis

This thesis of Bhupendra Bhatta, submitted for the degree of Master of Science with a Major in Plant Pathology and titled "*Globodera pallida* control using *Brassica juncea* seed meal extract and the trap crop *Solanum sisymbriifolium*," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____ Date: _____
Louise-Marie Dandurand, Ph.D.

Committee Members: _____ Date: _____
Matthew J. Morra, Ph.D.

_____ Date: _____
Edwin Lewis, Ph.D.

Department Administrator: _____ Date: _____
Edwin Lewis, Ph.D.

Abstract

Globodera pallida, the pale cyst nematode, is a regulated potato pest with the potential for detrimental economic impacts. Restrictions on use of the soil fumigant, methyl bromide, and lack of resistant russet type varieties for US markets have led to investigations of alternative strategies to control this potato pest. The efficacy of *Brassica juncea* seed meal extract (SME) was evaluated under greenhouse conditions (0, 0.14, 0.28, 0.56, 1.12, and 2.24 t SME/ha) and field conditions (0, 1.12, and 2.24 t SME/ha in year 2019 and 0, 0.14, 0.28, 0.56, and 1.12 t SME/ha in year 2020) either applied alone or in combination with the trap crop *Solanum sisymbriifolium*. The impact of the application of SME pre- or post-planting *S. sisymbriifolium* was determined. *Solanum sisymbriifolium* alone reduced the number of encysted eggs compared to the untreated control by up to 60% in greenhouse and 67% in field trials. When SME was applied post-planting, *S. sisymbriifolium* induced hatch of *G. pallida* and significantly fewer encysted eggs remained at termination of the experiment whether or not SME was applied. When SME was applied pre-plant, the remaining encysted eggs were not decreased by planting *S. sisymbriifolium* which may indicate that SME inhibited egg hatch in the presence of *S. sisymbriifolium*. Treatments with SME either alone or with *S. sisymbriifolium* provided a significant reduction of *G. pallida* egg viability, hatch, and reproduction compared to the untreated control at all rates tested under greenhouse conditions. Combined treatment with *S. sisymbriifolium* and SME at lower rates, 0.14 t/ha for pre-plant or 0.56 t/ha or less for the post-plant experiment, further reduced egg hatch and reproduction than each treatment alone. SME alone applied at higher rates of 1.12 and 2.24 t/ha whether or not combined with *S. sisymbriifolium* eliminated *G. pallida* reproduction on potato in both pre-plant and one post-plant greenhouse trials. In the second post-plant greenhouse trial, SME at 1.12 t/ha and 2.24 t/ha when applied alone highly reduced the reproduction factor compared to the untreated control by 64 and 89%, respectively, and eliminated reproduction when used in combination with *S. sisymbriifolium*. Collectively, our results indicated that a combination of SME and *S. sisymbriifolium* allows an opportunity to reduce the amount of SME required to control *G. pallida* and further decrease the potential reserve of the viable population remaining after individual treatment with each strategy.

Acknowledgments

I would like to thank my major professor- Dr. Louise-Marie Dandurand for the invaluable advice and for steering me in the right direction during my graduate studies at the University of Idaho. I would like to acknowledge my committee members, Dr. Matthew J. Morra, and Dr. Edwin Lewis, for their inputs and understanding over these past three years. I wish to express my sincere thanks to Dr. Inna Popova for providing the necessary seed meal extract for this research and reviewing the manuscript. I take this opportunity to acknowledge the entire PCN lab for training and supporting me to complete this research project. I would also like to thank Dr. William Price for his help with the statistical analysis.

This research was funded by Methyl Bromide Transition Program under award number 2017-51102-27271, USDA-APHIS, and Idaho potato commission.

Dedication

This thesis is dedicated to my grandfather, Patiram Bhatta. You are no longer with us, but your belief in my ability has made this journey possible.

Table of Contents

List of Tables

Chapter 1: Introduction

Potato Cyst Nematodes

Potato cyst nematodes include two different nematode species: *Globodera rostochiensis* (Skarbilovich 1959; Wollenweber 1923) and *Globodera pallida* (Behrens 1975; Stone 1972) classified under order Tylenchida. The order Tylenchida includes most of the economically important plant-parasitic nematodes affecting agricultural crops. The potato cyst nematodes coevolved with potato in the Andes region of South America (Grenier et al. 2010). They spread to Europe from the center of origin in South America, and from Europe to other parts of the world with the seed tubers of improved varieties developed in Europe. At present, potato cyst nematodes are present in temperate areas worldwide and are of regulatory significance.

Potato cyst nematodes were considered a single species until 1970 and were placed under the genus *Heterodera* until 1975. Guile (1970) observed golden-yellow and pale creamy-yellow-colored females emerging from the roots and noted the difference between the species of potato cyst nematodes distinguished by cyst color. Later, two species were described, *Heterodera rostochiensis*, commonly known as the golden cyst nematode, and *Heterodera pallida*, commonly known as pale cyst nematode. A few years later, Behrens (1975) suggested that potato cyst nematodes are different from *Heterodera* species, and they were placed under the genus *Globodera*. Unlike *Heterodera* species with lemon-shaped cysts, *Globodera* species have round cysts and lack a terminal cone (Fleming and Powers 1998).

The Pale Cyst Nematode *Globodera pallida*

Globodera pallida or the pale cyst nematode (PCN) is a globally regulated and economically important potato pest. Solanaceous crops, potato, tomato, and eggplant, constitute the major hosts of *G. pallida* (CABI 2020). In highly infested soils, *G. pallida* can cause up to 80% loss of tuber yield (Talavera et al. 1998; Contina et al. 2019). *Globodera pallida* has been detected in 55 countries across the globe (CABI 2020). The ability to survive adverse conditions for an extended period and high reproduction potential under favorable conditions make *G. pallida* one of the most damaging plant-parasitic nematodes (Turner and Evans 1998).

Globodera pallida is a sedentary endoparasite that survives in the soil as encysted eggs in the absence of a suitable host. *Globodera pallida* hatch only in response to the chemical hatching factor released by a suitable host (Byrne et al. 2001; Farnier et al. 2012; Perry and Clarke 1977). Once the eggs receive chemical cues from the host plant's roots known as hatching factors, they hatch into second-stage juveniles (J2s) (Widdowson and Wiltshire 1958). One of the hatching factors released from potato roots has been identified as solanoeclepin A (Schenk et al. 1999). Brief exposure to the potato root diffusate (PRD) can stimulate a hatch of *G. pallida* eggs. Forrest and Perry (1980) observed 43% hatch after *G. pallida* eggs were exposed to the PRD for 4 weeks. Hatching factors are responsible for a calcium-mediated change in egg membrane permeability, leading to loss of trehalose from the perivitelline fluid and facilitating hydration of J2s. The hydrated J2s begin movement, pierce the eggshell with their stylet, and subsequently hatch (Perry 1998). *Globodera pallida* is known to have a greater hatch rate at lower temperatures than *G. rostochiensis* (Franco 1979; Kaczmarek et al. 2014). Upon hatching, the infective J2s need to locate and invade the host roots before their food reserve drains out. Orientation of hatched J2 towards host roots results from various stimuli, including carbon dioxide gradient around the roots. Root tips produce more active diffusate than other portions of the root and are metabolically active. The infective juveniles, therefore, prefer invasion at root tips. The second stage juvenile uses its stylet to penetrate the epidermal cells and migrate intracellularly to reach vascular tissues, where they form a syncytium which serves as a feeding site (Turner and Evans 1998).

Jones and Northcote (1972) have described the structure and function of syncytia induced by potato cyst nematodes. Using light microscopy, they found changes in the root cells from the cortex to the vascular tissue and longitudinal spread of a syncytium along with the vascular tissue. The noticeable changes consist of a breakdown of the cell wall, protuberances formed in the syncytia wall, dense cytoplasm, loss of vacuole, and nuclear and cell enlargement. These changes facilitate the continuous supply of nutrients to the juveniles for their development and reproduction. The J2s remain sedentary while feeding from the syncytium and molt through the third and fourth stage (Koenning and Sipes 1998). At this stage, sex differentiation occurs, and the juveniles develop into either males or females. A high number of juveniles develop into females when the food supply is adequate (Trudgill 1967). The vermiform-shaped males stop feeding and exit the root, whereas females remain sedentary to continue feeding and enlarge in size. Due to their growing size, globose-shaped females rupture the root system exposing their posterior body to the soil. The pheromones produced by females attract males, and fertilization occurs. After fertilization, eggs are developed inside the female body. The female dies, and her cuticle is hardened to form the protective cyst

containing 200-500 eggs (Turner and Evans 1998). The encysted eggs undergo a dormancy period that allows for survival until conditions are favorable for hatching (Muhammad 1994).

Globodera pallida in Idaho

In the United States, *G. pallida* was first detected in Idaho in 2006 (Hafez et al. 2007). *Globodera pallida* is listed as a quarantine pest for Idaho under Title 7 CFR 301.86 Federal Regulation by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) and by the Idaho State Department of Agriculture (ISDA). As of December 2020, the regulated area is limited to 7,354 hectares, of which 3,446 hectares are infested with *G. pallida* (USDA-APHIS 2021). This infested area represents less than 1% of the annual potato production area of Idaho. Since its detection in 2006, a program to contain and eradicate *G. pallida* by USDA-APHIS has been implemented. Regulations to contain *G. pallida* prohibit growing potato and other host crops, require sanitization of farm equipment, and restrict the movement of soil and plant materials from the infested fields (USDA-APHIS 2009). Idaho is the largest producer of potatoes in the U.S. with a production of 6.9 million tons worth $1.03 billion in 2018 (USDA-NASS 2019). The economic impact of removing potatoes from the cropping system in Idaho's infested area was estimated to be a loss of $24.56 million in 2016 (Koirala et al. 2020). Therefore, the presence of *G. pallida* creates a significant threat to the U.S. potato industry. Current research efforts to control *G. pallida* in Idaho are focused on developing resistant russet potato varieties, investigating trap crops, biofumigation, risk analysis on yield and economic impact, and developing tools for improved detection and viability assessment (Dandurand et al. 2019).

Strategies to Control *Globodera pallida*

Methyl Bromide (MeBr), a broad-spectrum soil fumigant, was widely used to control plant-parasitic nematodes and other soilborne pests. However, due to its ability to deplete the stratospheric ozone, MeBr was phased out under the Montreal Protocol (Ristaino and Thomas 1997; Martin 2003). In Idaho, USDA-APHIS discontinued the use of MeBr in 2015 due to safety concerns (USDA-APHIS 2015). Currently, infested fields in Idaho are fumigated with Telone II (1,3 dichloropropene). The use of host resistance and rotation with the non-host crops are non-chemical strategies for controlling

plant-parasitic nematodes. For *G. rostochiensis*, commercial potato cultivars with a major gene, *H1*, are available with durable resistance. Unlike *G. rostochiensis*, resistance against *G. pallida* is polygenic, conferred by the additive action of multiple genes (Rigney et al. 2017). Currently, russet-skinned potatoes resistant to *G. pallida* are not available (Dandurand et al. 2017; Whitworth et al. 2018). Rotation with the non-host crops is a method to control the nematodes with a narrow host range. However, the annual decline rate of *G. pallida* may be as low as 10% in the absence of a suitable host (Turner 1996). Therefore, rotation with non-host crops is not an effective control measure for *G. pallida* despite its narrow host range. Loss of MeBr, unavailability of resistance in russet-type potato varieties, and this nematode's ability to remain dormant in the soil in the absence of a suitable host make control of this pest challenging in Idaho and elsewhere. The use of trap crops (Scholte 2000b; Timmermans et al. 2007; Dandurand et al. 2019) and biofumigation (Lord et al. 2011; Ngala et al. 2015; Dandurand et al. 2017) has proven to be effective non-chemical strategies to control *G. pallida*.

Biofumigation: Definition and Mechanism

Biofumigation involves use of glucosinolate-containing *Brassica* plants to suppress diseases and pests with the suppressive effect is specifically attributed to the biocidal properties of the isothiocyanates (ITCs) released by the hydrolysis of glucosinolates (GSLs) (Angus et al. 1994; Kirkegaard and Sarwar 1998; Lazzeri et al. 2004). *Brassica* is economically the most important genus of the family *Brassicaceae* with several crops of agronomic importance, such as cabbage (*Brassica oleracea* var. *capitata*), oilseed rape (*B. napus*), cauliflower (*B. oleracea* var. *botrytis*), black mustard (*B. nigra*), and yellow or Indian mustard (*B. juncea*). A limited number of plant families other than Brassicaceae biosynthesize GSLs. The glucosinolate molecule consists of a β-thioglucose moiety, a sulphonated oxime moiety, and a variable side chain. GSLs differ in structure by the side chain associated with more than 100 different side chains having been identified (Brown and Morra 1997; Mithen 2001).

The type and amount of GSLs vary by *Brassica* crop species, cultivars, and plant parts used, and such variations are the result of both genetic and environmental control (Kirkegaard and Sarwar 1998; Mithen 2001; Van Dam, Tytgat, and Kirkegaard 2009; Bhandari et al. 2015). For example, Bellostas et al. (2007) compared GSL profiles of four *Brassica* species (*B. juncea*, *B. carinita*, *B. napa*, and *B. rapa*) and found differences in the type of GSLs. *B. juncea*, *B. carinita*, and *B. napa* showed higher

concentrations of 2-propenylglucosinolate, while in *B. rapa*, 3-butenylglucosinolate, 2-hydroxy-3-butenylglucosinolate, and 2-hydroxy-4-pentenylglucosinolate were the major GSLs. Furthermore, GSL concentration may vary in different plant parts at different developmental stages. Total GSL concentration increased, but the concentration in roots decreased due to plant growth in *B. juncea*, *B. carinita*, and *B. napa*. However, the opposite trend was found in *B. rapa* (Bellostas et al. 2007). The highest GSL concentration was found in reproductive tissues of *B. juncea*, *B. carinita*, and *B. napa*, while roots produced the highest GSL concentration in *B. rapa* (Bellostas et al. 2004, 2007). The efficacy of biofumigation is also dependent on the timing of plant growth and plant tissue incorporation. Ngala et al. (2015) reported reduced *G. pallida* egg viability and reproduction with a summer cultivated and fall incorporated *B. juncea* cover crop but not when fall cultivated, and overwintered crop was incorporated in the spring.

