

The Role of Cover Crops with Biofumigation Potential for the Suppression of Plant-Parasitic Nematodes in Vineyards

by
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Declaration

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Abstract

Plant-parasitic nematodes, consisting of a wide range of species, can cause severe economic losses in most agricultural food crops. *Meloidogyne* spp. (root-knot nematodes), *Criconeoides xenoplax* (ring nematode), *Xiphinema index* (dagger nematode) and *Pratylenchus* spp. (lesion nematodes) are some of the economically important plant-parasitic nematodes that pose a threat to viticulture and other perennial crops in South Africa. Worldwide there is ever-increasing pressure on pre-plant synthetic soil fumigants and post-plant nematicides. For sustainable nematode management, it is important to have a holistic approach; taking into consideration cultural, biological and chemical options as part of an integrated management approach.

Biofumigation has the potential to fit into such an integrated management system and previous research indicates the positive response on soil-borne diseases, nematodes and weeds. Biofumigation occurs where certain plant species, containing glucosinolates (GSL) in the vacuole of the plant cells, come into contact (after cell maceration), with the enzyme myrosinase (MYR) situated in the cytoplasm of the cell, to form active compounds such as isothiocyanate (ITC). When this green manure is applied to infested soil, the ITC has the potential to have a direct suppressive effect on the soil-borne pathogens and there is also an indirect effect that can be expected after green manure soil amendment, because microbial activity is enhanced in the soil. Brassicas are known to possess GSL and MYR in their cells and thus have the potential to be utilized as biofumigation crops. There are, however, differences in the potential within the Brassicaceae family, based on different types and concentrations of GSL present in the different species. To ensure effective biofumigation it is important to use the correct brassica species and have a good understanding of the factors that have a positive impact on the biofumigation action.

Laboratory bioassays were done to determine the potential of different cover crops to suppress *Meloidogyne javanica* and *C. xenoplax*, when applied as a green manure. The cover crops used for the bioassays included Oats (*Avena sativa* cv. Pallinup), White mustard (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente 199) and Nemat (*Eruca sativa* cv. Nemat). The plant material was cut into small pieces and mixed with sterilised soil inoculated with either *M. javanica* or *C. xenoplax*. Results from the bioassays showed a significant suppression of *M. javanica* by the three biofumigation species: White mustard, Caliente

199 and Nemat. These results supported previous research, indicating the nematode suppressing effect due to the biofumigation action of certain brassica crops. Canola did not have the same suppressing impact on the *M. javanica* and gave comparable results to the control, indicating that Canola is not a good biofumigation crop for *M. javanica* suppression. In terms of biofumigation effect oats did not differ significantly from the control or the three brassicas: White mustard, Caliente 199 and Nemat. In the bioassays done for *C. xenoplax* no significant differences were found between the green manure treatments and the control. These results indicate that the different crops tested, including the three well known biofumigation crops, did not suppress the *C. xenoplax* at the applied biomass concentrations used in the bioassay.

Crops can also be classified according to their host status for certain plant parasitic nematodes. Crop host trials were conducted to determine the crop host status of the five different cover crops, to *M. javanica* and *C. xenoplax*. The crops were planted in sterilised soil, inoculated with the latter plant-parasitic nematodes and left for 60 days, after which, a root gall index analysis was done for *M. javanica* and for 85 or 92 days after which *C. xenoplax* was extracted from the soil. All the crops evaluated had a significantly lower root gall index for *M. javanica* than the control. Nemat and Oats was classified as poor hosts for *M. javanica*. A visual inspection of the root systems of all the crops was performed to determine whether *M. javanica* managed to complete its lifecycle in the different root systems. On all root systems, *M. javanica* managed to form root galls and produce egg masses, from which (J2) juveniles emerged. This indicates that *M. javanica* did complete its lifecycle in the different root systems of the crops evaluated and that all the cover crops acted as hosts. The expression of the gall symptoms were, however, less severe on Nemat and Oats, compared to the others. In the *C. xenoplax* crop host trials, all except the Nemat treatment showed a significant difference, compared to the Tomato treatment, with lower *C. xenoplax* numbers being present in the other crops. The nematode numbers in the different crops, compared well with the control (only inoculated soil), indicating that the crops did not stimulate the reproduction of *C. xenoplax*. Canola had the lowest numbers of *C. xenoplax* present after the growing cycle and Caliente 199 also showed a declining trend.

In South Africa, the use of annual cover crops in vineyards is an established soil cultivation practice. In a field study, Oats, White mustard, Canola, Caliente 199 and Nemat were established in a vineyard as cover crops for three growing seasons (2009/10, 2010/11, 2011/12), and evaluated for

their biofumigation impact, as well as their host impact on the suppression of certain economically important plant-parasitic nematodes. Two cover crop management practices, namely mechanical incorporation (MC) into the top soil and chemical removal of the cover crop (CC) were applied to the different cover crops. Nematode samples were taken in the work row and in the vine row at different times to determine the nematode status. These periods were April/May, before planting the cover crops, as well as 0, 15, 30 and 60 days after the management practices were performed. The crop biomass, measured as dry matter production (DMP) in tons/ha, differed significantly between the different crops, but also showed substantial increases during the three cover crop growing seasons for most crops. During the three consecutive seasons, Canola (CC) and Caliente 199 (CC) showed a constant reduction in the *C. xenoplax* population in the vine row based on the 60 day analysis. This trend was also observed for the total plant-parasitic nematode population in the vine row for the three seasons, based on 60 day analysis. The same trend took place during the three-year trial period for all the different sampling periods (0, 15, 30 and 60 days). The results can be attributed to the host status of these crops and not primarily because of the biofumigation effect. Both the Canola (CC) and the Caliente (CC) had a substantial increase in DMP during the three growing seasons that might have played a role in this trend. White mustard (CC and MC) showed a significant increase in the *C. xenoplax* population in the vine row, over the three year period, based on the 60 day analysis. The same trend was found Nemat (CC) and weeds and nematicide (CC) measured at the same period. A positive result from the *Meloidogyne* sp. analysis was that there was no significant increase in *the Meloidogyne* sp. in the vine row during the three growing seasons based on the 60 day analysis. This trend was seen in all the different treatments. The results from this study opens the possibility to apply these cover crops as part of a crop rotation programme without expecting an increase in the *Meloidogyne* sp. population to occur in the vine row through time.

Uittreksel

Plantparasitiese nematodes, wat bestaan uit 'n wye verskeidenheid van spesies, kan lei tot ernstige ekonomiese verliese in die meeste landbou gewasse. *Meloidogyne* spp. (knopwortel nematode), *Criconemoides xenoplax* (ring nematode), *Xiphinema index* (dolk nematode) en *Pratylenchus* spp. (letsel nematode) is van dié belangrikste plantparasitiese nematodes wat 'n bedreiging inhou vir wingerd en ander meerjarige gewasse in Suid-Afrika. Wêreldwyd is daar tans toenemende druk op die uifasering van voor-plant chemiese grondberoking middels en so ook op na-uitplant nematisiede. Vir volhoubare nematode bestuur, is dit belangrik om 'n holistiese benadering te volg, in ag genome kulturele, biologiese en chemiese maatreëls as deel van 'n geïntegreerde benadering. Bioberoking het die potensiaal om deel uit te maak van so 'n geïntegreerde benadering en baie vorige navorsing bevestig hierdie positiewe reaksie, in terme van onderdrukking, wat bioberoking op grond-gedraagde siektes, nematodes en onkruid kan hê. Bioberoking kan beskryf word as die reaksie, wat plaasvind wanneer glukosinolaat (GSL), wat teenwoordig is in die vakuool van die plantselle, in kontak kom met die ensiem mirosinase (MYR), nadat selbreking plaasgevind het en die aktiewe verbinding isothiosianaat (ITC) en ander sekondêre metaboliete gevorm word. Wanneer hierdie groen plantmateriaal in die grond ingewerk word, kan 'n direkte onderdrukkings effek, as gevolg van die ITC, asook 'n indirekte onderdrukkings effek as gevolg van die stimulasie van mikrobe aktiwiteit, verwag word. Brassica gewasse is bekend daarvoor dat daar GSL en MYR in die plantselle teenwoordig is en hulle besit dus die potensiaal om ITC te vorm. Daar is egter verskille in hierdie potensiaal binne die Brassicaceae familie, wat gebaseer is op verskillende tipes en konsentrasies GSL. Die keuse van 'n brassica spesie is dus belangrik, tesame met 'n verskeidenheid van ander faktore, om optimale bioberoking te verseker.

Laboratorium biotoetse is gedoen om die bioberokings effek van verskillende dekgewasse op *Meloidogyne javanica* en *C. xenoplax*, wanneer dit aangewend word as groenbemesting, te bevestig. Die dekgewasse wat gebruik is sluit in: Hawer (*Avena sativa* cv. Pallinup), Wit mosterd (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente 199) en Nemat (*Eruca sativa* cv. Nemat). Die plantmateriaal is fyn opgesny en ingewerk in gesteriliseerde grond wat met onderskeidelik *M. javanica* en *C. xenoplax* geïnokuleer is. Resultate van die biotoetse vir *M. javanica* toon dat die drie gewasse; Wit mosterd, Caliente 199 en Nemat, wat alombekend is vir hul bioberoking potensiaal, 'n betekenisvolle onderdrukkings op *M. javanica* tot gevolg gehad het.

Hierdie biotoetse ondersteun vorige navorsing, waar effektiewe onderdrukking van sekere *Meloidogyne* spesies as gevolg van bioberoking verkry is. Die resultate dui ook aan dat Canola nie 'n goeie opsie is vir effektiewe bioberoking om *M. javanica* onderdrukking te verkry nie. Die Hawer behandeling het nie betekenisvol van die kontrole of van die ander bioberokings gewasse verskil nie. Daar is geen betekenisvolle verskille verkry tussen die kontrole en die ander gewasse tydens die *C. xenoplax* biotoetse nie. Die resultate dui aan dat die dekgewasse, insluitende die drie bekende bioberokings gewasse, nie *C. xenoplax* onderdruk teen die toegediende biomassa konsentrasies nie.

Gewasse kan ook geklassifiseer word op grond van hul gasheer status vir sekere nematode. Gasheer toetse is gedoen om die gasheer status van die verskillende dekgewasse vir *M. javanica* en *C. xenoplax* te bepaal. Dieselfde vyf verskillende dekgewasse is geplant in grond, wat vooraf onderskeidelik met *M. javanica* en *C. xenoplax* geïnkuleer is. Plante is gelos om vir 'n spesifieke periode te groei waarna 'n galindeks evaluasie is gedoen om die gasheer status vir *M. javanica* te bepaal en 'n nematode ontleding gedoen is om die gasheer status vir *C. xenoplax* te bepaal. In die *M. javanica* gasheer toetse was die galindeks van al die gewasse betekenisvol laer as die kontrole. Nemat kan geklassifiseer word as 'n swak gasheer vir *M. javanica* en het betekenisvol minder galle as al die ander gewasse, behalwe die Hawer, waarvan dit nie betekenisvol verskil het nie. Nemat pas dus goed in 'n dekgewas program waar die doel is om die *M. javanica* populasie te onderdruk tydens die groei van die gewas. 'n Visuele inspeksie van die wortelstelsels is ook gedoen ten einde te bepaal of die lewensiklus van *M. javanica* voltooi is. Wortelgalle en eiersakkies was teenwoordig in die wortels van al die verskillende gewasse en larwes het uit die eiers uitgebroei. Dit dui aan dat *M. javanica* daarin geslaag het om sy lewensiklus op al die dekgewasse suksesvol te voltooi. Daar was aansienlik minder eiersakke by Nemat en Hawer; wat hul swak gasheer status bevestig. In die biotoetse vir die gasheerstatus van *C. xenoplax* het al die gewasse, behalwe Nemat, betekenisvol laer *C. xenoplax* getalle, in vergelyking met die Tamatie behandeling, tot gevolg gehad. Die nematode getalle was soortgelyk aan die kontrole (slegs geïnkuleerde grond), waar geen gewas in medium geplant is nie, en dui dus aan dat die getalle op die verskillende gewasse nie vermeerder het nie. Die Canola behandeling het die laagste *C. xenoplax* getalle gehad, gevolg deur Caliente 199. Hierdie gewasse toon dus die meeste potensiaal om aangewend te word in 'n rotasie stelsel of dekgewas program, waar die doel is om die *C. xenoplax* populasie te onderdruk.

In Suid-Afrika is die aanwending van spesifieke eenjarige gewasse, as dekgewasse in wingerde, reeds 'n standaard praktyk met verskeie voordele. In veldproewe oor 'n tydperk van drie jaar (2009/10, 2010/11, 2011/12) is Hawer, Wit mosterd, Canola, Caliente 199 en Nemat aangeplant as dekgewasse in 'n wingerd proefperseel. Die doel van die veldproewe was om die effek van dekgewasse op die plantparasitiese nematodes, wanneer dit aangewend word as bioberokings gewasse, te bepaal. Die gasheer status van die gewasse is ook ondersoek om te bepaal wat die effek sal wees op die nematode getalle. Twee dekgewas bestuurspraktyke is toegepas; meganiese inwerk van die dekgewasse in die bogrond (MC) en chemiese beheer van die dekgewasse (CC) en nematode monsters is op verskillende tye in die werksry en in die wingerdry geneem. Hierdie periodes sluit in April/Mei, voor die vestiging van die dekgewasse en 0, 15, 30 en 60 dae nadat die bestuurspraktyk toegepas is. Die dekgewas se biomassa produksie is, op grond van die droë massa produksie (DMP), in ton/ha gemeet, wat betekenisvol verskil het vir die verskillende dekgewas. Daar het ook 'n duidelike toename in DMP plaasgevind oor die drie seisoene vir meeste gewasse. Gedurende die drie jaar periode het die Canola (CC) en Caliente 199 behandelings, gemeet 60 dae na die bestuurspraktyk, 'n konstante afname getoon in die *C. xenoplax* in die wingerd ry. Dieselfde tendens het ook voorgekom gedurende hierdie periode in die totale plantparasitiese nematodes teenwoordig in die wingerd ry. Daar is ook 'n geleidelike afnemende tendens in die *C. xenoplax* in die wingerd ry, oor die verskillende periodes 0, 15, 30 en 60 dae vir die drie opeenvolgende seisoene, waargeneem. Hierdie resultate kan primêr toegeskryf word aan die gasheer status van die dekgewasse, wat in die gasheer proewe as swak gasheer vir *C. xenoplax* aangetoon is. Nog 'n faktor wat hier 'n rol speel is die feit dat beide die Canola (CC) en die Caliente 199 (CC) 'n toename in DMP van meer as 2 ton, gedurende die drie jaar periode, gehad het; wat op sigself ook 'n bydraende rol kon speel. Wit mosterd (CC en MC) het oor die drie seisoene 'n betekenisvolle verhoging in die *C. xenoplax* populasie tot gevolg gehad, gebaseer op die 60 dae ontleding. Dieselfde tendens is ook opgemerk vir die ander behandelings, onder andere Nemat (CC) en die onkruid en aalwurmdoder (CC) behandeling. 'n Baie positiewe resultaat na afloop van die drie seisoene is die feit dat daar nie 'n betekenisvolle verhoging in die *Meloidogyne* sp. populasie in die wingerdry, op grond van die 60 dae onledings, plaasgevind het nie. Dit was ook die geval vir al die ander behandelings. Hierdie resultate ondersteun die moontlikheid om hierdie bioberokings gewasse deel te maak van 'n geïntegreerde dekgewas benadering, sonder om in die proses die *Meloidogyne* sp. in die wingerd ry te verhoog.

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CHAPTER 1

Cover crops with biofumigation properties for the suppression of plant-parasitic nematodes

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Abstract

Plant-parasitic nematodes are a problem in vineyards worldwide, with some species acting as vectors of grapevine soil-transmitted viruses. Global pressure on the use of soil-applied chemical nematicides has led to a search for new control options or for alternative methods for the suppression of plant-parasitic nematodes as part of integrated pest management. This paper provides valuable background information on the use of cover crops with biofumigation properties for the suppression of plant-parasitic nematodes in vineyards.

Introduction

High population densities of plant-parasitic nematodes cause an economically significant crop reduction in most agricultural crops, including grapevine production in South Africa. In Australia, it is estimated that nematodes might cause a 7% production loss in the grapevine industry (Stirling *et al.*, 1992) and in California the grape production losses as a result of *Meloidogyne* spp. (root-knot nematode) damage alone are estimated to be approximately 20% (Raski, 1986). In South Africa, plant-parasitic nematodes have a negative impact on the production of good quality and economically viable grapes.

There are 162 species of plant-parasitic nematodes from 35 different genera that have been identified from root and soil samples collected in vineyards (Lamberti, 1988). Plant-parasitic nematodes present in South African vineyards include *Criconemoides xenoplax* (Raski, 1952) Loof & De Grisse, 1989 (ring nematode), *Longidorus* spp. (needle nematode), *Meloidogyne* spp. (root-knot

nematode), *Paratrichodorus* spp. (stubby root nematode), *Pratylenchus* spp. (root lesion nematode), spiral nematodes from different genera, *Tylenchulus semipenetrans* Cobb 1913 (citrus nematode) and *Xiphinema* spp. (dagger nematode) (Addison & Fourie, 2007; Storey, 2007).

In the past few decades, producers relied heavily on chemical fumigation for the control of soil-borne pathogens (Gamliel *et al.*, 2000), using products such as dichlorodiphenyltrichloroethane (DDT), which has been withdrawn from the market and methyl bromide that is currently still used, but in the process of being withdrawn. Currently, the global focus on sustainability in the agricultural environment is increasing in order to produce healthy, safe and good-quality crops and food. This focus includes the implementation of 'integrated pest (including disease and weed) management' (IPM), 'integrated production of wine', 'sustainable farming', 'farming for the future' (Woolworths) and 'from field to fork' (European Food Safety Commission), to name a few.

Multinational agricultural companies seem to have a bigger drive towards the development and funding of alternative management tools that are more target-specific, have a lower impact on natural predators and the environment, and have a favourable toxicological profile. The focus is not limited to one specific crop or disease, but includes all the different crops, diseases, pests, weeds and nematodes. Research is also focusing on the development of alternative management practices, including cultural and biological control options (Akhtar & Mahmood, 1996).

In the process of identifying alternative, more environmentally friendly control options for the control of soil-borne plant pests and diseases, the interest in biofumigation has increased (Lazzeri *et al.*, 2004). The purpose of this review is to investigate the potential of cover crops with biofumigation properties for the suppression and control of plant-parasitic nematodes in South African vineyards.

Principles of chemical soil fumigation

The primary aim of soil fumigation is to suppress soil-borne problems such as diseases, nematodes and weeds, which might otherwise have a negative economic impact on the production of crops (Louvét, 1979). The first application of fumigation for the control of nematodes was recorded as early as the 1870s (Van Berkum & Hoestra, 1979). In the years after 1945, several soil fumigants reached the market, including products such as chloropicrin, methyl bromide, 1,3-dichloropropene, ethylene-dibromide, 1,2-dibromo-3-chloropropane and methyl isothiocyanate (ITC) (Lembright, 1990).

However, for soil fumigation to be effective in the control of soil-borne pest and diseases intensive research on the application rate and a sound knowledge of the soil and of the environmental conditions involved are required. It is also necessary to keep the secondary negative impacts of the use of this method on the soil in mind (Louvet, 1979). Soil fumigation should be used as part of a holistic programme that forms part of a long-term approach (Louvet, 1979). Products such as methyl bromide, chloropicrin and combinations of chloropicrin and 1,3-dichloropropene must be applied by trained pest control operators to lower of the risk involved in using fumigation products.

Fumigation of the soil is done before planting of seed or transplanting of seedlings to prevent a negative impact of the product on the crops planted. To increase the efficacy of soil fumigation, factors such as a knowledge of the crop involved, its correct seeding or planting date, the presence of soil-borne pests and diseases that might pose a problem on the specific crop involved, availability of cultivars with resistance to certain soil-borne pest and diseases and soil preparation should be taken in consideration before applying the product. Furthermore, knowledge of the pest or disease and its survival in the soil is also imperative for the success in fumigation (Louvet, 1979).

Principles of soil biofumigation

Biofumigation takes place when certain soil-borne pests and diseases are suppressed as a result of the biocidal activity of glucosinolate (GSL)-containing plants when they are incorporated into the soil (Kirkegaard *et al.*, 1993; 1998). The fumigant action of the volatile compounds that are released during the biodegradation of organic matter suppresses plant pathogens (Piedra Buena *et al.*, 2007).

GSLs (glucose- and sulphur-containing organic anions) and ITCs are the main active compounds involved in biofumigation. The first observations of the unique properties of GSLs and ITCs were recorded at the beginning of the 17th century during efforts that were made at the time to understand the reason for the sharp taste of mustard seeds (Challenger, 1959). GSLs are sulphur-containing secondary metabolites produced by certain crops that are hydrolysed by the enzyme myrosinase (MYR) to form ITCs, in a process that is known as the GL-MYR system (Wathelet *et al.*, 2004). The ITCs have a toxic effect on many soil-borne pathogens (Sarwar *et al.*, 1998). Breakdown products, including the active compound ITC, are released when the plant cell walls are damaged or broken during maceration of the plant biomass (Sarwar *et al.*, 1998; Wathelet *et al.*, 2004).

