Control of *Globodera pallida* by combining *Sinapis alba* seed meal extract or 4- hydroxybenzyl alcohol with *Brassica juncea* seed meal extract or the trap crop *Solanum sisymbriifolium*

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Prativa Chhetri

Approved by:

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

Committee Members: Inna Popova, Ph.D.; Edwin Lewis, Ph.D.

Department Administrator: Edwin Lewis, Ph.D.

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Abstract

The pale cyst nematode, *Globodera pallida,* is a highly specialized, economically important pest for potato production. The specialized hatching requirements, ability to adapt, and the loss of effective control strategies such as methyl bromide fumigation increase the challenge to eradicate *G. pallida* in Idaho. Without a suitable host, this nematode can remain dormant as encysted eggs in soil for up to 20 years. The specificity of *G. pallida* hatching makes this life stage a potential target for designing efficient integrated control strategies. In this study, first, we demonstrated that *Sinapis alba* seed meal extract (SME) or 4-hydroxy benzyl alcohol (HBA), under laboratory and greenhouse conditions, enhances *G. pallida* egg hatch when exposed to potato root diffusate (PRD). This hatch enhancement in the presence of PRD is speculated to be due to an increase in egg-shell permeability. This study also aims to determine the efficacy of non-host trap crop *Solanum sisymbriifolium* or biofumigant *Brassica juncea* SME to control *G. pallida* when combined with the hatch enhancement properties of HBA or *S. alba* SME. For this study, we tested the efficacy of i) *S. sisymbriifolium* following prior treatment with *S. alba* SME (0, and 4.48 t/ha) or HBA (0, and 0.12 t/ha) and ii) *B. juncea* SME (0, 0.14, 0.56, and 1.12 t/ha) following HBA treatment (0, and 4.48 t/ha) on viability and hatch of *G. pallida* encysted eggs. *S. sisymbriifolium* alone reduced the number of encysted eggs compared to non-treated control by up to 67%, indicating that this trap crop triggered *G. pallida* egg hatch. When combined with *S. alba* SME or HBA, *S. sisymbriifolium* further significantly reduced egg count, hatch, and viability than *S. sisymbriifolium* alone. The combination of *S. sisymbriifolium* with HBA or *S. alba* SME eliminated *G. pallida* reproduction on the susceptible potato cultivar, Russet Burbank. Treatments with all the tested rates of *B. juncea* SME alone or with HBA reduced egg hatch and viability compared to the non-treated control. Combining HBA and *B. juncea* SME further reduced egg hatch and viability than *B. juncea* SME alone at rates 0.14, 0.56, and 1.12 t/ha. A lower egg hatch and viability with these combinations indicates that *S. alba* SME or HBA was able to increase the susceptibility of PCN eggs to the trap crop *S. sisymbriifolium* or nematicidal effect of *B. juncea* seed meal extract thus, decreasing *G. pallida* populations more effectively and rapidly.

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Dedication

This thesis is dedicated to my parents Sita Thapa and Durga Chhetri. Thank you for always believing in my ability.

Table of Contents

List of Tables

Chapter 1: Introduction

Potato cyst nematodes: *Globodera pallida* and *Globodera rostochiensis*

Globodera pallida (Behrens 1975; Stone 1972) *and Globodera rostochiensis* (Skarbilovich 1959; Wollenweber 1923), members of the *Heterodoridae* family, are internationally recognized regulated plant-parasitic nematodes. Collectively known as potato cyst nematodes, they are classified under the order Tylenchida, which includes most of the economically important plant-parasitic nematodes. The two species of potato cyst nematodes were initially considered a single species, *Heterodera rostochiensis*; however, Stone (1972) distinguished pale or white-colored from golden or yellow female bodies (cysts) developing from infected roots. These were later distinguished and described as *Heterodera rostochiensis* for golden cyst nematode and *Heterodera pallida* for pale cyst nematode in the subgenus *Globodera.* Then, Behrens (1975), based on morphological and biological distinction, placed these two potato cyst nematode species in the genus *Globodera*, with characteristics of round cysts lacking a terminal cone.

Potato cyst nematodes have a limited host range which includes potato (*Solanum tuberosum*) as the major host and several other solanaceous species (Nicol et al. 2011). Potato cyst nematodes have coevolved with their potato host in the Andean region of South America and were introduced to Europe in the 1850s on infested potato breeding materials, then subsequently spread to other parts of the world (Thorpe et al. 2014).

Globodera pallida

The pale cyst nematode, *G. pallida*, is a major plant-parasitic nematode of potato and is a threat to global potato production. *Globodera pallida* is regulated in most potato-growing areas worldwide and is currently reported in 55 countries (CABI 2020). The yield losses attributed to *G. pallida* are usually proportional to initial population density, resulting in up to 80% if no control measures are implemented (Hodda and Cook 2009; Contina et al. 2019). In addition, nematode feeding causes root damage and unthrifty aboveground growth, which ultimately leads to yield reduction and premature senescence.

Globodera pallida is a highly specialized obligate pest whose lifecycle is synchronized with its host (Perry 1989). Because of its high reproductive capacity and ability to remain dormant as encysted eggs until hatching stimulus is received, this nematode's chances of successful invasion are optimized (Turner and Evans 1998). In the absence of a suitable host, *G. pallida* eggs remain inside the dead female body, forming a melanized cyst structure that can survive in the soil for over 20 years (Evans and Stone 1977). Moreover, not all the viable eggs are subjected to hatching at once but rather require restimulation; this strategy renders population persistence throughout the growing season while also reducing competition among hatched juveniles (Perry 1989).

Hatching mechanism

Globodera pallida egg hatching encompasses three main steps: increased permeability of the eggshell lipid layer, activation of the juvenile, and eclosion (Perry and Moens 2011). In response to a hatching stimulus exuded from host roots, hatching is initiated by a change in the concentration of trehalose sugar with Ca^{2+} involved permeability changes of the eggshell lipid layer (Clarke et al. 1978). The trehalose sugar present in the perivitelline fluid plays a vital role in the dormancy and hatching of *G. pallida*. The accumulated sugar inhibits movement and induces dormancy by creating osmotic pressure inside the egg, making juveniles almost impervious to external factors (Atkinson et al. 1987). In potato root diffusate (PRD), the eggshell becomes more permeable, resulting in an influx of water following the loss of trehalose sugar (Clarke and Perry 1985). The subsequent loss of osmotic stress allows juveniles to rehydrate and become mobile. The activated juvenile then cuts the eggshell with its specialized mouthpart known as stylet and proceeds to hatch. The bimodal action of PRD on encysted eggs, i.e., change in eggshell permeability and metabolic activation of juveniles, usually occurs within 24 h of exposure to PRD; however, juveniles generally hatch three days after exposure to the hatching stimulus (Perry and Feil 1986).

Globodera pallida life cycle

The active part of the lifecycle starts with the infective second-stage juveniles (J2s) locating host roots (Byrne et al. 2001; Farnier et al. 2012). As with most nematodes, *G. pallida* have four juvenile stages and an adult stage. The infectious juvenile invades the root near the growing point (Evans and Stone 1977). It then moves intracellularly until it reaches

the inner cortex layer to form a feeding site known as a syncytium (Sobczak and Golinowski 2011). The syncytium, where the nematode remains until its lifecycle is complete, is formed by partial dissolution of the initial feeding cell (Jones et al. 2013). The now sedentary J2s develop into third and fourth-stage juveniles (Koenning and Sipes 1998). At this stage, sex differentiation occurs in developing juveniles depending on nutrient availability such that more adult females develop with adequate nutrient availability (Trudgill 1967). Females enlarge and assume the globose shape and eventually rupture through the root system, exposing their posterior bodies to the soil. Males, however, become vermiform and exit roots to find and fertilize females (Sobczak and Golinowski 2011). After fertilization, the female dies, and her cuticle forms a protective cyst that holds 200-500 embryonated eggs which can easily be dispersed in soil clinging to tubers, or farm equipment (Evans and Stone 1977). The cysts eventually detach from roots as the plant dies and undergo dormancy (Hominick 1986; Muhammad 1994).

Dormancy in *G. pallida* can be differentiated as quiescence or diapause. Quiescence refers to reversible arrested development induced by unfavorable conditions. However, in diapause state, hatching cannot be resumed until specific requirements are met even with favorable conditions (Perry 1997; Wright and Perry 2006). For some of the *G. pallida* encysted eggs, diapause may be broken after a period of cold temperature, such as an overwintering period; however, if a suitable host is not present, diapause is followed by quiescence (Wright and Perry 2006). The quiescent state allows encysted *G. pallida* eggs to persist in the soil for extended periods, broken only by host root diffusate (Jones et al. 1998). This state may allow encysted eggs to survive in the soil for 20 years or more (Evans and Stone 1977).

Globodera pallida **in Idaho**

In the United States, *G. pallida* was discovered in 2006 through a routine soil survey from a potato processing facility in eastern Idaho (Hafez et al. 2007). Since its detection, *G. pallida* is regulated by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) and Idaho State Department of Agriculture (ISDA) and is listed as a quarantine pest. Following *G. pallida* detection in Idaho, an embargo was established on importing Idaho fresh potato products and nursery stocks by some of Idaho's

most important trading partners, Canada, Mexico, and South Korea. Consequently, containment and eradication programs are in place by USDA-APHIS and ISDA. These regulations established restrictions on planting potato or other hosts, movement of soil, equipment and tubers, and requirement for sanitization of farm equipment (USDA-APHIS 2009). With extensive surveys and stringent phytosanitary measures, the *G. pallida* infestation has been limited to less than 1% of Idaho's total potato production area, with 1395 hectares infested of 2976 hectares regulated area (USDA-APHIS 2021). The estimated loss inflicted due to the removal of potatoes from the cropping system of infested fields in Idaho is $25.56 million (Koirala et al. 2020). *Globodera pallida* poses a significant threat to the U.S. potato industry as Idaho is the largest potato producer with production worth $1.03 billion in 2018 (USDA-NASS 2019). Eradication efforts as a part of stringent regulatory measures entail millions of dollars investment; thus, novel strategies to protect this investment and stop potential infestation are of utmost importance. Current research efforts are directed towards biofumigation, trap crops, development of resistance in russet potato varieties, predictive risk analysis on yield and economic impact, and improved detection and viability assays (Dandurand et al. 2019a).

Control strategies for *Globodera pallida*

The control of *G. pallida* is quite challenging because of its peculiar hatching requirements. Moreover, *G. pallida* is found to have a slower hatch than the closely related golden cyst nematode, *G. rostochiensis*, which was considered a reason for the low effectiveness of nematicides like oxamyl for the control of *G. pallida* on application than *G. rostochiensis* (Whitehead et al. 1984; Whitehead 1992). Methyl bromide had been an integral part of *G. pallida* eradication efforts by USDA-APHIS in Idaho. However, in 2015, based on safety concerns, the use of the fumigant MeBr was discontinued and replaced with 1,3-dichloropropene fumigation (USDA-APHIS 2015).