Soil properties such as soil texture, organic matter content, moisture content, pH, and temperature influence the biofumigation effect. Price et al. (2005) performed a comprehensive study of the effects of different soil conditions on *B. juncea* degradation products. They showed higher allyl ITC concentration in the sandy loam soil compared to the clay loam soil. The GSL content of *B. juncea* leaves incorporated into soil declined faster in sandy-loam soil than clay loam soil. The maximum amount of 2-propenyl-ITC produced in sandy-loam soil was approximately six times higher than that in clay-loam soil (Bending and Lincoln 1999). Soil organic matter has been demonstrated as the main sorbent of aliphatic ITCs in soil (Brown and Morra 1997; Gimsing et al. 2009). Borek et al. (1995) reported a decrease in allyl isothiocyanate half-life with an increase in soil organic carbon content. Such rapid dissipation of allyl ITC in soil results in short-lived pesticidal activity.

Because water is required for GSL hydrolysis and aliphatic ITCs are volatile, incorporating plant tissues followed by watering and soil sealing enhances the biofumigation effect (Morra and Kirkegaard 2002; Kirkegaard and Matthiessen 2004). On the other hand, the product of GSL degradation is pH-dependent. ITCs are generally produced at neutral pH, whereas conversion to nitriles occurs at lower pH (Uda et al.1986). However, soil temperature affects the half-life of allyl ITCs which are decreased from 35 h at 10^{o}C to 25 h at 25 ^{o}C (Borek et al. 1995). Volatilization of ITCs increase with increasing soil temperature and may result in a corresponding reduction in biological activity (Price et al. 2005).

Glucosinolate Degradation

Glucosinolates are present in the vacuole of sulfur-rich S-cells, whereas the enzyme myrosinase or *β*-thioglucosidase (EC 3.2.1.147), the catalyst required for hydrolysis of GSLs, is present in the myrosin grains of the myrosin cells (Pocock et al. 1987; Höglund et al. 1991). Since the vacuole containing GSLs and the enzyme myrosinase is present in different cells, tissue disruption is required to mix them. The enzymatic hydrolysis of GSLs occur in the presence of myrosinase to produce glucose, sulfate, and aglycone- an unstable intermediate. The aglycone undergoes rearrangement to produce several products- including ITCs, nitriles, and thiocyanates (Cole 1976; Brown and Morra 1997). ITCs are produced most frequently, and the type of ITCs depends upon the type of GSLs hydrolyzed. Conversion of the unstable intermediate to nitriles occurs at lower pH, and ferrous ions enhance the process (Uda et al. 1986).

Control of *Globodera pallida* with Biofumigation

Biofumigation with *Brassicaceae* has gained attention as one of the alternatives to control plant-parasitic nematodes in recent years due to the ban of synthetic pesticides, especially soil fumigants such as methyl bromide and ethylene dibromide. ITCs, the hydrolysis products of GSLs, are general biocides and have properties similar to synthetic fumigant nematicides such as metam sodium and dazomet (Brown and Morra 1997; Kirkegaard and Sarwar 1998; Matthiessen and Kirkegaard 2006). A plethora of research has demonstrated successful nematode suppression by biofumigation using several *Brassica* species and biofumigation methods. Potter et al. (1998) observed suppression of *Pratylenchus neglectus* by 95.2% and 48.3% after amendment with leaf tissues of *B. oxyrrhina* and *B. juncea*, respectively, and reported that suppression is more significant with leaf tissue amendments than with the similar amount root tissues from the same plants. Investigation of the activity of *Brassica* green manures on *G. pallida* showed over 95% egg mortality when *B. juncea* lines containing high sinigrin (2-propenyl glucosinolate) were incorporated in soil (Lord et al. 2011). *Raphanus sativus and Eruca sativa* grown as winter cycle crops and incorporated in the soil after 4 months of sowing reduced *Meloidogyne arenaria* root galls and egg masses on a tomato crop (Aydınlı and Mennan 2018). In addition to rotation and cover crops, the use of seed meal derived from *Brassica* species is also a proven promising method to control plant-parasitic nematodes. Soil amendment with *B. carinata* seed meal at a rate of 2.5 tons/ha reduced *Meloidogyne*

chitwoodi densities in soil (Henderson et al. 2009). Curto et al. (2016) demonstrated the potential of mustard seed meal to reduce *M. incognita* populations. It has been reported that 100% reduction of *M. incognita* and *P. penetrans* can be achieved using *B. juncea* cv. Pacific Gold seed meal and the suppression was due to the GSL degradation products of *B. juncea* (Zasada et al. 2009). Leaf extracts (Lord et al. 2011), liquid formulations of seed meal (De Nicola et al. 2013), and formulated seed meal extracts (Popova et al. 2017) have been developed and tested against plant-parasitic nematodes. Formulated *B. juncea* seed meal extract at rates 1.1 t/ha suppressed egg hatch of *G. pallida* and *G. ellingtonae* and eliminated reproduction of *G. pallida* on potato (Dandurand et al. 2017). For PCN control, SME can be applied at a much lower rate than when seed meal is used; 50% less SME is required to achieve similar efficacy (Dandurand et al. 2017).

Conventionally, biofumigation involves soil incorporation of *Brassica* tissues. However, with a better understanding of ITC production mechanisms, biofumigation strategies at field scale include tissue maceration, irrigation and/or soil sealing to maximize the biofumigation effect. The half-life of allyl ITC in 6 different soils ranged from 20 to 60 h (Borek et al. 1995). Because allyl ITC is short-lived, sufficient concentration is needed in soil to inhibit or kill the target pest (Borek et al. 1995). The rate of application of GSL-containing plant materials based on ITC lethal concentration values provides consistent and repeatable suppression of the plant-parasitic nematodes (Zasada and Ferris 2004). Seed meal extracts formulated in powder form have a longer shelf life, and less material required for efficacy reduces the associated transportation and handling costs. Furthermore, consistent results are more achievable with the seed meal extracts than with seed meal and green manures (Popova et al. 2017; Dandurand et al. 2017).

The Trap Crop *Solanum sisymbriifolium*

A non-host trap crop that stimulates egg hatching but does not support subsequent development and reproduction of the nematode offers a potential alternative for *G. pallida* control. The use of potato itself as a trap crop to control *G. pallida* is not desirable because the timing of destruction before new females are developed is difficult to determine (Scholte 2000a). Furthermore, in Idaho, the planting of potatoes is prohibited in infested fields. Scholte (2000c) investigated the potential of non-tuber bearing solanaceous plants for use as a trap crop to control the potato cyst nematodes and observed that *Solanum sisymbriifolium* (Lamarck) strongly stimulated hatching but provided complete resistance to both *G. pallida* and *G. rostochiensis*. *Solanum sisymbriifolium*,

commonly known as litchi tomato or sticky nightshade, is native to South America. It is naturalized in Africa, Asia, Australia, Europe, New Zealand, North America, and the Caribbean (CABI 2021). In the United States, *S. sisymbriifolium* is found naturalized in 17 states (USDA-APHIS 2013).

Control of *Globodera pallida* with *Solanum sisymbriifolium*

Solanum sisymbriifolium induces a hatch of *G. pallida*, but unlike in potatoes, the hatched juveniles cannot establish a feeding site in the roots of *S. sisymbriifolium* and die before maturity (Scholte 2000b; Kooliyottil et al. 2016). No live J2s were detected in *S. sisymbriifolium* 8 weeks post-inoculation; however, juveniles developed into third and fourth stages within 16 days of inoculation in potato roots (Kooliyottil et al. 2016). The dead *G. pallida* J2s surrounded by the necrotic cells were observed as early as 2 to 4 days post-infection in *S. sisymbriifolium* (Kooliyottil et al. 2019). This ability of *S. sisymbriifolium* to induce hatch and arrest reproduction provides a tool to reduce *G. pallida* populations.

The *Globodera pallida* hatch rate 6 weeks after planting *S. sisymbriifolium* was 47%,increasing to 75% after 21 weeks (Timmermans et al. 2006). Scholte (2000b) found the greatest reduction in encysted eggs (80% fewer), which is a reflection of the numbers that have hatched, to occur 11 weeks after planting *S. sisymbriifolium*. A higher cumulative hatch of both- *G. pallida,* and *G. rostochiensis*- occurred after exposure to the root exudates of *S. sisymbriifolium* compared to the tap water control (Dias et al. 2017). Similar to Kooliyottil (2016), reproduction of *G. rostochiensis* or *G. pallida* was not observed on *S. sisymbriifolium* after 10 weeks of growth (Dias et al. 2017). *Solanum sisymbriifolium* reduced soil infestation with the potato cyst nematodes by up to 74% under field conditions (Scholte and Vos 2000). Under greenhouse conditions, reproduction of *G. pallida* was reduced by 99% after potato-following-*S. sisymbriifolium* compared to the potato-following-fallow and potato-following-potato treatment (Dandurand and Knudsen 2016). Under Idaho field conditions, a 12-week exposure to *S. sisymbriifolium* reduced the number of *G. pallida* encysted eggs by up to 50% (Dandurand et al. 2019). The number of progeny cysts and encysted eggs in progeny cysts were reduced by 88% and 98%, respectively, after potato-following-*S. sisymbriifolium* compared to potato-following-fallow treatment (Dandurand et al. 2019). The reduction in reproduction of encysted eggs remaining after exposure to *S. sisymbriifolium* may be attributed to the nematicidal activity of glycoalkaloids present in *S. sisymbriifolium* (Dias et al. 2012; Sivasankara and Dandurand 2021).

Application of the Trap Crop *Solanum sisymbriifolium* on a Field Scale

As discussed above, the potential of *S. sisymbriifolium* to control *G. pallida* has been established by several researchers. Of the three *G. pallida*-infested fields in Idaho planted with *S. sisymbriifolium* by USDA-APHIS, egg viability was reduced by 100% and 95% in two fields, and no cysts were found in the third field (Dandurand et al. 2019). Although proven highly effective in controlling *G. pallida*, the risk of this trap crop becoming invasive remains in the areas to which it is not native. For Idaho, ISDA has listed *S. sisymbriifolium* as an invasive species. Therefore, ISDA requires a detailed permitting process to be followed for *S. sisymbriifolium* planting in Idaho fields (USDA-APHIS 2017). To prevent *S. sisymbriifolium* from becoming a weed, herbicides such as bromoxynil (Buctril), fluroxypyr (Starane), and clopyralid (Curtail, Stinger) and glyphosate can be used (Dandurand et al. 2014). Additionally, it is essential to control other solanaceous plants such as *S. physalifolium* that may host *G. pallida* while growing this trap crop. *Solanum sisymbriifolium* can be planted using a grain drill and grows well in the southern Idaho climate, with roots reaching the gravel layer at up to 152 cm below the soil surface (Dandurand et al. 2014).

Integrated Strategies to Control *Globodera pallida*

An integrated approach to control plant diseases that provide consistent suppression of pests by utilizing multiple strategies could be vital as the regulations on the use of agricultural chemicals becomes more restrictive (Noling 2002; Rosskopf et al. 2005). In Europe, *Globodera* species are managed by using resistant varieties when possible, soil testing, chemical treatment, and rotation with non-hosts for at least 6 years (Hockland 2002). However, growers are reticent to accept longer rotations of 6 to 7 years due to the loss of income. Integrating one well-managed trap crop or one application of fumigant nematicide to reduce *G. pallida* populations more rapidly (by up to 80%) can shorten the rotation period (Haydock and Evans 1998). Two-year rotation with non-hosts including *Pisum sativum* (first crop) and *Vicia faba* (second crop), combined with nematophagous fungus *Paecilomyces* sp. reduced *G. rostochiensis* population by 89.2%, which was greater than the reduction provided by *Paecilomyces* sp-fallow treatment (84.4%) and rotation only (30.7%) (López-Lima et al. 2013). A combination of soil biofumigation by cabbage residue and *Streptomyces rubrogriseus* HDZ-9-47 showed more significant control of *Meloidogyne incognita* than each treatment alone (Jin et al.

2019). Studying the integrated use of *G. pallida* control strategies such as rotation with non-hosts and trap crops, host-plant resistance, biofumigants, and fumigant nematicides rather than focusing on a single strategy could help to develop an effective control plan for this potato pest.

Rationale of the Study

The efficacy of individual treatment with *B. juncea* seed meal extract and the trap crop *S. sisymbriifolium* to control *G. pallida* was tested in Idaho field conditions (Dandurand et al. 2017; Dandurand et al. 2019). Each of these strategies has shown high efficacy in reducing *G. pallida* populations. However, the efficacy of seed meal extract at rates less than 1.1 t/ha has not been assessed. Reduced application rates of the seed meal extract would reduce associated handling and application costs. Therefore, testing the efficacy of lower rates to control *G. pallida* is vital. Also, a viable population of *G. pallida* remaining after *S. sisymbriifolium* may serve as potential inoculum to rebuild the population in the subsequent growing season. Elimination of such a population is vital to achieving the eradication goal, which may require additional years of trap cropping or combination with other control strategies. Combining both strategies may provide an opportunity to control *G. pallida* by using less *B. juncea* seed meal extract and eliminate the viable population of the nematode that may remain after *S. sisymbriifolium* alone.

Goal of the Study

This study aimed to test the efficacy of combining biofumigation using *B. juncea* seed meal extract and the trap crop *S. sisymbriifolium* to control *G. pallida* under greenhouse and field conditions. The specific objectives were to: (i) optimize the amount of *B. juncea* seed meal extract needed to control *G. pallida* and (ii) test the efficacy of pyramiding *B. juncea* seed meal extract and the trap crop *S. sisymbriifolium* to control *G. pallida* compared to individual treatment with each.