The role played by biofumigation in integrated pest management (IPM)

The positive biological activity of the GSL degradation products used for the suppression of some pathogenic fungi (Manici *et al.*, 1997) and nematodes (Lazzeri *et al.*, 1993) serves to open up new perspectives on IPM (Lazzeri *et al.*, 2004), because it has been proven to be effective against weeds, plant diseases and nematodes (Van Dam *et al.*, 2009). Numerous studies in literature confirmed the ability of certain plants to suppress nematodes through the nematicidal activity of the secondary metabolites (Chitwood, 2002; Zasada & Ferris, 2004). Research has furthermore proved that many *Brassica* spp. show nematicidal activity on such plant-parasitic nematode species as *M. incognita*, *M. javanica*, *Heterodera schachtii* and *Pratylenchus neglectus* (Thierfelder & Friedt, 1995; Potter *et al.*, 1998; Monfort *et al.*, 2007).

Plants containing GSL

The Family Brassicaceae contains more than 350 genera with 3 000 species of which many are known to contain GSL. However, GSLs are not confined to brassicas alone. At least 500 species of non-brassica dicotyledonous angiosperms have also been reported to contain one or more of the over 120 known GSLs (Fahey *et al.*, 2001). Each of the GSLs has its own chemical property and can be placed in one of three different classes, namely aliphatic, aromatic or indole forms (Zasada & Ferris, 2004; Padilla *et al.*, 2007).

Most GSL-containing genera, however, are clustered within the Brassicaceae, Capparaceae and Caricaceae families (Rodman, 1981). The GSL concentration in the cells of the various plants in the families differs substantially. Therefore, it is crucial to identify species that will be effective in suppressing soil-borne pests and diseases, including nematodes. Rotation crops tested for the presence of GSLs are provided in Table 1, which show that it is mostly the brassicas that contain GSLs and that different levels of GSL exist within different genera (Larkin & Griffin, 2007). The plant species that are therefore generally considered for biofumigation are found mostly in the family Brassicaceae and include *Brassica oleracea* (broccoli, cabbage, cauliflower, kale), *Brassica rapa* (turnip), *Raphanus sativus* (radish), *Brassica napus* (canola, rapeseed) and various mustards, such as *Sinapis alba* (White mustard) and *Brassica juncea* (Indian mustard) (Sarwar *et al.*, 1998; Ploeg, 2007).

Table 1.1. Relative glucosinolate content of selected rotation crops used in potato rotation systems.

Crop/Cultivar	Scientific name	Glucosinolate content
Oats	<i>Avena sativa</i>	None
Ryegrass - 'Lemtal'	<i>Lolium multiflorum</i>	None
Barley	<i>Hordeum vulgare</i>	None
Canola - 'Hyola 401'	<i>Brassica napus</i>	Low
Rapeseed - 'Dwarf Essex'	<i>Brassica napus</i>	Moderate
Turnip - 'Purple top'	<i>Brassica rapa</i>	Moderate
Radish (oilseed)	<i>Raphanus sativa</i>	Moderate
Yellow mustard - 'Ida Gold'	<i>Sinapis alba</i>	Moderate
Indian mustard (unknown)	<i>Brassica juncea</i>	High

(Adapted from Larkin & Griffin, 2007)

Four cultivars with biofumigation potential are currently commercially available in South Africa, namely Nemat (*Eruca sativa* cv. Nemat), White mustard (*Sinapis alba* cv. Braco), Caliente 199 (*Brassica juncea* cv. Caliente 199), and Canola (*Brassica napus* cv. AV Jade (Fig. 1.1). For the purpose of this paper, the agronomical aspects of these so-called 'biofumigation crops' will be discussed.

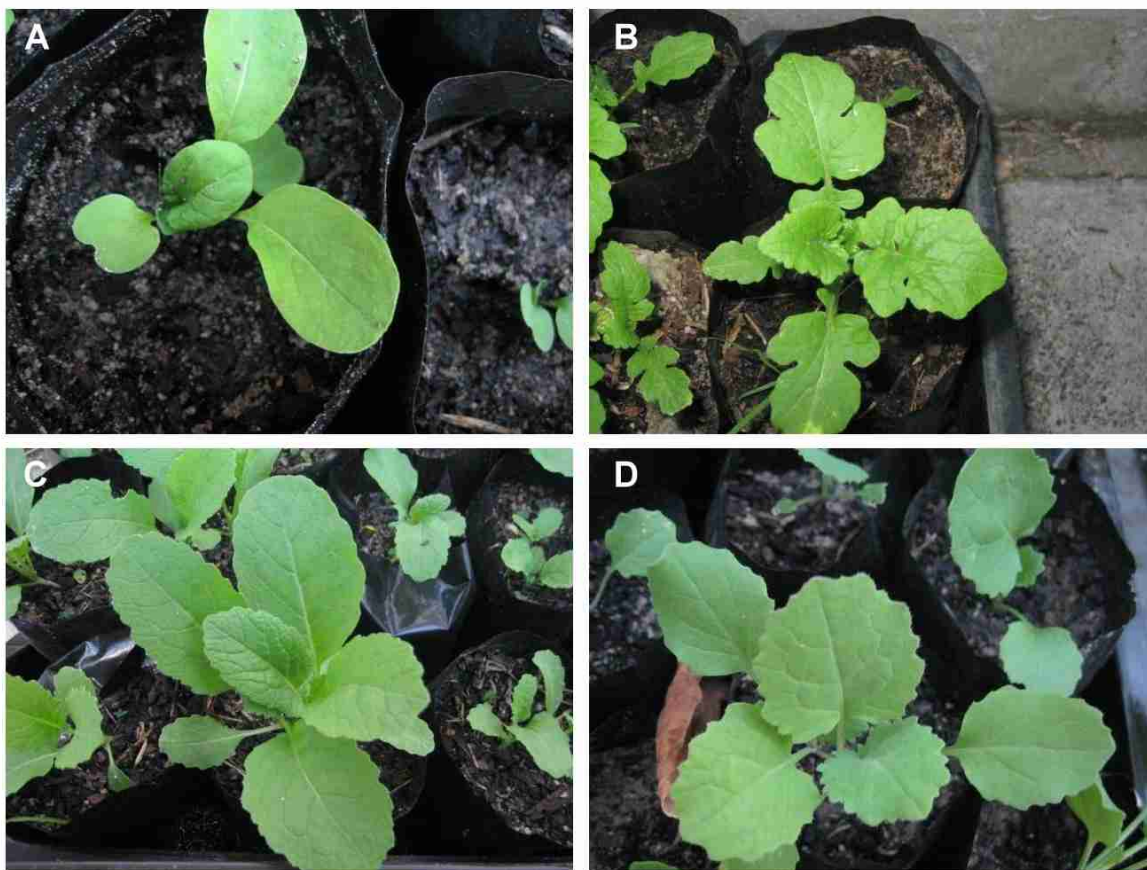


Fig. 1.1 Seedlings of Nemat (*Eruca sativa* cv. Nemat) (A), White mustard (*Sinapis alba* cv. Braco) (B), Caliente 199 (*Brassica juncea* cv. Caliente 199) (C), and Canola (*Brassica napus* cv. AV Jade) (E).

Nemat (*Eruca sativa* cv. *Nemat*)

Nemat reduce plant-parasitic nematode populations and therefore can be included in a crop rotation programme. Nemat is a fast-growing year-round crop, with leaves that have a distinct spicy, pungent flavour. Nemat is more drought-tolerant than mustard, and hence can be grown in dry land conditions. Nemat is unique in its mode of action of suppressing certain nematodes by functioning as a trap crop that also has the ability to form ITC when it is applied as a green manure (Riga & Collins, 2004; Riga *et al.*, 2004; Curto *et al.*, 2005; Melakeberhan *et al.*, 2006).

White mustard (*Sinapis alba* cv. *Braco*)

White mustard shows potential as a cover crop in vineyards and as a rotation crop in rotation programmes that include annual crops. Nematodes are suppressed by this crop when the active compound is released during the incorporation process 60-75 days after planting. It also has an effect

on the life cycle of certain nematodes by slowing down or preventing the completion of their life cycle in the roots (DLF International Seed, s.d.).

Caliente 199 (*Brassica juncea* cv. *Caliente 199*)

Caliente 199 is an annual, cool season herb that requires a short growing season. Initial germination is quick, but then plant growth slows down for three to five weeks before 'exploding' with very rapid growth and biomass production. To maximise biomass production, adequate soil moisture and sufficient nutrient levels should be maintained throughout the growing season (Gies, 2004).

Caliente 199 is primarily included in of a crop rotation programme during the season just before the planting of the cash crop. *Caliente 199* is planted mainly to suppress certain soil-borne diseases and weeds, but can also have a suppressive effect on certain nematodes. It is specifically efficient when combined with *E. sativa* (L. Lazzeri, personal communication, 2007).

Canola (*Brassica napus* cv. *AV Jade*)

Canola is primarily planted in a crop rotation system that includes wheat (*Triticum aestivum*) in the winter rainfall areas of South Africa. The inclusion of Canola as a rotation crop has economic benefits since it has a positive impact on the alternating wheat (Le Roux, 2012). Depending on cultivar and planting date, Canola flowers within 70 to 120 days after planting. Canola is a cool season crop and performs best under climatic conditions of approximately 21°C and within rainfall of approximately 300 mm. The species should preferably be established on clay-loam soils with pH levels of between 5.5 and 7.

Canola should be planted at a density of between 4 and 6 kg per ha. Similar to the other *Brassica* spp., Canola is also a heavy nitrogen feeder and requires approximately 55 kg of nitrogen for every ton of seed produced. Sulphur is also a very important nutrient, with between 15 and 20 kg per ha being required (Republic of South Africa, Department of Agriculture, Forestry and Fisheries, 2010). Root-knot nematode reproduction on 14 Canola cultivars has been investigated and all cultivars were found to be a poor host that maintains low root-knot nematode numbers (Mojtahedi *et al.*, 1991).

Aspects that influence GSL release and ITC activity

Techniques that ensure the maximum rupturing/maceration of the plant cells involved, as well as effective incorporation ensure the best release of ITC in the soil. This aspect, together with a variety with high GSL content and enough water present for hydrolysis to take place, ensure optimum biofumigation (Brown *et al.*, 1991; Poulton & Moller, 1993; Morra & Kirkegaard, 2002; Matthiessen *et al.*, 2004). One way to ensure the effective release of ITC is to cut the leaves with a slasher and then to plough the slashed residues into the soil as soon as possible thereafter using a rotavator or disc harrow (Fig. 1.2). A flail chopper ensures the best maceration results and consequently, a good GL-MYS interaction for the release of ITC (D. Gies, personal communication, 2011). The latter technique is applicable particularly for the *Brassica* spp. such as the mustards, which have a high GSL concentration in the above-ground parts of the plant.



Fig. 1.2. Slashing of crops with slasher (A). Texture of slashed crops (B). Slashed green material on the soil (C). Rotavating the green material into the soil (D).

The growth stage of the crop (emergence, rosette, flowering, seed filling, ripening), the amount of biomass produced and the correct incorporation into the soil all contribute towards the success of biofumigation (Bellostas *et al.*, 2004) (Fig 1.3). The flowering stage of the plant maintains a higher GSL content than in the vegetative plant parts. The GSL-MYS interaction can be expected to take place more effectively later in the growing season, prior to seed set. In the root tissue, the concentration of GSL is higher in the earlier root growth stage, with decreasing concentrations during the root growth cycle.

Different types of GSLs are present in the roots and shoots of different plant species (Van Dam *et al.*, 2009). Studies conducted by Van Dam *et al.* (2009), in which the root and shoot GSL of 29 plant species were evaluated for their GSL concentration and profiles, showed that the roots had a higher GSL concentration, as well as more diversity than did the shoots. The root and shoot concentration of specific GSLs was found to differ from one another, with the most prominent indole GSL in the shoots being indol-3-yl GSL and with the roots having higher concentrations of aromatic 2-phenylethyl GSL.

Low soil temperature slows down the enzymatic reaction during biofumigation and therefore, incorporation of green manure is not recommended at soil temperatures close to 0°C. The presence of organic matter seems to have an immobilising effect on the degradation products, thus preventing them from reaching the target pests (L. Lazzeri, personal communication, 2007).

The inclusion of sulphur fertilisers may improve the nutritional value of *Brassica* spp. Sulphur forms part of the process that takes place in the formation of secondary metabolites, *inter alia* GSLs. The level of GSLs is dependent on the genetic factors of the plant, but can also vary, according to environmental conditions and the availability of soil sulphur (De Pascale *et al.*, 2007).

Although the above-mentioned factors can be regarded as the most important, there are other parameters that also have an influence on the successful outcome of biofumigation. In Figure 1.3, the complex concept of biofumigation, with different variables that may have an influence on the expected effectivity, is indicated. Knowing the effect of biofumigation on beneficial microorganisms is also of importance (Bellostas *et al.*, 2004).

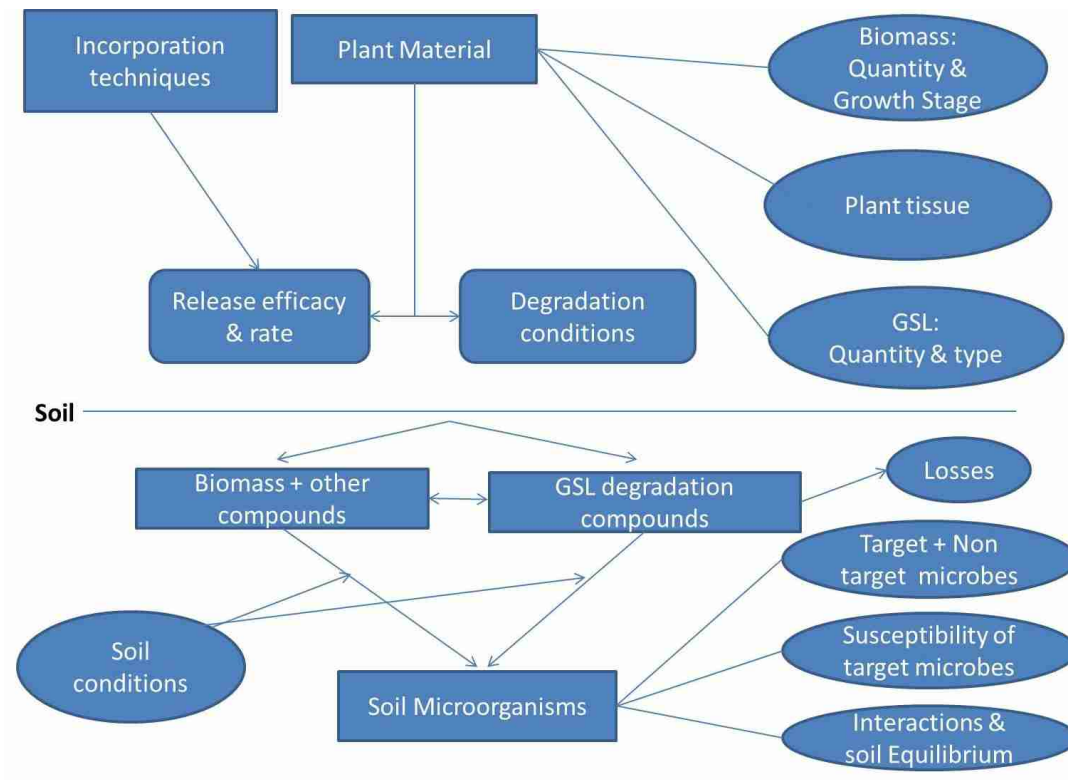


Fig. 1.3. Interlinking of factors affecting the success of soil biofumigation (adapted from Bellostas *et al.*, 2004).

Control of plant-parasitic nematodes in vineyards

The three most important plant-parasitic nematode genera in South African vineyards, measured in terms of their presence and potential damage, are *Meloidogyne* spp. (root-knot nematode), *C. xenoplax* (ring nematode) and *Xiphinema* spp. (dagger nematode) (Storey, 2007).

Root-knot nematodes (Heteroderidae) have a wide host range, are widely distributed in agricultural soils, and can cause extensive loss in terms of the yield quality of numerous crops (Kleynhans *et al.*, 1996). Damage symptoms on root-knot nematode infested vines include stunted growth, poor vigour and substandard yields (Loubser & Meyer, 1987).

In terms of the ring nematodes (Criconeematidae), only *C. xenoplax* is present in cultivated soil in South Africa. These ectoparasites are often found on woody perennials such as vines. They feed on the epidermal cells of the feeder roots, where they cause root stunting and collapsed roots, thereby influencing the uptake of nutrients and water through the root system (Kleynhans *et al.*, 1996).

Dagger nematodes (Longidoridae) are ectoparasites that feed on the root tips of mostly woody perennials. Their feeding behaviour slows down the root development of susceptible cultivars (Malan & Meyer, 1993). For vines, *X. index* is the most economically important dagger nematode in South Africa as they not only damage the roots of susceptible vine cultivars, but are also able to transmit grapevine viruses (Malan & Meyer, 1992; Kleynhans *et al.*, 1996; Nicol *et al.*, 1999; Malan & Hugo, 2003; Van Zyl *et al.*, 2012).

Plant-parasitic nematodes can be present in the soil of the vine inter-row, or in the vine row, although most spp. are present in the vine row soil (Ferris & McKenry, 1976; Rahman *et al.*, 2000), where they can infect the young, active feeder roots (Loubser & Meyer, 1986). Nematodes are controlled in South African vineyards using such chemical control products as fenamiphos, cadusafos and furfuraldehyde registered on grapevine or by planting nematode-resistant rootstocks. The resistance of some of the rootstocks that are used in the South African grapevine industries is listed in Table 1.2. Inter-row cover cropping also has the potential of having a suppressing effect on the plant-parasitic nematode population and can potentially form part of a holistic IPM approach to control nematodes in vineyards (Rahman & Somers, 2005).

Table 1.2. Nematode resistance on certain vine rootstocks (Storey, 2007).

Rootstock	Root-knot nematode	Ring nematode	Dagger nematode	Root-lesion nematode	Citrus nematode
Ramsey	R	-	-	R	R
SO4	R	-	-	-	-
Dog Ridge	R	S	S	MR	MR
Freedom	R	S	S	MR	S
Harmony	R	S	S	S	S
Paulsen 775	R	-	-	-	-
Richter 99	MR	S	S	S	MR
101-14 Mgt	MR	-	-	-	-
143-B-Mgt	MR	-	-	-	-
Paulsen 1103	MR	-	-	-	-
Richter 110	MS	-	-	-	-
US 8-7	MS	-	-	-	-
Paulsen 1447	MS	-	-	-	-
Metallica	S	-	-	-	-
140 Ruggeri	S	-	-	-	-
Jacquez	S	-	-	-	-

Resistance scale: R-Resistant; MR-Mildly resistant; MS-Mildly susceptible; S-Susceptible, - unknown

The use of cover crops, which is standard practice in South African vineyards, has many advantages, including the reduction of water run-off and erosion (Khan *et al.*, 1986; Roth *et al.*, 1988; Louw & Bennie, 1992), the preservation of soil moisture (Buckerfield & Webster, 1996), reduction of evaporation from the soil (Myburgh, 1998), temperature regulation of the soil (Fourie & Freitag, 2010), improvement of soil organic matter (Fourie *et al.*, 2007; Fourie, 2012) and the suppression of weeds (Fourie *et al.*, 2005, 2006; Fourie, 2010). The choice of cover crop is determined by the climatic conditions that are prevalent in the different grapevine regions, as well as by the requirements of the grapevines concerned (Fourie *et al.*, 2001). The inclusion of biofumigation crops as a cover crop in the cover crop management strategies employed in grapevines requires further research in South Africa, as the benefits thereof have to be determined.

Most of the scientific literature that has been cited focuses on the role that biofumigation can play in the suppression of root-knot nematodes, although there are also indications of the effect that biofumigation can have on other nematode species such as on *Paratrichodorus allius* (stubby root

nematode) (Riga & Collins, 2004). The effect of biofumigation on plant-parasitic nematodes has been tested on different crops including grapes (McLeod *et al.*, 1995, 1998). ITC suppressed fungi, bacteria, nematodes and weeds in numerous *in vitro* experiments (Brown & Morra, 1997). The question arises as to whether biofumigation green manures growing in the grapevine inter-row can have an effect on the nematode population in the vine row area after being incorporated mechanically into the soil. Rahman and Somers (2005) indicate that the application of *B. juncea* cv. Nemfix (Indian mustard) as a green manure is able to suppress *M. javanica* when it is incorporated into the inter-row, or the vine row. The effect of the green manure on the root-knot nematode population was more pronounced when it was applied in the vine row area (Rahman *et al.*, 2009).

According to Rahman *et al.* (2009) the use of *Brassica* spp. as cover crops planted in the grapevine inter-row reduced the root-knot nematode population over a period of three years. Biofumigation with the cover crops was observed to be as effective as were mustard seed meal and fenamiphos applications over the three-year period.

Nematode biofumigation bioassays

Green manure

In vitro studies showed that brassica green manures were more effective in suppressing plant parasitic nematodes than were non-brassica green manures (Mojtahedi *et al.*, 1991, 1993; Potter *et al.*, 1998). The brassica green manures suppressed root-knot nematodes significantly under controlled environments (McLeod & Steel, 1999). Not only is the GSL content of the brassica green manures thought to cause the suppression but in addition, other secondary metabolites that are released during the biofumigation process might also play a role in the process. The effect of biofumigation on the biological activity of the soil is also indicated, as well as is a possible increase in the population of antagonistic organisms, which can lead to the suppressing of plant-parasitic nematodes in the soil (Piedra Buena *et al.*, 2006).