Another potential control is to plant resistant potato cultivars against potato cyst nematodes. Although some level of resistance to *G. pallida* is present in European varieties, no commercially grown russet-skinned cultivars in Idaho are resistant (Dandurand et al. 2017; Whitworth et al. 2018). Also, despite having a narrow host range, rotation with non-host crops is quite impractical for growers because of the very low annual population decline

rate (as low as 10 %) and prolonged survival of *G. pallida* in the absence of a suitable host (Turner 1996; Trudgill et al. 1987). Therefore, there is a need for alternative environmentally safe and efficient novel control strategies that can disrupt *G. pallida* lifecycle by interfering with the hatching and reproduction process.

Biofumigation: a potential *Globodera pallida* control strategy

In the light of the loss of effective chemical control, a potential approach that has been extensively researched is biofumigation. This strategy suppresses soilborne pathogens by incorporating brassicaceous plant or plant products such as green manure or seed meal amendments into soil (Kirkegaard and Matthiessen 2004). Glucosinolates (GSLs), sulfur-containing compounds found in tissues of plants from the *Brassicaceae*, on hydrolysis with endogenous enzyme myrosinase, produce a variety of biologically active compounds, such as isothiocyanates (ITCs), ionic thiocyanates, and nitriles (Angus et al. 1994; Brown and Morra 1997; Fahey et al. 2001; Bones and Rossiter 2006). More than 120 GSLs, differentiated in structure by variable side chains (R groups), are found within *Brassicaceae* (Brown and Morra 1997; Fahey et al. 2001). The biocidal effect of the *Brassica* spp. as fumigants is attributed to the production of the volatile toxic GSLs catabolites, of which ITCs are most toxic. Isothiocyanates are general biocides that irreversibly interact with sulfhydryl groups in proteins, disulfide bonds, and amines, which interfere with various metabolic pathways (Keppler et al. 2014).

The first study reporting the efficacy of isothiocyanates on nematodes was conducted by Ellenby in 1945 while testing the effect of different brassicaceous leachate on *G. rostochochiensis* hatching. Of the species tested, *Brassica nigra* leachate significantly reduced hatch of the nematode (Ellenby 1945). Many species of *Brassica* have been studied for their nematicidal effect against different species, such as *Meloidogyne chitwoodi* (Mojtahedi et al. 1991), *Pratylenchus neglectus* (Potter et al., 1999), *G. rostochiensis* (Buskov et al. 2002), *M. incognita* (Roubtsova et al. 2007), *Steinernema feltiae and S. riobrave* (Henderson et al. 2009), *G. pallida* (Lord et al. 2011; Ngala et al. 2015c; Dandurand et al. 2017). The findings showed the use of these organic amendments generally had potential for management of plant-parasitic nematodes.

Factors affecting biofumigation

The effectiveness of *Brassica* spp. as biofumigants depends on many factors such as plant species and cultivar, type of tissue, and growth stage of the plant (Kirkegaard and Sarwar 1998; Van Dam et al. 2009; Bhandari et al. 2015). Bhandari (2015) found the highest GSLs concentration in seeds of most *Brassica* species. However, the type and concentration of the GSL differ for each brassicaceous species. For instance, 2-propenyl GSLs (sinigrin) is found in abundance in *Brassica juncea, B. napa,* and *B. carinita* (Bellostas et al. 2004); whereas the 2-phenylethyl GSL is predominant in *B. napus* (Potter et al. 1999) and *p*-hydroxy benzyl (sinalbin) is predominant in *Sinapis alba* (Lazzeri et al. 1993). The breakdown product of sinigrin, 2-propenyl ITC, is highly toxic to nematodes (Lazzeri et al. 1993; Buskov et al. 2002; Zasada and Ferris 2003). Not only the type of GSL but timing and method used to incorporate the GSL into the soil will affect the biofumigation potential of crops. As an example, cultivating *B. juncea* in the summer for fall incorporation provides greater nematode suppression for *G. pallida* than fall planting with spring incorporation (Ngala et al. 2015a).

Glucosinolate hydrolysis

Glucosinolate hydrolysis refers to myrosinase (EC 3.2.1.147) mediated enzymatic breakdown of the thioglucoside linkage, which releases glucose and an unstable aglycone which then initiates a reorganization of a series of catabolites such as ITCs, nitriles, or ionic thiocyanates (SCN^-) (Brown and Morra 1997; Bor et al. 2009). The enzyme myrosinase is present in myrosin organelles in close proximity with GSL containing cells which are localized in vacuoles (Bennett et al. 2006; Yan and Chen 2007; Kissen et al. 2009). The loss of this compartmentalization by tissue maceration or physical damage in the presence of water causes hydrolysis of GSLs (Song et al. 2005).

The effectiveness of biofumigation may also be influenced by edaphic factors such as soil type, organic matter content, pH, temperature, and moisture content (Borek et al. 1995; Brown and Morra 1997; Morra and Kirkegaard 2002; Price et al. 2005; Gimsing et al. 2009). Degradation of GSLs in soil requires water to facilitate hydrolysis reaction after plant tissue damage (Morra and Kirkegaard 2002). Higher concentration and faster degradation of 2-propenyl ITC occurs in sandy-loam soil compared to clay-loam soil (Price et al. 2005). In

addition, incremental changes in soil temperature from 10 to 25° C reduced the half-life of 2-propenyl ITC (Borek et al. 1995). Greater loss of 2-propenyl ITC is correlated with high organic carbon content in soil (Borek et al. 1995) as sorption of 2-propenyl ITC is higher with increased soil organic matter (Brown and Morra 1997; Gimsing et al. 2009). Moreover, production of GSL catabolites is strongly pH-dependent as neutral pH generally favors the production of ITCs while nitrile production occurs at lower pH (Borek et al. 1994; Bones and Rossiter 2006).

Impact of sinigrin hydrolysis products on G. pallida

The major GSL present in *B. juncea,* sinigrin, upon hydrolysis produces volatile and bioactive 2-propenyl ITC (Dai and Lim 2014; Popova et al. 2017). This sinigrin metabolite has a vapor pressure of approximately 500 Pa at 25°C, making it highly volatile (Lim and Tung 1997). The short exposure with an adequate concentration of 2-propenyl ITC (half-life ranging 20-60 h) was found suppressive on target pests (Borek et al. 1995). By keeping volatiles in the soil air space, plastic mulch increases the efficacy of biofumigation as does application at optimal soil moisture and temperature as these factors influence the movement of ITCs through the soil profile (Snyder et al. 2009).

The nematicidal effect of biofumigation with *B. juncea* green manures, seed meal, or seed meal extract for *G. pallida* control has been reported (Lord et al. 2011; Ngala et al. 2015b; Dandurand et al. 2017). The use of brassicaceous plants as green manure is common in Europe (Valdes et al. 2011). When incorporated into the soil, the crop residues from *B. juncea* lines with a high concentration of 2-propenyl GSL (sinigrin) showed 85% and 95% mortality of encysted eggs, respectively, in uncovered and polyethylene covered soil (Lord et al. 2011). Seed meal derived from *B. juncea* also contains a high level of 2-propenyl GSL and is a potential alternative for synthetic nematicides (Curto et al. 2016). *Brassica juncea* seed meal applied at 0.1% of soil weight eliminated *G. pallida* reproduction under greenhouse conditions (Dandurand et al. 2017). The use of *B. juncea* seed meal as a biofumigant reduced hatch and reproduction of *G. pallida* (Dandurand et al. 2017).

Despite being highly effective, biofumigation with green manure or seed meal has some limitations. These include the higher cost and time for green manure crop planting and

handling, higher application dose of seed meal required for efficacy may result in a potential negative impact from a large amount of materials (Dandurand et al. 2017). Moreover, these biofumigation sources have variable concentrations of bioactive 2-propenyl GSL and may provide inconsistent nematode control (Zasada and Ferris 2004). To overcome the challenges and provide more consistent results from biofumigation, a shelf-stable GSL extract was formulated (Popova et al. 2017). The formulated *B. juncea* seed meal extract at the rates 1.1 t/ha or higher suppressed *G. pallida* and *G. ellingtonae* hatch and inhibited the reproduction of *G. pallida* at a lower application dose than the seed meal (Dandurand et al. 2017). This could potentially be advantageous because of ease in application, handling, transport, and storage of a lesser amount of material required for efficacy.

Impact of sinalbin hydrolysis products on G. pallida

Sinalbin, a predominant GSLs in *Sinapis alba* (white mustard), is enzymatically hydrolyzed to produce unstable 4-hydroxybenzyl ITC which is then non-enzymatically transformed into 4- hydroxybenzyl alcohol (HBA) and phytotoxic ionic thiocyanate (SCN^-) (Borek and Morra 2005; Hansson et al. 2008; Popova et al. 2017). 4-Hydroxybenzyl alcohol (HBA) was described as the main sinalbin degradation product (Buskov et al. 2002).

Unlike *B. juncea*, previous studies showed *S. alba* has no nematicidal impact on *G. pallida* or *G. rostochiensis* (Ellenby 1945; Forrest and Farrer 1983; Valdes et al. 2011). In addition, in an *in vitro* study to determine the effect on *Heterodera schachtii* second-stage juveniles, sinalbin did not exhibit any nematicidal effect (Lazzeri et al. 1993). Ellenby (1945) discovered that the prior treatment with *S. alba* root diffusate did not affect the hatching of *G. rostochiensis* encysted eggs in potato root diffusate (PRD). However, previous exposure to root diffusate from *S. alba* resulted in a more rapid hatch for encysted eggs of *G. pallida* than water only when subsequently treated with PRD (Forrest and Farrer 1983). The effect of root diffusate, plant extracts, or a crop of *S. alba* green manure did not suppress *G. rostochiensis* hatching (Valdes et al. 2011). Although *S. alba* did not stimulate hatch with root diffusate alone, subsequent exposure to tomato root diffusate increased *G. rostochiensis* hatch (Valdes et al. 2011). Potato cyst nematode hatch in the presence of PRD is mediated by an increase in eggshell permeability followed by subsequent activation of juveniles and puncture of eggshell (Perry and Moens 2011). However, treatment with *S. alba* or sinalbin without host

root diffusate did not stimulate the hatch of *G. pallida and G. rostochiensis* (Forrest and Farrer 1983; Valdes et al. 2011), which leads to speculation that pretreatment could increase eggshell permeability sufficiently to readily activate juveniles in the presence of hatching factor from host root diffusate. Based on this assumption with the hatch enhancing ability, *S. alba* or sinalbin could be an effective tool for *G. pallida* control when pyramided with various control strategies like biofumigation or trap crops.

Trap crop: a potential *G. pallida* control strategy

Scholte (2000c) first reported the potential of non-tuber bearing solanaceous species as trap crops for *G. pallida*, from which *Solanum sisymbriifolium* Lamarck was considered the most promising candidate. *Solanum sisymbriifolium*, commonly known as sticky nightshade or litchi tomato, triggers rapid *G .pallida* hatch and provides complete resistance (Scholte 2000b; Timmermans et al. 2006; Dias et al. 2012; Dandurand and Knudsen 2016; Dandurand et al. 2019b). *Solanum sisymbriifolium* is an annual plant species originating from South America, but is now found throughout the world, naturalized in Africa, Asia, Australia, Europe, New Zealand, North America, and the Caribbean, and is considered a noxious invasive weed in some countries (CABI 2021).