Chapter 2: Control of *Globodera pallida* Using *Brassica juncea* Seed Meal Extract and the Trap Crop *Solanum sisymbriifolium* Under Greenhouse Conditions

Introduction

Globodera pallida (Behrens 1975; Stone 1972), commonly known as the pale cyst nematode (PCN), is a globally regulated, economically important pest of potato (Hodda and Cook 2009; Turner and Rowe 2006). In highly infested soils, *G. pallida* can cause up to 80% reduction in tuber yields (Talavera et al. 1998; Contina et al. 2019). The potato cyst nematodes *Globodera pallida* and *Globodera rostochiensis* (Skarbilovich 1959; Wollenweber 1923) coevolved with potato and closely related *Solanum* species in the Andes region of South America (Grenier et al. 2010). *Globodera pallida* was transported to Europe during the 1850s along with contaminated seed potatoes. Since then, it has spread to 55 countries (CABI 2020).

In the United States, *G. pallida* was first detected in Idaho in 2006 (Hafez et al. 2007). Idaho is the largest producer, processor, and packer of potato in the US, with a production of 6.9 million tonnes worth $1.03 billion in 2018 (USDA-NASS 2019). The presence of *G. pallida* constitutes a significant threat to the US potato industry. The US Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) and the Idaho State Department of Agriculture (ISDA) have implemented a containment and eradication program to prevent its spread.

Globodera pallida is a sedentary endoparasitic nematode that modifies the host root to form feeding sites. *Globodera pallida* survives as eggs inside cysts, a protective covering formed by the dead female body. A single cyst may contain 200 to 500 eggs (Turner and Evans 1998). In response to the specific chemicals known as hatching factors released by potato or other suitable hosts, second-stage juveniles (J2s) hatch from the eggs (Arntzen et al. 1993; Schenk et al. 1999), migrate into host roots to form feeding sites known as syncytia. Provided that enough nutrients are available from the syncytium, the J2s undergo a series of molts to develop into either males or females and reproduce. The ability of *G. pallida* to survive adverse conditions for an extended period, its host specificity, and high reproduction potential make it a highly damaging plant-parasitic nematode (Turner and Evans 1998).

The use of host resistance and rotation with the non-host crops are non-chemical alternatives for controlling plant-parasitic nematodes. However, resistance to *G. pallida* is not currently available in russet-skinned potato varieties (Whitworth et al. 2018), which represent the majority of potatoes grown in Idaho (USDA-NASS 2019). Although *G. pallida* has a relatively narrow host range, crop rotation with non-hosts is not an effective control measure because *G. pallida* hatching occurs only in response to the hatching factors released by a suitable host (Byrne et al. 2001; Farnier et al. 2012; Perry and Clarke 1977). The annual decline rates of *G. pallida* populations may be as low as 10% in the absence of a host (Turner 1996).

Biofumigation with brassicaceous seed meal extract is one of the potential alternatives to control *G. pallida*. The use of brassicaceous crop amendments has been tested against a wide range of soil-borne pests of agricultural crops, including plant-parasitic nematodes (Brown and Morra 1997; Morra 2004; Lord et al. 2011; Mazzola et al. 2014). Brassicaceous materials in the form of a cover crop, green manure, seed meal, or seed meal extract produce volatile toxic isothiocyanates upon hydrolysis of glucosinolates catalyzed by the enzyme myrosinase (Brown and Morra 1997; Zasada and Ferris 2004). The efficacy of biofumigation to control plant-parasitic nematodes varies depending upon the predominant glucosinolate type the brassicaceous source contains. Of the Brassicaceae species evaluated, *Brassica juncea* varieties with high concentrations of 2-propenyl glucosinolate (sinigrin) were most effective in controlling *G. pallida* (Lord et al. 2011). *Brassica juncea* seed meal extract (SME) is a shelf-stable powdered product containing increased concentrations of sinigrin formulated from *B. juncea* seed meal (Popova et al. 2017). Because of the increased concentration of sinigrin found in SME, a relatively lower rate of *B. juncea* SME has been found to be effective against *G. pallida* and *G. ellingtonae* (Dandurand et al. 2017).

Trap crops, which promote hatch of nematodes but do not support subsequent nematode development and reproduction, offer another alternative to control the plant-parasitic nematodes. Although potato itself could be used as a trap crop to control *G. pallida*, it is not desirable because the timing of destruction before developing new females is difficult to determine (Scholte 2000a). Non-tuber-bearing solanaceous plants have been investigated as potential trap crops to control *G. pallida. Solanum sisymbriifolium* is one of the trap crops of interest that induces egg hatching of potato cyst nematodes without allowing reproduction (Scholte 2000b; Timmermans et al. 2007; Dias et al. 2017; Dandurand and Knudsen, 2016; Kooliyottil et al. 2019). *Solanum sisymbriifolium* induced up to 75% hatching of *G. pallida* juveniles after 12 weeks after planting (Timmermans et al., 2006) and reduced soil infestation of *G. pallida* by up to 77% (Scholte and Vos 2000). The *Globodera pallida* reproduction factor was reduced by 99% (Dandurand and Knudsen 2016), and J2s in progeny cysts

were reduced by 23 to 50% (Dandurand et al. 2019) in potato-following-*S. sisymbriifolium* compared to potato-following-fallow treatment. Rapid localized necrosis of cells in response to *G. pallida* infection resulting in the nematode death has been observed (Kooliyottil et al., 2019).

An integrated approach to control plant diseases that provide consistent suppression of pests by utilizing multiple alternatives could be vital as regulations on the use of agricultural chemicals may become more restrictive in the future (Noling 2002; Rosskopf et al. 2005). As discussed above, individually, biofumigation with *B. juncea* SME and the trap crop *S. sisymbriifolium* effectively control *G. pallida*. However, the efficacy of SME at rates less than 1.1 t/ha has not been assessed. Also, a viable population of *G. pallida* remaining after *S. sisymbriifolium* may serve as potential inoculum in a subsequent growing season. In this study, greenhouse experiments were conducted with the objectives to (i) optimize the amount of *B. juncea* SME needed to control *G. pallida* and (ii) test the efficacy of pyramiding *B. juncea* SME and the trap crop *S. sisymbriifolium* to control *G. pallida* compared to individual treatment with each.

Methods and Materials

Rearing of Globodera pallida

Due to quarantine status in Idaho, *Globodera pallida* is reared under greenhouse conditions. *Globodera pallida* cysts were initially obtained from infested fields in Shelly, ID, and reared in a USDA-APHIS approved facility at the University of Idaho, Moscow, ID on the susceptible potato cultivar 'Désirée' under greenhouse conditions of day temperature of 18°C and night temperature of 10°C at a 16:8-h light: dark period (Dandurand and Knudsen 2016; Dandurand et al. 2017; Dandurand et al. 2019). After 16 weeks of growth, the cysts were extracted using a USDA-type elutriator (USDA-APHIS 2009) and picked by hand under a stereomicroscope (Leica Microsystems, Wetzlar, Germany). The identity of *G. pallida* was confirmed by morphological and molecular methods (Skantar et al. 2007). All cysts were incubated at 4°C for a minimum of 16 weeks before experimental use.

Mustard Seed Meal Extract

Mustard seed meal extract, extracted from seed meal of *B. juncea* variety 'Pacific Gold', was produced and obtained as a shelf-stable powder from a facility at the University of Idaho, Moscow, ID (Popova et al. 2017). The formulated *B. juncea* SME with a sinigrin concentration of 264 μmol/g was used.

Plant Material

Solanum sisymbriifolium seeds obtained from Chuck Brown, USDA-ARS, Prosser, WA were germinated, and 4-week-old seedlings were used. For the bioassay, potato cv. 'Désirée' grown from tissue culture in standard media (Murashige and Skoog 1962) was used, and 4-week-old tissue culture plantlets of potato were transplanted.

Greenhouse Experiment Set-up

Experiments were conducted under greenhouse conditions (18°C and 16:8-h light: dark period) at the University of Idaho, Moscow, ID. *Globodera pallida* cysts were placed into 2.54 cm^2 wear-resistant nylon mesh (248.92 µm) bags (McMaster-Carr, Elmhurst, IL). The cysts contained in the nylon mesh bags were hydrated in sterile distilled water for 3 days before use. Air-dried Prosser fine sandy loam soil was sieved through a mesh (5 mm opening), mixed with sand (Lane Mountain Company, Valley, WA), and a 2:1 soil: sand mixture (56% sand, 35% silt, 8% clay, pH 7.0) was prepared. The soil mix was autoclaved twice (at the interval of 24-h) at 121°C for 90 minutes before use. Experiments were conducted in Terra cotta clay pots with a 15-cm diameter (The Home Depot, Atlanta, GA). Each pot contained a 1.5 kg soil mix and an initial nematode density of 5 eggs/g soil. Six replicates of each treatment were arranged in a split-plot design on a greenhouse bench with trap crop (*S. sisymbriifolium* vs. bare soil) allocated to the main plots, and different rates of *B. juncea* SME were randomly distributed within each main plot. In separate greenhouse experiments, the efficacy of the combination of *B. juncea* SME with *S. sisymbriifolium* was tested as a pre-plant or post-plant application of the SME.

Biofumigant Applied Pre-plant Experiment

For the pre-plant greenhouse experiment, *G. pallida* cysts were exposed to five rates of *B. juncea* SME (0, 0.14, 0.28, 0.56, and 1.12 t/ha) before *S. sisymbriifolium* or bare soil treatment. The experiment was repeated with six rates of *B. juncea* SME (0, 0.14, 0.28, 0.56, 1.12, and 2.24 t/ha). There were six replicates for each treatment. The pots were filled with 8-cm of soil mix, two nylon mesh bags containing 10 *G. pallida* cysts each were placed on the top and covered with an additional 3-cm of soil. *Brassica juncea* SME at appropriate rates was applied in a band over the entire surface of pots containing cysts and 3-cm soil added on the top of the extract. The non-amended control did not receive SME but remained fallow for 2 weeks until *S. sisymbriifolium* was planted or for the duration of the experiment. To facilitate the hydrolysis of sinigrin, 240-ml water was added to each pot, and the top of the pot was sealed with aluminum foil to simulate a closed system. The aluminum foil was removed 2 weeks post-amendment with the SME, and each rate of the SME was followed by *S. sisymbriifolium* or bare soil (unplanted). A 4-week-old *S. sisymbriifolium* transplant was planted in

the treatments including trap crop. Pots were watered once daily with 75 ml of water and fertilized once a week using Jack's classic all-purpose fertilizer 20-20-20 (JR Peters Inc., Allentown, PA, USA) at a rate of 0.5 g/liter water. After 12 weeks of growth, aboveground parts of *S. sisymbriifolium* were removed, cysts were retrieved, and one cyst bag from each pot was removed for determining the effect of treatments on the number of encysted eggs, egg viability, and egg hatch. Pots with the remaining (one) cyst bag were placed at 4°C for at least 8 -weeks chilling period (Perry and Gaur 1996; Perry and Moens 2011; Palomares-Rius et al. 2013) before planting susceptible potato in the greenhouse.

Biofumigant Applied Post-plant Experiment

To test the impact of post-plant application of SME, *G. pallida* cysts were exposed to six rates of *B. juncea* SME (0, 0.14, 0.28, 0.56, 1.12, and 2.24 t/ha) after a 12 week growing cycle with or without (bare soil) *S. sisymbriifolium*. Pots were filled with 8-cm of soil followed by placing two nylon mesh bags containing 10 *G. pallida* cysts each. An additional 6-cm of soil was added to cover the cyst bags. For the trap crop treatments, one 4-week-old *S. sisymbriifolium* was transplanted per pot, whereas the bare soil treatment remained unplanted. Thirty-six pots were used for each *S. sisymbriifolium* and bare soil treatment. Watering and fertilizer application were the same as described above for the pre-plant experiment. Aboveground parts of *S. sisymbriifolium* were removed after 12 weeks of growth. The pots were allowed to dry under greenhouse conditions for 1 week before SME application. The top 3-cm of soil was removed from pots, SME was applied at appropriate rates later over the entire surface, and the soil was replaced to each pot. In the non-amended control, only soil was added. All pots were watered with 240 ml of water per pot, and the top was sealed with aluminum foil. After 2 weeks, the aluminum foil was removed, cysts were retrieved, and one cyst bag from each pot was removed for determining the number of remaining encysted eggs, egg viability, and egg hatching. Pots with the remaining (one) cyst bag were placed at 4°C for at least 8 weeks chilling period before planting susceptible potato in the greenhouse.

Effect of B. juncea Seed Meal Extract and S. sisymbriifolium on G. pallida Egg Densities

To determine the effect of treatments on the number of *G. pallida* encysted eggs, which will give an indication of attrition or hatching rates, eggs from 3 cysts were released in 300 µl sterile distilled water. All released eggs were enumerated in a 100 µl aliquot using a stereomicroscope (Leica Microsystems). The average number of eggs per cyst was reported as the total number of eggs/total number of cysts.

Effect of B. juncea Seed Meal Extract and S. sisymbriifolium on G. pallida Egg Viability and Egg Hatch

The effect of the treatments on *G. pallida* egg viability was determined by staining *G. pallida* eggs with acridine orange, which stains non-viable eggs only (Sivasankara and Dandurand 2019). Briefly, the eggs were released from cysts and stained for 4 hours with acridine orange (10 µg/ml) (Thermo Fisher Scientific, Eugene, OR, USA). The number of stained and non-stained eggs was counted using a Lecia DMi8 fluorescent microscope (Lecia microsystems CMS GmbH, Wetzlar, Germany) equipped with a metal halide light source (Lumen 200 Fluorescence Illumination Systems, Prior Scientific Inc., Rockland, MA, USA). Percent egg viability was calculated using the formula: egg viability (%) = (Non-stained Eggs/ (Stained eggs + Non-stained eggs)) × 100.