Another possibility regarding the suppressing effect of biofumigation on plant-parasitic nematodes lies in the stimulation of competition for food sources that can occur after incorporating green manure into the soil. The main focus is, however, on the role that volatiles and non-volatiles play during the decomposition of plant residues in the soil (Piedra Buena *et al.*, 2006). Research into

the role of green manures has included *Capsicum* spp. (pepper), *Fragaria ananassa* (strawberry), *Solanum lycopersicum* (tomato), *Cucumis sativus* (cucumber) and *Citrus sinensis* (orange) residues. The treatments were evaluated using medium in plastic bags that were infested with large numbers of the root-knot nematode, *M. incognita*. The biofumigation action was simulated by incorporating the crop by-products at a specific rate, correlating to field dosages, in the infested soil. Root galling was used as an indicator of the efficacy of the different crops as a biofumigant. In both bioassays, there was a reduction in the amount of root galling caused by *M. incognita* in comparison to the amount that occurred in the untreated control (Piedra Buena *et al.*, 2006).

Ploeg and Stapleton (2001) investigated the effect of time and temperature in combination with brassica soil residues on the suppression of *M. incognita* and *M. javanica*. Soil temperature and the length of exposure to such temperatures played an important role in the efficacy of soil solarisation treatments. The addition of broccoli residues to the soil at a temperature of 20°C was not effective in suppressing root galling on melon plants but at a temperature of 30-35°C for a period of 10 days, the amendment of broccoli to the soil almost eliminated the galling on the roots.

A pot trial with vines that was conducted by Rahman *et al.* (2011) compared the root-knot nematode suppression effect of fenamiphos and two *Brassica* spp. as green manure and of Indian mustard seed meal. No statistical difference was found between the effects of the brassica green manures, the mustard seed meal or the fenamiphos treatments after their application over a period of three consecutive years. All of the treatments showed significantly different effects when compared with the untreated control.

Nematode host status of different biofumigation crops

The ideal cover crop to be planted in vineyards for nematode suppression should either be resistant to or have a poor nematode host status, in addition to having a biofumigation suppressing effect on the target nematode when applied as a green manure to the soil (Vianene & Abawi, 1998). The possibility exist that *Brassica* spp., if used as cover crops in vineyards, can also be susceptible to a specific nematodes species that require suppressing. If the target pest manages to reproduce on the cover crop species before it is ploughed in as a green manure these *Brassica* spp. cannot recommended as a cover crop (McLeod & Warren, 1993).

Root-knot nematode species can complete their life cycle on several *Brassica* spp. but there are major differences in the susceptibility of such crops to these nematodes (McLeod & Steel, 1999). In a glasshouse study, Curto *et al.* (2005) evaluated the host status of different brassicas for *M. incognita*. Although all of the brassicas act as hosts, the life cycle of the latter nematode was in general much slower in comparison to tomato. They rated certain brassicas as poor or non-hosts (resistant), maintenance hosts (tolerant) or good host (susceptible). *Eruca sativa* cv. Nemat was evaluated for its potential as a trap crop for root-knot nematode. No eggs were produced in 80% of the plants indicating it to have the potential to act as trap crop for *M. hapla* (Melakeberhan *et al.*, 2006).

Conclusion

With the increasing pressure on chemical control options for nematode management in most crops, as well as the limited fumigation options that are available for use prior to the planting of crops, there is a growing need for more biological control options for plant-parasitic nematodes as well as for other soil-borne diseases. Biofumigation is a concept that has been well studied, with definite potential and good results being shown where the method has been applied correctly for the management of plant-parasitic nematodes, soil-borne diseases and weeds. The challenge is to understand the complex interactions during biofumigation, and to ensure that the different factors that play a role in optimal biofumigation are applied. The main factors concerned include the basic principles of fumigation, *Brassica* spp. selection and biomass production, GSL concentration and spectrum, ITC concentration and spectrum, and the maceration and incorporation process.

The potential for biofumigation as part of an IPM approach consists both of the role of the active compounds, primarily ITC, in the direct suppression of soil-borne diseases, plant-parasitic nematodes and weeds, and also the secondary effect that can be expected during the application of green manure in the soil. The secondary effect plays a very important role in promoting microbial and other microorganism diversity in the soil and can therefore be expected to have a positive impact on the stimulation of competition among soil-borne diseases in the rhizosphere. Another important factor that can have a positive impact on the suppression of the plant-parasitic nematode populations is the susceptibility or resistance of the brassica crops used. With good management practices and proper medium to long term planning, biofumigation, together with all the other beneficial aspects mentioned,

could play a substantial role as part of a rotation/cover crop system for annual and perennial crops, and specifically as part of a cover crop rotation programme in vineyards.

The overall aim of this study was to investigate the use of cover crops to suppress plant-parasitic nematodes in vineyards:

The specific objectives of the study were to:

1. Use laboratory bioassays to determine the biofumigation potential of five cover crops when applied as a green manure to control *Meloidogyne javanica* and *Criconemoides xenoplax*.
2. Use glasshouse trials to determine the reproduction potential *M. javanica* and *C. xenoplax* on five cover crops.
3. Do field trials to determine the long term effect of cover crops and management practices on the plant-parasitic nematode numbers.

The chapters of this study have been written as separate publishable papers, and, for this reason, some repetition in the different chapters has been unavoidable.

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CHAPTER 2

Bioassays to determine the potential of cover crops to control *Meloidogyne javanica* and *Criconemoides xenoplax* when applied as green manure

Abstract

Previous research indicates the positive effect of biofumigation in both laboratory bioassays and field applications in the suppression of soil borne diseases, plant-parasitic nematodes as well as certain weeds. Many factors can influence the efficacy of the biofumigation action and consideration should be given to utilise this concept successfully in a farming system. Laboratory bioassays were done to determine the potential of different cover crops to suppress *Meloidogyne javanica* (root-knot nematode) and *Criconemoides xenoplax* (ring nematode), when applied as green manure. Crop biomass, used in the bioassays included those harvested from Oats (*Avena sativa* cv. Pullinup), White mustard (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente 199) and Nemat (*Eruca sativa* cv. Nemat). The green plant material of the different crops was cut into pieces and mixed with soil inoculated with *M. javanica* or *C. xenoplax* respectively. After a period of 14 and 28 days, respectively, susceptible tomato plants were planted in the *M. javanica* inoculated soil and left to grow in a glasshouse before doing a root gall index analysis for all the crops evaluated. The same was done with the *C. xenoplax*, where a soil analysis was conducted, after 14 and 28 days, to determine the impact of the plant biomass on the *C. xenoplax* population. The results, obtained from the bioassays, showed a significant suppression of *M. javanica*, due to biofumigation using green material of White mustard, Caliente 199 and Nemat, however, no significant differences were found in the *C. xenoplax* bioassays.

Introduction

Plant-parasitic nematode management is complicated and the complexity of the soil, as well as the effect of the different plant-parasitic nematode species on different crops, makes nematode control a challenging part of crop protection (Nusbaum & Ferris, 1973). It is estimated that the root-knot nematode (*Meloidogyne hapla*) has a host status of more than 550 different crops and weeds, making the implementation of an effective cover crop rotation system, as part of the cultural

management component of integrated nematode management, very challenging (Jepson, 1987). The other components, of the integrated approach to nematode management, consist of chemical and biological practices. There is conservation pressure on the chemical control options for nematode management and therefore there is a need for non-chemical alternatives to assist in managing soil-borne diseases and plant-parasitic nematodes (Gamliel *et al.*, 2000; Lazzeri *et al.*, 2004b). As part of an integrated approach, the use of resistant cultivars; the choice of cover crops in a crop rotation system; the use of organic matter and the use of green manure play an important role (Barker & Koenning, 1998; Westphal, 2011; Widmer *et al.*, 2002).

The definition of green manure basically encompasses the incorporation of above ground crop biomass, while the crop is still growing or in the green stage, into the soil; as a supplement to the soil either where it is cultivated, on the site, or brought in from another site (Pieters, 2006). This practice has been used for thousands of years, but recent studies, started in the nineteenth century, are during the past few years, focusing on the actual benefit that the follow-up crop can have after incorporation of a green manure (Pieters, 2006). Green manure can be classified into four different groups, depending on the purpose of implementation on the farm, namely: main, companion, catch or cover crop (Pieters, 2006). In most cases in South Africa, green manure will be categorized in the cover crop class, as it is planted in a rotation system before the planting of the cash crop, or as a cover crop in the case of grapes. There is also potential to utilize cover crops as part of an integrated approach, before the establishment of perennial crops, to make use of biological amendments in suppressing disease complexes; like apple replant disease (Mazzola *et al.*, 2007). Previous research indicates different crops that have been tested or used for cover crop purposes and include a wide range of legume crops, grain crops and brassica crops (Widmer *et al.*, 2002; Pieters, 2006).

Another well documented role that green manure can play in a production system is the biocidal effect it has on soil borne diseases, nematodes and weeds. This is the result of certain biological active compounds, released during the maceration and incorporation processes of green manures, with specific reference to Brassicaceae plants and biofumigation (Brown & Morra, 1997; Sarwar *et al.*, 1998; Lazzeri *et al.*, 2004a; Matthiessen & Kirkegaard, 2006). This technique, defined as biofumigation, relies on the fumigant action of volatile compounds, released during biodegradation, for the suppression of plant pathogens (Piedra Buena *et al.*, 2007).

Brassica crops contain the active compound glucosinolate (GSL), which is sulphur containing secondary metabolites, which occur in the vacuole of the plant cells. When brassica plant cells are ruptured by means of maceration, or any sort of mechanism that breaks the plant cells, the GSL comes into contact with the enzyme myrosinase (MYR), which is present in the cytoplasm of the cell. Glucosinolate can be divided in different chemical classes, namely: aliphatic, aromatic and indolyl GSLs (Fenwick *et al.*, 1983). The GSL- MYR system is present in most Crucifereae crops, but the concentration, as well as the type of GSL varies in different *Brassica* spp. as does the distribution in different plant organs (Kirkegaard & Matthiessen, 2005). When these two active compounds come into contact; the GSL is hydrolysed to form a range of end products, with isothiocyanate (ITC) being the most important, but thiocyanates, nitriles and oxazolidinethions are also formed (Sarwar *et al.*, 1998; Bending & Lincoln, 1999). The interest in the possible role that ITC can play, as a biological compound, released during the biofumigation process, arose from the widely used synthetic ITC, namely metam sodium (methyl isothiocyanate), which is a well-known broad spectrum soil fumigant used to control soil-borne diseases including soil pathogens, nematodes and weeds (Matthiessen & Kirkegaard, 2006). The role that the biological ITC can play in an integrated approach, to suppress soil borne diseases, have been evaluated extensively and the biofumigation concept can definitely contribute to effective soil borne disease, nematode and weed suppression (Kirkegaard & Sarwar, 1998; Lazzeri *et al.*, 2004a; Larkin & Griffin, 2007).

There are many factors that play a role in chemical soil fumigation. These factors include chemical, physical and biological factors and can have an impact on the efficacy of the fumigation process (Munnecke & Van Gundy, 1979). These factors are also involved when green manure is applied to soil and secondary metabolites are released during the decomposing process of the material to form volatile compounds.

Ploeg & Stapleton (2001) indicated that both time and temperature have an impact on broccoli plant residues against *M. incognita* and *M. javanica* populations. They found that the application of broccoli to infested soil, at higher temperatures for a longer period, gave a good suppressing effect on the nematodes. The lethal dose needed to control certain soil borne diseases declines with a rise in temperature, because the distribution of the volatile products are better, keeping the other limiting factors in mind (Munnecke & Van Gundy, 1979). Looking at biofumigation, it is important to realize that this is a biological approach and that the amount of active compounds

released into the soil is not constant and can differ based on certain cultivation practices, soil conditions and climatic conditions. Another role of green manure, applied for biofumigation purposes, is the soil building properties of the organic matter that brings about a total new perspective with regards to the application of green manure aimed at biofumigation (Roubtsova *et al.*, 2007).

The objective of this study was to evaluate the suppressing effect of different *Brassica* crops, when applied as green manure to soil, infected with *Meloidogyne javanica* (root-knot nematodes) and *Criconemoides xenoplax* (ring nematodes), in a controlled environment.

Materials and methods

Cover crops for green manure application

Five different cover crops were selected to evaluate their potential when applied as green manure on the suppression of *M. javanica* and *C. xenoplax*. The cover crops included Oats (*Avena sativa* cv. Pullinup), White mustard (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente 199) and Nemat (*Eruca sativa* cv. Nemat).

In the first bioassays the cover crop biomass used, was grown as part of the field trial in Stellenbosch, Western Cape. The cover crops were collected at the late flower, early pod formation stage and some of the cultivars, oats and canola, were slightly later in the physiological development stage. For the repetition of each bioassay the crop biomass was grown in pots at 25 ± 2 °C. Seeds of the five different cover crops were sowed in six 4 l black plastic growing bags. The plants were fertilized on a weekly basis with Chemicult, consisting of a balanced N.P.K ratio as well as micro nutrients. Plants were irrigated on a daily basis.

Meloidogyne javanica inoculum

Tomato plants, inoculated with eggs of *M. javanica*, were grown in a glasshouse for four months. To obtain *M. javanica* eggs, the roots were carefully removed from the soil and washed. The roots were then cut into 2 cm pieces and added to 250 ml of 0.5% sodium chloride solution (NaOCl) in a 500 ml Schott bottle and shaken vigorously for 4 min. The contents of the bottle was passed through a 75- μ m pore (200-mesh) sieve, nested in a 38 μ m-pore sieve (500-mesh), and washed with a stream of water. The eggs, collected on the 38 μ m-pore sieve, were washed into a beaker. Roots

were returned to the bottle, water added and the process repeated. The nematode egg concentration was determined using the technique of Navon and Ascher (2000). Five drops of 10 µl each of a suspension of nematodes in a specific volume were put on a glass slide and the number of nematodes counted in 50 µl. This was repeated five times and the volume of water was diluted to the concentration used as inoculum.

Criconemoides xenoplax inoculum

The peach rootstock Atlas was planted in 25 L plastic pots and plants were inoculated with *C. xenoplax* approximately 24 months earlier. The plants were kept in a glasshouse at a temperature of < 25 °C. A soil auger was used to take a 100 ml soil sample from the roots of 25 pots. The soil was washed through a 200 µm sieve into a 10 litre bucket. While stirring, the bucket was filled to ¾ of the volume, left for one minute and then poured through two nested sieves of 53 µm-pores and a 45 µm-pore size. The content was then washed into a glass beaker. This process was repeated, but left for 15 seconds to settle and again poured through the sieves; as described above. The content, washed from the soil, was centrifuged for 5 min at 3 000 rpm, the supernatant discarded, and each tube was filled with a sugar solution and centrifuged for 1 min. The content of the tubes was poured through a 45 µm sieve, washed to free it from the sugar solution and the nematodes were then collected from the sieve and washed into a 100 ml beaker. The suspension was left for 30 min for the nematodes to settle to the bottom, after which the supernatant was siphoned off to a volume of 20 ml. The contents of the beaker was brought into suspension by using an air pump and two ml of the contents were counted out, using Peter's slides and a Leica 2000 research microscope. A soil concentration of *C. xenoplax* was determined to get the desired amount of nematodes for inoculation of the bags used in the two bioassays. The concentration used to inoculate both bioassays was 2500 juveniles per root system of each plant.

Experimental layout

The experimental method used in this assay is indicated in Figure 2.1 and is based on a protocol, as described by Piedra Buena *et al.* (2006), and was developed by the Agro-ecology Department of Centro de Ciencias Medioambientales – CSIC, Madrid, Spain.

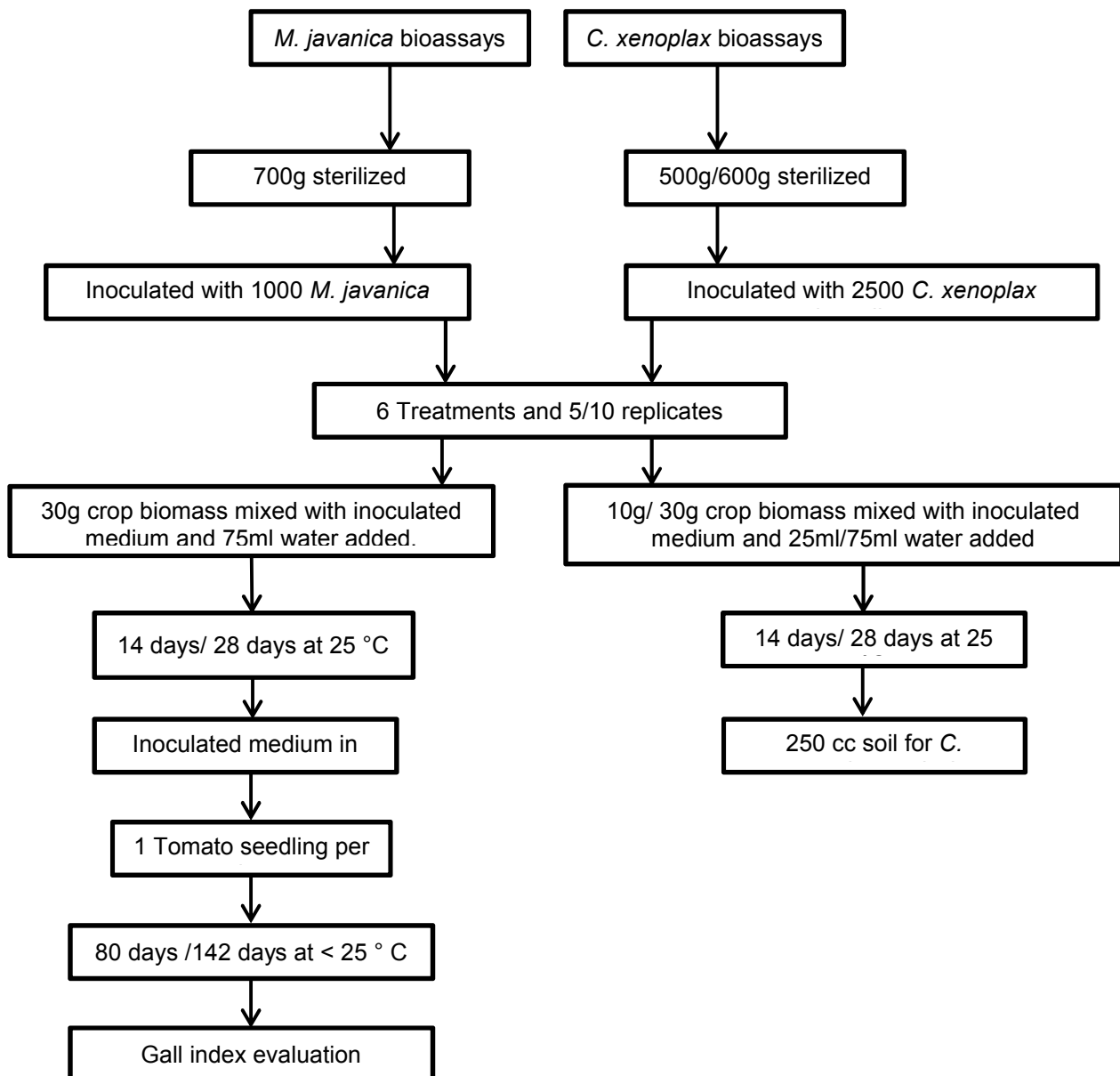


Fig. 2.1. Graphical layout of the protocol used for the *Meloidogyne javanica* and *Criconeoides xenoplax* bioassays that were conducted to determine the impact that green manure of Oats (*Avena sativa* cv. Pullinup), White mustard (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente 199) and Nemat (*Eruca sativa* cv. Nemat) will have on nematode suppression.

Effect of green manure on Meloidogyne javanica

A total of 700 g sterilized medium, consisting of bark and sand, was added to sealable plastic bags. The medium was inoculated with 1000 *M. javanica* eggs and mixed to obtain an even distribution of the eggs in the medium. The green manure (biomass of the cover crops) was added to the medium just after the inoculation was performed. The control treatment was only inoculated with nematode eggs and did not receive any green manure. For each treatment there were ten repetitions. A total of 30 g of the plant material, consisting of roots, stems and leaves and 75 ml water, was macerated in a food blender for 10 sec. The plant material was then added to the inoculated medium in the plastic bags. The bags were mixed to get an even distribution of the biomass within the bags. The bags were then placed in a growth chamber at 25 °C for 14 days, after which the contents from the bags was placed in growing pots. Susceptible tomato (*Solanum lycopersicum* cv. Moneymaker) seedlings were planted in the content of the bags. The pots were placed in a glasshouse < 25 °C in a completely randomised design. After 80 days the experiment was terminated and each plant was carefully removed from the bags and the roots were washed with water. Each root system was inspected and a root galling index was used to determine the *M. javanica* infestation in the roots. This gall evaluation was done on a scale of 0-5 adapted from the technique used by Hussey and Janssen (2002), where 0 = no galls, 1 = 1-10 galls, 2 = 10-50 galls, 3 = 50-100 galls, 4 = > 100 galls and 5 = covered with galls.

The same protocol as indicated in Figure 2.1 was followed during the repeat bioassay. During the flowering, early pod formation stage, 30 g of the biomass, consisting in this case of only leaves and stems, was macerated in a food blender for approximately 10 sec and was then applied to the inoculated sealable plastic bags, together with 75 ml water; that was added to the medium just before the inoculation with the root-knot eggs. All the treatments were left in a temperature controlled chamber at 25 °C. After 28 days, the content of the bags was placed in growing pots and susceptible tomato plants were planted in the growing medium. The pots were placed in a glasshouse at a maximum of 25 °C in a completely randomised design. The pots were left for 142 days and then evaluated for *M. javanica* root gall formation on the tomato roots. This period was longer than the protocol suggested; but as root gall formation had not taken place in the control pots the decision was made to leave the plants until sufficient root gall formation could be evaluated in the control treatment.

Effect of green manure on Criconemoides xenoplax

The soil used for the *C. xenoplax* bioassay was collected at the field trial site. The soil was sieved and heat sterilized (55°C for 24 h) to make sure that there is no contamination of other plant-parasitic and non-parasitic nematodes in the medium. A total of 500 g of the sterilized medium was placed in sealable plastic bags.