Impact of S. sisymbriifolium on G. pallida

Although *S. sisymbriifolium* stimulates the hatch of *G. pallida*, second-stage juveniles quickly die following invasion into roots (Scholte 2000b; Kooliyottil et al. 2016), and localized root cell necrosis is observed within 24 hours of invasion (Kooliyottil et al. 2016). *Solanum sisymbriifolium* as a trap crop has been found to reduce *G. pallida* population densities by up to 80% (Scholte 2000b; Scholte and Vos 2000; Timmermans et al. 2007). Furthermore, under greenhouse conditions, the reproduction rate of *G. pallida* (Idaho population) was decreased by 99% in potato-following-*S. sisymbriifolium* compared to the potato-following fallow or potato-following-potato rotation (Dandurand and Knudsen 2016). Moreover, under Idaho field conditions, the number of encysted eggs of *G. pallida* after trap cropping with *S. sisymbriifolium* was reduced by up to 50%, along with the reduction in reproduction by 99 to 100% on potato compared to fallow treatment (Dandurand et al. 2019b). *Solanum sisymbriifolium* contains biologically active glycoalkaloids, such as α-solamargine and solasodine; exposure to either glycoalkaloids showed a suppressive effect

on *G. pallida* hatch and reproduction, thus indicating potential nematicidal properties of *S.* sisymbriifolium (Dias et al. 2012; Pillai and Dandurand 2021).

Despite being an ideal trap crop for *G. pallida*, incorporating *S. sisymbriifolium* in Idaho's eradication efforts for *G. pallida* is quite challenging. The trap crop is not native to Idaho, which risks it being invasive in local flora. Therefore, ISDA has listed *S. sisymbriifolium* as an invasive species for Idaho and entails a comprehensive permitting process for approval of planting of *S. sisymbriifolium* in Idaho (USDA-APHIS 2017).

Integrated *Globodera pallida* control strategies

Potato cyst nematode control has been mainly based on integrating crop rotation, chemical control, and the use of resistant cultivars (Trudgill et al. 1987). In Europe, the prevalent mixed population of *G. rostochienesis* and *G. pallida* are controlled by 6-year rotations with non-hosts, soil testing, use of resistant cultivars, trap cropping, and nematicides (Hockland 2002). Integrating these different control measures is most effective for potato cyst nematodes (Haydock and Evans 1998). Also, because of the limitations of the strategies to provide efficient control of *G. pallida* individually and the capacity of this nematode to adapt, there is a need for continued development of control strategies by integrating different methods. For example, by integrating *S. sisymbriifolium* with biofumigant *B. juncea* SME, the reserve of viable *G. pallida* population was reduced compared to the application of individual strategies alone (Bhatta 2021).

Globodera pallida encysted eggs undergo induced dormancy, making juveniles almost impervious to external factors until they receive hatching stimulus from the host roots. *Globodera pallida* eggs after pretreatment with PRD for 24 h were more sensitive to desiccation than untreated eggs, possibly due to permeability change of the exposed eggs as an initial step in the hatching process (Forrest and Farrer 1983). *Globodera rostochiensis* hatch was subsequently reduced in PRD after being treated by the mixture of aldicarb and PRD compared to the prior treatment with aldicarb alone; the differences with the impact of aldicarb was discussed in relation to metabolic activation of juveniles by root diffusate (Osborne 1970). Clarke and Hennessy (1981) also showed that ruthenium red effectively inhibited PRD-initiated hatching, presumably by inhibiting nematode movement with inactivation of the hatching factor. Thus, incorporating the hatch-inducing factors with

nematicidal properties of control strategies could provide a novel medium for integrated control of *G. pallida*.

Rationale of the study

Sinapsis alba enhances hatch of *Globodera spp.* after exposure to host root diffusate, which may increase eggshell permeability. 4-Hydroxybenzyl alcohol (HBA), the product of enzymatic hydrolysis of sinalbin (Buskov et al. 2002; Popova et al. 2017), could be responsible for the observed hatch enhancement. Thus, pretreatment with *S. alba* seed meal or SME or HBA alone could enhance *G. pallida* hatch with PRD and potentially be a part of integrated control strategies.

Unlike sinalbin, *B. juncea* SME as an individual treatment inhibits *G. pallida* hatch and reproduction (Dandurand et al. 2017). However, with *B. juncea* SME, rates of 1.1 t/ha or more were required for efficacy. Therefore, we hypothesize that combining HBA with *B. juncea* SME may increase eggshell permeability, which may amplify the nematicidal activity of sinigrin found in *B. juncea* SME.

Although *S. sisymbriifolium* alone reduced *G. pallida* by inducing hatch without supporting progressive development and reproduction under Idaho field conditions (Dandurand et al. 2019b), a small remnant viable population remains and could act as a potential reservoir for the subsequent growing season. Thus, *S. sisymbriifolium*, when pyramided with hatch enhancement effects of *S. alba* SME or HBA, could potentially enhance more *G. pallida* hatch compared to trap crop alone, which could, in turn, deplete potential inoculum for the next season.

Objectives of the study

Our goal from this study was to determine the efficacy of combining *S. alba* SME or HBA with biofumigation using *B. juncea* SME or the trap crop, *S. sisymbriifolium* for control of *G. pallida* under laboratory or greenhouse conditions. Our specific objectives were to i) assess the effect of *S. alba* seed meal or SME or HBA on *G. pallida* hatch ii) test the efficacy of *B. juncea* SME on *G. pallida* egg hatch, viability, and reproduction when pyramided with HBA iii) determine the impact of *S. sisymbriifolium* on *G. pallida* egg densities, hatch, viability, and reproduction when combined with *S. alba* SME or HBA.

Chapter 2: Control of *Globodera pallida* using trap crop *Solanum sisymbriifolium* combined with *Sinapis alba* seed meal extract or 4-hydroxy benzyl alcohol

Introduction

Globodera pallida (Behrens 1975; Stone 1972), the pale cyst nematode, is an economically important plant-parasitic nematode of potato and a threat to global potato production. *Globodera pallida* is regulated in most potato-growing areas worldwide and is currently reported in 55 countries (CABI 2020). *Globodera pallida*-caused yield losses are generally proportionate to initial population density, resulting in up to 80% losses if no control measures are adopted (Hodda and Cook 2009; Contina et al. 2019). The nematode causes root damage and poor aboveground growth, resulting in early senescence and decreased tuber production. *Globodera pallida,* with its high reproductive capacity, host specificity, and ability to remain dormant as encysted eggs in soil for extended periods, optimizes the likelihood of a successful infection on its host (Turner and Evans 1998). The dormant state, making juveniles almost impervious to external factors, may allow encysted eggs to survive in the soil without a suitable host for periods of 20 years or more (Evans and Stone 1977).

In the United States, *G. pallida* was first discovered in Idaho in 2006 (Hafez et al. 2007). Since its detection, *G. pallida* has been regulated by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) and Idaho State Department of Agriculture (ISDA), and containment and eradication programs are in place. Soil fumigation with methyl bromide (MeBr) was an integral part of *G. pallida* eradication efforts in Idaho; however, based on restrictions in use, MeBr fumigation has been stopped. Soil fumigation has been replaced by 1,3-dichloropropene (USDA-APHIS 2015). The use of partial-resistant cultivars and rotation with non-host crops are popular *G. pallida* control strategies in Europe, but no commercially grown russet-skinned cultivars in the US carry *G. pallida* resistance (Dandurand et al. 2017; Whitworth et al. 2018). The prolonged survival and low annual decline rate (as low as 10%) make rotation with non-host crop quite impractical for *G. pallida* control (Turner 1996; Trudgill et al. 1987). The hatching requirements of *G. pallida*, and lack of available control strategies make this nematode quite challenging to control which indicates the need for alternative approaches.

One potential alternative *G. pallida* control strategy is the non-host trap crop *Solanum sisymbriifolium*, commonly known as litchi tomato. *Solanum sisymbriifolium* is the most promising non-tuber bearing solanaceous trap crop species for *G. pallida* control (Scholte 2000b). *Solanum sisymbriifolium* triggers *G. pallida* hatch and provides complete resistance (Scholte 2000a; Timmermans et al. 2006; Dias et al. 2012; Dandurand and Knudsen 2016; Dandurand et al. 2019b). The second-stage juveniles (J2) die shortly after root invasion (Scholte 2000a; Kooliyottil et al. 2019), and localized cell death is observed within 24 h of invasion (Kooliyottil et al. 2019). *Solanum sisymbriifolium* reduced *G. pallida* population densities by up to 80% (Scholte and Vos 2000; Timmermans et al. 2007). Under Idaho field conditions, following *S. sisymbriifolium*, the number of encysted eggs was reduced by 50%, along with 99 to 100% reduction in the reproduction of remaining encysted eggs on susceptible potato compared to fallow treatment (Dandurand et al. 2019b). Biologically active glycoalkaloids with potential nematicidal effects are known to reduce infection and reproduction of *G. pallida* and may be present in *S. sisymbriifolium* (Sivasankara Pillai and Dandurand 2021). Although *S. sisymbriifolium* reduced the *G. pallida* population by inducing hatch without supporting progressive development and reproduction, a small remnant viable population exists in soil which may act as a potential reservoir for the next cropping season.

Studies to test the effect of *Sinapis alba* or its predominant glucosinolate, sinalbin, on *G. rostochiensis* (Ellenby 1945; Valdes et al. 2011), *Heterodera schachtii* (Lazzeri et al. 1993), or *G. pallida* (Forrest and Farrer 1983) have been conducted, and *S. alba* or sinalbin did not display any nematicidal effect. However, the subsequent exposure of *G. pallida* or *G. rostochiensis* encysted eggs in host root diffusate resulted in enhanced hatch (Ellenby 1945; Forrest and Farrer 1983; Valdes et al. 2011). The reason behind this hatch enhancement is unknown. We speculate that the pretreatments may increase eggshell permeability as the *S. alba* treatments did not induce hatch by themselves. This study aims to investigate the effect of *S. alba* seed meal or seed meal extract (SME), a shelf-stable powder prepared from seed meal with highly concentrated sinalbin (Popova et al. 2017), on *G. palllida*. Sinalbin on hydrolysis produces 4-hydroxy benzyl alcohol (HBA) and ionic thiocyanate (SCN^-). HBA has been described as the main myrosinase-mediated sinalbin degradation product (Buskov et al. 2002) and could be responsible for the observed hatch enhancement.

Due to the limitations of individual control strategies and the capacity of *G. pallida* to adapt, the continual evolution of control strategies by integrating different novel methods is required for efficient control. Thus, incorporating the hatch enhancing factors with nematicidal properties of control strategies could provide a novel medium for integrated control of *G. pallida*. We hypothesize that *Solanum sisymbriifolium,* when pyramided with hatch enhancement effects of *Sinapis alba* SME or HBA, may enhance *G. pallida* hatch compared to *S. sisymbriifolium* alone, which may, in turn, deplete potential inoculum for the subsequent growing season. Our specific objectives for this study were to: (i) assess the effect of *S. alba* seed meal or SME on *G. pallida* hatch and viability under laboratory conditions (ii) determine the impact of *S. sisymbriifolium* on *G. pallida* egg densities, hatch, viability, and reproduction when combined with *S. alba* SME or HBA under greenhouse conditions.