The effect of treatments on *G. pallida* egg hatch was determined by exposing *G. pallida* eggs to potato root diffusate (PRD) for 2 weeks. Potato root diffusate (PRD) was collected from the potato cultivar 'Désirée' after 4 weeks of growth under greenhouse conditions. Diffusate was collected by pouring 200 ml of distilled water through the pots with potato plants, and the diffusate collected was filter-sterilized using 0.45 and 0.22 µm bottle top filter (Corning Incorporated, Corning, NY). *Globodera pallida* cysts were crushed in sterile distilled water, aliquots containing approximately 100 eggs were dispensed into 96-well plates, and sterile DI water was added to each well to achieve a total 100 µl solution in each well. The number of eggs and J2s in each well were counted, and 100 µl PRD (collected and filter sterilized as described above) was added to each well. After 2 weeks, the number of hatched J2 was counted, and the percent hatch was calculated by using the formula: egg hatch (%) = ((number of 2-week J2 − number of initial J2)/ Number of initial eggs) × 100.

Effect of B. juncea Seed Meal Extract and S. sisymbriifolium on G. pallida Reproduction in Greenhouse Bioassays

A potato bioassay was conducted under greenhouse conditions to determine the treatment effects on *G. pallida* reproduction. For the bioassay, 4- week-old (approximately 8-cm in height) tissue culture plantlets of potato cv. 'Désirée' were planted in pots (1 plantlet per pot). Watering and fertilizer application were the same as described previously for pre-plant greenhouse experiment. Aboveground parts of potato plants were terminated after 12 weeks of growth. *Globodera pallida* cysts were extracted from soil (entire pot) using an elutriator (USDA-APHIS 2009). The number of progeny cysts per pot was determined, and for the treatments that produced new cysts, eggs were enumerated as described previously. The reproduction factor was determined as the final egg population (*Pf*)/initial egg population (*Pi*).

Data Analysis

Data were analyzed by analysis of variance (ANOVA) using the general linear mixed model (PROC GLM) statement in Statistical Analysis Software (SAS) (SAS Institute Inc., Cary, NC). To ensure a normal distribution and constant variation, log transformation was used for the number of encysted eggs and reproduction data. Arcsine transformations were used for viability and hatching data. Pairwise comparisons were performed using Student's t-test to assess the treatment differences and means were considered significantly different at at $P \leq 0.05$.

Results

Biofumigant Applied Pre-plant Experiment

In pre-plant greenhouse trials, the number of encysted eggs was significantly reduced compared to the untreated control after exposing *G. pallida* to only *S. sisymbriifolium* ($P \leq 0.05$) (Table 2.1, 2.2, and 2.3). However, no significant egg reduction was observed after treatment with *B. juncea* SME at all rates tested whether followed by *S. sisymbriifolium* or the bare soil only ($P > 0.05$) which indicates that SME did not induce further attrition than a bare soil. *Brassica juncea* SME at all rates tested and *S. sisymbriifolium* whether applied individually or in combination reduced *G. pallida* egg viability, egg hatch, and reproduction factor (*Pf/Pi*) compared to the untreated control ($P \leq 0.05$).

In greenhouse trial 1 (Table 2.1), the number of encysted eggs was significantly reduced by *S. sisymbriifolium* only compared to the untreated control by 53% ($P < 0.0001$). Treatment with only *Solanum sisymbriifolium* significantly reduced egg viability, egg hatch and reproduction factor (*Pf/Pi*) compared to the untreated control by 38, 32, and 90%, respectively ($P < 0.0001$). Treatment with only SME at 0.14, 0.28, 0.56, and 1.12 t/ha significantly reduced *G. pallida* egg viability compared to the untreated control by 43, 55, 61, and 61%, respectively ($P < 0.0001$). Similarly, egg hatch was significantly reduced compared to the untreated control by 34, 54, 60, and 67 %, respectively ($P < 0.0001$). No significant difference in egg viability was observed whether the SME was applied alone or in combination with *S. sisymbriifolium* ($P > 0.05$). However, a combination of SME at 0.14 t/ha with *S. sisymbriifolium* further significantly reduced hatch rate by 31 or 33% compared to the individual treatments with SME at 0.14 t/ha ($P < 0.0001$) or *S. sisymbriifolium* ($P < 0.0001$), respectively. The reproduction factor (*Pf/Pi*) was significantly reduced compared to untreated control by 97% and 98% after treatment with SME alone at 0.14 t/ha ($P < 0.0001$) and 0.28 t/ha ($P < 0.0001$), respectively. Also, a combination of SME at rates 0.14 and 0.28 t/ha with *S. sisymbriifolium* further

significantly reduced the reproduction factor (*Pf/Pi*) compared to the treatment with *S. sisymbriifolium* only ($P < 0.0001$). SME with or without *S. sisymbriifolium* eliminated the reproduction of *G. pallida* on potato when applied at rates ≥ 0.56 t/ha ($P < 0.0001$).

Table 2.1. Effect of *Brassica juncea* seed meal extract (SME) and *Solanum sisymbriifolium* (litchi tomato) on *Globodera pallida* in pre-plant greenhouse trial 1.

Treatment	**Pre-plant greenhouse trial 1**			
	Eggs per cyst	**Egg viability (%)**	**Egg hatch (%)**	***Pf/Pi*[y]**
Untreated control	305 ± 20 a[z]	69.38 ± 3.66 a	28.43 ± 1.14 a	25.04 ± 7.49 a
Litchi tomato (LT) only	143 ± 20 b	43.18 ± 2.89 b	19.29 ± 0.79 b	2.55 ± 0.86 b
0.14 t/ha SME + Bare soil	242 ± 29 a	39.67 ± 6.70 bc	18.77 ± 0.96 b	0.68 ± 0.53 c
0.14 t/ha SME + LT	257 ± 44 a	30.21 ± 2.04 cd	12.99 ± 0.95 c	0.08 ± 0.08 c
0.28 t/ha SME + Bare soil	246 ± 12 a	31.40 ± 5.22 cd	13.20 ± 0.72 c	0.54 ± 0.51 c
0.28 t/ha SME + LT	230 ± 27 a	26.53 ± 2.28 d	11.55 ± 1.14 cd	0.08 ± 0.08 c
0.56 t/ha SME + Bare soil	277 ± 35 a	27.02 ± 2.76 d	11.40 ± 0.54 cde	0 ± 0 c
0.56 t/ha SME + LT	287 ± 18 a	23.41 ± 2.16 d	9.67 ± 0.54 def	0 ± 0 c
1.12 t/ha SME + Bare soil	243 ± 37 a	27.19 ± 2.97 d	9.39 ± 0.72 ef	0 ± 0 c
1.12 t/ha SME + LT	253 ± 14 a	24.43 ± 3.96 d	8.69 ± 0.46 f	0 ± 0 c

[z]Values ± standard errors are the average of six replicates. Values within the same column followed by a common letter are not significantly different ($P \leq 0.05$).

[y] Initial population during potato bioassay (*Pi*)= 2.5 eggs/gram soil.

In greenhouse trial 2 (Table 2.2), the effect of treatments on *G. pallida* reduction followed a similar trend to trial 1 with a significant reduction in the number of encysted eggs compared to the untreated control by 36% after treatment *S. sisymbriifolium* alone ($P = 0.0019$). Treatment with *S. sisymbriifolium* only significantly reduced the egg viability, egg hatch, and reproduction factor (*Pf/Pi*) compared to the untreated control by 40%, 33%, and 99%, respectively ($P < 0.0001$). Compared to the untreated control, treatment with SME only at 0.14, 0.28, 0.56, 1.12, and 2.24 t/ha significantly reduced *G. pallida* egg viability by 49, 63, 67, 75, and 82%, respectively ($P < 0.0001$). Similarly, egg hatch compared to the untreated control was also significantly reduced by 41, 61, 71, 73, and 79%, respectively ($P < 0.0001$). Combined treatment with SME at 0.14 t/ha and *S. sisymbriifolium* further significantly reduced *G. pallida* egg viability and egg hatch compared to the individual treatments with 0.14 t/ha of the SME ($P = 0.0223$ for viability, $P = 0.0081$ for hatch) and *S. sisymbriifolium* ($P = 0.0003$ for viability, $P < 0.0001$ for hatch). SME at 0.14 t/ha significantly reduced the reproduction factor (*Pf/Pi*) compared to untreated control by 95% when applied alone ($P < 0.0001$) and eliminated reproduction on potato when in combination with *S. sisymbriifolium* ($P < 0.0001$). At rates ≥ 0.28

t/ha, SME with or without *S. sisymbriifolium* eliminated the reproduction of *G. pallida* on potato ($P < 0.0001$).

Table 2.2. Effect of *Brassica juncea* seed meal extract (SME) and *Solanum sisymbriifolium* (litchi tomato) on *Globodera pallida* in pre-plant greenhouse trial 2.

Treatment	**Pre-plant greenhouse trial 2**			
	Eggs per cyst	**Egg viability (%)**	**Egg hatch (%)**	***Pf/Pi*[y]**
Untreated control	376 ± 26 a[z]	61.71 ± 0.98 a[a]	32.08 ± 2.76 a	6.45 ± 1.88 a
Litchi tomato (LT) only	240 ± 26 b	36.91 ± 2.23 b	21.57 ± 1.44 b	0.04 ± 0.03 bc
0.14 t/ha SME + Bare soil	358 ± 46 a	31.80 ± 1.94 b	19.04 ± 1.34 b	0.34 ± 0.22 b
0.14 t/ha SME + LT	349 ± 24 a	24.61 ± 2.88 c	14.62 ± 0.96 c	0 ± 0 d c
0.28 t/ha SME + Bare soil	361 ± 29 a	23.03 ± 4.04 c	12.56 ± 0.78 c	0 ± 0 c
0.28 t/ha SME + LT	363 ± 32 a	24.14 ± 1.55 c	12.05 ± 0.69 cd	0 ± 0 c
0.56 t/ha SME + Bare soil	360 ± 24 a	20.25 ± 2.14 cd	9.19 ± 0.92 e	0 ± 0 c
0.56 t/ha SME + LT	326 ± 32 a	23.65 ± 1.58 c	9.58 ± 0.56 de	0 ± 0 c
1.12 t/ha SME + Bare soil	345 ± 36 a	15.46 ± 1.90 de	8.61 ± 0.60 ef	0 ± 0 c
1.12 t/ha SME + LT	333 ± 43 a	12.29 ± 1.37 e	8.17 ± 0.69 ef	0 ± 0 c
2.24 t/ha SME + Bare soil	316 ± 30 ab	10.91 ± 0.73 e	6.70 ± 0.70 f	0 ± 0 c
2.24 t/ha SME + LT	310 ± 21 ab	12.81 ± 1.26 e	7.33 ± 0.48 ef	0 ± 0 c

[z]Values ± standard errors are the average of six replicates. Values within the same column followed by a common letter are not significantly different ($P \leq 0.05$).

[y] Initial population during potato bioassay (*Pi*)= 2.5 eggs/gram soil.

Biofumigant Applied Post-plant Experiment

In both post-plant trials, *S. sisymbriifolium* with or without SME significantly reduced the number of *G. pallida* encysted eggs compared to the untreated control ($P < 0.0001$) (Table 2.3 and 2.4). However, no significant egg reduction was observed after treatment with only SME at all rates tested ($P > 0.05$). As observed in the pre-plant experiment, SME at all rates tested and *S. sisymbriifolium* reduced *G. pallida* egg viability, egg hatch, and reproduction factor (*Pf/Pi*) compared to the untreated control in both post-plant trials ($P \leq 0.05$).

In greenhouse trial 1 (Table 2.3), treatment with only *S. sisymbriifolium* significantly reduced the number of encysted eggs compared to the untreated control by 60% ($P < 0.0001$). A similar reduction of the encysted eggs was observed after a combined *S. sisymbriifolium* and SME treatment at all rates tested. Compared to the untreated control, reduction of the encysted egg numbers by combined treatments ranged from 52% for *S. sisymbriifolium* combined with 0.28 t/ha of SME ($P < 0.0001$) to 62% for *S. sisymbriifolium* combined with 2.24 t/ha of SME ($P < 0.0001$). Also, *S. sisymbriifolium* alone significantly reduced egg viability, egg hatch and reproduction factor (*Pf/Pi*)

compared to the untreated control by 43, 52, and 95%, respectively ($P < 0.0001$). Treatment with only SME at 0.14, 0.28, 0.56, 1.12 and 2.24 t/ha significantly reduced *G. pallida* egg viability compared to the untreated control by 18, 24, 46, 54, and 61%, respectively ($P = 0.0013$ for SME at 0.14 t/ha, $P < 0.0001$ for SME at ≥0.28 t/ha). Similarly, egg hatch significantly decreased compared to the untreated control by 43, 55, 62, 83, and 92%, respectively ($P < 0.0001$). A combination of SME at 0.14 and 0.28 t/ha with *S. sisymbriifolium* further significantly reduced egg viability compared to the individual treatments with SME at respective rates ($P < 0.0001$ for SME at 0.14 t/ha, $P = 0.0002$ for SME at 0.28 t/ha). Similarly, egg hatch was also further reduced significantly by a combination of SME at 0.14, 0.28, and 0.56 t/ha with *S. sisymbriifolium* compared to the individual treatments with SME at respective rates ($P < 0.0001$ for all SME rates) or *S. sisymbriifolium* ($P < 0.0001$). The reproduction factor (*Pf/Pi*) was significantly reduced compared to untreated control by 69, 74 and 86% after treatment with SME alone at 0.14 t/ha ($P < 0.0001$), 0.28 ($P < 0.0001$) and 0.56 t/ha ($P < 0.0001$), respectively. Also, a combination of SME at 0.14 and *S. sisymbriifolium* further significantly reduced the reproduction factor (*Pf/Pi*) compared to the treatment with only SME at 0.14 t/ha ($P < 0.0001$). SME at 0.28 and 0.56 t/ha in combination with *S. sisymbriifolium* also further significantly reduced the reproduction factor (*Pf/Pi*) compared to treatment with only SME at respective rates ($P < 0.0001$ for both SME rates) and only *S. sisymbriifolium* ($P < 0.0001$). SME with or without *S. sisymbriifolium* eliminated the reproduction of *G. pallida* on potato when applied at rates ≥ 1.12 t/ha ($P < 0.0001$).