A total of 200 ml of growing medium, representing an estimate amount of 2500 *C. xenoplax* juveniles, was placed in the same plastic bags and mixed thoroughly. There were six treatments, consisting of five cover crops and one control. The green manure was added to the inoculated medium. The control treatment consisted of only sterilized medium, inoculated with the *C. xenoplax*.

Plant material (10 g) consisting of roots, stems and leaves was cut into fine pieces, using scissors and then added to the plastic bags containing the sterilized medium and *C. xenoplax*. Water (25 ml) was added to the plastic bags. The plastic bags were then placed in a temperature controlled chamber at 25° C for 14 days after which the evaluation was done; using the same extraction technique as described above but with 250 ml soil. For each treatment there were five replicates.

In the second bioassay sterilized medium, consisting of bark and sand, was used as the medium for inoculation of *C. xenoplax*. A total of 600 g of the sterilized medium was placed in sealable plastic bags. A total of 75ml water was added to the medium before the inoculation of the nematodes. Thereafter 100ml of growing medium, representing 2500 *C. xenoplax*, was placed in the same plastic bags and mixed thoroughly. The same treatments were conducted, as in the first bioassay, but for each treatment there were 10 replicates. Plant biomass, consisting of leaves and stems, was harvested after approximately 2 months, during the flowering or early pod formation period, 30 g of the plant material was cut into fine pieces, smaller than 1 x 1 cm with a food processor for approximately 10 sec and applied to the 600g inoculated medium. The cut up green plant material was thoroughly mixed with the inoculated soil. The bags were then placed in a temperature controlled chamber at 25°C for 28 days and afterwards the *C. xenoplax* numbers present were determined; using the same extraction technique as described above, but with 250 ml soil.

Statistical analyses

All laboratory experiments were repeated on different test dates. All statistical analyses were performed using the STATISTICA ver. 10 data analysis software system (Statsoft Inc., 2011). Data, obtained from the bioassays, were analysed using ANOVA with trial test date and relevant treatments as separate factors. If the data were not normally distributed a non-parametrically analysis, using the Kruskal-Wallis test, was performed.

Results

Meloidogyne javanica bioassays

No significant differences ($F_{(5,108)} = 1.800$; $p = 0.118$) were found between interaction effects of the two bioassays (test-date and treatment) when analysed using a two-way ANOVA. Results from the two trial dates were then pooled and analysed, using a one-way ANOVA, with significant differences ($F_{(5,108)} = 3.862$; $p < 0.005$) found among treatments.

There were no significant differences found between the root gall index of Oats, Canola and the control. All three crops obtained a gall index of 3, with between 50 to 100 galls. There was a significantly lower gall index found between the White mustard and Canola ($p = 0.0188$). In addition, a significantly lower gall index was found on Caliente 199 ($p = 0.0248$) and Nemat ($p = 0.0188$) compared to the Canola. White mustard, Caliente 199 and Nemat did not differ significantly from each other and there were also no significant differences between these three treatments and the Oats treatment.

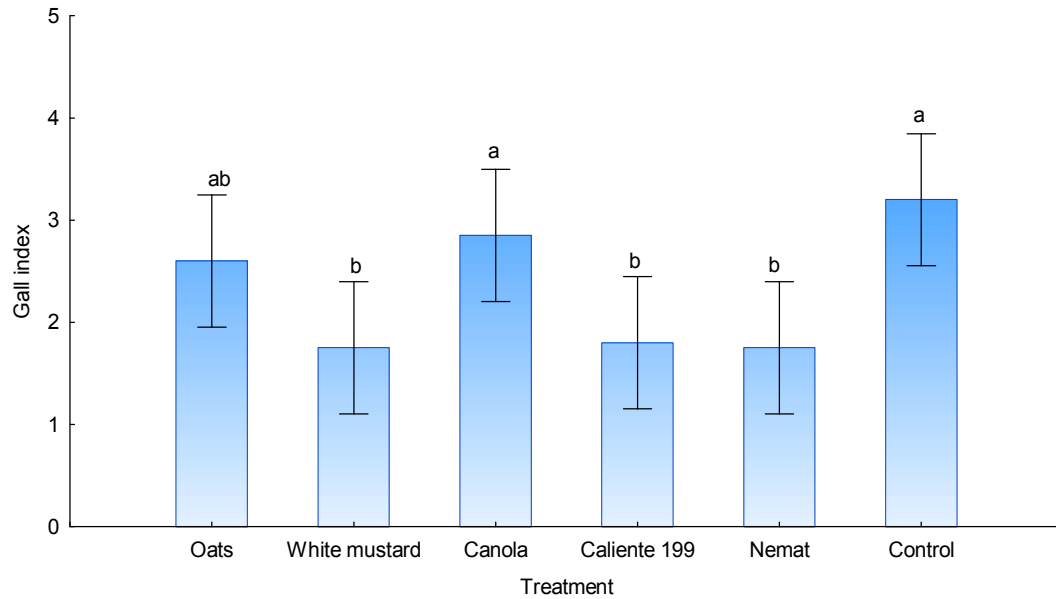


Fig. 2.2. *Meloidogyne javanica* (root-knot nematode) gall index (95% confidence interval) on susceptible tomato plants (*Solanum lycopersicon* cv. Moneymaker), treated with green manure of five different cover crops: Oats (*Avena sativa* cv. Pallinup), White mustard (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente 199) and Nemat (*Eruca sativa* cv. Nemat), incorporated into *M. javanica* inoculated soil (one-way ANOVA; ($F_{(5,108)} = 3.862$; $p < 0.005$). Bars with the same letter did not differ significantly.

Criconemoides xenoplax bioassays

A two-way ANOVA was performed and the interaction was not significant ($F_{(5, 78)} = 0.746$; $p=0.591$) and main effects could be interpreted. However, no significant difference was found between the different treatments ($F_{(5,78)} = 0.463$; $p = 0.802$) (Fig. 2.3).

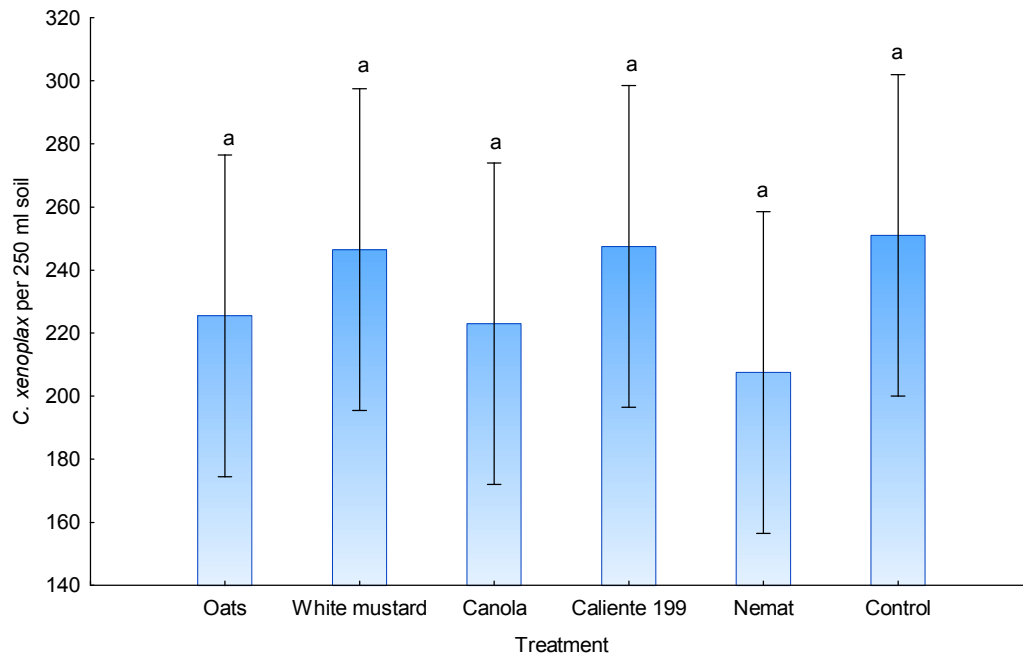


Fig. 2.3. *Criconemoides xenoplax* numbers (95% confidence interval) after treatment with green manure of five different cover crops, Oats (*Avena sativa* cv Pallinup), White mustard (*Sinapis alba* cv.Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente 199) and Nemat (*Eruca sativa* cv. Nemat), incorporated into a *C. xenoplax* inoculated soil. Bars with the same letter did not differ significantly.

Discussion

The results, obtained from the two laboratory bioassays, indicate that the three brassica crops; White mustard, Caliente 199 and Nemat, known as good biofumigation crops, suppressed *M. javanica* gall formation. This correlates well with previous studies, where *in vitro* tests showed that, in most cases, there was a reduction of root knot nematodes with the application of brassica crops as green manure, in comparison with non-brassica crops (Mojtahedi *et al.*, 1993). This effect is most probably due to the GSL in the tissue of brassicas (Brown & Morra, 1997). The formation of the active ingredients, with the most emphasis on the ITC, was believed to give the suppressing effect (Lazzeri *et al.*, 1993). An example, of such an experiment, was conducted by McLeod & Steel (1999), where different *Brassica* cultivars were sown during two sowing periods and the harvested green manure was chopped into pieces and 1000 *M. javanica* J2 was added to a one kilogram potting soil. After being left for 2 weeks at temperatures between 10°C and 25°C the 10 g application rate and 20 g

application rate had a significant reduction on the *M. javanica* population. The nematode suppressing effect of different brassica and other biofumigation crops, after incorporation into the soil, can however differ drastically between crops and not all crops have the potential to be utilized in this manner (McLeod & Steel, 1999; Piedra Buen *et al.*, 2006).

Rahman *et al.* (2009) performed a trial with one year old Semillon grapevines planted in pots and after 3 months they were inoculated with 500 *M. javanica* larvae and left for 6 months for establishment. For three consecutive years, *brassica* seeds were sown under the vines and after 3 months they were slashed and incorporated into the soil at the correct growing stage. Results indicate a gradual decline in *M. javanica* population in the pots, with the best results obtained in the third year. There was also a growth response of the vines in the pots that received the green manure, indicating the secondary effect of the green manure applications.

In another trial, done by Stirling & Stirling (2003), *Brassica* species were sown in field soil. Plants were grown for approximately 10 weeks before incorporating the green manure into the soil at a depth of 18 cm. Afterwards the soil was inoculated with *M. javanica* and put into pots. After 4 and 9 weeks a root gall index indicated that there was a significant reduction in the *M. javanica* root galls, where brassicas was incorporated at an earlier stage.

In the current study, the Canola treatment did not show the same response to *M. javanica*, with regard to the root gall index, as the other brassica species. There are different types and concentrations of GSL present in different brassica crops and Canola is not considered to have a very active composition of GSL. Therefore it can be expected that Canola crop residues would not have the same biofumigation effect on *M. javanica* as the other brassica species, well known for their active biocidal role when applied for biofumigation. The results obtained in this study support previous work that was done in this research field, therefore, the great interest shown in the incorporation of these cover crops into the soil as part of an integrated approach for *Meloidogyne* spp. suppression in the field.

Since there was no significant difference in the *C. xenoplax* population, where the crop residues were applied to the inoculated medium, the results from this study indicates that in these specific bioassays, biofumigation cannot be considered as effective in suppressing *C. xenoplax*. It is, however, important to note that there is also a dose response that must be taken into consideration

with biofumigation. Future research should consider the application of higher concentrations of biomass as part of the trials. For any fumigation action to be successful, contact time and concentration are key factors that must be considered and by enhancing this, can potentially have a more positive impact on the suppression of *C. xenoplax*.

It is also well known that *C. xenoplax* are in general considered more difficult to control than most of the other plant-parasitic nematodes. One of the reasons is the thick cuticle that gives the nematode its descriptive name, which makes the contact action of most control measures a challenge. One can also expect that the concentration of ITC that will be needed to effectively suppress *C. xenoplax* will be higher than the concentration needed to suppress *M. javanica*; because of the factors mentioned above.

There is numerous research done, indicating the specific type of GSL present in certain brassica species as well as the types of ITC formed after the MYR-GSL reaction. Research has also been conducted on the efficacy of biofumigation of *Meloidogyne* spp. suppression. In future research it will be advantageous, if a specific lethal concentration can be determined, for constant effective suppression of *Meloidogyne* species, as well as to determine a lethal concentration of ITC that will be effective in a constant suppression of *C. xenoplax*, keeping in mind all the factors, mentioned above, that can play a role in effective biofumigation.

Biofumigation is a definite option as part of an integrated approach for the management of plant-parasitic nematodes and must be implemented as part of a rotation system as well as cover crop systems, as a biological alternative in combination with certain chemical options. The biological interactions that take place, when incorporating green manure, is also a very beneficial aspect, which can in itself have a positive secondary impact on the suppression of plant-parasitic nematodes; by means of biological diversity stimulation.

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CHAPTER 3

Host status of Brassicaceae cover crops to *Meloidogyne javanica* and *Criconemoides xenoplax* in glasshouse trials

Abstract

Cover crops form an integral part of an integrated approach for managing certain soil borne diseases, plant-parasitic nematodes and weeds. If implemented correctly, it can have a positive impact on the optimization of soil nutrients and crop protection strategies, thus ensuring economical sustainability. A good cover crop, specifically for nematode management, can be classified as a crop that has a poor host status for a specific nematode species involved and also has suppressing effects on plant-parasitic nematodes, when applied as a green manure in the soil. In this study, crop host trials were conducted to determine the host status of Oats (*Avena sativa* cv. Pullinup), White mustard (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente 199), Nemat (*Eruca sativa* cv. Nemat) and Tomato (*Solanum lycopersicum* cv. Moneymaker), as control for *Meloidogyne javanica* (root-knot nematode) and *Criconemoides xenoplax* (ring nematode). In the *M. javanica* host trials, Nemat was classified as a poor host while Oats and White mustard also showed promising results as poor hosts. In the *C. xenoplax* host trials, Canola had a suppressing effect on *C. xenoplax* and can therefore be considered as a poor host. Caliente 199 also showed promising results as a poor host for *C. xenoplax*.

Introduction

Plant-parasitic nematodes can have a significant impact on most crops. Of the different plant-parasitic nematodes, *Meloidogyne* spp. (root-knot nematodes) is considered to be the most important genus (Nyczepir & Tomas, 2009). Some of the factors that makes this genus so succesfull, as a economically important plant-parasitic nematode, include their widespread distribution internationally, several lifecycles per season and their wide host range (Nyczepir & Meyer, 2010). *Criconemoides xenoplax* (ring nematode) (Raski, 1952; Loof & De Grisse, 1989) on the other hand, is also an economically important plant-parasitic nematode on crops like stone fruit and grapes and combined with *Meloidogyne* spp. they are considered to play an important role in the so called peach tree short

life disease (Hugo & Meyer, 1995; Nyczepir *et al.*, 1997). According to Pinkerton *et al.* (2004) *C. xenoplax* are widely distributed throughout vineyards in most countries such as the United States and Europe. In South Africa *C. xenoplax* can have a negative growth response, as well as a reduction in yields in crops like vineyards (Storey, 2007). In a study, conducted by McKenry (1992), it was found that there can be a reduction in yield of grapes of between 10% and 25% if *C. xenoplax* are present in numbers of more than 500 per kg⁻¹ soil.

It is not easy to control plant-parasitic nematodes in the soil; as soil is a very dynamic and complex entity, with biological, physical and chemical interactions (Norton, 1978; Starr & Roberts, 2004). The use of an integrated pest management (IPM) approach, to manage nematode pests, is seen as the most sustainable long-term practice (McKenry, 1992). This IPM approach considers all the different aspects that can give a long term sustainable solution to the nematode problem and includes aspects like, resistant cultivars and the choice of winter cover crops (Westpal, 2011). Knowing the cropping system on a farm can lead to decisions that may have a beneficial long-term impact. By using cover crops that are poor hosts to certain plant-parasitic nematodes and by implementing these crops as part of a crop rotation system, intercropping system or cover cropping approach it can have the benefit of suppressing the development of the specific plant-parasitic nematode population involved (Westphal, 2011). Certain brassica species have shown potential as poor or intermediate hosts, rather than good hosts for *M. javanica*, as well as having the potential to be utilised for their biofumigation potential (Kirkegaard & Sarwar, 1998; McLeod & Steel, 1999; McLeod *et al.*, 2001). Brassicas are not necessary non hosts for plant-parasitic nematodes, but in a study by McLeod & Steel (1999) it was found that a wide range of brassica crops are not suitable hosts for *M. javanica* and they also indicated that there were differences between the various brassica crops, with regard to their host status. Brassicaceae crops are seen as maintenance or intermediate hosts in most cases, either because penetration of the roots by *M. javanica* larvae is less or because egg production is reduced (McLeod *et al.*, 2001).

Cover crops can play an important role in the suppression of root-knot nematodes if it has a poor host status and therefore has a suppressing impact on the development of the nematode population. The latter can be seen as an indirect suppression of the nematode population. Furthermore, it will also be beneficial if the cover crop, when applied as a green manure, can have a suppressing effect on the root-knot nematode species involved. This can be described as a direct

suppressing effect on the nematode population (Viaene, 1998). Another aspect that can play a role in the suppression of nematodes, with specific reference to *Meloidogyne hapla*, is the trap cropping potential of certain crops. The term trapcrop can be defined as a crop, where nematode penetration takes place, but further development of the lifecycle does not take place (Melakeberhan *et al.*, 2006).

If cover crops in the family Brassicaceae are to be utilised for biofumigation purposes by incorporation into the soil, it is important to know the nematode status of these crops. This will ensure that that they will not cause an increase in specific economically important nematode species in the soil. In this study, five different cover crops were evaluated in glasshouse trials for their host status to *M. javanica* and *C. xenoplax*.

Materials and methods

Host plants production

Five different cover crops were evaluated as hosts for *M. javanica* and *C. xenoplax*, namely: Oats (*Avena sativa* cv. Pallinup), White mustard (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente 199) and Nemat (*Eruca sativa* cv. Nemat). Tomato (*Solanum lycopersicum* cv. Moneymaker) was used as control. Seeds of the different cover crops were sown in black bags with a volume of 4 L for trial 1 and 700 ml for trial 2. Steam sterilized potting medium was used as growing medium, consisting of bark and sand. After the seeds germinated they were thinned to one plant per bag. Plants were grown in a glasshouse at a temperature < 25 °C. Plants were hand irrigated on a daily basis and fertilized on a weekly basis with Chemicult, a balanced plant nutrition supplement.

Meloidogyne javanica inoculum

Tomato plants, inoculated with eggs of *M. javanica*, were grown in a glasshouse for four months. To obtain *M. javanica* eggs, the roots were carefully removed from the soil and washed. The roots were then cut into 2 cm pieces and added to 250 ml of 0.5% sodium chloride solution (NaOCl) in a 500 ml Schott bottle and shaken vigorously for 4 min. The contents of the bottle was passed through a 75- μ m pore (200-mesh) sieve nested in a 38 μ m-pore sieve (500-mesh) and washed with a stream of water. The eggs collected on the 38 μ m-pore sieve were washed into a beaker. Roots were

returned to the bottle, water added and the process repeated. The nematode egg concentration was determined by using the technique of Navon and Ascher (2000). Five drops of 10 μ l each of a suspension of nematodes in a specific volume were put on a glass slide and the number of nematodes counted in 50 μ l. This was repeated five times and the volume of water was diluted or added to obtain the correct egg concentration used as inoculum.

Criconemoides xenoplax inoculum

The peach rootstock Atlas, planted in 25 L plastic pots was inoculated with *C. xenoplax* approximately 24 months earlier. The plants were kept in a glasshouse with a temperature of < 25 °C. A soil auger was used to take a 100 ml soil sample from soil around the roots of 25 pots and the number of nematodes per 100 ml soil was determined by using the sugar flotation technique. A soil concentration of *C. xenoplax* was determined to get the desired amount of nematodes for inoculation of the bags used in the two trials.

Bioassay protocol

The bioassay, for each nematode species, consisted of the five cover crop species with a tomato treatment as control. A graphical presentation of the experimental layout is indicated in Figure 3.1. For each host there were 10 replicates. After the plants were grown for \pm 40 days after which they were inoculated with either eggs of *M. javanica* or soil infested with *C. xenoplax*, according to a predetermined concentration. The plants were arranged in a completely randomised design.