Materials and methods

Globodera pallida

Globodera pallida, being a quarantined pest in Idaho, is reared and maintained under a bio-secure environment at the University of Idaho, Moscow, ID. *Globodera pallida* cysts used in experiments were initially isolated from an infested field in Shelly, ID, identified by morphological and molecular characterization (Skantar et al. 2011) and reared under greenhouse conditions on susceptible potato cultivar 'Russet Burbank', maintained at 18°C day temperature and night temperature of 10°C at a 16:8-h (light: dark) photoperiod (Dandurand et al. 2017; Dandurand and Knudsen 2016; Dandurand et al. 2019b). After 16 weeks, cysts were recovered from soil using a USDA-type elutriator (USDA-APHIS 2009). The recovered cysts were stored at 4° C for a minimum of 16 weeks before being used for experiments. For all the experiments, *G. pallida* cysts were sealed in a 250 µm mesh size wear-resistant nylon mesh bag (McMaster-Carr, Elmhurst, IL) (3 x 3 cm^2) with a hand sealer (Sealer 8" F-200; Sealer sales Inc., Northridge, CA). The sealed cyst bags were then surface sterilized in 0.3% sodium hypochlorite solution for 5 min and rinsed five times in sterile deionized water. After surface-sterilization, the cysts in sealed nylon mesh bags were hydrated for 3d before each experiment.

Treatments used

Sinapis alba seed meal from the variety 'IdaGold' was obtained from a cold press facility at (University of Idaho facility, Moscow, ID) (Popova et al. 2017). *Sinapis alba* seed meal extract, extracted from seed meal, was concentrated and formulated as a shelf-stable powder (Popova et al. 2017). The concentration of sinalbin in the seed meal and formulated SME used for the experiments was 190 and 464 µmol/g, respectively. In addition, 4-hydroxy benzyl alcohol (HBA) (Sigma-Aldrich, Inc., St. Louis, MO), equivalent to 464 µmol/g sinalbin hydrolysis, was used for the experiments.

Plants used

For all experiments, *S. sisymbriifolium* seeds, obtained from USDA-ARS, Prosser, WA, USA, were sown into nursery trays and transplanted after four weeks into 4-inch clay pots. To assess reproduction on a susceptible host, a bioassay was conducted with potato plantlets (*Solanum tuberosum* L. cv. 'Russet Burbank') grown from tissue culture in standard media (Murashige and Skoog, 1962). The plantlets were grown for four weeks.

Effect of S. alba seed meal or seed meal extract on G. pallida hatch and viability in laboratory experiments

Experiments were conducted in an incubator (Thermo Fisher Precision™ Low-Temperature BOD Refrigerated Incubator, Thermo Fisher Scientific LLC, Marietta, OH) maintained at 20°C. *Globodera pallida* cysts were exposed to two rates of *S. alba* seed meal (0 or 4.48 t/ha) or SME (0 or 1.8 t/ha) in a sealed magenta GA-7 tissue culture vessel (Sigma Aldrich, St. Louis, MO). First, the tissue culture vessel was lined with 1-cm of autoclaved gravel (Moscow Building Supply, Moscow, ID). Next, 250 g of autoclaved 2:1 ratio of silica sand (Lane Mountain Company, Valley, WA) mixed with air-dried Prosser fine sandy loam soil (56% sand, 35% silt, 8% clay, pH 7.0) was added. Ten cysts (with an average of 305 eggs/cyst for an inoculation density of 10 eggs/g soil) enclosed in nylon mesh bags were then placed on the soil mix and covered with 50 g of soil. A band of *S. alba* seed meal or SME was applied at an appropriate rate on the soil surface, and an additional 50 g of soil was added at the top. The magenta vessel was sealed with a lid after applying 30 ml of sterile deionized water. Six replicates for each treatment were arranged in a complete randomized

design and left for 14 d. After two weeks, cyst bags were retrieved from the vessels, surface-sterilized, and used for hatching and viability assay. The experiment was repeated.

Hatching assay: For the hatching assay, six cysts were removed from each nylon mesh bag, and the eggs were released in 600 µl of 1.5 mg/ml gentamycin solution. First, 100 µl of eggs aliquots was dispensed into each of six wells of 96-well plates. The initial number of eggs and J2s in each well were counted using a stereomicroscope. Next, a 100 µl of potato root diffusate (PRD), collected from 4-wk grown potato cultivar 'Russet Burbank', was added to each of the three wells, and 100 µl of bare soil diffusate (BSD) was added to the remaining three wells. Bare soil diffusate was used as a negative control. Diffusate was collected by pouring 100 ml sterile deionized water through the pots with potato plants or fallow soil, then was filter-sterilized respectively with 0.45 and 0.22 µm bottle-top-vacuum filters (Corning Incorporated, Corning, NY). The eggs were exposed to the diffusates for two weeks, then the number of hatched J2 was counted, and the percentage egg hatch was calculated as ((number of hatched J2 – number of initial J2)/number of initial eggs) x 100.

Viability assay: Globodera pallida egg viability was assessed by the acridine orange (A.O.) staining method (Pillai and Dandurand 2019). Three cysts from each treatment replicate were exposed to A.O. (25 µg/ml) (Thermo Fisher Scientific, Eugene, OR) for 16h, then were washed thoroughly. The cysts were then crushed in sterile deionized water, centrifuged for three minutes at 4000 rpm, the supernatant was removed, and the residuals were mixed with 40 µl of sterile deionized water. Three samples were taken from each aliquot. The stained and non-stained eggs were counted under a Lecia DMi8 fluorescent microscope (Lecia microsystems CMS GmbH, Wetzlar, Germany) equipped with a metal halide light (Lumen 200 Fluorescence Illumination Systems, Prior Scientific Inc., Rockland, MA, USA) source. Percentage egg viability was calculated as (non-stained eggs/ (stained eggs+ Non-stained eggs)) x 100.

Effect of S. alba SME or HBA combined with S. sisymbriifolium on G. pallida egg densities, hatch, viability, and reproduction in greenhouse experiments

Experiments were conducted under greenhouse conditions at the University of Idaho, Moscow, ID. *Globodera pallida* cysts were exposed to *Sinapis alba* SME (0, 4.48 t/ha) or

HBA (0, 0.12 t/ha), which was followed by *Solanum sisymbriifolium* or bare soil treatment in Terra cotta clay pots (10-cm diameter) (The Home Depot, Atlanta, GA). Two nylon mesh bags with 20 *G. pallida* cysts (305 eggs/ cyst; inoculation density of 10 eggs/ g soil) were placed on top of 300g of autoclaved 2:1 soil mix, and an additional 150g of soil mix was added to cover the mesh bags. Then, a band of *S. alba* SME or HBA at the appropriate rates was thoroughly applied to the pots, followed by a 150 g soil mix added on top. Each pot was then watered with 75 ml of water to activate the treatments. For trial 1, cysts were treated with HBA and *S. alba* SME for two weeks. *Solanum sisymbriifolium* (4-wk-old, approximately 8 cm ht.; 1 plant/pot) was transplanted or left fallow for each treatment. We found a phytotoxic effect on transplants after SME treatment, possibly because of the residual ionic thiocyanate (SCN^-), a sinalbin metabolite produced after enzymatic hydrolysis (Hansson et al. 2008). Therefore, for trials 2 and 3, *S. sisymbriifolium* was planted after four weeks rather than two weeks post SME or HBA application. Additionally, the pots were rinsed with 100 ml of water every other day to leach out the residual SCN^-, two-week-post treatment of HBA or *S. alba* SME to avoid phytotoxicity. After four weeks, for each treatment, half of the pots remained fallow while *Solanum sisymbriifolium* was transplanted in the remaining. Pots were watered daily with 50 ml of water and fertilized using 20N-20P-20K all-purpose fertilizer (J.R. Peters Inc., Allentown, PA) weekly as per label instructions. Six replicates for each treatment were arranged in a split-plot design where *S. sisymbriifolium* or bare soil had been allocated as the main plots and different rates of *S. alba* SME or HBA as randomized split plots. After 12-wk-growth, all the aboveground plant material was removed, and one mesh bag containing cysts was retrieved from each pot. The retrieved cysts were surface-sterilized and used to determine egg densities, egg hatch, and egg viability. Pots containing the remaining nylon mesh bag were transferred to a cold room and maintained at 4^o C for eight weeks (Perry and Gaur 1996; Perry and Moens 2011) before conducting a reproduction assay. This experiment was repeated.

Egg count, egg hatch, and egg viability: The effect of treatments on *G. pallida* egg densities was assessed by enumerating the remaining encysted eggs. Eggs were released from three cysts in 300 µl sterile deionized water, and 100µl of aliquot was dispensed into each of three wells in 96 well-plates. Then, the number of encysted eggs was counted in each well under a

stereomicroscope (Lecia Microsystems). Finally, egg hatch and viability were determined as described previously in the hatching and viability assay of the laboratory experiment.

Reproduction assay: Potato bioassays were conducted to determine the effect of treatments on *G. pallida* multiplication by growing on the susceptible potato cultivar 'Russet Burbank' under greenhouse conditions. One 4-wk-old tissue culture potato plantlet was transplanted in each Terra cotta clay pots (10-cm diameter) and allowed to grow for 12 weeks. Plants were watered and fertilized as described previously for *S. sisymbriifolium*. After 12 weeks, the bioassay was terminated by removing the aboveground plant parts. The soil in each pot was dried prior to extracting cysts using an elutriator (USDA-APHIS 2009). Cysts per pot were enumerated under a Lecia S6E stereomicroscope, and egg counts (as described previously) were determined for the new generation of cysts. The final egg population (P*f*) was calculated as the number of progeny eggs per gram of soil. For treatments with the new generation of cysts, the reproduction rate (R*f)* was calculated as final egg population (P*f*) / initial egg population (P*i*).

Data Analysis

Data were analyzed by analysis of variance (ANOVA) using a generalized linear mixed model (GLIMMIX) statement for egg count, hatch, and viability in Statistical Analysis Software (SAS) (SAS Institute Inc., Cary, NC). In addition, a general linear model (GLM) with log transformation to ensure normal distribution and constant variation was used for reproduction data analysis. Data were considered significantly different at $P \leq 0.05$.

Results

Effect of S. alba seed meal or seed meal extract on G. pallida hatch and viability in laboratory experiments

In the laboratory experiments to determine the effect of *S. alba* seed meal or SME on *G. pallida* percentage egg hatch and viability, a similar result was observed across trials (Table 1 and 2). Both seed meal and SME led to significantly higher *G. pallida* egg hatch in the presence of PRD compared to non-treated control ($P \leq 0.05$). No significant hatch with both treatments was noticed in BSD compared to non-treated control regardless of pretreatment with *S. alba* seed meal or SME ($P > 0.05$). Egg hatch in PRD was significantly higher than BSD ($P < .0001$). However, egg viability was significantly reduced for both *S. alba* seed meal and SME compared to the non-treated control. The decrease in viability indicates the number of A.O.-stained eggs was greater with *S. alba* seed meal or seed meal extract treatment than non-treated control ($P < .0001$).