Table 2.3. Effect of *Brassica juncea* seed meal extract (SME) and *Solanum sisymbriifolium* (litchi tomato, LT) on *Globodera pallida* in post-plant greenhouse trial 1.

Treatment	**Post-plant greenhouse trial 1**			
	Eggs per cyst	**Egg viability (%)**	**Egg hatch (%)**	***Pf/Pi*[y]**
Untreated control	287 ± 17 a[z]	67.40 ± 2.71 a	27.40 ± 2.15 a	16.92 ± 1.95 a
Litchi tomato (LT) only	116 ± 8 b	38.46 ± 3.68 c	13.21 ± 1.38 bc	0.81 ± 0.10 d
0.14 t/ha SME + Bare soil	242 ± 22 a	55.19 ± 3.73 b	15.62 ± 0.91 b	5.25 ± 0.60 b
0.14 t/ha SME + LT	124 ± 8 b	34.34 ± 2.52 cd	7.68 ± 1.30 d	0.58 ± 0.08 d
0.28 t/ha SME + Bare soil	278 ± 28 a	50.96 ± 3.66 b	12.32 ± 0.73 bc	4.47 ± 0.34 b
0.28 t/ha SME + LT	139 ± 12 b	36.38 ± 1.96 cd	4.41 ± 1.00 e	0.17 ± 0.08 e
0.56 t/ha SME + Bare soil	270 ± 39 a	36.24 ± 2.41 cd	10.51 ± 1.27 cd	2.46 ± 0.73 c
0.56 t/ha SME + LT	122 ± 27 b	33.69 ± 2.21 cd	4.05 ± 0.29 e	0.11 ± 0.07 e
1.12 t/ha SME + Bare soil	258 ± 28 a	31.27 ± 1.22 cde	4.59 ± 1.04 e	0 ± 0 e
1.12 t/ha SME + LT	116 ± 14 b	30.18 ± 1.66 de	4.58 ± 1.11 e	0 ± 0 e
2.24 t/ha SME + Bare soil	227 ± 21 a	25.96 ± 1.04 e	2.08 ± 0.59 f	0 ± 0 e
2.24 t/ha SME + LT	108 ± 6 b	25.28 ± 1.71 e	2.81 ± 0.52 ef	0 ± 0 e

[z]Values ± standard errors are the average of six replicates. Values within the same column followed by a common letter are not significantly different ($P \leq 0.05$).

[y] Initial population during potato bioassay (*Pi*)= 2.5 eggs/gram soil.

Table 2.4. Effect of *Brassica juncea* seed meal extract (SME) and *Solanum sisymbriifolium* (litchi tomato) on *Globodera pallida* in post-plant greenhouse trial 2.

Treatment	**Post-plant greenhouse trial 2**			
	Eggs per cyst	**Egg viability (%)**	**Egg hatch (%)**	***Pf/Pi*[y]**
Untreated control	287 ± 19 a[z]	66.37 ± 2.41 a	24.66 ± 1.70 a	13.05 ± 1.35 a
Litchi tomato (LT) only	140 ± 9 b	39.59 ± 1.24 de	7.17 ± 1.97 bc	0.60 ± 0.19 f
0.14 t/ha SME + Bare soil	279 ± 12 a	54.62 ± 1.95 b	8.81 ± 1.01 b	8.04 ± 0.76 b
0.14 t/ha SME + LT	159 ± 4 b	42.66 ± 1.41 d	1.75 ± 0.51 de	0.43 ± 0.16 fg
0.28 t/ha SME + Bare soil	255 ± 22 a	47.68 ± 1.97 c	8.01 ± 1.48 b	6.81 ± 0.91 bc
0.28 t/ha SME + LT	139 ± 13 b	39.87 ± 1.61 de	2.37 ± 1.00 de	0.14 ± 0.07 gh
0.56 t/ha SME + Bare soil	253 ± 6 a	39.76 ± 0.71 de	3.55 ± 0.96 d	5.50 ± 0.66 cd
0.56 t/ha SME + LT	145 ± 10 b	37.91 ± 2.39 e	2.95 ± 0.83 de	0.21 ± 0.06 fgh
1.12 t/ha SME + Bare soil	254 ± 11 a	39.79 ± 1.43 de	3.61 ± 0.91 cd	4.76 ± 0.52 d
1.12 t/ha SME + LT	146 ± 17 b	36.00 ± 1.20 e	1.65 ± 0.44 de	0 ± 0 h
2.24 t/ha SME + Bare soil	283 ± 24 a	38.48 ± 0.67 de	2.62 ± 1.13 de	1.50 ± 0.36 e
2.24 t/ha SME + LT	134 ± 9 b	36.55 ± 1.47 e	1.12 ± 0.52 e	0 ± 0 h

[z]Values ± standard errors are the average of six replicates. Values within the same column followed by a common letter are not significantly different ($P \leq 0.05$).

[y] Initial population during potato bioassay (*Pi*)= 2.5 eggs/gram soil.

In greenhouse trial 2 (Table 2.4), *S. sisymbriifolium* only significantly reduced the number of encysted eggs compared to the untreated control by 51% ($P < 0.0001$), and a similar reduction was observed for the combined treatment of *S. sisymbriifolium* and SME at all rates tested. Compared to the untreated control, reduction of the encysted egg numbers by combined treatments ranged from 45% for *S. sisymbriifolium* combined with 0.14 t/ha of SME ($P < 0.0001$) to 53% for *S. sisymbriifolium* combined with 2.24 t/ha of SME ($P < 0.0001$). *Solanum sisymbriifolium* also significantly reduced egg viability, egg hatch and reproduction factor (*Pf/Pi*) compared to the untreated control by 40, 71, and 95%, respectively ($P < 0.0001$). SME only at 0.14, 0.28, 0.56, 1.12 and 2.24 t/ha significantly reduced *G. pallida* egg viability compared to the untreated control by 18, 28, 40, 40 and 42%, respectively ($P < 0.0001$). Similarly, egg hatch significantly decreased compared to the untreated control by 64, 68, 86, 85, and 89%, respectively ($P < 0.0001$). As observed in trial 1, a combination of SME at 0.14 and 0.28 t/ha with *S. sisymbriifolium* further significantly reduced egg viability compared to the individual treatments with SME at respective rates ($P < 0.0001$ for SME at 0.14 t/ha, $P = 0.0011$ for SME at 0.28 t/ha). The combined treatment with SME at 0.14 and 0.28 t/ha and *S. sisymbriifolium* also further significantly reduced egg hatch compared to the individual treatment with *S. sisymbriifolium* ($P < 0.0001$) or respective rates of SME ($P < 0.0001$ for SME at 0.14 t/ha, $P = 0.0046$ for SME at 0.28 t/ha). The reproduction factor (*Pf/Pi*) was significantly reduced

compared to untreated control by 38, 48, 58, 64 and 89% after treatment with SME alone at 0.14 t/ha, 0.28, 0.56, 1.12 and 2.24 t/ha ($P = 0.0028$ for SME at 0.14 t/ha, $P < 0.0001$ for SME at ≥0.28 t/ha), respectively. A combination of SME at 0.14 and 0.56 t/ha with *S. sisymbriifolium* further significantly reduced the reproduction factor (*Pf/Pi*) compared to the treatment with only SME at 0.14 and 0.28 t/ha ($P < 0.0001$). SME at 0.28 t/ha in combination with *S. sisymbriifolium* also further significantly reduced the reproduction factor (*Pf/Pi*) compared to treatment with only SME at 0.28 t/ha ($P < 0.0001$) and only *S. sisymbriifolium* ($P = 0.03$). SME at 1.12 and 2.24 t/ha combined with *S. sisymbriifolium* eliminated the reproduction of *G. pallida* on potato ($P < 0.0001$).

Discussion

This is the first report on the efficacy of *B. juncea* SME combined with the trap crop *S. sisymbriifolium* to control *G. pallida*. Our results show that treatment with *B. juncea* SME reduced *G. pallida* egg viability, hatch, and reproduction on potato at rates ranging from 0.14 to 2.24 t/ha tested across greenhouse trials. The highest rates of *B. juncea* SME tested provided *G. pallida* reduction similar to the level reported previously for *G. pallida* (Dandurand et al. 2017). Two weeks of exposure to *B. juncea* SME at the rate of 1.1 t/ha reduced *G. pallida* egg hatch by 97 to 99.7% under greenhouse conditions (Dandurand et al. 2017). Reproduction of *G. pallida* was also significantly reduced by 99% with the application of *B. juncea* SME at a rate of 1.1 t/ha (Dandurand et al. 2017). When applied in a less concentrated form, *B. juncea* seed meal at 0.1% of soil weight (corresponding to approximately 2 t/ha) resulted in a 97% reduction of *Meloidogyne incognita* and *Pratylenchus penetrans* (Zasada et al. 2009). Similar to *B. juncea* seed meal, SME produces 2-propenyl isothiocyanate upon enzymatic hydrolysis of 2-propenyl glucosinolate, the predominant glucosinolate found in *B. juncea* seed (Borek et al. 1994; Popova and Morra 2014). The nematicidal property of *B. juncea* seed meal and SME is primarily due to the activity of isothiocyanate (Brown and Morra 1997). The pre-plant experiment results indicate that treatment with *B. juncea* SME alone at a rate of 0.56 t/ha eliminates *G. pallida* reproduction.

That fewer encysted eggs remained after treatment with only *S. sisymbriifolium* and *S. sisymbriifolium* planted before SME treatment indicates that *G. pallida* hatched in the presence of the trap crop *S. sisymbriifolium.* Previous reports have shown a 23 to 50% reduction in the number of *G. pallida* encysted eggs after 12 weeks (Dandurand et al. 2019) and up to 75% 21 weeks after planting (Timmermans et al. 2006). The reduction in the number of encysted eggs is attributed to the ability of

S. sisymbriifolium root diffusate to stimulate hatch of *G. pallida* (Scholte 2000b; Scholte 2000c). In our experiments, we observed a high level of suppression in viability and hatch of the remaining *G. pallida* eggs after treatment with *S. sisymbriifolium*. *Globodera pallida* reproduction on potato following *S. sisymbriifolium* was reduced by 90 to 99 %. *Solanum sisymbriifolium* may contain glycoalkaloids with nematicidal properties (Dias et al. 2012; Sivasankara and Dandurand, 2021) which may reduce the viability and hatch of the remaining encysted eggs. Reproduction (*Pf/Pi*) of *G. pallida* on a susceptible potato following rotation with *S. sisymbriifolium* was reduced by 99% compared to potato following a bare soil rotation (Dandurand and Knudsen, 2016).

Application of either *B. juncea* SME or *S. sisymbriifolium* individually reduced *G. pallida* viability, hatch, and reproduction on potato. However, integrating SME with *S. sisymbriifolium* provides an opportunity to suppress *G. pallida* hatch and reproduction further. When combined with *S. sisymbriifolium*, SME at lower rates, 0.56 t/ha or lower, provided *G. pallida* reduction at similar levels to that provided by SME alone at higher rates of 1.12 and 2.24 t/ha. In our pre-plant experiment, combining *S. sisymbriifolium* with the lowest SME rate, 0.14 t/ha, provided a greater reduction of *G. pallida* hatch than individual treatments. In the post-plant experiment, the impact of combining SME with *S. sisymbriifolium* on *G. pallida* control was more evident. A combination of SME at rates ≤0.56 t/ha and *S. sisymbriifolium* provided a higher level of *G. pallida* reduction than individual treatments. This greater reduction may be due to the activity of the subsequent treatments, *S. sisymbriifolium* in pre-plant or SME in post-plant trials, on the nematodes weakened by the nematicidal effect of the initial SME treatment in pre-plant trials or *S. sisymbriifolium* treatment in post-plant trials (Dandurand et al. 2017; Sivasankara and Dandurand, 2021). Whether applied as a pre- or post-plant, SME, when combined with *S. sisymbriifolium*, further reduced *G. pallida* populations suggesting that combining both strategies can be an effective strategy for nematode control.

When combined, biofumigation with cabbage residue and application of the biocontrol agent *Streptomyces rubrogriseus* HDZ-9-47 resulted in greater control of *Meloidogyne incognita* compared to each treatment alone (Jin et al. 2019). An integrated use of a nematophagous fungus *Metarhizium carneum* with a 2 year crop rotation (non-host *Vicia faba* followed by a susceptible potato) or 2 year fallow period provided a higher reduction of *G. rostochiensis* than only *M. carneum* followed by a susceptible potato (López-Lima et al. 2020). Non-host rotation only (*V. faba* only followed by a susceptible potato) did not reduce *G. rostochiensis* density (López-Lima et al. 2020). The *Globodera pallida* population in a non-host/non-trap crop or continuous fallow is estimated to take 15 years to decline below a density of 1 egg/gram soil (Timmermans et al. 2006). However, the length of time

needed to reduce *G. pallida* to those same levels is 11 years when *S. sisymbriifolium* is planted in only one season (Timmermans et al. 2006). Our results show that a higher reduction of *G. pallida* populations can also be achieved in a single growing season when integrating the trap crop *S. sisymbriifolium* with *B. juncea* SME. Incorporating these strategies in a cropping system to control *G. pallida* could shorten the length of a rotation prior to potato.

In summary, our results support the previous finding that biofumigation using *B. juncea* SME or planting the trap crop *S. sisymbriifolium* effectively control *G. pallida*. Also, our experiments indicate a further advantage to combining these two strategies for *G. pallida* control. Integrated use of both strategies may serve as a component of the *G. pallida* eradication plan in Idaho. Our future experiments will test the efficacy of the *B. juncea* SME combined with the trap crop under field conditions.