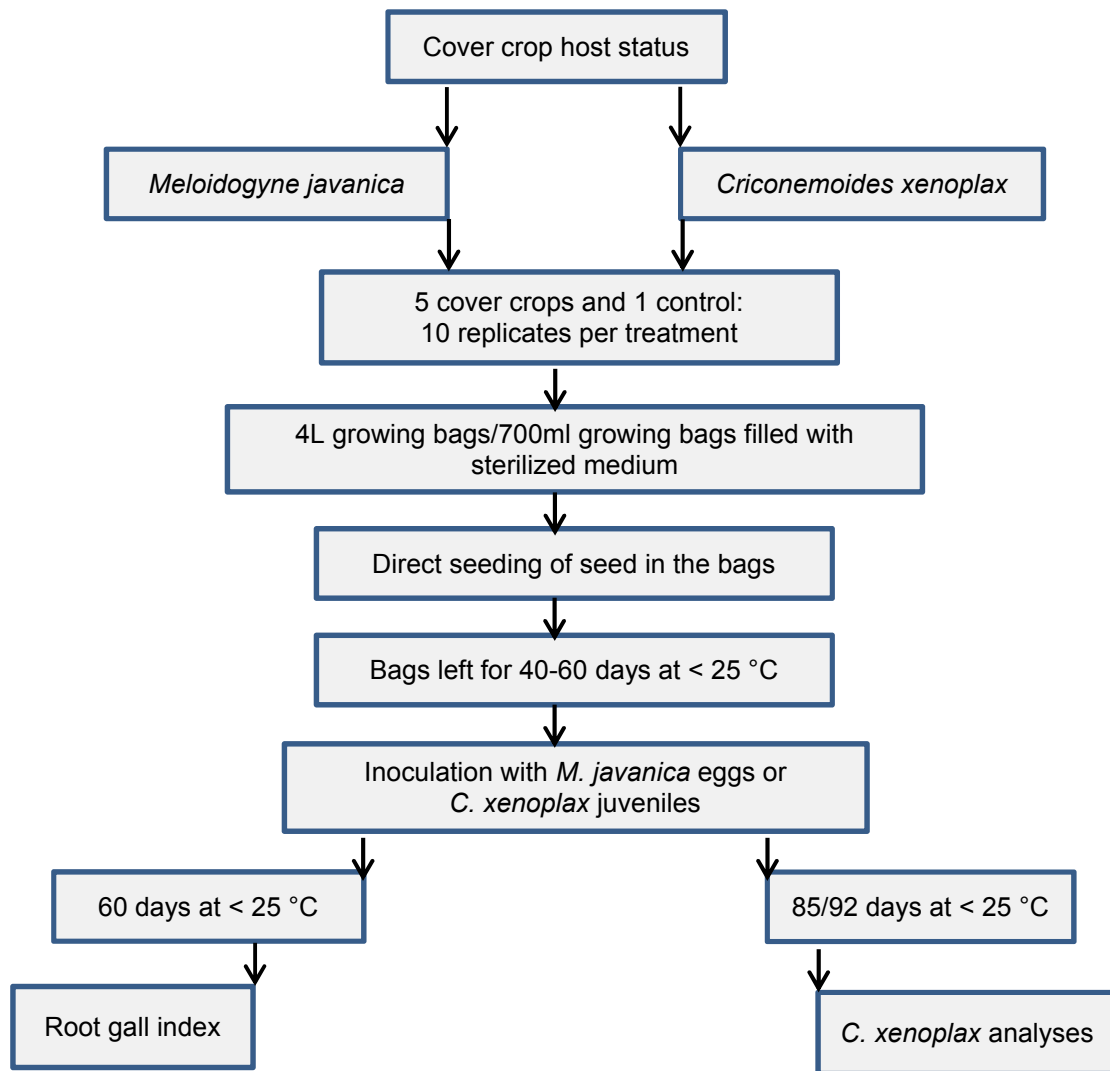


Fig. 3.1. Flow diagram of cover crop host trials for *Meloidogyne javanica* and *Criconemoides xenoplax* to determine the susceptibility of the different cover crops for these nematodes.

Host status of cover crops for Meloidogyne javanica

The bioassay protocol and experimental layout, as described above, were followed for both the *M. javanica* crop host bioassay repetitions; except in the first trial, 4 L growing bags were used and in the second trial 700 ml growing bags. Plants were inoculated with 4000 *M. javanica* eggs in trial 1 and 1000 eggs in trial 2. In both trial repetitions, the plants were left to grow for 60 days before a root gall evaluation was conducted.

Host status of cover crops for Criconemoides xenoplax

The same trial layout as described in Figure 3.1 was used in the *C. xenoplax* bioassays. In trial 1, 200 ml of soil, representing 2500 *C. xenoplax* was used to inoculate the 4 L growing bags. The crops were grown for 85 days before the *C. xenoplax* evaluation was done. In the second trial 700 ml growing bags were used to grow the cover crops and were inoculated with 100 ml of medium, representing 2 500 *C. xenoplax*. In each trial, bags filled with sterilized medium, inoculated with *C. xenoplax*, but without any cover crop planted in the soil, were used as a control. An additional treatment was with tomato as host and was also inoculated with *C. xenoplax*. The tomato treatment was only part of the second trial. After inoculation, the plants were grown for 92 days after which the evaluation was done.

Meloidogyne javanica evaluation

After termination of the experiment, each plant was carefully removed from the bags and the roots washed with water. Each root system was carefully inspected and a root galling index was used to determine the *M. javanica* infestation in the roots. This gall evaluation was done on a scale of 0 - 5 adapted from the technique used by Hussey and Janssen (2002), where 0 = no galls, 1 = 1-10 galls, 2 = 10-50 galls, 3 = 50-100 galls, 4 = > 100 galls and 5 = covered with galls. According to the mean gall classification, the cover crops were then classed as good hosts, maintenance host or poor hosts for *M. javanica*. Between 0-2 was classified as poor host, between 2 and 4 as maintenance crops and between 4 and 5 as good hosts. The root systems were visually inspected by using a Leica MZ7 stereo microscope, fitted with a camera, to determine the formation of egg masses. Egg masses were removed and left for 24 h in a glass crusibel to determine hatching.

Criconemoides xenoplax evaluation

For the extraction of *C. xenoplax* the method of Jenkins (1964) was used. Soil from each plant was mixed and 250 ml soil was washed through a 200 µm sieve into a 10 L bucket. While stirring, the bucket was filled with water to $\frac{3}{4}$ of the volume, left for one minute and then poured through two nested sieves of 53 µm and a 45 µm-pore size, respectively. The content was then washed into a glass beaker. This process was repeated, but first it was left for 15 seconds to settle and then poured through the sieves again, as described above. The content, washed from the soil,

was centrifuged for 5 min at 3000 rpm, the supernatant discarded and each tube filled with a sugar solution and centrifuged for 1 min. The content of the tubes were poured through a 45 µm sieve, washed to free the nematodes from the sugar solution and the nematodes were then collected from the sieve and washed into a 100 ml beaker. The suspension was left for 30 min for the nematodes to settle to the bottom, after which the supernatant was siphoned off to a volume of 20 ml. The content of the beaker was brought into suspension by using air from a fish pump and two ml of the contents were counted, using Peter's slides and a Leica 2000 research microscope.

Statistical analyses

All laboratory experiments were repeated on different test dates. Statistical analyses were performed using the STATISTICA ver. 10 data analysis software system (Statsoft Inc., 2011). Data, obtained from the two trials, were analysed by using a two-way analysis of variance (ANOVA), with date and treatment as the main effects. If there were no trial test data-treatment interactions when main effects were interpreted, data from the two trails were pooled and a one-way ANOVA used for final analysis. If the data were not normally distributed a non-parametrical analysis, using the Kruskal-Wallis test, was performed.

Results

Host status of cover crops for Meloidogyne javanica

No significant differences ($F_{(5, 104)} = 2.155$; $p = 0.065$) were found between the interaction effects (test-date and galling) when analysed, using a two-way ANOVA. Data from the two trial dates were pooled and analysed, using a one-way ANOVA, with significant differences found among treatments ($F_{(5, 110)} = 64.454$; $p < 0.005$) (Fig. 3.2).

All the cover crops differed significantly ($p < 0.05$) from the tomato control, with regards to its host status for *M. javanica*; with the control resulting in a severe expression of galls on the roots with a gall index of 5. The gall index for the Oats treatment was significantly lower than Canola ($p < 0.01$) and Caliente 199 ($p=0.01$), but did not differ significantly from White mustard ($p=0.4$) and Nemat ($p=0.8$). Canola was also significantly higher in its gall index expression than the Nemat treatment ($p < 0.01$), but there were no statistical differences with respect to White mustard ($p=0.08$) and Caliente

199 ($p=1$). The gall index for White mustard was also significantly higher ($p < 0.01$) than expressed by Nemat, while Nemat was significantly lower in its *M. javanica* gall expression than all the other treatments; except for Oats.

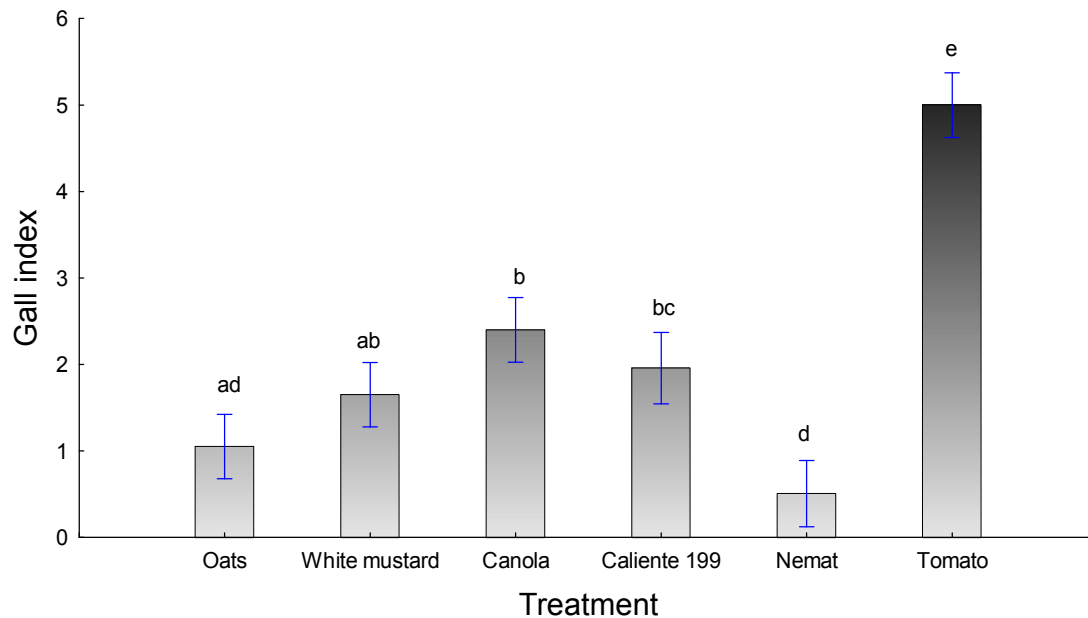


Fig. 3.2. Gall index of *Meloidogyne javanica* (95% confidence interval) 60 days after inoculation of five different cover crops Oats (*Avena sativa* cv. Pallinup), White mustard (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente), Nemat (*Eruca sativa* cv. Nemat) and tomato (*Solanum lycopersicum* cv. Moneymaker) as control (one-way ANOVA; ($F_{(5,104)} = 68.919$; $p < 0.05$). Bars with the same letter did not differ significantly.

Visual inspection of the different root systems

In Figure 3.3 the root systems as, well as the gall-and egg mass formation on the roots of the different crops, were depicted and described as follows: The root gall symptoms on the Canola roots were very prominent and were comparable to the symptoms on the control roots. Egg masses were prominent and the distribution of the symptoms was uniform throughout the root system. The females were well imbedded in the root system and enclosed by the root cells. Prominent root galls and egg masses were also present in the root system of Caliente 199. The females were deeply imbedded in the root system and well protected by the root cells. Fewer galls were present on the total root system of Oats and the galls, that were present, were less prominent and more like a slight enlargement of the root tissue. The female body was not totally imbedded in the Oats root system, with a part of the

body still visible outside the root. Egg masses were more visual than the galls on the roots. The distribution of the egg masses were not uniform throughout the root system and seemed to be situated closer to the soil surface. Very few galls or egg masses were present on the root system of Nemat. The galls, that were present, were only a slight enlargement of the root tissue, with few egg masses showing on the roots. Less galls and egg masses were present on the roots of the White mustard in comparison with Caliente 199 and Canola and the distribution through the root system was not uniform. The females were not fully imbedded in the root system; but more protected in comparison with the females present in the Oats treatment. The roots of the control plants were totally covered with galls and egg masses were very prominent. The females were totally imbedded in the root tissue and well protected by the root cells.

Canola (*Brassica napus* cv. AV Jade)Caliente (*Brassica juncea* cv. Caliente 199)Oats (*Avena sativa* cv. Pallinup)Nemat (*Eruca sativa* cv. Nemat)White mustard (*Sinapis alba* cv. Braco)Tomato (*Solanum lycopersicum* cv. Moneymaker)

Fig. 3.3. *Meloidogyne javanica* galls and egg masses present on the different cover crops roots.

Host status of cover crops for *Criconemoides xenoplax*

No significant differences ($F_{(5,107)} = 1.075$; $p = 0.105$) were found between interaction effects (test-date and treatment) when analysed by using a two-way ANOVA. Results from the two trial dates were pooled and analysed, using a one-way ANOVA, with significant differences ($F_{(6,122)} = 8.233$; $p < 0.005$) found among treatments (Fig. 3.4).

The tomato treatment had significantly higher ($p < 0.01$) *C. xenoplax* numbers, than all other treatments, except for Nemat, with no significant differences ($p = 1$). The final number of nematodes in the tomato treatment was significantly higher than all the other treatments. The Oats, White mustard and Caliente 199 cover crop treatments did not differ significantly from any of the other cover crop treatments and also did not differ from the control (soil only). Canola had the lowest *C. xenoplax* numbers at the time of evaluation and it was significantly lower than those of Nemat ($p = 0.003$); but was not significantly lower than the other crops.

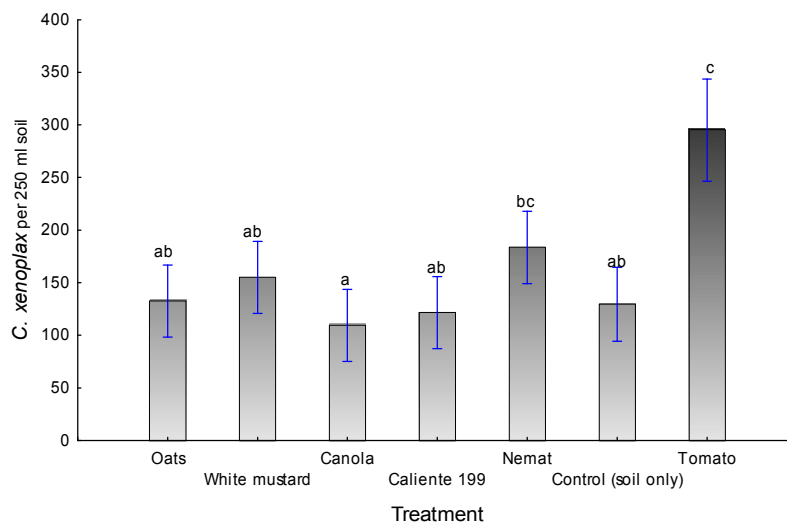


Fig. 3.4. *Criconemoides xenoplax* numbers (95% confidence interval) on five different cover crops, Oats (*Avena sativa* cv. Pallinup), White mustard (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*B. juncea* cv. Caliente 199) and Nemat (*Eruca sativa* cv. Nemat) 60 days after inoculation with nematodes. Inoculated soil was used as control and Tomato (*Solanum lycopersicon* cv. Moneymaker) were used as an additional treatment (one-way ANOVA; $F_{(6,122)} = 8.2325$; $p < 0.005$). Bars with the same letter did not differ significantly.

Discussion

In the *M. javanica* host trials, the control gall index was significantly higher than the rest of the cover crops tested. This can be expected as the tomato cultivar, chosen as part of this study, is not known to be resistant to *M. javanica* and was therefore suitable as a control treatment. The gall symptom expression on the tomato plants were also very severe and gave a good impression on what a crop will look like when heavily infected with *M. javanica*. This also illustrates the impact of the wrong crop, planted as part of a crop rotation, inter cropping or crop rotation system, on the *M.*

javanica population, as such a crop will host the full development of its lifecycle and also cause a population build-up in the soil.

Brassicaceae crops are seen as crops that have biofumigation properties, where glucosinolate (GSL), in the presence of the enzyme myrosinase (MYR), reacts to form the active compound isothiocyanate (ITC) and certain other secondary metabolites (Lazzeri *et al.*, 2004). The work done by Lazzeri *et al.* (2004) shows that the biofumigation properties of certain brassicas have a biocidal effect on *M. incognita*, when applied as a green manure. The glucosinolate-isothiocyanate interaction may also play a role in classifying the brassica crops, depending on species, as maintenance crops or even poor or non-host crops (Lazzeri *et al.*, 2004). This can be due to the fact that not only the above ground plant parts (green manure) that play a role in biofumigation and have the potential to suppress nematodes when applied as green manure, but also the roots. In a literature study, done by van Dam *et al.*, 2009, it was found that on average roots of brassica plants had a higher concentration of GSL than shoots and also that the diversity of the GSL were more in the roots compared to the shoots. This is an important factor when looking at the crop host results for the specific brassica crops involved in this study.

The results, obtained from this study, indicate that all cover crops tested were hosts for *M. javanica* as galling, egg mass production and egg hatching was observed for all cultivars. The severity of the infection, as well as the expression of the symptoms, were different, however, and because of this there will also be differences in the *M. javanica* population build-up where these cover crops are planted. The gall index of Nemat was significantly lower than all the other brassica crops including, White mustard, Canola and Caliente 199, with a gall index of less than 1. Nemat can therefore be classified as a poor host for *M. javanica*. Nemat are also known as a trap root host (Melakeberhan *et al.*, 2006). In this study, however, *M. javanica* did complete its life cycle and Nemat did not act as a catch crop in preventing the development of a new generation of J2 larvae.

In research done by Melakeberhan *et al.* (2006) it was shown that Nemat reduce the development and reproduction of *Meloidogyne hapla* in pot trials, where the evaluation was based on root galls present on the roots, but also in the suppression of all the development stages of *M. hapla*. These studies also showed that there was a limiting effect in the development of the females and thus in the reproduction on Nemat roots with no egg production. The current study indicated Nemat to be a poor host for *M. javanica*, which could have a significant suppressing impact on the population

development in the field. A study, conducted by Curto *et al.* (2005), also showed that Nemat reduced *M. incognita* reproduction, because of the interruption of the lifecycle or slowing down the reproduction rate. The potential thus exist for Nemat to be used in an integrated root-knot nematode management approach from the perspective as a trap crop, but also the positive contribution that it makes through biofumigation (Curto *et al.*, 2005).

The other three brassica species White mustard, Canola and Caliente 199, did not differ significantly from each other and had a low root gall index. These three crops can be classified as maintenance crops for *M. javanica*. This result correlates with work that was done by Curto *et al.*, 2005, where certain *Brassicaceae* and *Capparaceae* crops were selected and tested for their crop host status for *M. incognita*. Their study indicated that *Rapistrum rugosum* sel. ISCI 15, *Eruca sativa* cv. Nemat, *Barbare averna* sel. ISCI 50 and *Raphanus sativus* cv. Boss can all be classified as poor to non-hosts, while *Brassica juncea* sel. ISCI 99 is classified as a maintenance crop and *B. juncea* sel. ISCI 20, *Lepidium campestre* sel. ISCI 103 and *Eruca strumgallicum* are classified as good hosts for *M. incognita*.

A study conducted by Stirling & Stirling (2003) reproduction of *M. javanica*, on certain brassica crops, was compared to other crops that are not known to have biofumigation properties. The crops, that were included in the trials, were Indian mustard (*B. juncea* cv. Nemfix), canola (*B. napus* cv. Dunkeld), rape (*B. napus* cv. Rang), forage sorghum (*Sorghum bicolor* x *Sorghum* Sudanese cv. Jumbo) and tomato (*L. esculentum* cv. Tiny Tim). They found that the brassica crops were hosts (maintenance crops) for *M. javanica*, but significantly less so than the tomato plants and that with the forage sorghum the number of eggs was the lowest. These results are all comparable to the results provided in this study.

Canola, although not significantly different to the Caliente 199 and White mustard, had the highest gall index rating of the brassica crops. It could, therefore, over the medium to longer-term sustain a population build-up of *M. javanica* better in comparison to other brassica crops. Canola is considered to be a poor biofumigation crop, because of its GSL spectrum and the possible impact that this might have on the root susceptibility. Also, Canola has a lower biofumigation potential when applied as a biofumigation crop. Canola will therefore not be seen as the best option for the suppression of *M. javanica* and this must be taken into consideration when the exact aims of the cover crop programme is planned.

Oats is not a brassica crop, but it is widely accepted as a crop with a poor host status for a wide range of soil borne problems, including *M. javanica*. This was also found in this study, as it showed the second lowest root gall index and did not differ significantly from the Nemat treatment. It can however not be classified as a non-host or trap crop as there was root gall formation and egg mass production on the roots. These egg masses seemed to be situated closer to the soil surface and closer to the point of inoculation and can indicate that a high population of *M. javanica* must be present, before root gall symptoms and egg masses will develop on the root system. It is, nevertheless, clear that, from a cover crop or rotation crop perspective focusing on *M. javanica* population suppression, that Oats is a viable option and can be used as part of a cover crop rotation programme, without the risk of stimulating the *M. javanica* population in the specific soil where it is planted.

The results obtained in this study indicate that Nemat and Oats can successfully be used as part of an IPM programme to help suppress the population build-up of *M. javanica* in the soil. These crops can be considered as cover crops in perennial crops, as rotation crops in annual crops, like vegetables or in an intercropping system. It is important in the latter application, to keep in mind the other aspects, like nutrition competition, might play a role. The above mentioned factors are all focused on the crop host status and trap crop effect of Nemat, but there is also the possibility to implement Nemat as a biofumigation crop, and by doing this have a three-way positive impact; 1) the impact that the cover crop host status have in preventing a population build-up as discussed, 2) a direct *M. javanica* suppression effect that can be expected to take place due to the biofumigation effect and 3) the secondary effect that the application of the green manure biomass can have on the general health and biodiversity of the soil, when applied as a biological soil amendment.