Table 2.1 Effect of *Sinapis alba* seed meal or seed meal extract (SME) on *Globodera pallida* hatch and viability in laboratory trial 1

Treatment	**Egg hatch (%)**		**Egg viability (%)**
	PRD	**BSD**	
Nontreated control	33.14 ± 1.6 B[y]	3.6 ± 0.6 C	62.07 ± 0.6 A
1.8 t/ha *S. alba* SME	40.85 ± 1.7 A	3.8 ± 0.7 C	51.36 ± 0.6 B
4.48 t/ha *S. alba* seed meal	39.71 ± 1.7 A	4.5 ± 0.7 C	52.04 ± 0.6 B

[y]Values ± standard errors are the average of six replicates. Values for egg hatch (%), and egg viability (%) followed by a common letter are not significantly different ($P \leq 0.05$).

In trials 1 and 2, a lower rate of SME with sinalbin concentration of 464 µmol/g, 1.8 t/ha, resulted in no significant difference in percentage hatch ($P = 0.67$) in PRD or viability (%) ($P = 0.45$) compared to seed meal with a sinalbin concentration of 190 µmol/g at 4.48 t/ha.

Compared to non-treated control, treatment with SME or seed meal enhanced hatch by 23 % (*P = 0.0095*) or 20 % (*P = 0.0223*) in trial 1 and 21% (*P = 0.0254*) or 20% (*P = 0.0329*) in trial 2, respectively in PRD.

Table 2.2. Effect of *Sinapis alba* seed meal or seed meal extract (SME) on *Globodera pallida* hatch and viability in laboratory trial 2

Treatment	**Egg hatch (%)**		**Egg viability (%)**
	PRD	**BSD**	
Nontreated control	30.83 ± 1.8 B[y]	4.3 ± 0.7 C	60.17 ± 1.9 A
1.8 t/ha *S. alba* SME	37.32 ± 1.9 A	5.6 ± 0.9 C	47.89 ± 1.9 B
4.48 t/ha *S. alba* seed meal	36.96 ± 1.9 A	5.1 ± 0.9 C	49.39 ± 1.9 B

[y]Values ± standard errors are the average of six replicates. Values for egg hatch (%), and egg viability (%) followed by a common letter are not significantly different ($P \leq 0.05$).

Effect of S. alba SME or HBA combined with S. sisymbriifolium on G. pallida egg densities, hatch, viability, and reproduction in greenhouse experiments

In the greenhouse trials, no significant differences were found in the number of encysted eggs when treated with *S. alba* SME or HBA alone compared to the non-treated control ($P > 0.05$), which indicates these treatments did not stimulate egg hatch by themselves. Similar to the laboratory experiments, *S. alba* SME or HBA significantly enhanced egg hatch in PRD and reduced viability (%) under greenhouse conditions compared to non-treated control ($P \leq 0.05$). As observed in laboratory experiments, the effect of treatments on *G. pallida* egg hatch when exposed to BSD ($P > 0.05$) was not significantly different than the non-treated control. However, the number of encysted eggs, hatch (in PRD), viability, and reproduction were significantly reduced with *S. sisymbriifolium,* whether as an individual treatment or combined with *S. alba* SME or HBA compared to the non-treated control ($P \leq 0.05$). Encysted egg counts, hatch, and viability were further significantly reduced with *S. sisymbriifolium* after the cysts were pretreated with HBA or *S. alba* SME than *S. sisymbriifolium* alone ($P \leq 0.05$). No significant differences were observed in the number of

encysted eggs, hatch, and viability with *S. sisymbriifolium* when combined with HBA or *S. alba* SME (*P > 0.05*) across trials 2 and 3.

Table 2.3. Effect of *Sinapis alba* seed meal extract (SME) or 4-hydroxybenzyl alcohol (HBA) combined with *Solanum sisymbriifolium* on *Globodera pallida* egg count, hatch, and viability in greenhouse trial 1

Treatment	**Eggs per cyst**	**Egg hatch (%)**		**Egg viability (%)**
		PRD	**BSD**	
Nontreated control	269.89 ± 6.71 A[y]	27.88 ± 1.02 B	3.96 ± 0.44 E	54.77 ±1.2 A
HBA	266.89 ± 6.67 A	38.34 ± 1.11 A	4.64 ± 0.48 E	42.99 ±1.19 B
S. alba SME	256.72 ± 6.54 A	36.36 ± 1.1 A	4.76 ± 0.49 E	43.34 ±1.19 B
Solanum sisymbriifolium (SS)	72.28 ± 3.47 B	17.18 ± 0.86 C	3.58 ± 0.42 E	33.85 ±1.13 C
HBA+ SS	35.22 ± 2.42 C	13.65 ± 0.78 D	3.87 ± 0.44 E	24.60 ±1.01 D
S. alba SME + SS	77.33 ± 3.59 B	18.67 ± 0.89 C	4.54 ± 0.48 E	26.73 ±1.04 D

[y]Values ± standard errors are the average of six replicates. Values for egg count, egg hatch (%), and egg viability (%) followed by a common letter are not significantly different (*P ≤ 0.05*).

In trial 1, the number of encysted eggs (*P = 0.28)*, hatch in PRD (*P = 0.21)*, and viability (*P = 0.82)* (%) with HBA treatment were not significantly different compared to the *S. alba* SME. Compared to the non-treated control, *S. alba* SME or HBA significantly increased the percentage hatch in PRD by 37 (*P < 0.0001)* and 35% (*P < 0.0001)*, respectively. Compared to non-treated control, *S. sisymbriifolium* significantly reduced number of encysted eggs, hatch, and viability (%) by 73 (*P < 0.0001)*, 38 (*P < 0.0001)* and 38% (*P < 0.0001)*. *Solanum sisymbriifolium* when combined with HBA significantly reduced the number of

encysted eggs, hatch, and viability (%) by 87 (*P < 0.0001)*, 51 (*P < 0.0001*), and 55 % (*P < 0.0001)* compared to non-treated control and 51 (*P < 0.0001)*, 20 (*P = 0.0038)*, and 27% (*P < 0.0001)* compared to *S. sisymbriifolium* treatment alone, respectively. *Solanum sisymbriifolium*, following the two-week *S. alba* SME treatment, did not significantly reduce egg count and hatch compared to *S. sisymbriifolium* alone. However, when combined with HBA, egg count and hatch was significantly lower than *S. sisymbriifolium* individual treatment. Moreover, % viability was significantly reduced for the combination compared to *S. sisymbriifolium* alone.

Table 2.4. Effect of *Sinapis alba* seed meal extract (SME) or 4-hydroxybenzyl alcohol (HBA) combined with *Solanum sisymbriifolium* on *Globodera pallida* reproduction in greenhouse trial 1

Treatment	**Cyst per pot (progeny)**	**Eggs per cyst (progeny)**	***Pf***	***Pf/Pi*** [*]
Nontreated control	35.17 ± 2.36 A[y]	199.61 ± 8.45 A	11.64 ± 0.56 A	2.33 ± 0.11A
HBA	37 ± 2.44 A	198.5 ± 8.35 A	12.4 ± 1.27 A	2.48 ± 0.25 A
S. alba SME	34.50 ± 1.88 A	193.89 ± 6.73 A	11.18 ± 0.62 A	2.24 ± 0.12 A
Solanum sisymbriifolium (SS)	3.17 ± 1.56 B	66.33 ± 30.13 B	0.72 ± .37 B	0.14 ± 0.07 B
HBA+ SS	0 C	0 C	0 C	0 B
S. alba SME + SS	1.33 ± 0.88 BC	42.06 ± 26.7 BC	0.28 ± 0.18 BC	0.06 ± 0.04 B

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

[*]Initial population for reproduction assay (*Pi*) = 5 eggs per gram soil.

In trial 1, the results from the reproduction assay followed the same trend as hatching and viability assays. No significant difference was found in reproduction following *S. alba* or HBA treatment when applied alone compared to the non-treated control. However, the number of cysts per pot, eggs per cysts, and final population (*Pf*) with *S. sisymbriifolium* alone was significantly reduced compared to the non-treated control by 91 ($P < 0.0001$), 67, and 94%, respectively. Similarly, the reproduction factor (R*f*, *Pf/Pi*) was significantly reduced by 94% compared to non-treated control for *S. sisymbriifolium* treatment alone. Following HBA pretreatment, reproduction was eliminated in *S. sisymbriifolium.* However, no significant difference was observed with *S. sisymbriifolium* treatment alone compared to the combination with *S. alba* SME.

Table 2.5. Effect of *Sinapis alba* seed meal extract (SME) or 4-hydroxybenzyl alcohol (HBA) combined with *Solanum sisymbriifolium* on *Globodera pallida* egg count, hatch, and viability in greenhouse trial 2

Treatment	**Egg count**	**Egg hatch (%)**		**Egg viability (%)**
		PRD	**BSD**	
Nontreated control	266.59 ± 10.80 A[y]	26.09 ± 0.84 B	2.61 ± 0.27 E	51.95 ± 0.88 A
HBA	269.97 ± 10.91 A	34.09 ± 0.90 A	1.91 ± 0.26 E	46.52 ± 0.88 B
S. alba SME	253.20 ±10.36 A	33.66 ± 0.90 A	1.74 ± 0.25 EF	46.52 ±0.88 B
Solanum sisymbriifolium (SS)	85.83 ±4.67 B	19.60 ± 0.76 C	1.62 ± 0.24 EF	26.29 ±0.78 C
HBA+ SS	32.69 ± 2.55 C	16.19 ± 0.70 D	0.99 ± 0.19 G	9.51± 0.52 D
S. alba SME + SS	31.56 ± 2.50 C	15.86 ± 0.70 D	1.11 ± 0.20 FG	9.21± 0.51 D

[y]Values ± standard errors are the average of six replicates. Values for egg count, egg hatch (%), and egg viability (%) followed by a common letter are not significantly different ($P \leq 0.05$).

In trial 2, *G. pallida* egg hatch in PRD, and viability ($P = 0.89$) when treated with HBA was not significantly different than *S. alba* SME. *Sinapis alba* SME or HBA significantly increased the percentage hatch in PRD by up to 30% compared to the control, respectively. However, *S. sisymbriifolium* alone reduced the number of encysted eggs, hatch, and viability by 68($P < 0.0001$), 25, and 50 ($P < 0.0001$), respectively, compared to the non-treated control. *S. sisymbriifolium*, when combined with *S. alba* or HBA, further significantly reduced egg count, hatch, and viability by up to 88 ($P < 0.0001$), 39 and 82 ($P < 0.0001$), compared to non-treated control and 63 ($P < 0.0001$), 19 and 65 ($P < 0.0001$) compared to *S.*

sisymbriifolium alone. No significant differences in the number of encysted eggs ($P = 0.75$), hatch, and viability ($P = 0.68$) were observed when *S. sisymbriifolium* was combined with HBA or *S. alba* SME.

Table 2.6. Effect of *Sinapis alba* seed meal extract (SME) or 4-hydroxybenzyl alcohol (HBA) combined with *Solanum sisymbriifolium* on *Globodera pallida* reproduction in greenhouse trial 2

Treatment	**Cyst per pot (progeny)**	**Eggs per cyst (progeny)**	***Pf***	***Pf/Pi****
Nontreated control	41.67 ± 1.15 A[y]	193.72 ± 7.4 A	13.45 ± 0.9 A	2.69 ± 0.18 A
HBA	45.17 ± 3.18 A	208.78 ± 12.08 A	15.74 ± 1.61 A	3.15 ± 0.32 A
S. alba SME	46 ± 2 A	193.89 ± 6.73 A	14.65 ± 1.01 A	2.93 ± 0.2 A
Solanum sisymbriifolium (SS)	2.5 ± 1.15 B	65.81 ± 29.49 B	0.55 ± 0.25 B	0.11 ± 0.05 B
HBA+ SS	0 C	0 C	0 C	0 B
S. alba SME + SS	0 C	0 C	0 C	0 B

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

*Initial population for reproduction assay (*Pi*) = 5 eggs per gram soil.