Chapter 3: Control of *Globodera pallida* Using *Brassica juncea* Seed Meal Extract and the Trap Crop *Solanum sisymbriifolium* Under Field Conditions

Introduction

In Idaho, measures to contain and eradicate *Globodera pallida* have been implemented by USDA-APHIS since its detection. The containment regulations prohibit growing host crops, require sanitization of farm equipment, and restrict the movement of soil and plant materials from the infested fields (USDA-APHIS 2009). Soil fumigation has been a chief focus of the eradication efforts in Idaho. Initially, MeBr and 1,3 dichloropropene (Telone II) were used for soil fumigation. However, methyl bromide (MeBr) was discontinued in 2015 due to safety concerns (USDA-APHIS 2015). Due to the loss of MeBr, an effective soil fumigant, novel strategies to control *G. pallida* that are effective under Idaho field conditions are needed to control existing and potential new infestations.

Brassica juncea seed meal extract formulated in powder form has a longer shelf life and a higher concentration of 2-propenyl glucosinolate than the seed meal, which can be advantageous for a field-scale application (Popova et al. 2017; Dandurand et al. 2017). Separate field studies conducted in southern Idaho have demonstrated the potential of *B. juncea* seed meal extract (Dandurand et al. 2017) and *S. sisymbriifolium* (Dandurand et al. 2019) in reducing *G. pallida* hatch and reproduction. Also, field studies to test the agronomic performance of *Solanum sisymbriifolium* have shown that *S. sisymbriifolium* grows well in southern Idaho (Dandurand et al. 2014). Our greenhouse experiments suggested that a combination of *B. juncea* SME at lower rates (≤ 0.56 t/ha) and *S. sisymbriifolium* may provide a more effective reduction of *G. pallida* populations compared to the individual treatment with only seed meal extract (SME) (Chapter 2). We tested the efficacy the *B. juncea* SME when applied alone versus when followed by *S. sisymbriifolium* under field conditions in this study.

Methods and Materials

Field Experiment Set-up

For the field experiments, microplot trials were conducted in Shelly, ID in 2019 and 2020. To contain *G. pallida*, cysts were placed in sealed nylon mesh bags as described above, and plastic buckets (18.9 liters) were used as microplots as described previously (Dandurand et al. 2017; Dandurand et al. 2019). In 2019, three rates of *B. juncea* SME (0, 1.12 and 2.24 t/ha) were tested alone or in combination with *Solanum sisymbriifolium*. The microplots consisted of 5-gallon buckets, that were placed in a hole in the ground and then back-filled with field soil which was Bannock silt loam. Each bucket contained 15-kg of soil. Six cyst bags containing a total of 102 *G. pallida* cysts were placed in each microplot to establish an initial nematode population of 2.5 eggs/gram soil. *Brassica juncea* SME at appropriate rates was applied in a layer over the entire surface of each microplots, and covered with 3-cm soil. In the non-amended control, only soil was added. Five hundred milliliters of water were added to each microplot and covered with lids for 2 weeks. In 2019, due to regulatory concerns, we were not allowed to plant *S. sisymbriifolium* and potato in the field. The buckets were transported to USDA-APHIS approved greenhouse facility at the University of Idaho, Moscow, ID, 2 weeks post amendment with the *B. juncea* SME in the field. The lids were removed, and 4-week-old *S. sisymbriifolium* transplants were planted (6 plants per microplot) in the treatments including the trap crop. The microplots with only SME treatments remained fallow for the duration of the experiment. The experiment was arranged in a split-plot design with trap crop (*S. sisymbriifolium* vs. fallow) as the main effect and different rates of *B. juncea* SME were randomly distributed within each main plot. Microplots were watered daily with 200 ml water and fertilized once a week using Jack's classic all-purpose fertilizer 20-20-20 (JR Peters Inc., Allentown, PA, USA) at a rate of 0.5 g/liter water. After 12 week growth under greenhouse conditions (18°C and 16:8-h light: dark period), all aboveground parts of *S. sisymbriifolium* were removed, cysts were retrieved, and one cyst bag (containing 17 cysts) from each microplot was removed to determine the number of remaining encysted eggs, viability, and hatching. Microplots with the remaining cyst bags were placed at 4°C for 8 weeks to break the dormancy of the remaining population of encysted eggs. (Perry and Gaur 1996; Perry and Moens 2011; Palomares-Rius et al. 2013; Dandurand et al. 2017). After eight weeks, six-week-old tissue culture potato plantlets cv "Desiree" was trans-planted to the buckets and were grown for 12 weeks to assess reproduction of *G. pallida* under the different treatment.

In 2020, five rates of *B. juncea* SME (0, 0.14, 0.28, 0.56, and 1.12 t/ha) were tested alone or in combination with *S. sisymbriifolium*. Six replicates of each treatment were arranged in a split-plot design with trap crop (*S. sisymbriifolium* vs. fallow) as the main effect. *B. juncea* SME at appropriate rates were randomly distributed within each main plot. The microplots were filled with Bannock silt loam field soil, seven cyst bags containing a total of 116 *G. pallida* cysts were placed in each microplot to establish an initial nematode population of 3 eggs/gram soil and covered with an additional 3-cm soil layer. Treatments (SME and/or *S. sisymbriifolium*) were applied to the microplots as described above for field trial 2019. After 12 weeks of growth under field conditions, all aboveground parts of *S. sisymbriifolium* were removed, cysts were retrieved, and one cyst bag (containing 20 cysts) from each microplot was removed to determine the number of remaining encysted eggs, egg viability, and egg hatching. Microplots with the remaining cyst bags were brought back to the USDA-APHIS approved facility at the University of Idaho, Moscow, ID, and placed at 4°C before planting susceptible potato in the greenhouse.

Effect of B. juncea Seed Meal Extract and S. sisymbriifolium on G. pallida Egg Densities, Egg Viability, Egg Hatch and Reproduction

To determine the effect of treatments on the number of *G. pallida* encysted eggs, eggs from 5 cysts were released in 500 µl sterile distilled water and all released eggs were enumerated in a 100 µl aliquot using a stereomicroscope (Leica Microsystems). The average number of eggs per cyst was reported as the total number of eggs/total number of cysts. The effect of treatments on *G. pallida* egg viability and egg hatch was determined as described for greenhouse trials (Chapter 2). For egg viability, *G. pallida* eggs were stained with acridine orange (10 µg/ml) for 4 hours and the number of stained and non-stained eggs was counted (Sivasankara and Dandurand 2019). Percent egg viability was calculated using the formula: egg viability (%) = (Non-stained Eggs/ (Stained eggs + Non-stained eggs)) × 100. To determine the impact on *G. pallida* egg hatch, *G. pallida* eggs were exposed to potato root diffusate (PRD) collected from the 4-weeks-old potato cultivar 'Désirée'. After 2 weeks of exposure to PRD, the number of hatched J2 was counted, and the percent hatch was calculated by using the formula: egg hatch (%) = ((number of 2-week J2 − number of initial J2)/ Number of initial eggs) × 100.

A potato bioassay was conducted under greenhouse conditions to determine the effect of treatments on *G. pallida* reproduction. For the bioassay, 4- week-old (approximately 8-cm in height) tissue culture plantlets of potato cv. 'Désirée' were planted in microplots (6 plantlets per microplot). Watering and fertilizer application were the same as described previously for the greenhouse

experiment. Aboveground parts of potato plants were terminated after 12 weeks of growth. The soil was sampled (1 kg per microplot), and *G. pallida* cysts were extracted using an elutriator (USDA-APHIS 2009). The number of progeny cysts per microplot was determined, and for the treatments that produced new cysts, eggs were enumerated as described previously. The reproduction factor was determined as the final egg population (*Pf*)/initial egg population (*Pi*).

Data were analyzed by analysis of variance (ANOVA) using the general linear mixed model (PROC GLM) statement in Statistical Analysis Software (SAS) (SAS Institute Inc., Cary, NC) as described previously for greenhouse trials (Chapter 2). To ensure a normal distribution and constant variation, log transformation was used for the number of encysted eggs and reproduction data. Arcsine transformations were used for viability and hatching data. Means were separated at $P \leq 0.05$.

Results

Brassica juncea SME at rates ≥0.28 t/ha and ≥ 0.56 t/ha significantly reduced *G. pallida* egg viability and egg hatch, respectively, compared to the untreated control under field conditions ($P < 0.05$). Similarly, *S. sisymbriifolium* significantly reduced *G. pallida* egg viability and egg hatch compared to the untreated control under field conditions ($P < 0.05$) (Table 3.1 and 3.2).

In field trial 2019 (Table 3.1), the effect of *B. juncea* SME and/or *S. sisymbriifolium* on *G. pallida* reduction was similar to that in greenhouse trials. Treatment with only *Solanum sisymbriifolium* significantly reduced the number of encysted eggs by 67% compared to the untreated control ($P < 0.0001$). Similarly, treatment with only *S. sisymbriifolium* significantly reduced egg viability, egg hatch, and reproduction factor (*Pf/Pi*) compared to the untreated control by 58% ($P < 0.0001$), 72% ($P < 0.0001$) and 96% ($P < 0.0001$), respectively. Compared to the untreated control, treatment with only SME at 1.12 and 2.24 t/ha significantly decreased *G. pallida* egg viability by 68 and 77%, respectively ($P < 0.0001$ for both SME rates) and egg hatch by 92 and 97% ($P < 0.0001$ for both SME rates), respectively. As observed for greenhouse trials, no significant difference in *G. pallida* egg viability and egg hatch reduction was observed when SME at 1.12 and 2.24 t/ha was applied alone or combined with *S. sisymbriifolium* ($P > 0.05$). SME at 1.12 t/ha reduced the reproduction factor (*Pf/Pi*) compared to the untreated control by 97% when applied alone ($P < 0.0001$) and eliminated reproduction on potato when in combination with *S. sisymbriifolium* ($P <$

0.0001). SME at 2.24 t/ha with or without *S. sisymbriifolium* eliminated *G. pallida* reproduction on potato.

Table 3.1. Effect of *Brassica juncea* seed meal extract (SME) and *Solanum sisymbriifolium* (litchi tomato) on *Globodera pallida* in field trial 2019.

Treatment	**Field trial 2019**			
	Eggs per cyst	**Egg viability (%)**	**Egg hatch (%)**	***Pf*/*Pi*[y]**
Untreated control	221 ± 5.78 a[z]	63.87 ± 3.19 a	28.41 ± 3.43 a	1.52 ± 0.21 a
Litchi tomato (LT) only	94 ± 10 b	26.68 ± 4.09 b	8.10 ± 1.14 b	0.06 ± 0.04 b
1.12 t/ha SME + Bare soil	199 ± 7 a	20.73 ± 3.12 bc	2.16 ± 0.32 c	0.05 ± 0.05 b
1.12 t/ha SME + LT	206 ± 14 a	17.59 ± 1.15 cd	1.23 ± 0.59 c	0 ± 0 b
2.24 t/ha SME + Bare soil	209 ± 5 a	14.77 ± 1.52 cd	0.92 ± 0.31 c	0 ± 0 b
2.24 t/ha SME + LT	220 ± 7 a	13.21 ± 2.78 d	0.75 ± 0.23 c	0 ± 0 b

[z]Values ± standard errors are the average of six replicates. Values within the same column followed by a common letter are not significantly different ($P \leq 0.05$).

[y] Initial population during potato bioassay (*Pi*)= 2.2 eggs/gram soil.

In the 2020 field trial (Table 3.2), treatment with only *Solanum sisymbriifolium* significantly reduced the number of encysted eggs remaining in cysts at the end of the experiment by 57% compared to the untreated control ($P < 0.0001$). In comparison, when followed by 0.14 t/ha SME, *S. sisymbriifolium* reduced the number of encysted eggs remaining at the end of the growing season by only 25% compared to the untreated control ($P < 0.0001$). When *S. sisymbriifolium* was grown after treatment with 0.28 t/ha or higher rates of SME, a significant reduction in the number of encysted eggs remaining at the end of the growing season was not observed compared to the control. Similarly, treatment with only *S. sisymbriifolium* significantly reduced egg viability and hatch compared to the untreated control by 34% ($P < 0.0001$) and 91% ($P < 0.0001$), respectively. Compared to the untreated control, treatment with only SME at 0.28, 0.56, and 1.12 t/ha significantly decreased *G. pallida* egg viability by 22, 41, and 47%, respectively ($P < 0.0001$ for both SME rates). *Globodera pallida* egg hatch was reduced by 85 and 94% compared to the untreated control after treatment with SME only at rates 0.56 and 1.12 t/ha, respectively ($P < 0.0001$ for both SME rates). SME at 0.14 and 0.28 t/ha combined with *S. sisymbriifolium* significantly reduced *G. pallida* egg viability by 23 and 25%, respectively, compared to the treatment with SME only at respective rates ($P= 0.0003$ and $P= 0.0006$, respectively). Similarly, treatment with SME at rate 0.14 and 0.28 t/ha combined with *S.*

sisymbriifolium significantly reduced egg hatch by 94 and 93%, respectively, compared to only SME at respective rates ($P < 0.0001$ for both SME rates). No significant difference in *G. pallida* egg viability or hatch reduction was observed with the SME application at 0.56 and 1.12 t/ha whether applied alone or combined with *S. sisymbriifolium* ($P > 0.05$).

Table 3.2. Effect of *Brassica juncea* seed meal extract (SME) and *Solanum sisymbriifolium* (litchi tomato) on *Globodera pallida* in field trial 2020.

Treatments	**Field trial 2020**		
	Eggs per cyst	**Egg viability (%)**	**Egg hatch (%)**
Untreated control	209 ± 7 a[z]	67.72 ± 2.35 a	21.16 ± 2.91 a
Litchi tomato (LT) only	89 ± 8 c	44.60 ± 2.64 cd	1.97 ± 0.50 bc
0.14 t/ha SME + Bare soil	198 ± 2 a	61.90 ± 2.28 a	15.49 ± 2.45 a
0.14 t/ha SME + LT	157 ± 5 b	47.44 ± 2.65 bc	0.90 ± 0.37 bc
0.28 t/ha SME + Bare soil	202 ± 9 a	52.72 ± 1.63 b	15.89 ± 4.42 a
0.28 t/ha SME + LT	193 ± 6 a	39.32 ± 2.97 def	1.18 ± 0.40 bc
0.56 t/ha SME + Bare soil	196 ± 7 a	40.07 ± 2.79 de	3.24 ± 1.20 b
0.56 t/ha SME + LT	191 ± 9 a	39.47 ± 2.84 def	0.50 ± 0.15 bc
1.12 t/ha SME + Bare soil	199 ± 7 a	36.23 ± 2.96 ef	1.33 ± 0.59 bc
1.12 t/ha SME + LT	195 ± 6 a	32.50 ± 1.97 f	0.48 ± 0.14 c

[z]Values ± standard errors are the average of six replicates. Values within the same column followed by a common letter are not significantly different ($P \leq 0.05$).