The practical application of using Nemat as a cover crop (planting, slashing and incorporation) suggest that will be implemented before the planting of the next cash crop (slashing and incorporation at least 21 days before planting the follow-up crop), to reduce the population of *M. javanica*, during its growing period, as well as through biofumigation after incorporation. By doing this, it will also lower the pressure on chemical nematicide application. The potential also exists to combine Nemat and chemical fumigation and, in work done by Riga (2011), it is illustrated that there was a significant reduction in the *Meloidogyne chitwoodi* population in comparison with Nemat alone and other treatments with the exception of fumigant applied alone at the full rate.

Among all the cover crop treatments, Oats, White mustard, Canola, Caliente 199 and Nemat; the *C. xenoplax* numbers were the highest for the Nemat treatment and were significantly higher than the Canola treatment. There was however not a significant difference between the Nemat and the control, indicating that even though there is a trend that the Nemat increased the *C. xenoplax* population, it does not indicate that Nemat is a good host for *C. xenoplax*, but can rather be classified as a maintenance crop for *C. xenoplax*. From a crop protection perspective; it is, however, not recommended to plant Nemat as a cover crop and to expect a *C. xenoplax* population decline to take place over time. A positive aspect that comes out of this data is the fact that the Canola treatment resulted in the lowest number of *C. xenoplax*, and can therefore be classified as a poor host for *C. xenoplax*. It can be expected that if Canola is planted in a cover crop system; it will not stimulate a *C. xenoplax* population build-up, neither maintain the population, but will rather have a suppressing effect on the population. Caliente 199, Oats and White mustard shows a similar, but not as strong trend.

Cover crops can play an essential role in an integrated management programme for a wide range of soil borne diseases, weeds as well as plant-parasitic nematodes. It is widely expected that a well-planned rotation programme, where different crops with different characteristics are rotated with each other, can have a suppressing effect on a wide range of economically important soil borne diseases, including plant-parasitic nematodes and weeds. One of these characteristics, that is very important to keep in mind, is the host status of the specific crop. Whether it is applied as a cover crop in vineyards or orchards, during the dormant stage of the crop, or as a rotation crop in a cash cropping system, or used before replanting trees, where the replant disease complex plays a role, the host status of the crop used is a critical factor to break the lifecycle of certain soil borne biotic problems. The use of Nemat as a cover crop or rotation crop can be beneficial in suppressing *M. javanica*. In the case of *C. xenoplax* one can be expect to see a declining effect in the population over time when Canola is implemented in a cover crop system.

As cover crops can play a very important role in IPM, in future research it will be beneficial to assess the crop host status for most cover crops that forms part of cover crop or rotation systems and also investigate the possibility to combine this with other chemical and biological options in establishing a long-term solution for nematode management.

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CHAPTER 4

The effect of cover crops and the management thereof on plant-parasitic nematodes in vineyards

Abstract

Plant-parasitic nematodes impact negatively on the production of grapevines in South Africa. Most of the effective synthetic nematicides are presently under pressure of being phased out, creating an ever-growing need for a more biological approach. In South Africa the use of annual cover crops is an established soil cultivation practice in vineyards that is environment-friendly and financially sustainable in the long term. Species from the Brassicaceae family are well-known for their biofumigation potential. In this study, White mustard (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente 199), Nemat (*Eruca sativa* cv. Nemat) and Oats (*Avena sativa* cv. Pallinup) were established as cover crops in a vineyard for three consecutive growing seasons and evaluated for their biofumigation impact, as well as the crop host impact on the suppression of economically important plant-parasitic nematodes. Two cover crop management practices, viz. mechanical incorporation (MC) into the top soil and chemical removal of the cover crop (CC) were applied. The effect of these treatments on different plant-parasitic nematodes, with specific focus on *Criconemoides xenoplax* (ring nematode) and on *Meloidogyne* sp. (root-knot nematode) numbers, was determined prior to the start of the cover crop season, as well as 0, 15, 30 and 60 days after the management practices were applied for three growing seasons. In the field trial, 60 days after the management practices, Canola (CC) and Caliente 199 (CC), showed a constant reduction during the three seasons in the *C. xenoplax* numbers present in the vine row. This same trend existed during the three-year trial period for all different sampling periods (0, 15, 30 and 60 days). This trend was also observed for the total plant-parasitic nematode numbers for the three-year trial period measured at 60 days after the management practice sampling period. Results can mainly be attributed to the crop host status of the two crop species in terms of *C. xenoplax*. White mustard (CC and MC) showed a constant increase in the *C. xenoplax* numbers in the vine row over the three-year period, compared to weeds (CC) treatment. The same was also the case for some of the other

treatments, with the trend being less obvious and more of a maintenance type of effect, with no build-up of plant-parasitic nematodes.

Introduction

Soil-borne pests and diseases have a negative impact on food production in most agricultural crops. The phasing out of effective soil fumigation options, in addition to the pressure on other chemical control options, causes the control of these soil-borne pests and-diseases to become an even greater challenge. Therefore, alternative methods have to be pursued to meet the challenge that these soil-borne diseases pose to agriculture (Matthiessen & Kirkegaard, 2006). These alternatives need to have a low environmental impact; yet must be effective in the management of soil-borne pathogens (Lazzeri *et al.*, 2004).

Crucifer tissue, when applied to the soil as a green manure, after maceration of the plant tissue, has shown biocidal effects that can be defined as a form of biofumigation (Angus *et al.*, 1994; Kirkegaard & Matthiessen, 2004). The concept of biofumigation is not new, and has been used in other parts of the world for a period of time. The first observations of this technique were already recorded at the beginning of the 17th century (Challenger, 1959). There are three areas in which biofumigation could have a positive effect, in terms of integrated pest management (IPM), namely: nematode control (Zasada & Ferris, 2004; Monfort *et al.*, 2007), the control of soil-borne diseases (Lazzeri *et al.*, 1993; Manici *et al.*, 1997) and weed control (Brown & Morra, 1997).

Certain crops, *inter alia* the brassica species., produce sulphur-containing secondary metabolites with the main focus on isothiocyanate (ITC). Glucosinolates (GSL) present in the cells are hydrolysed by the enzyme myrosinase (MYR) to form the highly active ITC, which has a toxic effect on many soil-borne pathogens (Sarwar *et al.*, 1998). There are also other secondary metabolites that form during the degradation of the crop tissues, including nitriles and thiocyanates (Cole, 1976; Fenwick *et al.*, 1983). GSL can be grouped into three main groups, including aliphatic, aromatic and heterocyclic (Indole) (Fahey *et al.*, 2001). The presence of GSL differs between the different plant parts; such as the roots, leaves, stems and seeds (Fahey *et al.*, 1997; Van Dam *et al.*, 2009). The release of the active compound, ITC, takes place when the cell walls of these plants are ruptured (Van Etten & Tookey, 1983; Matthiessen *et al.*, 2004) and the GSL inside the vacuole of the cell

comes in contact with the enzyme MYR, which is situated inside the cytoplasm of the cell (Poulton & Moller, 1993).

To maximise the presence of the ITC in the soil during the biofumigation process, *Brassica* spp., with high GSL concentration, must be selected and optimal cellular disruption during the maceration and incorporation process should be achieved, whilst ensuring that there is sufficient soil moisture present, both during and after incorporation (Brown *et al.*, 1991; Poulton & Moller, 1993; Morra & Kirkegaard, 2002; Mathiessen *et al.*, 2004). Water plays a role in the hydrolysis process, with GSL being hydrolysed to ITC and it is essential that enough soil moisture is present in the soil for the desired reaction to take place (Tyagi, 2002; Lazzeri, *et al.*, 2004; Mathiessen *et al.*, 2004).

A principle that forms part of IPM, and which is successfully used to suppress soil-borne diseases, is crop rotation. Green manuring, with selected *Brassica* spp., has delivered promising results in suppressing soil-borne pests and diseases (Larkin & Griffin, 2007). *Brassica napus* (Canola) residues suppressed certain wheat diseases; most probably because of the fungicidal compounds, like ITC, that are released during the breakdown process of the canola residues (Kirkgaard *et al.*, 1996 a, b; Sarwar *et al.*, 1998). Canola and rapeseed (another *Brassica napus* cultivar) applied in a crop rotation system before the planting of *Solanum tuberosum* (potato) have also suppressed certain potato diseases, such as *Rhizoctonia solani* (Larkin & Honeycutt, 2006). Different *Brassica* spp. have been evaluated for their capacity to suppress potato diseases, but *Brassica juncea* (Indian mustard), has been found to be the most effective in inhibiting fungal growth in *in vitro* tests (Larkin & Griffin, 2007).

In grape production, cover crops, established in the inter row, reduced water runoff and erosion (Khan *et al.*, 1986; Roth *et al.*, 1988; Louw & Bennie, 1992), restricted evaporation from the soil surface (Van Huyssteen *et al.*, 1984; Myburgh, 1998), conserved soil water (Buckerfield & Webster, 1996) as well as reduced temperature fluctuations in the soil (Van Huyssteen *et al.*, 1984; Fourie & Freitag, 2010). It also facilitated effective suppression of both winter and summer growing weeds (Fourie *et al.*, 2005; Fourie *et al.*, 2006; Fourie, 2010). Cover crops in vineyards have also been studied for their effect on plant-parasitic nematodes (Addison and Fourie, 2008). It was observed that certain grass species, as well as certain broad-leaf species, had the potential to suppress nematode pests. The ability of certain *Brassica* spp. to suppress nematodes is well documented (Mojtahedi *et al.*, 1991, 1993; McLeod & Steel, 1999; Melakeberhan *et al.*, 2006).

Worldwide, the most important nematodes associated with grapevines are *Meloidogyne* spp. (root-knot nematode), *Pratylenus* spp. (root lesion nematode), *Criconemoides xenoplax* (Raski, 1952) Loof and De Grisse, 1976 (ring nematode) and *Xiphinema* spp. (dagger nematode) (McKenry, 1992; Pinkerton *et al.*, 1999; Walker & Stirling, 2008). In Australia, plant-parasitic nematodes are problematic in all the different grape-growing regions (Nicol *et al.*, 1999), and in South Africa grapevines are also host to a wide range of plant-parasitic nematodes (Smith, 1977; Loubser & Meyer, 1987a,b).

The symptomatic effect of plant-parasitic nematodes on crops is very clear; especially on vegetables such as *Solanum lycopersicum* (tomatoes) or potatoes. The economic impact of plant-parasitic nematodes on a variety of crops is estimated to be approximately, US \$100 billion/year (Koenning *et al.*, 1999; Sasser & Freckman, 1987). It is estimated that the economic impact of plant parasitic nematodes on crops in South Africa is approximately R1.9 Billion. The impact of plant-parasitic nematodes on grapevines is less obvious and normally manifest when the grapevines are under some sort of stress; such as water stress (Ferris & McKenry, 1975). Symptoms of vines infected with *M. javanica* spp include: poor vigour, stunted growth and poor yields (Seinhorst & Sauer, 1956). It is, however, very important to note that *Xiphinema index* (Thorn & Allen 1950), which is one of the nematode species that is present, in high numbers in some viticulture regions in South Africa, not only damages the roots of susceptible grapevine cultivars; but is also a vector for the grapevine fan-leaf virus (Malan & Meyer, 1992; Malan & Meyer 1994; Malan & Meyer 1999).

The management of plant-parasitic nematodes in viticulture in South African is currently based on chemicals, registered under the Fertilisers, Farm Feeds, Agricultural Remedies and Stock Remedies Act 36 of 1947. The following active ingredients are registered: cadusafos, fenamifos and furfural (ProCrop Database, 2012). The use of rootstocks resistant to specific plant-parasitic nematode spp. also forms part of an integrated approach to manage nematode pest in South African vineyards. The rootstocks available are classified as: resistant, moderately resistant, moderately susceptible and susceptible, with most of the resistance being developed for root-knot nematodes (Loubser & Meyer, 1987b). There are, currently, no rootstocks available that are resistant against *C. xenoplax* (Storey, 2012).

Brassica cover crops used in vineyards, known for their biofumigation potential, showed promising results as part of an integrated approach for *M. javanica* suppression. Indian mustard cv

Nemfix, grown in the grapevine inter-row and applied as a green manure, either to the vine row, or to inter-row, suppressed the *M. javanica* numbers (Rahman & Somers, 2005).

The main objective of this study was to obtain scientifically based guidelines for the sustainable use of cover crops in vineyards, as part of a nematode management program. Firstly, cover crops were selected for their potential to biofumigate the soil, as a means of suppressing plant-parasitic nematodes in vineyards. Secondly, the two management practices (green manuring versus no till at grapevine bud break) could indicate the role that green manure itself can play on the suppression of plant-parasitic nematodes (cover crops with none or little biofumigation properties), as well as the effect that the cover crop host status has on the long-term suppression of plant-parasitic nematodes.

Materials and methods

Experiment vineyard and layout

The three-year study (2009/10, 2010/11 and 2011/12 seasons) was executed in a seven year (2009/10) old Shiraz/101-14 drip irrigated vineyard, that has been established on a sandy to sandy loam soil near Stellenbosch in the Western Cape, South Africa. The rootstock is classified as being mildly resistant to root-knot nematodes. Stellenbosch receives an average 673 mm of rain annually, of which approximately 73% precipitates from March to August. Before the start of the field trial, soil samples were collected on a random basis to determine whether the site was suitable as a nematode trial site. Grapevine cultivation practices applied on this site were in keeping with the standard practices applied in vineyards of South Africa. The vineyard was drip irrigated from December to March.

Fourteen treatments were applied as described in (Table 4.1). These treatments consisted of five cover crop species, managed according to two management practices (10 treatments), which were compared to two treatments in which no cover crop was sown and the weeds were managed according to the above-mentioned two management practices, as well as to two similar treatments in which a nematicide (Rugby 10ME, active ingredient cadusafos) was applied at 15 ml/m² to the vine row. The treatments were replicated five times in a randomised block design. Each plot (replicate) consisted of a surface area of approximately 83 m². A vine row functioned as a buffer zone between

treatments situated in different work rows and a buffer area, the length of five vines was left between the experimental vines of treatment plots situated in the same vine row (Figure 4.1).

Table 4.1. The cover crops management practices and seeding densities of the cover crops applied to a seven year old, Shiraz vineyard grafted on a 101-14 rootstock, situated in Stellenbosch in the Western Cape wine grape growing region of South Africa.

Treatment no.	Cover Crops	Management practice applied	Seeding density (kg/ha)
1	Oats (<i>Avena sativa</i> cv. Pallinup)	CC	100
2	Oats	MC	100
3	White mustard (<i>Sinapis alba</i> cv. Braco)	CC	8
4	White mustard	MC	8
5	Canola (<i>Brassica napus</i> cv. AV Jade)	CC	8
6	Canola	MC	8
7	Caliente 199 (<i>Brassica juncea</i> cv. Caliente 199)	CC	10
8	Caliente 199	MC	10
9	Nemat (<i>Eruca sativa</i> cv. Nemat)	CC	5
10	Nemat	MC	5
11	Weeds	CC	-
12	Weeds	MC	-
13	Weeds + nematicide (Rugby 10ME @15ml/m ²)	CC	-
14	Weeds + nematicide (Rugby 10ME @15ml/m ²)	MC	-

CC = Full surface chemical control of cover crop from just before bud break to grapevine harvest.

MC = Chemical control in the vine row and mechanical incorporation of the weeds/cover crops in the work row just before bud break, CC from berry set.

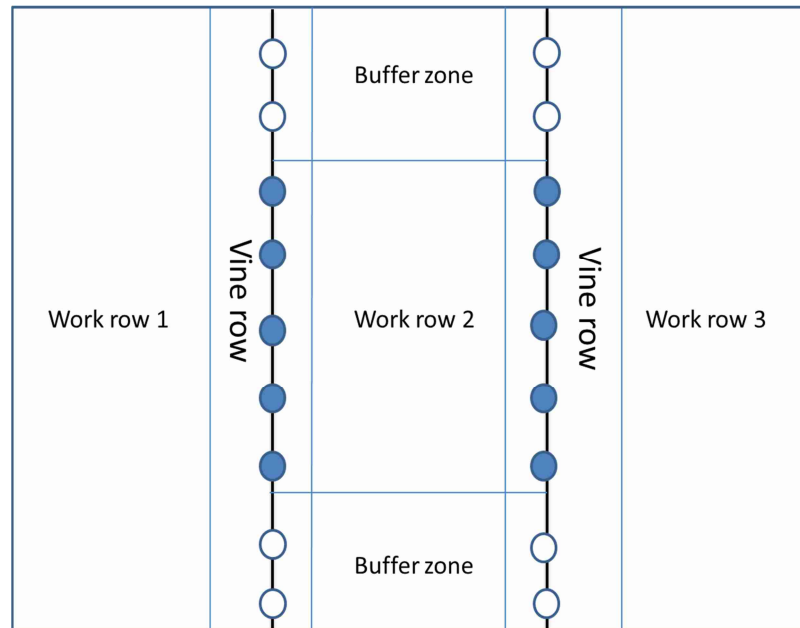


Fig. 4.1. Visual layout of the experimental plot where the cover crops were planted in the work row and nematode samples were taken in the work row and the vine row.

Soil preparation

Seedbed preparation in the work row was done with a disc harrow (two passes). The seeds were broadcasted by hand, at the seeding densities indicated in Table 4.1. The cover crops were established on 4 May (2009 and 2011) and 10 May (2010), after the onset of the first good winter rains. At the beginning of the trial in 2009, a proper fine seedbed could not be created, as a result of excessive weed growth in the work row and a slight furrow in the centre of the work row, causing the vine rows to be slightly ridged. After sowing, the seeds were covered by means of a light cultivation action to ensure good seed/soil contact. The slanted soil surface caused a large percentage of the seeds to accumulate in the centre of the work row. The mechanical cultivation, applied during the first year, levelled the soil in the work row to such an extent that seedbed preparation and the covering of the broadcasted seeds was achieved across the full surface from the second season onwards.

Table 4.2. Rainfall for the period May to August for the three cover crop growing seasons 2009, 2010 and 2011 measured at a weather station at Alto, close to the trial site in Stellenbosch.

Date	Rainfall mm		
	2009	2010	2011
1 May - 7 May	13.45	37.08	28.95
8 May - 14 May	5.84	75.43	0.00
15 May - 21 May	58.67	0.25	0.25
22 May - 28 May	7.11	27.94	49.53
29 May - 4 June	34.3	1.27	34.54
5 June - 11 June	27.94	32.5	10.15
12 June - 18 June	34.04	59.44	44.45
19 June - 25 June	46.23	5.84	49.77
26 June - 2 July	4.31	11.93	29.46
3 July - 9 July	2.03	6.60	2.29
10 July - 16 July	64.82	55.62	0.00
17 July - 23 July	10.16	5.08	0.00
24 July - 30 July	5.08	0.50	15.25
31 July - 6 August	32.76	0.25	29.72
7 August - 13 August	24.13	25.4	17.78
14 August - 20 August	33.02	6.35	10.41
21 August - 27 August	6.10	35.05	23.88
Total rainfall (mm) for period	409.99	386.53	346.43

Biofumigation crops are heavy feeders and require enough fertiliser for good biomass production. According to Dale Gies (personal communication, 2007) optimal biomass production with Caliente 199 and Nemat can be achieved with approximately 120 kg N/ha and 60-80 kg N/ha, respectively, depending on the fertility of the soil involved. However, any fertiliser applied to a cover crop in vineyards should not exceed the fertiliser needs of the grapevines. Therefore, soil nutrient status and grapevine nutrient status were monitored throughout the trial period, to ensure that the amounts of fertiliser applied to the cover crops did not cause a nutrient imbalance in the grapevines. The fertilisers that were applied to the cover crops are shown in Table 4.3.

Table 4.3. Fertiliser applications during the 2009, 2010 and 2011 cover crop growing seasons

Growing season	Timing 1	Timing 2	N (kg/ha)	P (kg/ha)	K (kg/ha)
2009/10	-	Two to six leaf stages of oats	28	-	-
2010/11	-	Two to six leaf stages of oats	28	-	-
		Flowering stage of grapevines	28	-	30
2011/12	Before sowing	-	28	30	-
		Two to six leaf stages of oats	28	-	-

The cover crops were controlled between late bloom and early seed/pod formation, depending on the climatic conditions during that specific growing cycle. This resulted in the management practice being applied at 25 August, 3 September and 10 September during the 2009/10, 2010/11 and 2011/12 grape growing seasons respectively. The mechanical maceration was done by making use of a standard weed slasher and a disc harrow and was incorporated to a depth of 200 mm. The incorporation was timed to coincide with high soil water content, as water plays an important role (hydrolysis) during biofumigation (Matthiessen *et al.*, 2004). According to Luca Lazzeri, (personal communication, 2012), if the soil moisture level is 66% of field water capacity by the time of incorporation, irrigation or rainfall after incorporation is not essential and biofumigation can still be expected to take place. In Table 4.4 the rainfall for the period end August to November are shown for the periods where soil moisture played an important role in the biofumigation process.

Table 4.4. Rainfall before and after the management practice were applied for the three cover crop seasons 2009, 2010 and 2011.

Date	Rainfall mm		
	2009	2010	2011
21 August - 27 August	6.1	35.05	23.88
1 September - 7 September	36.32	12.19	21.8
8 September - 14 September	28.2	2.54	8.89
15 September - 21 September	10.67	15.24	16.01
22 September - 28 September	27.94	12.44	0
29 September - 5 October	0.51	0	2.28
6 October - 12 October	48.77	24.64	0
13 October - 19 October	6.35	2.03	27.94
20 October - 26 October	9.14	8.63	0
27 October - 2 November	3.05	0	5.59
3 November - 9 November	84.84	23.62	7.37
10 November - 16 November	25.39	9.4	28.7
17 November - 23 November	0	20.58	24.38
24 November - 30 November	0.25	1.52	0
Total rainfall (mm) for period	281.43	132.83	142.96

Roundup Classic (360g/l glyphosate as active ingredient) was applied at a rate of 5 L/ha in both the CC (work row and vine row) and MC treatments (vine row). The standard pest and disease management programme, used by the farm, was applied.