The number of progeny cyst per pot, progeny eggs per cyst, final population (*Pf*), and reproduction factor (*Pf/Pi*) following the treatment with *S. sisymbriifolium* alone was significantly reduced by 94 ($P < 0.0001$), 66 ($P < 0.0001$), 99 ($P < 0.0001$), and 96 % ($P < 0.0001$), respectively, compared to the non-treated control. *Solanum sisymbriifolium*, when combined with *S. alba* SME or HBA, eliminated *G. pallida* reproduction on potato by 100 %. The number of progeny cyst per pot ($P = 0.0007$), progeny eggs per cyst ($P = 0.0006$), final

population (*Pf*) (*P = 0.0053)* for the combination was significantly lower than *S. sisymbriifolium* alone.

Table 2.7. Effect of *Sinapis alba* seed meal extract (SME) or 4-hydroxybenzyl alcohol (HBA) combined with *Solanum sisymbriifolium* on *Globodera pallida* egg count, hatch, viability in greenhouse trial 3

Treatment	**Egg count**	**Egg hatch (%)**		**Egg viability (%)**
		PRD	**BSD**	
Nontreated control	236.67 ± 8.07 A[y]	22.93 ± 0.39 B	0.77 ± 0.18 E	51.02 ± 1.5 A
HBA	239.42 ± 8.13 A	31.07 ± 0.77 A	0.85 ± 0.08 E	45.2 ± 1.5 B
S. alba SME	246.03 ± 8.29 A	30.75 ± 0.70 A	0.95 ± 0.13 E	46.7 ± 1.5 AB
Solanum sisymbriifolium (SS)	63.73 ± 3.53 B	11.91 ± 1.76 C	0.77 ± 0.16 E	25.9 ± 1.3 C
HBA+ SS	29.48 ± 2.30 C	3.94 ± 0.56 D	0.25 ± 0.16 F	8.4 ± 0.8 D
S. alba SME + SS	28.65 ± 2.27 C	3.55 ± 0.57 D	0.19 ± 0.12 F	8.9 ± 0.9 D

[y]Values ± standard errors are the average of six replicates. Values for egg count, egg hatch (%), and egg viability (%) followed by a common letter are not significantly different ($P \leq 0.05$).

In trial 3, like trial 2, encysted eggs pretreated with *S. alba* SME or HBA had significantly increased hatch in PRD by up to 35% ($P < 0.0001$) than non-treated control. Compared to non-treated control, *S. sisymbriifolium* alone significantly reduced encysted egg count, hatch, and viability by 73 ($P < 0.0001$), 48 ($P < 0.0001$) and 49 ($P < 0.0001$) respectively, and by up to 88 ($P < 0.0001$), 85 ($P < 0.0001$) and 84 ($P < 0.0001$) when combined with *S. alba* or HBA pretreatment. Similarly, the combination further significantly reduced the number of encysted eggs, hatch, and viability by 55, 70 ($P < 0.0001$) and 68 % ($P < 0.0001$) compared

to *S. sisymbriifolium* alone. Potato bioassay for this trial to determine the impact of treatments in *G. pallida* multiplication is underway.

Discussion

This study investigated the impact of *S. alba* seed meal or SME or HBA on *G. pallida* egg hatch and viability. After two weeks of treatment with *S. alba* seed meal, SME, or HBA, *G. pallida* eggs showed enhanced hatch when exposed to PRD in our experiments. These treatments did not stimulate significant egg hatch in BSD across all trials, which indicates that these three treatments did not induce *G. pallida* egg hatch by themselves but required subsequent exposure to PRD to stimulate enhanced hatch. These results agree with a previous report where exposure to *S. alba* root diffusate required subsequent treatment with PRD to enable a faster G. *pallida* hatch rate than pretreatment with water (Forrest and Farrer 1983). Similarly, prior treatment with *S. alba* root diffusate enhanced *G. rostochiensis* egg hatch in the presence of tomato root diffusate compared to the cysts treated with tomato root diffusate only (Valdes et al. 2011). The hatching process of potato cyst nematodes in the presence of PRD appears to be ‘bimodal'; permeability changes in the eggshell lipid layer followed by metabolic activation of juveniles (Perry and Moens 2011). Since *S. alba* seed meal, SME, or HBA without PRD did not stimulate *G. pallida* egg hatch; we speculate that these pretreatments could have increased eggshell permeability sufficiently to readily activate juveniles in the presence of hatching factor found in PRD.

Interestingly, compared to non-treated control, viability (%) following these pretreatments was significantly reduced across all the experiments, as indicated by the increased numbers of A.O.-stained eggs (Pillai and Dandurand 2019). We suspect the decrease in egg viability may have been caused by the increase in eggshell permeability which allowed the stain to penetrate but not necessarily damage the J2 since the pretreatments did not show any nematicidal effect on *G. pallida* encysted eggs, as evidenced by subsequent PRD hatch.

Hatch of *G. pallida* in PRD after treatment with *S. alba* seed meal at the rate of 4.48 t/ha did not differ significantly from SME at 1.8 t/ha. This result is similar to a previous study, which showed *Brassica juncea* SME with application dose 50% less than seed meal

showed similar efficacy on *G. pallida* control (Dandurand et al. 2017). Thus, the lesser amount of *S. alba* SME required for efficacy could be beneficial due to ease in application, handling, transport, and storage.

In addition, we also designed experiments to test the efficacy of combining hatch enhancing ability of *S. alba* SME or HBA with the trap crop, *Solanum sisymbriifolium* instead of PRD for control of *G. pallida*. HBA has been described as the main sinalbin degradation product (Buskov et al. 2002; Popova et al. 2017). Therefore, we suspected HBA might be the compound responsible for observed hatch enhancement with *S. alba* SME. For our greenhouse experiment, we used HBA at the rate equivalent to HBA produced by 464 µmol/g sinalbin hydrolysis. Our results showed no significant differences in the hatch for *S. alba* SME (464 µmol/g sinalbin) or HBA treatment under the experimental conditions, which provides evidence for our speculation. Further experiments using different doses of HBA and SME under greenhouse and field conditions need to be done to verify these results further.

This study is the first report to test the efficacy of combining *S. alba* SME or HBA with trap crop *Solanum sisymbriifolium.* Our results show that *S. sisymbriifolium* individually or in combination led to fewer encysted eggs compared to the control, indicating that the trap crop stimulated *G. pallida* egg hatch. In addition, *S. sisymbriifolium* provided a reduction in egg densities similar to previous reports (Timmermans et al. 2007; Dandurand et al. 2019b). Twelve weeks of exposure to *S. sisymbriifolium* in our experiment showed a substantial reduction in the hatch and viability of remaining *G. pallida* eggs. Furthermore, the reproduction factor (*Pf/Pi*) on a susceptible potato cultivar was reduced by up to 99% after the *S. sisymbriifolium* treatment alone. These suppressive effects on *G. pallida* may be because of the nematicidal properties of biologically active glycoalkaloids found in *S. sisymbriifolium* (Dias et al. 2012; Sivasankara Pillai and Dandurand 2021). In the previous report, *G. pallida* reproduction following *S. sisymbriifolium* was reduced by 99% on susceptible potato under greenhouse conditions compared to potato-following-potato or fallow rotation and by 99-100% under Idaho field conditions compared to fallow treatment (Dandurand and Knudsen 2016; Dandurand et al. 2019b).

Combining the hatch-enhancing ability of *S. alba* or HBA with trap crop *S. sisymbriifolium* further reduces egg densities, hatch, viability, and reproduction. After the

pretreatments, the number of encysted eggs after S. sisymbriifolium was further significantly reduced than *S. sisymbriifolium* alone. This reduction in egg count indicates that more nematodes were hatched with the combination than with *S. sisymbriifolium* only. Similarly, the combination significantly decreased hatch and viability for the remaining eggs and eliminated reproduction on a susceptible potato cultivar. These suppressive effects indicated that the pretreatments presumably made *G. pallida* encysted eggs more susceptible to the trap crop qualities of *S. sisymbriifolium* treatment (Timmermans et al. 2007; Dias et al. 2012; Dandurand and Knudsen 2016; Dandurand et al. 2019b). *Globodera pallida* eggs were found more prone to desiccation following a 24h pretreatment with PRD than untreated eggs (Forrest and Farrer 1983). Osborne (1970) noticed *G. rostochiensis* hatch suppression in PRD following prior exposure to a mix of aldicarb and PRD than aldicarb alone. When in rotation with *S. sisymbriifolium* planted for only one season, the *G. pallida* population is anticipated to take 11 years to decrease to a density of 1 egg/g soil (Timmermans et al. 2006). Our findings demonstrate that combining the trap crop *S. sisymbriifolium* with *S. alba* SME or HBA can better suppress *G. pallida* populations in a single growing season and deplete the potential inoculum for the next cropping season.

Overall, our results show that under laboratory and greenhouse conditions, *S. alba* seed meal, SME, or HBA enhances *G. pallida* hatch only in the presence of PRD; alone, they do not stimulate hatch. The findings from our greenhouse experiments indicate that *S. sisymbriifolium* is an effective tool in *G. pallida* control. Moreover, combining the hatch-enhancing ability of *S. alba* SME or HBA with trap crop *S. sisymbriifolium* could provide a novel medium for integrated control of *G. pallida*. Further study to test this approach under field conditions should be done for future assessments.

Chapter 3: Control of *Globodera pallida* using *Brassica juncea* seed meal extract combined with 4-hydroxy benzyl alcohol

Introduction

Globodera pallida is a highly specialized obligate potato pest whose lifecycle is synchronized with its host to optimize the chances of successful invasion (Perry 1989). Without a suitable host, *G. pallida* remains dormant as encysted eggs in soil for 20 years or more (Evans and Stone 1977). Thus, the requirement for hatching stimulus by this nematode is almost absolute, and the potato root diffusate has a 'bimodal action' on *G. pallida* hatching, which includes an increase in eggshell permeability and metabolic activation of the juveniles (Perry and Moens 2011). However, even with the hatch stimulation, some viable eggs do not hatch readily but require restimulation. This allows the nematode to persist throughout the growing season and reduces competition among hatched juveniles or hatch in a subsequent season (Perry 1989).

The stringent hatching requirements, ability to adapt, and the loss of effective control strategies like methyl bromide fumigation increase the challenge for controlling *G. pallida* in Idaho. Therefore, there is a need for novel control strategies that can disrupt *G. pallida*'s lifecycle by interfering with the hatching and reproduction process. One potential alternative strategy is biofumigation using *Brassica juncea* seed meal extract (SME), a shelf-stable powder formulated from the seed meal (Popova et al. 2017). The nematicidal activity of SME is attributed to a volatile 2-propenyl isothiocyanate, produced by enzymatic hydrolysis of 2-propenyl glucosinolate (sinigrin) found in the SME (Popova et al. 2017; Dandurand et al. 2017). *Brassica juncea* SME, used as a biofumigant, suppressed hatch and reproduction of *G. pallida* under greenhouse and field conditions (Dandurand et al. 2017; Bhatta 2021). However, with *B. juncea* SME, rates of 1.1 t/ha or more were required for increased efficacy (Dandurand et al. 2017).