Discussion

This experiment demonstrates the effectiveness of *B. juncea* SME and *S. sisymbriifolium* in reducing *G. pallida* egg viability and egg hatch under field conditions. Previously, we reported that *B. juncea* SME applied at lower rates, 0.14 t/ha in the pre-plant application and ≤ 0.56 t/ha in the post-plant application, when used in combination with *S. sisymbriifolium* provided a significant reduction of *G. pallida* population compared to only SME when experiments were conducted under greenhouse conditions (Chapter 2). The primary purpose of this study was to determine the field performance of *B. juncea* SME alone versus in combination with the trap crop *S. sisymbriifolium*.

At the termination of the experiment, *B. juncea* SME alone reduced egg viability when applied at rates ≥0.28 t/ha and egg hatch when applied at rates ≥0.56. No significant reduction in *G. pallida* egg numbers was observed after treatment with only SME at all rates tested. As discussed in Chapter 2, the reduction of *G. pallida* hatch by *B. juncea* SME is due to the release of 2-propenyl

isothiocyanate by the hydrolysis of 2-propenyl glucosinolate (Brown and Morra 1997; Lord et al. 2011; Dandurand et al. 2017). Previously, *B. juncea* SME reduced egg hatch by 97 to 99.7% after 2 weeks of exposure to the extract at the rates ranging from 1.1 t/ha to 4.5 t/ha under field conditions (Dandurand et al. 2017). Furthermore, our results which show a high reduction of the *G. pallida* reproduction by SME applied at 1.12 and 2.24 t/ha is within the range reported by Dandurand et al (2017).

As expected, treatment with only *S. sisymbriifolium* resulted in significantly fewer encysted eggs than the untreated control. When followed by 0.14 t/ha of SME, *S. sisymbriifolium* significantly reduced the number of encysted eggs compared to the untreated control. However, the egg numbers were approximately 76% higher than those remaining after only *S. sisymbriifolium* treatment indicating that SME at the lowest rate tested significantly reduced the proportion of nematode eggs that could hatch in the presence of *S. sisymbriifolium*. These results agree with the findings from our greenhouse experiments where *S. sisymbriifolium* induced and *B. juncea* SME at rates ranging from 0.14 to 2.24 t/ha inhibited *G. pallida* egg hatch (Chapter 2). Up to 75% hatch of *G. pallida* juveniles was observed after 21 weeks of planting *Solanum sisymbriifolium* (Timmermans et al. 2006). In field microplots, the number of *G. pallida* encysted eggs was reduced by 50% after 12 week growth of *S. sisymbriifolium* (Dandurand et al. 2019).

Brassica juncea SME at the lowest rate tested did not reduce *G. pallida* egg viability or hatch when applied alone; however, a significant reduction was observed when followed by *S. sisymbriifolium*. At higher rates, ≥0.56 t/ha, a significant difference in egg viability or hatch was not observed whether or not SME was combined with *S. sisymbriifolium* confirming our greenhouse trial findings that the higher rates of SME alone highly reduce nematode egg hatch such that the addition of the trap crop causes no further reduction. Also, *S. sisymbriifolium* reduced the viability and hatch of remaining *G. pallida* eggs at similar levels regardless of prior SME treatment. In sum, the results on egg numbers, egg viability, and egg hatch imply that 0.28 t/ha or higher rates of SME are needed to significantly reduce *G. pallida* egg viability and egg hatch under field conditions and *S. sisymbriifolium* regardless of the prior SME treatment strongly suppresses the egg hatch.

The efficacy of *B. juncea* SME may be different for the nematodes at varying depths in the soil profile. We applied *B. juncea* SME at 3 cm above the soil surface containing *G. pallida* cysts in our experiments. The addition of *S. sisymbriifolium* may provide a potential advantage to reduce *G. pallida* population situated at greater depths because *S. sisymbriifolium* can penetrate the soil at greater depths- up to 30 cm below the soil surface as observed by Scholte and Vos (2000). In Idaho

soil, *S. sisymbriifolium* roots were detected in the gravel layer at up to 152 cm below the soil surface (Dandurand et al. 2014). We assessed the impact of our treatments on *G. pallida* egg hatch by exposing the *G. pallida* eggs to the potato root diffusate for 2 weeks. However, the potential of the hatched juveniles to reproduce on a susceptible potato is not known yet for SME applied at a rate of 0.56 t/ha or lower. A bioassay with the susceptible potato cultivar 'Désirée' to determine the impact of treatments on *G. pallida* reproduction is underway. This will provide a better understanding of the efficacy of the treatment with lower SME rates applied with or without *S. sisymbriifolium* under field conditions.

Literature Cited

Angus, J. F., Gardner, P. A., Kirkegaard, J. A., and Desmarchelier, J. M. 1994. Biofumigation: Isothiocyanates released from brassica roots inhibit growth of the take-all fungus. Plant Soil. 162:107–112.

Arntzen, F. K., Visser, J. H. M., and Hoogendoorn, J. 1993. Hatching of *Globodera pallida* juveniles by diffusate of potato genotypes, differing in tolerance to *G. pallida*. Ann. Appl. Biol. 123:83–91.

Avato, P., D'Addabbo, T., Leonetti, P., and Argentieri, M. P. 2013. Nematicidal potential of Brassicaceae. Phytochem. Rev. 12:791–802.

Aydınlı, G., and Mennan, S. 2018. Biofumigation Studies by Using *Raphanus sativus* and *Eruca sativa* as a Winter Cycle Crops to Control Root-knot Nematodes. Brazilian Arch. Biol. Technol. 61.

Behrens, E. 1975. Globodera Skarbilovich, 1959, eine selbständige Gattung in der Unterfamilie Heteroderinae Skarbilovich, 1947 (Nematoda: Heteroderidae). Vor. zu Aktuellen Probl. der Phytonematologie. Rostock. :12–26.

Bellostas, N., Sørensen, J., and Sørensen, H. 2004. Qualitative and quantitative evaluation of glucosinolates in cruciferous plants during their life cycles. Agroindustria. 3:5–10.

Bending, G. D., and Lincoln, S. D. 1999. Characterisation of volatile sulphur-containing compounds produced during decomposition of *Brassica juncea* tissues in soil. Soil Biol. Biochem. 31:695–703.

Bhandari, S. R., Jo, J. S., and Lee, J. G. 2015. Comparison of glucosinolate profiles in different tissues of nine brassica crops. Molecules. 20:15827–15841.

Borek, V., Morra, M. J., Brown, P. D., and McCaffrey, J. P. 1994. Allelochemicals produced during sinigrin decomposition in soil. J. Agric. Food Chem. 42:1030–1034.

Borek, V., Morra, M. J., Brown, P. D., and McCaffrey, J. P. 1995. Transformation of the Glucosinolate-Derived Allelochemicals Allyl Isothiocyanate and Allylnitrile in Soil. J. Agric. Food Chem. 43:1935–1940.

Brown, P. D., and Morra, M. J. 1997. Control of Soilborne Plant Pests Using Glucosinolate-Containing Plants. Adv. Agron. 61:167–231.

Byrne, J. T., Maher, N. J., and Jones, P. W. 2001. Comparative responses of *Globodera rostochiensis* and *G. pallida* to hatching chemicals. J. Nematol. 33:195–202.

CABI. 2020. *Globodera pallida* (white potato cyst nematode). Online publication. Centre for Agriculture and Biosciences International. https://www.cabi.org/isc/datasheet/27033.

CABI. 2021. *Solanum sisymbriifolium* (sticky nightshade). Online publication. Centre for Agriculture and Biosciences International. https://www.cabi.org/isc/datasheet/117241.

Coelho, C. M. M., Bellato, C. de M., Santos, J. C. P., Ortega, E. M. M., and Tsai, S. M. 2007. Effect of phytate and storage conditions on the development of the 'hard-to-cook .' J. Sci. Food Agric. 1243:1237–1243.

Cole, R. A. 1976. Isothiocyanates, nitriles and thiocyanates as products of autolysis of glucosinolates in Cruciferae. Phytochemistry. 15:759–762.

Contina, J.-B., Dandurand, L.-M., and Knudsen, G. R. 2019. A Predictive Risk Model Analysis of the Potato Cyst Nematode *Globodera pallida* in Idaho . Plant Dis. :3117–3128.

Curto, G., Dallavalle, E., Matteo, R., and Lazzeri, L. 2016. Biofumigant effect of new defatted seed meals against the southern root-knot nematode, *Meloidogyne incognita*. Ann. Appl. Biol. 169:17–26.

Van Dam, N. M., Tytgat, T. O. G., and Kirkegaard, J. A. 2009. Root and shoot glucosinolates: A comparison of their diversity, function and interactions in natural and managed ecosystems. Phytochem. Rev. 8:171–186.

Dandurand, L.-M., Thornton, M., Hutchinson, P., Brown, C., Zasada, I. A., and Gresham, T. 2014. Litchi tomato: Trap crop for *Globodera pallida* control. Online publication.

https://www.uidaho.edu/-/media/UIdaho-Responsive/Files/cals/programs/potatoes/news-pubs/Litchi-tomato-Trap-crop-for-globodera-pallida-control.pdf?la=en&hash=65F2CCEFB7824C2E371E2FD2D8E41D242A614D9D#:~:text=Litchi%20tomato%20is%20preferred%20as,fumigants%20without%20the%20environmental%20consequences.

Dandurand, L.-M., Zasada, I. A., Wang, X., Mimee, B., De Jong, W., and Novy, R. 2019. Current Status of Potato Cyst Nematodes in North America. Annu. Rev. Phytopathol. 57:117–133.

Dandurand, L. M., and Knudsen, G. R. 2016. Effect of the trap crop *Solanum sisymbriifolium* and two biocontrol fungi on reproduction of the potato cyst nematode, *Globodera pallida*. Ann. Appl. Biol. 169:180–189.

Dandurand, L. M., Morra, M. J., Zasada, I. A., Phillips, W. S., Popova, I., and Harder, C. 2017. Control of *Globodera* spp. Using *Brassica juncea* seed meal and seed meal extract. J. Nematol. 49:437–445.

Dandurand, L. M., Zasada, I. A., and LaMondia, J. A. 2019. Effect of the trap crop, *Solanum sisymbriifolium*, on *Globodera pallida*, *Globodera tabacum*, and *Globodera ellingtonae*. J. Nematol. 51.

Dias, M. C., Conceição, I. L., Abrantes, I., and Cunha, M. J. 2012. *Solanum sisymbriifolium* - a new approach for the management of plant-parasitic nematodes. Eur. J. Plant Pathol. 133:171–179.

Dias, M. C., Perpétuo, L. S., Cabral, A. T., Guilherme, R., da Cunha, M. J. M., Melo, F., et al. 2017. Effects of *Solanum sisymbriifolium* on potato cyst nematode populations in Portugal. Plant Soil. 421:439–452.

Farnier, K., Bengtsson, M., Becher, P. G., Witzell, J., Witzgall, P., and Manduríc, S. 2012. Novel Bioassay Demonstrates Attraction of the White Potato Cyst Nematode *Globodera pallida* (Stone) to Non-volatile and Volatile Host Plant Cues. J. Chem. Ecol. 38:795–801.

Fleming, C., and Powers, T. 1998. Potato Cyst Nematode: species, Pathotypes and Virulence Concepts. In *Potato Cyst Nematode, Biology, Distribution and Control*, eds. R.J. Marks and B.B. Brodie. Wallingford, Oxfordshire, U.K. ; New York, NY, USA, p. 51–55.

Forrest, J. M. S., and Perry, R. N. 1980. Hatching of *Globodera pallida* eggs after brief exposures to potato root diffusate. Nematologica. 26:130–132.

Franco, J. 1979. Effect of temperature on hatching and multiplication of potato cyst-nematodes. Nematologica. 25:237–244.

Gimsing, A. L., Strobel, B. W., and Hansen, H. C. B. 2009. Degradation and sorption of 2-propenyl and benzyl isothiocyanate in soil. Environ. Toxicol. Chem. 28:1178–1184.

Grenier, E., Fournet, S., Petit, E., and Anthoine, G. 2010. A cyst nematode "species factory" called the Andes. Nematology. 12:163–169.

Guile, C. T. 1970. Further Observations on Cyst Colour Changes in Potato Cyst Eelworm Pathotypes. Plant Pathol. 19:1–6.

Hafez, S. L., Sundararaj, P., Handoo, Z. A., Skantar, A. M., Carta, L. K., and Chitwood, D. J. 2007. First Report of the Pale Cyst Nematode, *Globodera pallida*, in the United States. Plant Dis. 91:325.

Haydock, P. P. J., and Evans, K. 1998. Management of potato cyst nematodes in the U.K.: An integrated approach? Outlook Agric. 27:253–260.

Henderson, D. R., Riga, E., Ramirez, R. A., Wilson, J., and Snyder, W. E. 2009. Mustard biofumigation disrupts biological control by *Steinernema* spp. nematodes in the soil. Biol. Control. 48:316–322.

Hockland, S. 2002. Potato cyst nematodes - a technical overview for England and Wales. Sand Hutton, York, UK: CSL.

Hodda, M., and Cook, D. C. 2009. Economic impact from unrestricted spread of potato cyst nematodes in Australia. Phytopathology. 99:1387–1393.

Höglund, A. S., Lenman, M., Falk, A., and Rask, L. 1991. Distribution of myrosinase in rapeseed tissues. Plant Physiol. 95:213–221.