Dry matter production

The dry matter production (DMP) of the different cover crops was determined as described by Fourie *et al.* (2001). A sample of each treatment was taken by harvesting the above-ground vegetation in a 0.5 m² subplot in each experimental plot. The samples were oven-dried for 48 hours at 105 °C after which it was weighed.

Soil nematode status

To determine the effect of the selected cover crops and cover crop management practices on the nematode numbers, a composite soil sample was taken from the 0-250 mm soil layer of each plot at the beginning of April (before the re-establishment of the cover crops), as well as 0 days (just before the management practice), 15, 30 and 60 days after the cover crop management practice took place. The samples were taken in the work row or inter-row, as well as in the vine row and analysed

separately. Each sample consisted of five subsamples taken diagonally across the work row, as well as underneath the vines in the vine row (Figure 4.1). Nematodes were extracted from the soil, using a sugar centrifugation technique, based on the method used by Kleynhans *et al.* (1996).

Extraction and identification of nematodes

Soil samples were mixed in the laboratory, and the plant-parasitic and non-parasitic nematodes were extracted from a 250 ml subsample using a sugar flotation technique. The nematodes were then counted and identified, using a light microscope, according to the technique described by Kleynhans *et al.* (1996).

Statistical procedures

The experiment was arranged in a randomized block design with 14 treatments replicated five times (Table 4.1). The treatments were repeated for three consecutive seasons in 2009/10, 2010/11 and 2011/12. Ten experimental grapevines per plot were used for measurements. An analysis of variance was performed separately for each season, using SAS (SAS, 1990). Student's *t* least significant difference (LSD) was calculated at the 5% and 10% significance level to facilitate comparison between treatment means. The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965).

Results and discussion

Nematode population

The plant-parasitic nematode species identified in soil samples over the three seasons were: *Meloidogyne* sp. (Kofoid & White, 1919) Chitwood, 1949, *C. xenoplax* and *Xiphinema* spp. *Criconemoides xenoplax* and the *Meloidogyne* sp. had the highest number of nematodes in all extractions. The extraction of *Pratylenchus* spp. from the roots was only done for the first year, but it was discontinued because of low nematode numbers.

Dry matter production (DMP)

There are numerous factors that play a role in optimal cover crop production, with climatic conditions (rainfall and temperature), as well as correct fertilizer application, being some of the key factors. Work done by Fourie et al. (2001) indicates that the timing of rainfall after seeding a cover crop is crucial. Table 4.5 indicates significant differences between the DMP of the different cover crops species within the same year. Differences were also found between the years for the same cover crop species. In 2009 the DMP in the Oats CC treatment were significantly higher than the Canola (CC and MC) and the Nemat (CC and MC). The Nemat DMP for both the CC treatment and the MC treatment were the lowest for 2009. Overall the DMP of the cover crops were not good during the 2009 season and this can be attributed to the seedbed preparation that could not be performed optimally. Also, the low rainfall during the first 10 days after the seeding action might have had a possible impact on the early establishment and growth of the cover crops. All the cover crop treatments, with the exception of Oats in 2010 and White mustard in 2010, showed a constant, year by year, increase in the DMP from 2009 to 2011. This is a very important trend as it can be expected that, with a higher DMP, the potential of the biofumigation cover crops, to actively perform the biofumigation action after maceration and incorporation, are enhanced (Morra & Kirkegaard, 2002, Matthiessen *et al*, 2004).

The importance of DMP is also stressed in work done by Rahman and Somers (2005) who found that the suppression of *M. javanica* in vineyards were better where higher biomass were applied to the infested soil. Stirling and Potter (1998) also found that at least 2 tons DMP/ha of brassica biomass are needed for a significant biocidal impact, while Stirling and Stirling (2003) indicated the reduction of *M. javanica* after the application of 17 ton DMP/ha in comparison to the impact of lower DMP, but could not determine whether the reduction was specifically due to the ITC release or perhaps other secondary aspects.

The reason for the increasing DMP trend in this study can be ascribed to the better seedbed preparation from 2009-2011, but probably more specifically to the fertilizer applications that were altered from 2009-2011, as indicated in Table 4.2. From 2010, the amount of nitrogen applied to establish the cover crops were applied two times during the growing season. Furthermore in 2011, 30 kg/ha of phosphates were applied, before sowing the cover crops and this had a dramatic impact on the establishment and growth of the cover crops. Nemat (MC) DMP were below 2 tons/ ha for all three

seasons and, keeping in mind the work by Stirling and Potter (1998), this treatment is not expected to have a dramatic impact on the *Meloidogyne* sp. numbers, as the amount of biomass produced and therefore the potential to form ITC was very low.

Table 4.5. Dry matter production (DMP) of the different cover crops used, measured in August of 2009, 2010 and 2011.

No.	Treatment	DMP (t/ha)		
		August 2009	August 2010	August 2011
1	Oats (<i>Avena sativa</i> cv. Pallinup), CC	3.29	2.48	5.66
2	Oats, MC	2.10	2.49	2.52
3	White mustard (<i>Sinapis alba</i> cv. Braco), CC	2.70	4.96	4.48
4	White mustard, MC	2.57	3.21	5.12
5	Canola (<i>Brassica napus</i> cv. AV Jade), CC	1.81	2.36	4.39
6	Canola, MC	1.64	2.22	3.98
7	Caliente 199 (<i>Brassica juncea</i> cv. Caliente 199), CC	2.37	3.08	5.23
8	Caliente 199, MC	2.43	3.08	5.62
9	Nemat (<i>Eruca sativa</i> cv. Nemat), CC	0.71	3.33	5.13
10	Nemat, MC	0.95	1.62	1.79
11	Weeds, CC	-	-	-
12	Weeds, MC	-	-	-
13	Weeds, nematicide, CC	-	-	-
14	Weeds, nematicide, MC	-	-	-
	LSD ($p \leq 0.05$)	1.18	1.03	1.39

CC = Full surface CC from grapevine bud break.

MC = CC vine row, mechanical incorporation in work row from grapevine bud break, full surface CC from berry set.

Effect of cover crops, without management practices, on Criconemoides xenoplax in the vine and work row

In Table 4.6 the interaction year, time, position and practice in the vine row was found to be not significantly different ($p = 0.86$). The least significant difference (LSD) on a 95% confidence interval was found to be 208.26. Fluctuations did take place in *C. xenoplax* in the vine row from day 0-60 within each year and there is also fluctuation evident between the different years and cover crops. Two important periods to consider is 0-60 days and 60-0 days for the specific years. These periods indicate the impact of the cover crops on *C. xenoplax*, after the management practice was done (0-60

days) and also the impact that the cover crops had on the *C. xenoplax* numbers, during active growth (60-0 days).

The three treatments that showed a significant decrease in *C. xenoplax* (0-60 days) in the vine row for the three years were White mustard, Nemat and weeds plus the nematicide. This seems to be a positive result, but when the total impact 2009 (0 days) to 2011 (60 days) are considered, White mustard and Nemat were the only two crops indicating an increase in *C. xenoplax*. The weeds and nematicide treatment did not show the same increase over the total period. This is also illustrated in Figure 4.2 where the White mustard and Nemat shows a similar long term trend to the Weeds (control). An important reason for this is the fact that there was a significant increase for these two treatments from the (60-0 days) period for both 2009/10 and 2010/11. All the other crops, except Canola (2009/10 and 2010/11) and Caliente 199 (2010/11) showed a significant increase in *C. xenoplax* from 60-0 days for the 2009/10 and 2010/11 period. The increase that took place for White mustard and Nemat during this period was the most prominent and was significantly higher than Canola during both seasons. It can therefore be expected that *C. xenoplax*, for these two crops, start of from a higher number, because of a strong build up over the period 60-0 days and from there the higher *C. xenoplax* over time. The increase in *C. xenoplax* for Canola (2009/10 and 2010/11) and Caliente 199 (2010/11) over the period 60-0 days, was not significant. It is also these two crops that showed a constant decreasing trend (Figure 4.2), that compared well with the weeds and nematicide trend over the total period (2009-2011). A possible explanation for the above finding is shown in the *C. xenoplax* crop host trial (Chapter 3), in which Canola and Caliente 199 showed the lowest *C. xenoplax* numbers in comparison with the other crops tested.

The *C. xenoplax* in the work row were in all cases significantly lower than the numbers in the vine row for the specific year, crop and time (Table 4.6). The *C. xenoplax* present in the work row were very low and no specific conclusion or trend with regards to the impact of the cover crops on the *C. xenoplax* can be made. What is clear is that there was an increase in *C. xenoplax* in 2011, with constantly more nematodes present at the specific sampling times (0, 15, 30, 60 days after the management practice). The trend is, however, not restricted to a specific crop and the same trend occurs in the weeds (control). This can be ascribed to the total biomass produced for the crops that were higher during 2011 compared to 2010 and 2009, indicating that the growing conditions were

more favourable for the crops. With an increase in biomass, root biomass will also increase and provide the potential for better nematode establishment.

Table 4.6. The effect of the cover crop, without management practice, on *Criconemoides xenoplax* in the vine and work row. The interaction involved is Year (2009, 2010, 2011) x Time (0,15,30,60 days) x Position (vine row and work row) x Crops (Y x T x Pos x Crop).

		Number of nematodes in 250ml soil											
		Days after management practice											
		0	15	30	60	0	15	30	60	0	15	30	60
Cover crop		2009				2010				2011			
Vine row	Oats (<i>Avena sativa</i> cv. Pallinup)	649	334	524	282	609	624	670	410	788	626	259	426
	White mustard (<i>Sinapis alba</i> cv. Braco)	582	379	564	347	905	695	693	536	1093	907	616	739
	Canola (<i>Brassica napus</i> cv. AV Jade)	708	450	574	645	759	792	619	589	739	843	431	464
	Caliente 199 (<i>Brassica juncea</i> cv. Caliente 199)	679	506	749	463	865	1087	947	552	622	775	600	454
	Nemat (<i>Eruca sativa</i> cv. Nemat)	574	525	657	266.5	803	735	984	519	999	694	482	608
	Weeds (control)	548	422	556	347	646	740	821	483	840	587	766	397
	Weeds, nematicide	553	468	601	258	548	359	572	205	753	564	349	396
Work row	Oats (<i>Avena sativa</i> cv. Pallinup)	21	16	23	6	13	27	11	32	125	54	26	75
	White mustard (<i>Sinapis alba</i> cv. Braco)	19	35	11	7	66	90	17	17	37	169	239	128
	Canola (<i>Brassica napus</i> cv. AV Jade)	34	8	20	56	46	95	56	45	110	120	98	123
	Caliente 199 (<i>Brassica juncea</i> cv. Caliente 199)	23	15	18	13	46	92	32	101	85	127	159	200
	Nemat (<i>Eruca sativa</i> cv. Nemat)	23	9	66	46	14	40	17	72	75	73	113	177
	Weeds (control)	28	9	33	12	51	23	39	2	45	55	131	139
	Weeds, nematicide	35	26	25	52	87	3	19	7	70	72	138	95
LSD (p = 0.86)		208.26											

The interaction (Y x T x Pos x Crop) did not differ significantly (p=0.86), but the treatments differed significantly on a 95% confidence interval. Least significant difference (LSD) = 208.26

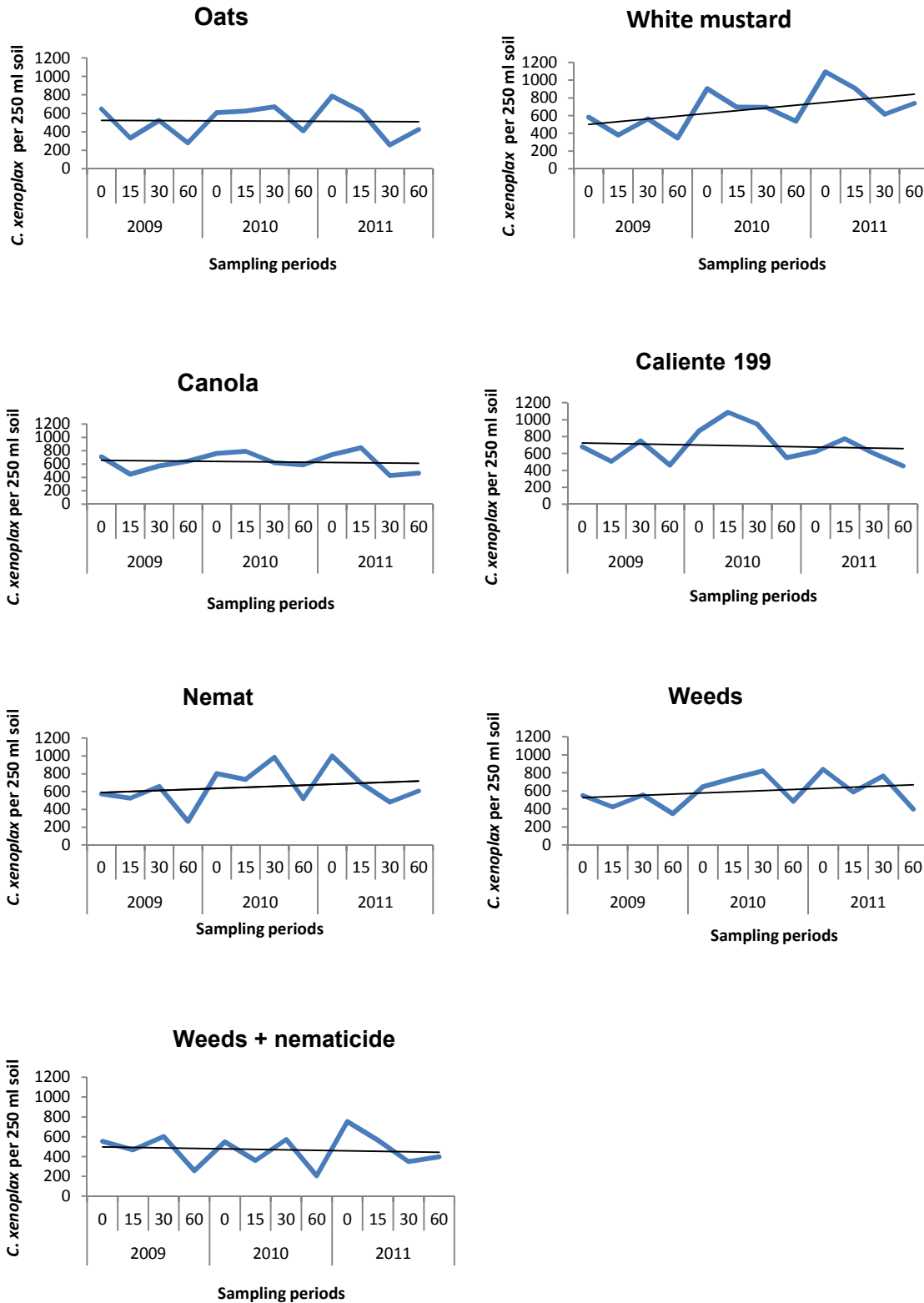


Fig. 4.2. *Criconeoides xenoplax* trends for the three year (2009, 2010 and 2011) when the effect of the cover crop alone is considered. The management practice is not taken into consideration.

Effect of cover crops, without management practices, on the Meloidogyne sp. in the vine and work row

The *Meloidogyne* sp. numbers in the vine and work row, for the three years, show that crops and sampling periods were low, with lower *Meloidogyne* sp. numbers present in the work row compared to the vine row (Table 4.7). These numbers are, however, not expected to pose an economic threat to the growth and yield of the vineyards (Sheila Storey, pers. Comm., 2013). The same trend, as is the case for the *C. xenoplax*, appears for the period 60-0 days, with both 2009/10 and 2010/11 showing a significant increase in the *Meloidogyne* sp. This trend can be attributed to the climatic conditions being favourable for the numbers to build up over these periods. The positive results that are indicated in Table 4.7, is that there was no economically important *Meloidogyne* sp. increasing trend for these crops over time, except for the weeds (control) treatment that do show an increasing trend (Figure 4.3).

Table 4.7. The effect of the cover crop, without management practice, on the *Meloidogyne* sp. in the vine and the work row. The interaction involved here is Year (2009, 2010, 2011) x Time (0, 15, 30, 60 days) x Position (vine and work row) x Crop (Y x T x Pos x Crop).

Cover crop		Number of nematodes in 250ml soil											
		Days after management practice											
		0	15	30	60	0	15	30	60	0	15	30	60
		2009				2010				2011			
Vine row	Oats (<i>Avena sativa</i> cv. Pallinup)	47	30	45	17	53	32	75	30	119	15	8	52
	White mustard (<i>Sinapis alba</i> cv. Braco)	55	32	53	23	72	54	55	33	81	43	60	41
	Canola (<i>Brassica napus</i> cv. AV Jade)	113	28	42	47	48	60	41	21	114	57	68	38
	Caliente 199 (<i>Brassica juncea</i> cv. Caliente 199)	73	48	119	57	65	90	56	35	78	110	89	27
	Nemat (<i>Eruca sativa</i> cv. Nemat)	63	16	40	48	22	58	60	21	92	72	77	37
	Weeds (control)	72	16	84	27	81	63	64	28	128	72	163	48
	Weeds, nematicide	93	22	28	41	39	80	22	19	103	66	27	30
	Work row	Oats (<i>Avena sativa</i> cv. Pallinup)	24	12	10	0	0	34	0	0	0	3	0
White mustard (<i>Sinapis alba</i> cv. Braco)		2	8	2	5	0	46	0	0	0	3	0	0
Canola (<i>Brassica napus</i> cv. AV Jade)		12	0	0	1	0	18	0	0	0	0	0	4
Caliente 199 (<i>Brassica juncea</i> cv. Caliente 199)		1	1	6	2	0	0	0	0	0	2	0	0
Nemat (<i>Eruca sativa</i> cv. Nemat)		2	1	1	0	22	0	0	0	0	0	2	0
Weeds (control)		0	1	0	1	0	0	0	0	0	4	6	0
Weeds, nematicide		7	3	11	3	0	0	2	0	1	16	3	0
LSD (p = 0.43)		41.25											

The interaction (Y x T x Pos x Crop) did not differ significantly ($p=0.43$), but the treatments differed significantly on a 95% confidence interval.

Least significant difference (LSD) = 41.25

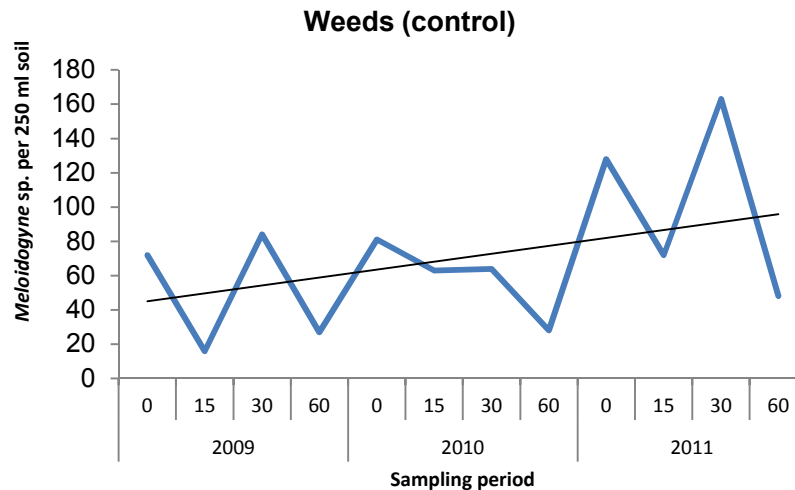


Fig. 4.3. The effect of the weeds treatment, without management practices, on the *Meloidogyne* sp. in the vine row for the period 2009 to 2011.

The overall effect of the cover crops and management practices on Criconemoides xenoplax in the vine and work row

The interaction between year, time, position and crop/practice (CP) were taken into consideration for the three growing seasons, with the results being shown in Table 4.8. The data showed significant differences in *C. xenoplax*, measured in the vine row, between the different sampling times within a specific treatment and between the different treatments. Comparing (time 0) and (time 60) for each treatment from 2009 to 2011 the White mustard (MC), Canola (MC), and weeds and nematicide (CC) treatments showed a similar trend, with a significant decrease in *C. xenoplax* for (time 0) to (time 60). All treatments, except Canola (CC), showed a similar, however not significant, trend. Canola (CC) showed a significant increase in *C. xenoplax* in 2009, time 0 compared to time 60 with no statistical decrease in 2010 and 2011. The times of the year that were represented by time 0 and time 60, were August and end October/beginning of November. From this trend, a natural decrease in *C. xenoplax* can be expected to take place during this time of the year, however in most cases this is not a constant decrease over the total period (0, 15, 30 and 60 days), with fluctuations in the nematode numbers on day 15 and 30. The reason for this decrease in the numbers can be attributed to the fact this is not the time of the year that much rain is expected. Keeping in mind that normally irrigation of the vineyard only started from December onwards and the fact that

nematodes move with water, it can be expected that *C. xenoplax* will decrease as the soil moisture content in the top 25 cm of soil decreases.