Our previous experiments from chapter 2 showed that *Sinapis alba* SME with predominant glucosinolate sinalbin, unlike *Brassica juncea* SME, enhances *G. pallida* egg hatch in the presence of PRD, possibly by increasing eggshell permeability. We also found 4-hydroxy benzyl alcohol (HBA), a sinalbin metabolite, has a similar effect on *G. pallida*. In

this study, we integrated the hatch enhancing ability of HBA with the biofumigant *B. juncea* SME. We hypothesized that combining HBA with *B. juncea* SME may increase eggshell permeability, which may amplify the nematicidal activity of isothiocyanate produced by hydrolysis of sinigrin found in *B. juncea* seed meal extract. Our objectives for this study were to i) test the effect of HBA on hatch stimulation and viability on *G. pallida* eggs under laboratory conditions, and ii) assess the efficacy of *Brassica juncea* SME on *G. pallida* egg hatch and viability when combined with HBA under laboratory and greenhouse conditions.

Materials and methods

Treatments used

The shelf-stable formulated *Brassica juncea* SME was obtained from Dr. Inna Popova at the University of Idaho, Moscow, ID (Popova et al. 2017). The concentration of sinigrin in the formulated SME used for all experiments was 360 µmol/g. 4-hydroxy benzyl alcohol (HBA) (Sigma-Aldrich, Inc., St. Louis, MO) was used for all the experiments.

Effect of HBA on G. pallida egg hatch and viability in the laboratory experiment.

The experimental set-up was similar to that described in chapter 2 to test the impact of *S. alba* seed meal or SME on *G. pallida* egg hatch and viability. Ten *G. pallida* cysts (with an average of 305 eggs/cyst for an inoculation density of 10 eggs/g soil) were exposed to 0, 4.48 t/ha (139 µmol/g), 2.24 t/ha (69.5 µmol/g), and 1.12 t/ha (34.75 µmol/g) HBA in sealed magenta GA-7 tissue culture vessel (Sigma Aldrich, St. Louis, MO). Cysts were enclosed in nylon mesh bags as described in Chapter 1. Six replicates for each treatment were arranged in a complete randomized design. Cysts were treated for two weeks with HBA, after which they were retrieved, surface-sterilized, and used for subsequent hatching and viability assay. The experiment was conducted twice.

Hatching and viability assay: For the hatching assay, eggs from three cysts were exposed to potato root diffusate (PRD) or bare soil diffusate (BSD) for two weeks. Then, the number of hatched J2s was counted for both PRD and BSD, and the percentage egg hatch was calculated as ((number of hatched J2 – number of initial J2)/number of initial eggs) x 100. For viability assays, three cysts were exposed to Acridine orange (A.O.) (25 µg/ml) for 16h,

and the number of stained and non-stained eggs were counted (Pillai and Dandurand 2019). The percentage egg viability was calculated as (non-stained eggs/ (stained eggs+ non-stained eggs)) x 100.

Effect of B. juncea SME when combined with HBA on G. pallida egg hatch and viability

Laboratory experiment:

Experiments were conducted under similar conditions and set-up as described in chapter one. Ten cysts enclosed in nylon mesh bags (an inoculation density of 10 eggs/g soil) were first exposed to 0 or 4.48 t/ha (139 µmol/g) for two weeks. After two weeks, four rates of *B. juncea* SME (0, 0.14, 0.56, and 1.12 t/ha) were applied for four weeks. The magenta vessels were sealed with a lid after applying 30-ml of sterile deionized water. Six replicates for each treatment were arranged in the spilt-plot design with HBA or bare soil as the main plot and split-plot as rates of *B. juncea* SME in the incubator. This experiment was conducted twice. After this, hatching and viability assays were performed as described above to determine the effect of HBA.

Greenhouse experiments:

To determine the effect of combining *B. juncea* SME (0, 0.14, 0.56, and 1.12 t/ha) with (0 or 4.48 t/ha) HBA, greenhouse experiments (conditions and set-up in 10-cm diameter Terra cotta clay pots as described in chapter 1 for greenhouse experiment to determine the effect of combining *S. alba SME* or HBA with *S. sisymbriifolium*) at the University of Idaho, Moscow, ID. Two nylon mesh bags filled with ten cysts on each mesh bag (initial population density ten eggs/g) were exposed to *B. juncea* SME treatment for four weeks before the two-week treatment with HBA. Six replicates of each treatment were placed on a greenhouse bench in a split-plot design with HBA or bare soil as the main plots and different rates of *B. juncea* SME as randomized split plots on a greenhouse bench. Following the treatments, one of the cyst bags was retrieved, surface-sterilized, and subsequent hatching and viability assays were conducted on them (as described in the previous experiments). This experiment was repeated.

Data Analysis

Statistical Analysis Software (SAS) (SAS Institute Inc., Cary, NC) was used to analyze the data with analysis of variance (ANOVA) with a generalized linear mixed model (GLIMMIX) statement for egg hatch and viability. Data were considered significantly different at $P \leq 0.05$.

Results

Effect of 4-hydroxy benzyl alcohol (HBA) on Globodera pallida egg hatch and viability in a laboratory experiment

In laboratory experiments to determine the impact of four different rates of HBA (0, 1.12, 2.24, and 4.48 t/ha) on *G. pallida* hatch and viability, trials 1 and 2 had similar trends. All the tested rates of HBA had significantly increased hatch when exposed to PRD ($P \leq 0.05$) than the non-treated control. Hatch with PRD was significantly enhanced with higher tested rates of HBA than lower rates ($P \leq 0.05$). However, the hatch in BSD was not significantly different irrespective of the HBA treatments ($P > 0.05$). Viability (%) was significantly reduced for all the doses of HBA compared to the non-treated control ($P \leq 0.05$). The reduction in viability (%) indicates that the number of stained eggs was increased by the HBA treatment (Pillai and Dandurand 2019).

In trial 1, HBA with rates 1.12, 2.24, and 4.48 t/ha significantly increased hatch by 16 % ($P = 0.0129$), 40 % ($P < 0.0001$), and 72% ($P < 0.0001$) and in trial 2 by 12 % ($P = 0.0129$), 33 % ($P < 0.0001$), and 68% ($P < 0.0001$) with PRD compared to the non-treated control. HBA at the rate of 4.48 t/ha significantly ($P < 0.0001$) enhanced hatch in PRD by 49 %, and 23% more than the rates of 1.12 and 2.24 t/ha of HBA, respectively. Similarly, HBA at the rate of 2.24 t/ha significantly increased *G. pallida* egg hatch by 22% compared to 1.12 t/ha in both trials 1 ($P = 0.0003$) and 2 ($P < 0.0001$). In trial 2, hatch in HBA at the rate of 4.48 t/ha was significantly ($P < 0.0001$) enhanced by 50 % and 26 %compared to the rates of 1.12 and 2.24 t/ha, respectively.

Table 3.1. Effect of 4-hydroxybenzyl alcohol (HBA) on *Globodera pallida* egg hatch and viability laboratory trial 1

Treatment	**Egg hatch (%)**		**Egg viability (%)**
	PRD	**BSD**	
Nontreated control	32.21 ± 1.32 A	4.54 ± 0.59 FG	60.72 ± 1.57 A
1.12 t/ha HBA	37.21 ± 1.47 B	5.92 ± 0.67 EF	55.31 ± 1.61 B
2.24 t/ha HBA	45.22 ± 1.40 C	3.60 ± 0.52 G	52.18 ± 1.61 BC
4.48 t/ha HBA	55.50 ± 1.41 D	7.68 ± 0.77 E	47.40 ± 1.60 C

[y]Values ± standard errors are the average of six replicates. Values within egg hatch (%) and viability (%) followed by a common letter are not significantly different ($P \leq 0.05$).

Table 3.2. Effect of 4-hydroxybenzyl alcohol (HBA) on *Globodera pallida* egg hatch and viability laboratory trial 2

Treatment	**Egg hatch (%)**		**Egg viability (%)**
	PRD	**BSD**	
Nontreated control	30.89 ± 0.94 A	8.81 ± 0.57 G	58.59 ± 0.50 A
1.12 t/ha HBA	34.59 ± 0.97 B	9.66 ± 0.60 FG	51.03 ± 0.50 B
2.24 t/ha HBA	41.02 ± 1.00 C	11.28 ± 0.64 EF	47.65 ± 0.50 C
4.48 t/ha HBA	51.79 ± 1.02 D	12.06 ± 0.66 E	43.97 ± 0.50 D

[y]Values ± standard errors are the average of six replicates. Values within egg hatch (%) and viability (%) followed by a common letter are not significantly different ($P \leq 0.05$).

Effect of Brassica. juncea seed meal extract (SME) when combined with HBA on Globodera pallida egg hatch and viability under laboratory and greenhouse experiments

Under both laboratory and greenhouse conditions, HBA at a rate of 4.48 t/ha significantly ($P \leq 0.05$) increased *G. pallida* egg hatch in PRD but reduced viability (%) compared to the non-treated control. As expected, the hatch in BSD did not differ significantly among the treatments used ($P > 0.05$). However, *B. juncea* SME at all the tested rates significantly reduced egg hatch (PRD) and viability (%) ($P \leq 0.05$) compared to the non-treated control. Furthermore, under laboratory conditions, *B. juncea* at the tested rates 0.56 t/ha or higher after HBA pretreatment further significantly reduced hatch (PRD) and viability (%) compared to those of *B. juncea* SME alone ($P \leq 0.05$). Similarly, under greenhouse conditions, combination at all the tested rates of *B. juncea* SME further significantly reduced hatch (PRD) and viability (%) ($P \leq 0.05$).

Table 3.3. Effect of *Brassica juncea* seed meal extract (SME), when combined with 4-hydroxybenzyl alcohol (HBA) on *Globodera pallida* egg hatch and viability for laboratory trial 1

Treatment	**Egg hatch (%)**		**Egg viability (%)**
	PRD	**BSD**	
Nontreated control	29.92 ± 0.90 B[y]	6.04 ± 0.47 FG	62.43 ± 0.79 A
HBA	48.82 ± 1.00 A	7.52 ± 0.52 F	46.56 ± 0.81 BC
0.14 t/ha *B. juncea* SME	21.56 ± 0.80 C	5.42 ± 0.44 FG	48.49 ± 0.82 B
0.14 t/ha *B. juncea* SME + HBA	19.82 ± 0.78 C	4.88 ± 0.42 G	45.81 ± 0.81 CD
0.56 t/ha *B. juncea* SME	15.69 ± 0.71 D	4.81 ± 0.42 FG	43.82 ± 0.81 C
0.56 t/ha *B. juncea* SME + HBA	12.52 ± 0.65 E	4.12 ± 0.39 FG	39.60 ± 0.80 E
1.12 t/ha *B. juncea* SME	12.42 ± 0.65 E	5.00 ± 0.42 G	40.91 ± 0.81 E
1.12 t/ha *B. juncea* SME + HBA	8.38 ± 0.54 F	3.97 ± 0.38 G	35.85 ± 0.78 F

[y]Values ± standard errors are the average of six replicates. Values within the same column for egg hatch (%) and viability (%) followed by a common letter are not significantly different ($P \leq 0.05$).