Jin, N., Lu, X., Wang, X., Liu, Q., Peng, D., and Jian, H. 2019. The effect of combined application of *Streptomyces rubrogriseus* HDZ-9-47 with soil biofumigation on soil microbial and nematode communities. Sci. Rep. 9:1–12.

Jones, M. G. K., and Northcote, D. H. 1972. Nematode-induced syncytium-A multinucleate transfer cell. J. Cell Sci. 10:789–809.

Kaczmarek, A., Mackenzie, K., Kettle, H., and Blok, V. C. 2014. Influence of soil temperature on *Globodera rostochiensis* and *Globodera pallida*. Phytopathol. Mediterr. 53:396–405.

Kirkegaard, J. A., and Sarwar, M. 1998. Biofumigation potential of brassicas: I. Variation in glucosinolate profiles of diverse field-grown brassicas. Plant Soil. 201:71–89.

Kirkegaard, J., and Matthiessen, J. 2004. Developing and refining the biofumigation concept. Agroindustria. 3:233–240.

Koenning, S. R., and Sipes, B. S. 1998. Biology. In *The Cyst Nematodes*, Springer Netherlands.

Koirala, S., Watson, P., McIntosh, C. S., and Dandurand, L. M. 2020. Economic Impact of *Globodera Pallida* on the Idaho Economy. Am. J. Potato Res. 97:214–220.

Kooliyottil, R., Dandurand, L.-M., Govindan, B. N., and R. Knudsen, G. 2016. Microscopy Method to Compare Cyst Nematode Infection of Different Plant Species. Adv. Biosci. Biotechnol. 07:311–318.

Kooliyottil, R., Dandurand, L. M., Kuhl, J. C., Caplan, A., Xiao, F., Mimee, B., et al. 2019. Transcriptome analysis of *Globodera pallida* from the susceptible host *Solanum tuberosum* or the resistant plant *Solanum sisymbriifolium*. Sci. Rep. 9:2–9.

Lazzeri, L., Curto, G., Leoni, O., and Dallavalle, E. 2004. Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita* (Kofoid et White) Chitw. J. Agric. Food Chem. 52:6703–6707.

López-Lima, D., Desgarennes, D., Lima-Rivera, D., and Carrión, G. 2020. Integrated management of *Globodera rostochiensis*: a novel biocontrol agent, crop rotation and fallow. J. Plant Dis. Prot. 127:633–640.

López-Lima, D., Sánchez-Nava, P., Carrión, G., and Núñez-Sánchez, A. E. 2013. 89 % Reduction of a Potato Cyst Nematode Population Using Biological Control and Rotation. Agron. Sustain. Dev. 33:425–431.

Lord, J. S., Lazzeri, L., Atkinson, H. J., and Urwin, P. E. 2011. Biofumigation for control of pale potato cyst nematodes: Activity of brassica leaf extracts and green manures on *Globodera pallida* in vitro and in soil. J. Agric. Food Chem. 59:7882–7890.

Martin, F. N. 2003. Development of Alternative Strategies for Management of Soilborne Pathogens Currently Controlled With Methyl Bromide. Annu. Rev. Phytopathol. 41:325–350.

Matthiessen, J., and Kirkegaard, J. 2006. Biofumigation and enhanced biodegradation: Opportunity and challenge in soilborne pest and disease management. CRC. Crit. Rev. Plant Sci. 25:235–265.

Mazzola, M., Hewavitharana, S. S., and Strauss, S. L. 2014. Brassica seed meal soil amendments transform the rhizosphere microbiome and improve apple production through resistance to pathogen reinfestation . Phytopathology. 105:460–469.

Mithen, R. 2001. Glucosinolates - Biochemistry, genetics and biological activity. Plant Growth Regul. 34:91–103.

Mojtahedi, H. 1993. Managing *Meloidogyne chitwoodi* on Potato with Rapeseed as Green Manure . Plant Dis. 77:42.

Morra, M. J., and Kirkegaard, J. A. 2002. Isothiocyanate release from soil-incorporated Brassica tissues. Soil Biol. Biochem. 34:1683–1690.

Muhammad, Z. 1994. Diapause in the nematode *Globodera pallida*. Eur. J. Plant Pathol. 100:413–423.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Ngala, B. M., Haydock, P. P., Woods, S., and Back, M. A. 2015. Biofumigation with *Brassica juncea*, *Raphanus sativus* and *Eruca sativa* for the management of field populations of the potato cyst nematode *Globodera pallida*. Pest Manag. Sci. 71:759–769.

De Nicola, G. R., D'Avino, L., Curto, G., Malaguti, L., Ugolini, L., Cinti, S., et al. 2013. A new biobased liquid formulation with biofumigant and fertilising properties for drip irrigation distribution. Ind. Crops Prod. 42:113–118.

Noling, J. W. 2002. The practical realities of alternatives to methyl bromide: Concluding remarks. Phytopathology. 92:1373–1375.

Palomares-Rius, J. E., Jones, J. T., Cock, P. J., Castillo, P., and Blok, V. C. 2013. Activation of hatching in diapaused and quiescent *Globodera pallida*. Parasitology. 140:445–454.

Perry, R. N. 1998. The physiology and sensory perception of potato cyst nematodes, *Globodera* species. In *Potato Cyst Nematodes: Biology, Distribution and Control*, eds. R J Marks and B B Brodie. Wallingford, Oxfordshire, U.K. ; New York, NY, USA: CAB International, p. 27–49.

Perry, R. N., and Clarke, A. J. 1977. Hatching of Cyst-Nematodes. Nematologica. 23:350–368.

Perry, R. N., and Clarke, A. J. 1977. Hatching of cyst-nematodes. Nematologica. 23:350–368.

Perry, R. N., and Gaur, H. S. 1996. Host plant influences on the hatching of cyst nematodes. Fundam. Appl. Nematol. 19:505–510.

Pillai, S. S., and Dandurand, L. 2021. Effect of steroidal glycoalkaloids on hatch and reproduction of the potato cyst nematode. Plant Dis. :1–29.

Pocock, K., Heaney, R. K., Wilkinson, A. P., Beaumont, J. E., Vaughan, J. G., and Fenwick, G. R. 1987. Changes in myrosinase activity and isoenzyme pattern, glucosinolate content and the cytology of myrosin cells in the leaves of heads of three cultivars of English white cabbage. J. Sci. Food Agric. 41:245–257.

Popova, I. E., Dubie, J. S., and Morra, M. J. 2017. Optimization of hydrolysis conditions for release of biopesticides from glucosinolates in *Brassica juncea* and *Sinapis alba* seed meal extracts. Ind. Crops Prod. 97:354–359.

Potter, M. J., Davies, K., and Rathjen, A. J. 1998. Suppressive impact of glucosinolates in Brassica vegetative tissues on root lesion nematode *Pratylenchus neglectus*. J. Chem. Ecol. 24:67–80.

Price, A. J., Charron, C. S., Saxton, A. M., and Sams, C. E. 2005. Allyl isothiocyanate and carbon dioxide produced during degradation of *Brassica juncea* tissue in different soil conditions. HortScience. 40:1734–1739.

Rigney, B., Blok, V., Griffin, D., Dalton, E., and Milbourne, D. 2017. Consistent action of two partially effective loci conferring resistance to *Globodera pallida* Pa2/3 across multiple nematode field populations. Plant Pathol. 66:1031–1040.

Ristaino, J., and Thomas, W. 1997. Agriculture, Methyl Bromide, and the Ozone Hole: Can We Fill the Gaps? Plant Dis. - PLANT DIS. 81:964–977.

Rosskopf, E. N., Chellemi, D. O., Kokalis-burelle, N., and T. Church, G. 2005. Alternatives to Methyl Bromide : A Florida Perspective Plant Health Progress Plant Health Progress. Plant Heal. Prog.

Schenk, H., Driessen, R. A. J., De Gelder, R., Goubitz, K., Nieboer, H., Brüggemann-Rotgans, I. E. M., et al. 1999. Elucidation of the Structure of Solanoeclepin A, a Natural Hatching Factor of Potato and Tomato Cyst Nematodes, by Single-crystal X-ray Diffraction. Croat. Chem. Acta. 72:593–606.

Scholte, K. 2000a. Effect of potato used as a trap crop on potato cyst nematodes and other soil pathogens and on the growth of a subsequent main potato crop. Ann. Appl. Biol. 136:229–238.

Scholte, K. 2000b. Growth and development of plants with potential for use as trap crops for potato cyst nematodes and their effects on the numbers of juveniles in cysts. Ann. Appl. Biol. 137:31–42.

Scholte, K. 2000c. Screening of non-tuber bearing solanaceae for resistance to and induction of juvenile hatch of potato cyst nematodes and their potential for trap cropping. Ann. Appl. Biol. 136:239–246.

Scholte, K., and Vos, J. 2000. Effects of potential trap crops and planting date on soil infestation with potato cyst nematodes and root-knot nematodes. Ann. Appl. Biol. 137:153–164.

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

Stone, A. R. 1972. The round cyst species of Heterodera as a group. Ann. Appl. Biol. 71:280–283.

Talavera, M., Andreu, M., Valor, H., and Tobar, A. 1998. Nematodos fitoparásitos en áreas productoras de patata de Motril y Salobreña. Investig. Agrar. Prod. y Protección Veg. 13:87–95.

Timmermans, B. G. H., Vos, J., Stomph, T. J., Van Nieuwburg, J., and Van Der Putten, P. E. L. 2007. Field performance of *Solanum sisymbriifolium*, a trap crop for potato cyst nematodes. II. Root characteristics: Research article. Ann. Appl. Biol. 150:99–106.

Timmermans, B. G. H., Vos, J., Stomph, T. J., Van Nieuwburg, J., and Van Der Putten, P. E. L. 2006. Growth duration and root length density of *Solanum sisymbriifolium* (Lam.) as determinants of hatching of *Globodera pallida* (Stone). Ann. Appl. Biol. 148:213–222.

Trudgill, D. L. 1967. The effect of environment on sex determination in *Heterodera rostochiensis*. Nematologica. 13:263–272.

Turner, S. J. 1996. Population decline of potato cyst nematodes (*Globodera rostochiensis*, *G. pallida*) in field soils in Northern Ireland. Ann. Appl. Biol. 129:315–322.

Turner, S. J., and Evans, K. 1998. The origins, global distribution and biology of potato cyst nematodes (*Globodera rostochiensis* (Woll.) and *Globodera pallida Stone*). In *Potato Cyst Nematodes: Biology, Distribution and Control*, eds. R.J. Marks and B.B. Broide. Wallingford, Oxfordshire, U.K. ; New York, NY, USA: CAB International, p. 7–26.

Turner, S. J., and Rowe, J. A. 2006. Cyst nematodes. In *Plant Nematology*, eds. Ronald N Perry and Maurice Moens. Wallingford, Oxfordshire, UK ; Cambridge, MA, USA: CABI Publishing, p. 91–122.

Uda, Y., Kurata, T., and Arakawa, N. 1986. Effects of ph and ferrous ion on the degradation of glucosinolates by myrosinase. Agric. Biol. Chem. 50:2735–3740.

USDA-APHIS. 2009. Pale potato cyst nematode national survey and diagnostic cyst sample forwarding protocols. Online publication. United States Department of Agriculture's Animal and Plant Health Inspection Service. https://www.aphis.usda.gov/plant_health/plant_pest_info/potato/downloads/pcndocs/PCN_NatlSurvey.pdf

USDA-APHIS. 2021. Pale Cyst Nematode (PCN) Eradication Program - 2020 4th Quarter Report (October 1- December 31). Idaho Falls, Idaho. Online publication. Online publication. United States Department of Agriculture's Animal and Plant Health Inspection Service. https://www.aphis.usda.gov/plant_health/plant_pest_info/potato/downloads/pcndocs/surveyupdates/2020/pcn-4th-quarter-2020.pdf

USDA-APHIS. 2015. Pale Cyst Nematode (PCN) Program Stakeholder Update. Online publication. United States Department of Agriculture's Animal and Plant Health Inspection Service. https://www.aphis.usda.gov/plant_health/plant_pest_info/potato/downloads/pcndocs/stakeholder/2015/06-2015.pdf.

USDA-APHIS. 2017. Pale Cyst Nematode in Bingham and Bonneville Counties, Idaho. Online publication. United States Department of Agriculture's Animal and Plant Health Inspection Service. https://www.aphis.usda.gov/plant_health/ea/downloads/2017/pcn-idaho-sea-march-2017.pdf.

USDA-APHIS. 2009. Potato Cyst Nematode; Quarantine and Regulations. Online publication. United States Department of Agriculture's Animal and Plant Health Inspection Service. https://www.federalregister.gov/documents/2009/04/29/E9-9724/pale-cyst-nematode-quarantine-and-regulations.

USDA-APHIS. 2013. Weed Risk Assessment for *Solanum sisymbriifolium* Lam . (Solanaceae) – Sticky nightshade. Online publication. United States Department of Agriculture's Animal and Plant Health Inspection Service. http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/wra/Solanum_sisymbriifolium_WRA.pdf.

USDA-NASS. 2019. Potatoes 2018 Summary. Online publication. United States Department of Agriculture's National Agriculture Statistics Service. https://downloads.usda.library.cornell.edu/usda-esmis/files/fx719m44h/7p88cv74d/b5645489z/pots0919.pdf.

Whitworth, J. L., Novy, R. G., Zasada, I. A., Wang, X., Dandurand, L. M., and Kuhl, J. C. 2018. Resistance of Potato Breeding Clones and Cultivars to Three Species of Potato Cyst Nematode. Plant Dis. 102:2120–2128.

Widdowson, E., and Wiltshire, G. H. 1958. The potato-eelworm hatching factor. 46.

Zasada, I. A., and Ferris, H. 2004. Nematode suppression with brassicaceous amendments: Application based upon glucosinolate profiles. Soil Biol. Biochem. 36:1017–1024.

Zasada, I. A., Meyer, S. L. F., and Morra, M. J. 2009. Brassicaceous Seed Meals as Soil Amendments to Suppress the Plant-parasitic Nematodes *Pratylenchus penetrans* and *Meloidogyne incognita*. J. Nematol. 41:221–227.