Laboratory experiment:

HBA significantly increased hatch (PRD) by 63 % and 68 % in trials 1 and 2, respectively, compared to the non-treated control. In both trials, *B. juncea* SME at the rates 0.14, 0.56, and 1.12 significantly reduced hatch (PRD) ($P < 0.0001$) by up to 28 %, 47 %, and 59 %, respectively, compared to the non-treated control. Similarly, egg viability ($P <$

0.0001) was reduced by 22 %, 30 %, and 34 %. Moreover, when *B. juncea* SME at rates 0.56 and 1.12 t/ha was combined with HBA pretreatment, there was a further reduction in hatch (*P < 0.0001*) by up to 23 %, and 48% than these rates of *B. juncea* SME alone across both trials, respectively. No significant differences were observed in hatch (*P = 0.9123*) and viability (*P = 0.2544*) for combination at the rate of 0.56 t/ha *B. juncea* SME compared to 1.12 t/ha of *B. juncea* SME alone.

Table 3.4. Effect of *Brassica juncea* seed meal extract (SME), when combined with 4-hydroxybenzyl alcohol (HBA) on *Globodera pallida* egg hatch and viability for laboratory trial 2

Treatment	**Egg hatch (%)**		**Egg viability (%)**
	PRD	**BSD**	
Nontreated control	31.05 ± 1.41 B	5.84 ± 0.71 FG	64.14 ± 0.86 A
HBA	52.43 ± 1.59A	7.84 ± 0.82 F	46.50 ± 0.90 C
0.14 t/ha *B. juncea* SME	23.71 ± 1.29 C	6.11 ± 0.73 FG	52.48 ± 0.90 B
0.14 t/ha *B. juncea* SME + HBA	20.45 ± 1.22 C	4.74 ± 0.64 G	47.43 ± 0.90 C
0.56 t/ha *B. juncea* SME	16.62 ± 1.13 D	6.07 ± 0.72 FG	45.14 ± 0.89 C
0.56 t/ha *B. juncea* SME + HBA	12.68 ± 1.01 E	5.89 ± 0.71 FG	40.48 ± 0.88 DE
1.12 t/ha *B. juncea* SME	12.53 ± 1.00 E	5.63 ± 0.69 G	41.16 ± 0.88 D
1.12 t/ha *B. juncea* SME + HBA	6.45 ± 0.74 F	5.33 ± 0.68 G	38.22 ± 0.87 E

[y]Values ± standard errors are the average of six replicates. Values within egg hatch (%) and viability (%) followed by a common letter are not significantly different (*P ≤ 0.05*).

Greenhouse experiment

Under greenhouse conditions, HBA significantly enhanced *G. pallida* hatch (PRD) (*P < 0.0001*) by up to 56 % in trial 1 and 46% in trial 2 compared to non-treated control. *Brassica juncea* SME treatment alone at the rates 0.14, 0.56, and 1.12 t/ha significantly reduced hatch (PRD) by 25 % (*P = 0.0010*), 48 % (*P < 0.0001*), and 63 % (*P < 0.0001*) in trial 1 and at *P < 0.0001* by 19 %, 36 %, and 58 % in trial 2, respectively, compared to the non-treated control. Similarly, for the rates 0.14, 0.56, and 1.12 t/ha of *B. juncea* SME viability (%) was significantly decreased by 7 % (*P = 0.0003*), 21 % (*P < 0.0001*), and 37 % (*P < 0.0001*) in trial 1 and 13 %, 23 %, and 39% in trial 2 at *P < 0.0001*, respectively. Compared to treatments of *B. juncea* SME alone at rates 0.14, 0.56, and 1.12 t/ha, SME at these rates when combined with HBA significantly reduced hatch (PRD) by 17% (*P = 0.0465*), 30% (*P = 0.0049*), and 37% (*P = 0.0046*) in trial 1 and 25%, 36%, and 59% in trial 2 at *P < 0.0001*, respectively. Also, there was no significant differences found in hatch (PRD) for combination of *B. juncea* SME at rate 0.14 t/ha with 0.56 t/ha *B. juncea* SME alone (*P = 0.1194*), and 1.12 t/ha of *B. juncea* SME individual treatment with 0.56 t/ha *B. juncea* SME treatment when combined with HBA (*P = 0.9248*).

Table 3.5. Effect of *Brassica juncea* seed meal extract (SME), when combined with 4-hydroxybenzyl alcohol (HBA) on *Globodera pallida* egg hatch and viability for greenhouse trial 1

Treatment	**Egg hatch (%)**		**Egg viability (%)**
	PRD	**BSD**	
Nontreated control	25.45 ± 1.35 B	1.17 ± 0.33 G	50.22 ± 0.62 A
HBA	39.65± 1.52 A	1.17± 0.33 G	43.53 ± 0.62 C
0.14 t/ha *B. juncea* SME	19.17 ± 1.22 C	1.33 ± 0.35 G	46.69 ± 0.62 B
0.14 t/ha *B. juncea* SME + HBA	15.79 ± 1.13 D	1.15 ± 0.33 G	43.95 ± 0.62 C
0.56 t/ha *B. juncea* SME	13.35 ± 1.06 D	1.19 ± 0.34 G	39.61 ± 0.61 D
0.56 t/ha *B. juncea* SME + HBA	9.3 ± 0.90 E	1.62 ± 0.40 G	34.90 ± 0.59 E
1.12 t/ha *B. juncea* SME	9.42 ± 0.91 E	099 ± 0.31 G	31.44 ± 0.58 F
1.12 t/ha *B. juncea* SME + HBA	5.98 ± 0.74 F	1.01 ± 0.31G	27.04 ± 0.55 G

[y]Values ± standard errors are the average of six replicates. Values within egg hatch (%) and viability (%) followed by a common letter are not significantly different ($P \leq 0.05$).

Table 3.6. Effect of *Brassica juncea* seed meal extract (SME), when combined with 4-hydroxybenzyl alcohol (HBA) on *Globodera pallida* egg hatch and viability for greenhouse trial 2

Treatment	**Egg hatch (%)**		**Egg viability (%)**
	PRD	**BSD**	
Nontreated control	22.91 ± 0.73 B	1.03 ± 0.18G	50.89 ± 0.52 A
HBA	33.38 ± 0.82 A	1.02± 0.18 GH	44.77 ± 0.51 B
0.14 t/ha *B. juncea* SME	18.48 ± 0.68 C	0.9 ± 0.17 GH	43.93 ± 0.51 B
0.14 t/ha *B. juncea* SME + HBA	13.85 ± 0.6 D	0.5 ± 0.13 HI	39.38 ± 0.5 C
0.56 t/ha *B. juncea* SME	14.71 ± 0.62 D	0.8 ± 0.16 GH	38.78 ± 0.5 C
0.56 t/ha *B. juncea* SME + HBA	9.38 ± 0.51 E	0.4 ± 0.11 I	30.62 ± 0.48 D
1.12 t/ha *B. juncea* SME	9.51 ± 0.51 E	0.3 ± 0.1 I	31.09 ± 0.48 D
1.12 t/ha *B. juncea* SME + HBA	3.87 ± 0.34 F	0.2 ± 0.08 I	21.13 ± 0.42 E

[y]Values ± standard errors are the average of six replicates. Values within egg hatch (%) and viability (%) followed by a common letter are not significantly different ($P \leq 0.05$).

Discussion

This study explores the impact of different rates of HBA on hatch stimulation and viability of *G. pallida* eggs under laboratory conditions. We previously demonstrated in chapter 2 that *Sinapis alba* SME treated *G. pallida* encysted eggs when subsequently exposed to PRD had significantly enhanced *G. pallida* hatch. We found similar results with the HBA treatment both under laboratory and greenhouse conditions which showed increased hatch at all the tested rates in PRD compared to non-treated control, but not in BSD. Also, viability (%) was significantly reduced by HBA, similar to what we observed with *S. alba* SME or HBA treatment in Chapter 2.

Based on this observed hatch-enhancing ability of HBA, we also designed experiments under laboratory and greenhouse conditions to determine the efficacy of *B. juncea* SME on *G. pallida* egg viability and hatch when combined with HBA pretreatment compared to *B. juncea* SME treatment alone. Experiments under both laboratory and greenhouse conditions demonstrated that *B. juncea* SME independently at all the tested rates significantly reduced *G. pallida* egg to hatch and viability than non-treated control. These results agree with a previous report where *B. juncea* SME at the rates ≥ 1.1 t/ha in the greenhouse experiments suppressed egg hatch by up to 99% and eliminated *G. pallida* reproduction (Dandurand et al. 2017). Furthermore, *Brassica juncea* SME, when applied at a rate of 0.56 t/ha, eliminated *G. pallida* reproduction on susceptible potato cultivar (Bhatta 2021). This suppressive effect of *B. juncea* SME is attributed to the bioactive 2-propenyl isothiocyanate, the breakdown product of 2-propenyl GSL (Lazzeri et al. 1993; Buskov et al. 2002; Zasada and Ferris 2003).

In the laboratory experiment, when combined with HBA, *B. juncea* SME at the rates 0.56 or higher further suppressed *G. pallida* egg hatch and viability compared to those rates of SME alone. However, no significant difference in the hatch was noticed with the lowest application dose of SME when applied alone or in combination with HBA. Egg hatch and viability of *G. pallida* were significantly reduced under greenhouse conditions when HBA was integrated with *B. juncea* SME at all the tested rates than individual SME treatment. 4-Hydroxy benzyl alcohol (HBA) treatment could be increasing eggshell permeability sufficiently to readily allow isothiocyanate to enter the encysted eggs. However, *G. pallida* encysted eggs without hatching factors remain almost impervious to external factors allowing this nematode to survive in the soil for extended periods (Evans and Stone 1977). Therefore, the higher application dose of *B. juncea* SME individual treatment was required to suppress the *G. pallida* population (Dandurand et al. 2017). However, when this strategy was integrated with trap crop *Solanum sisymbriifolium,* a greater reduction on viable *G. pallida* population that remains after individual treatment and reduction in rates of *B. juncea* SME required for the efficacy was observed (Bhatta 2021). In our experiments, the combination at the lower application dose of *B. juncea* SME showed similar efficacy in reducing egg hatch as the corresponding higher rate. This finding suggests that the nematicidal activity of 2-

propenyl GSL found in *B. juncea* SME was pronounced even at a lower application dose when the encysted eggs were pretreated with HBA. The impact of this combination on *G. pallida* reproduction on a susceptible potato cultivar is yet to be determined.

Overall, our study confirms that HBA enhances *G. pallida* egg hatch only when subsequently exposed to PRD. Thus, integrating HBA with biofumigant *B. juncea* SME was more effective in suppressing *G. pallida* egg hatch and viability under laboratory and greenhouse conditions. However, we only tested one rate of HBA in combination with *B. juncea* SME. Therefore, a possible future direction would be to test combinations of HBA at rates lower than 4.48 t/ha. In addition, field experiments to test the efficacy of this integrated strategy need to be conducted.

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