Another factor, that is involved, is the period from day 60 (end October/beginning of November) to day 0 (August). This period includes the very warm months of December, January, February and March and on this specific trial site irrigation was given during this period by means of drip irrigation. This supplied available moisture for the grapevine roots to absorb and normal growth and ripening of the grapes to take place. This is, however, also favourable for the *C. xenoplax* to flourish. All the factors that determine numbers build-up are present during this period (temperature, water and a favourable host). During the periods of 2009 (60 days) in comparison with 2010 (0 days) and 2010 (60 days) and with 2011 (0 days), in most cases there was a significant increase in the *C. xenoplax* numbers or at least an increasing trend. It was only Canola (CC) that once again showed a decline, compared to the rest of the treatments, with a significant decline in the *C. xenoplax* numbers during this period (2009, 60 days to 2010, 0 days) and showing a declining trend from 2010 (60 days) to 2011 (0 days). Because of the variances that occur between the two different periods, August to end October/beginning November and end October/beginning November to August, the effect over the three year period at 0 days (just before the management practice) and 60 days after the management practice, are considered to give the best indication of the impact of the crop host and the long-term impact of the treatments respectively. During the 0 day analysis for the three years, there was no significant decrease in any of the treatments during this period. White mustard (CC and MC), Nemat (CC and MC), Weeds (CC) and weeds and nematicide (CC) had significantly higher *C. xenoplax* in 2011 than in 2009. This may be an indication that White mustard and Nemat can be considered as good hosts for *C. xenoplax* and therefore can contribute to a numbers build-up during the cover crop growing season (April – August). All the other treatments, except Caliente 199 (CC), showed an increasing trend from 2009 to 2011, but not a significant increase.

For the 60 days after the management practice analyses, the Canola (CC) treatment, showed a decreasing trend in *C. xenoplax* in the vine row from 2009 to 2011 (Fig. 4.4). This is the only treatment where there was a significant difference between *C. xenoplax* for 2009 and 2010 and also between 2009 and 2011. Caliente 199, Weeds (MC), Weeds and nematicide (MC) showed a similar trend, however not significant. A similar trend for Canola (CC) and Caliente 199 was observed when the effect on the total plant-parasitic nematode numbers, measured 60 days after the management

practice, for the three seasons was considered (Figure 4.5). When the *C. xenoplax* numbers of the Canola (CC) treatment are compared with the Weeds (CC and MC), as well as the Weeds and nematicide (CC and MC) treatments, the starting numbers in 2009 (60 days) were significantly higher with a decreasing trend manifesting to such an extent that there was no significant difference between these treatments measured in 2011 (60 days). On the other hand, the *C. xenoplax* in the vine row, for the White mustard (CC and MC), Nemat (CC), Weeds and nematicide (CC) treatments, were significantly higher in 2011 than in 2009. The Oats (CC and MC), Canola (MC), Caliente 199 (MC), Nemat (MC) and Weeds (CC) treatments all showed similar increasing trends from 2009 (60 days) to 2011 (60 days).

Despite it being impossible to eradicate plant-parasitic nematodes from the soil, the aim remains to suppress their numbers to below the economical threshold level for the crop involved. In work done by Mckenry (1992), it was found that in Californian vineyards economic damage, in the sense of yield reduction, were prevalent where *C. xenoplax* were present in numbers of more than 500/kg soil. Thereafter, nematode pests should be managed continuously to prevent an increase in the numbers to the point where it can have a negative economic impact.

Figure 4.6 show the overall trend of Canola (CC) and Caliente 199 (CC) on *C. xenoplax* at all sampling dates over the three growing seasons. There were fluctuations in the *C. xenoplax* numbers within each season, as well as between the seasons and sampling periods. However, a long-term decreasing trend was established. Nemat (MC), weeds (CC) and Weeds and nematicide (MC) showed a similar long-term trend, although not as prominent. The results obtained, cannot be ascribed to the biofumigation effect on *C. xenoplax*, however; as, at that time, the biomass was not macerated and incorporated into the soil. So the effect concerned here should rather be attributed to the crop host status that suppressed the development of the *C. xenoplax* in the vine row.

In Table 4.9 the effect of the different treatments and management practices on the *C. xenoplax* numbers in the work row are indicated. The data of Table 4.8 and Table 4.9 were analysed together and the LSD value (247.13) is relevant for the data in both the tables. The data for the vine row (table 4.8) and the work row (Table 4.9) were separated to make the interpretation easier. It is clear, looking at the data for the work row, that there are significantly less *C. xenoplax* present in the work row area compared to the vine row area. This is the case for all the treatments measured at 0 and 15 days after the management practice for all three seasons. Factors that can play a role in this

trend demonstrated is: soil moisture; host status of the crops present in the work row in comparison with the vine grape host status and soil temperature. The biggest impact is, however, due to the compaction in the work row caused by tractor movement in this area. The difference between the *C. xenoplax* numbers in the work row, compared to the *C. xenoplax* numbers in the vine row, are less prominent in 2011 (30 and 60 days after the management practice), with Oats (CC), White mustard (MC), Nemat (MC), Weeds (MC) and Weeds and nematicide (CC) not differing significantly 30 days after the management practice took place, and Caliente 199 (CC), Weeds (MC) and Weeds and nematicide (MC) also not differing significantly 60 days after the management practice were applied. It is also important to note that the DMP of the cover crops in 2011 (Table 4.5) were higher compared to 2009 and 2010, with the exception of White mustard (CC) 2010 and this would have had a dramatic impact, from a root and shoot biomass perspective, on the potential role that these cover crops can play in nematode suppression. Except for White mustard (CC) analysed in 2011 (30 days after the management practices), that differed significantly from some of the other periods, there were no significant differences between the *C. xenoplax* numbers present at the different time periods for the different treatments.

Table 4.8. The effect of different cover crops and management practices on the suppression of *Criconemoides xenoplax*, in the vine row over the three seasons. The interaction takes the following into consideration: Year (2009, 2010, and 2011); Time (0, 15, 30, and 60 days); Position (vine and work row); Crops and Practice (mechanical control or chemical control) (Y x T x Pos x CP).

Treatments			0	15	30	60	0	15	30	60	0	15	30	60
Cover crop	Management practice	2009				2010				2011				
		1	Oats (<i>Avena sativa</i> cv Pallinup)	CC	620	358	578	212	494	486	510	474	828	536
2	Oats	MC	678	310	470	352	724	762	830	346	748	716	274	508
3	White mustard (<i>Sinapis alba</i> cv. Braco)	CC	700	476	590	522	1098	886	966	792	1200	996	834	894
4	White mustard	MC	464	282	538	172	712	504	420	280	986	818	398	584
5	Canola (<i>Brassica napus</i> cv. AV Jade)	CC	574	318	650	900	612	910	694	682	634	872	480	426
6	Canola	MC	842	582	498	390	906	674	544	496	844	814	382	502
7	Caliente 199 (<i>Brassica juncea</i> cv. Caliente 199)	CC	626	626	772	602	1006	998	894	520	540	780	712	372
8	Caliente 199	MC	732	386	726	324	724	1176	1000	584	704	770	488	536
9	Nemat (<i>Eruca sativa</i> cv. Nemat)	CC	656	612	620	207	732	458	1038	716	1018	604	676	740
10	Nemat	MC	492	438	694	362	874	1012	930	322	980	784	288	476
11	Weeds	CC	590	444	632	362	744	892	864	590	942	758	1118	518
12	Weeds	MC	506	400	480	332	548	588	778	376	738	416	414	276
13	Weeds + nematicide	CC	530	636	648	136	536	312	682	192	998	600	234	526
14	Weeds + nematicide	MC	576	300	554	380	560	406	462	218	508	528	464	266
LSD (p=0.2)			247.13											

The interaction (Y x T x Pos x CP) did not differ significantly ($p=0.2$), but the treatments differed significantly on a 95% confidence interval. Least significant difference (LSD) = 247.13

Table 4.9. The effect of different cover crops and management practices on the suppression of *Criconemoides xenoplax* in the work row, over the three growing seasons. The interaction takes the following into consideration: Year (2009, 2010, and 2011); Time (0, 15, 30, and 60 days); Position (Vine row and work row); Crops and Practice (mechanical control or chemical control) (Y x T x Pos x CP).

Treatments			0	15	30	60	0	15	30	60	0	15	30	60
Cover Crop	Management practice	2009				2010				2011				
		1	Oats (<i>Avena sativa</i> cv. Pallinup)	CC	28	4	46	0	14	14	2	6	194	62
2	Oats	MC	14	28	0	12	12	40	20	58	56	46	8	62
3	White mustard (<i>Sinapis alba</i> cv. Braco)	CC	8	26	10	12	4	134	10	4	36	196	50	196
4	White mustard	MC	30	44	12	2	128	46	24	30	38	142	428	60
5	Canola (<i>Brassica napus</i> cv. AV Jade)	CC	20	14	16	10	54	186	56	2	108	146	92	136
6	Canola	MC	48	2	24	102	38	4	56	88	112	94	104	110
7	Caliente 199 (<i>Brassica juncea</i> cv. Caliente 199)	CC	36	10	4	18	56	156	42	112	154	168	174	212
8	Caliente 199	MC	10	20	32	8	36	28	22	90	16	86	144	188
9	Nemat (<i>Eruca sativa</i> cv. Nemat)	CC	30	16	96	62	18	4	4	78	6	30	18	148
10	Nemat	MC	16	2	36	30	10	76	30	66	144	116	208	206
11	Weeds	CC	8	6	10	10	16	20	4	4	32	72	46	220
12	Weeds	MC	48	12	56	14	86	26	74	0	58	38	216	58
13	Weeds + nematicide	CC	26	42	16	86	134	2	4	14	120	42	102	78
14	Weeds + nematicide	MC	44	10	34	18	40	4	34	0	20	102	174	112
LSD (p = 0.2)			247.13											

The interaction (Y x T x Pos x CP) did not differ significantly (p=0.2), but the treatments differed significantly on a 95% confidence interval. Least significant difference (LSD) = 247.13

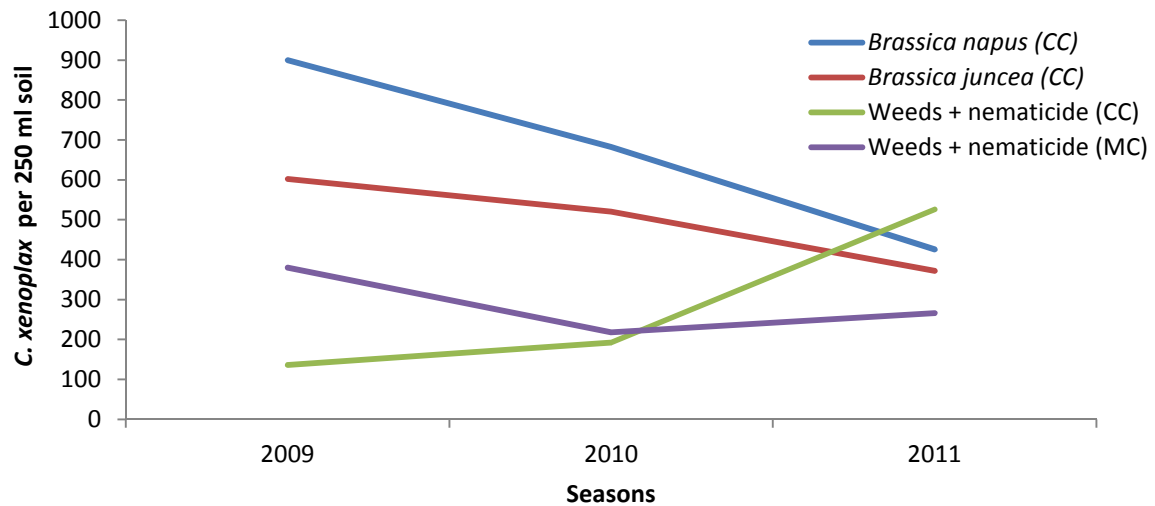


Fig. 4.4. *Criconemoides xenoplax* numbers in the vine row, measured at 60 days after the management practice for the 2009, 2010 and 2011 seasons.

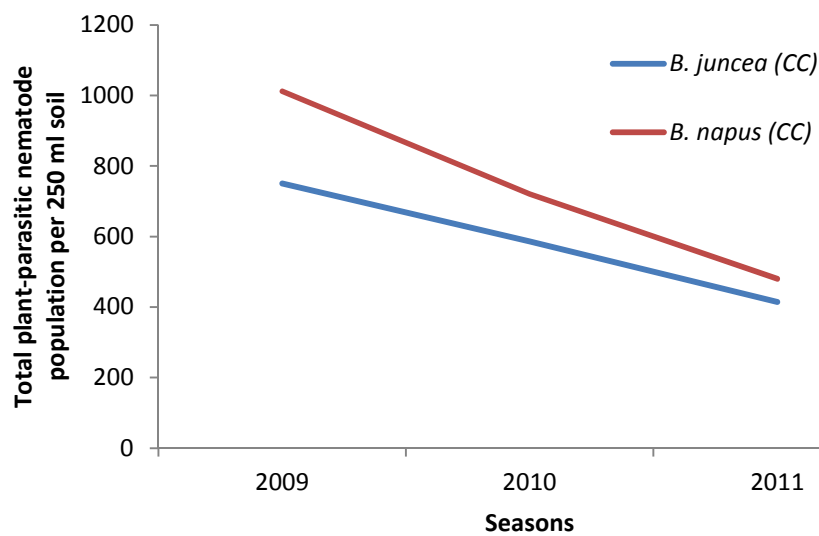


Fig. 4.5. Total plant-parasitic nematode numbers measured in the vine row, 60 days after the management practice.

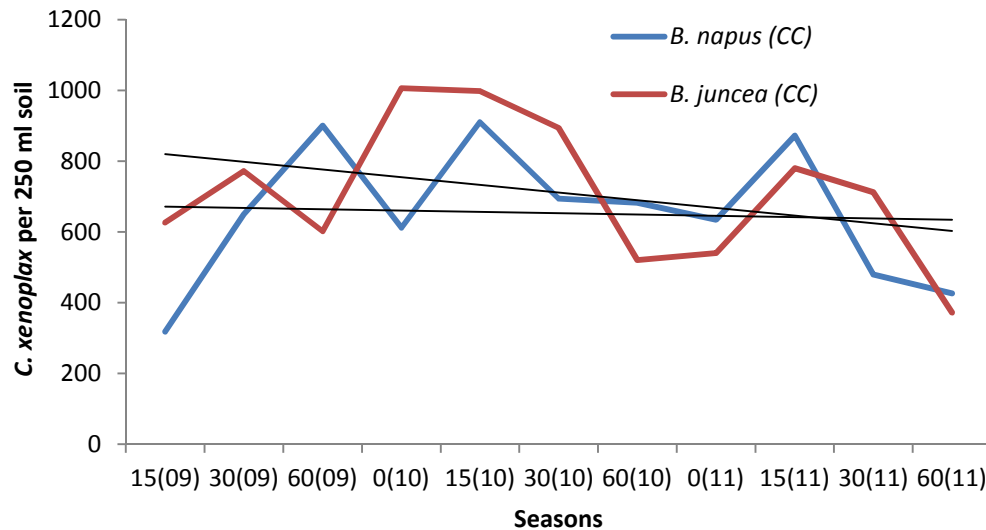


Fig. 4.6. The effect of Caliente 199 (*Brassica juncea* cv. Caliente 199) chemical control (CC) and Canola (*Brassica napus* cv. AV Jade) chemical control (CC) on the suppression of *Crictonemoides xenoplax* in the vine row, over a three season period and at different sampling periods (0, 15, 30 and 60 days) after the management practice was applied.

The overall effect of the cover crops and management practices on the Meloidogyne sp. in the vine and work row

The interaction the year (2009, 2010 and 2011); time (0, 15, 30 and 60); position (vine row and work row); crops (different crops involved) and (mechanical control or chemical control practices (Y x T x Pos x CP) were not significant, but significant differences were established within the treatments and time periods (LSD = 58.13).

In Table 4.10, it was shown that the overall presence of *Meloidogyne* sp. in this trial site was lower than the *C. xenoplax* numbers. There were also lower numbers of the *Meloidogyne* sp. in the work row compared to the vine row. Overall there were no significant differences between *Meloidogyne* sp., recorded in the work row, with the exception of 2010 (15 days after the management practice) for White mustard (CC) treatment; results were significantly higher than most of the other work row numbers over all the time periods. Once again the two periods day 0-60 (August to end of October/beginning of November) and day 60-0 (end of October/beginning of November to

August) were considered. This indicates the period after the management of the cover crops and the period during active growth.

The period 0-60 days for 2009, 2010 and 2011 indicated a decreasing trend every year and as is the case for all the treatments and year intervals except for Nemat in 2009. It therefore indicates, as was the case for the *C. xenoplax*, that this is a normal decrease in the nematode numbers in the vine row that can be expected over this period. For the period day 60-0, there was a significant increase in numbers in the vine row for the weeds (MC) treatment in 2009/10 and 2010/11 with a significant increase in 2010/11 occurring also for Oats (MC), White mustard (CC and MC), Nemat (CC and MC), Weeds (CC) and Weeds and nematicide (CC and MC). The 2010/11 data demonstrated more treatments, with a significant increase, can be ascribed to an increase in biomass production for the 2010 cover crop growing season, compared to the 2011 growing season. This data does not correlate to what would have been expected from Nemat. Nemat was found to be a poor host for *M. javanica* in the glasshouse trials for cover crop host status.

There were no significant increase in the *Meloidogyne* sp. in the vine row, comparing the starting numbers at day 0 (2009) with the end numbers day 60 (2011). No significant difference was found between the different cover crops with regard to the suppression of *Meloidogyne* sp., measured at the 60 day sampling period for 2009, 2010 and 2011. Even though this data does not show any clear reaction on the *Meloidogyne* sp. in a specific time period or over the whole time spectrum, the fact that there was no significant increase, when the total period was considered, is an indication that these cover crops do not enhance the numbers to such an extent that it may have an economic impact.

Table 4.10. The effect of the different cover crops and management practices, on the suppression of the *Meloidogyne* sp. in the vine and work row, over three growing seasons. The interaction takes the following into consideration: Year (2009, 2010 and 2011); Time (0, 15, 30 and 60 days); Position (vine row and work row); Crops and Practice (mechanical control or chemical control)(Y x T x Pos x CP).

Treatment			2009								2010								2011							
Cover crop	Management practice		Vine row				Work row				Vine row				Work row				Vine row				Work row			
			0	15	30	60	0	15	30	60	0	15	30	60	0	15	30	60	0	15	30	60	0	15	30	60
1	Oats (<i>Avena sativa</i> cv Pallinup)	CC	50	26	40	8	46	24	18	0	24	30	78	42	0	26	0	0	82	16	10	20	0	0	0	0
2	Oats	MC	44	34	50	26	2	2	0	82	34	72	18	0	42	0	0	156	14	6	84	0	6	0	0	
3	White mustard (<i>Sinapis alba</i> cv. Braco)	CC	86	20	68	36	4	2	2	8	90	74	80	38	0	72	0	0	68	74	30	36	0	6	0	0
4	White mustard	MC	24	44	38	10	0	14	2	2	54	34	30	28	0	20	0	0	94	12	90	46	0	0	0	0
5	Canola (<i>Brassica napus</i> cv AV Jade)	CC	176	20	30	80	0	0	0	0	36	38	30	16	0	16	0	0	176	56	54	44	0	0	0	4
6	Canola	MC	50	36	54	14	24	0	0	2	60	82	52	26	0	20	0	0	52	58	82	32	0	0	0	4
7	Caliente 199 (<i>Brassica juncea</i> cv Caliente 199)	CC	92	56	116	90	2	2	12	0	86	160	46	32	0	0	0	62	128	64	38	0	4	0	0	
8	Caliente 199	MC	54	40	122	24	0	0	0	4	44	20	66	38	0	0	0	94	88	114	16	0	0	0	0	
9	Nemat (<i>Eruca sativa</i> cv Nemat)	CC	56	20	24	14	0	2	2	0	12	84	72	26	0	0	0	94	72	76	32	0	0	0	0	
10	Nemat	MC	70	12	56	82	4	0	0	0	32	32	48	16	0	0	0	90	72	78	42	0	0	4	0	
11	Weeds	CC	64	20	106	30	0	0	0	0	66	52	74	24	0	0	0	108	82	152	26	0	0	0	0	
12	Weeds	MC	80	12	62	24	0	2	0	2	96	74	54	32	0	0	0	148	62	174	70	0	8	12	0	
13	Weeds + nematicide	CC	76	14	32	38	2	0	6	6	40	126	12	24	0	0	0	82	42	6	18	0	6	4	0	
14	Weeds + nematicide	MC	110	30	24	44	12	6	16	0	38	34	32	14	0	0	4	0	124	90	48	42	2	26	2	0
LSD (p = 0.95)			58.33																							

The interaction (Y x T x Pos x CP) did not differ significantly ($p=0.95$), but the treatments differed significantly on a 95% confidence interval. Least significant difference (LSD) = 58.33

Effect of the management practice on Criconemoides xenoplax numbers in the vine and work row

In Figure 4.7 the effect of the management practice on *C. xenoplax* is shown for the specific time periods. The *C. xenoplax* numbers in the vine row, for each time period, where the chemical control was applied to remove the cover crops, were always higher than the *C. xenoplax* numbers where mechanical control was applied. The differences within the periods were however not always significant and it was only in the 30 day and the 60 day period that significant differences between *C. xenoplax* numbers and the management practices were found. A possible explanation for this trend is the impact of the green manure incorporation into the soil, the biodiversity in the soil which indirectly influenced the *C. xenoplax* numbers in the vine row. The more acceptable reason for this is most probably the role that physical mechanical impact has on the nematode numbers.

A positive result was indicated in the comparison between the 0 day (CC and MC) and the 60 day (CC and MC); in both cases there was a significant decline in *C. xenoplax*. These results indicate that the management practice alone will not have a dramatic decreasing impact on the nematode numbers. The negative impact of mechanical control in the vineyards should also be considered when taking a holistic farming approach. There were significant differences between the vine row numbers of *C. xenoplax* and the work row numbers during all periods. The work row numbers did not differ significantly over the three years and between the sampling periods for the different management practices as shown in Figure 4.7.

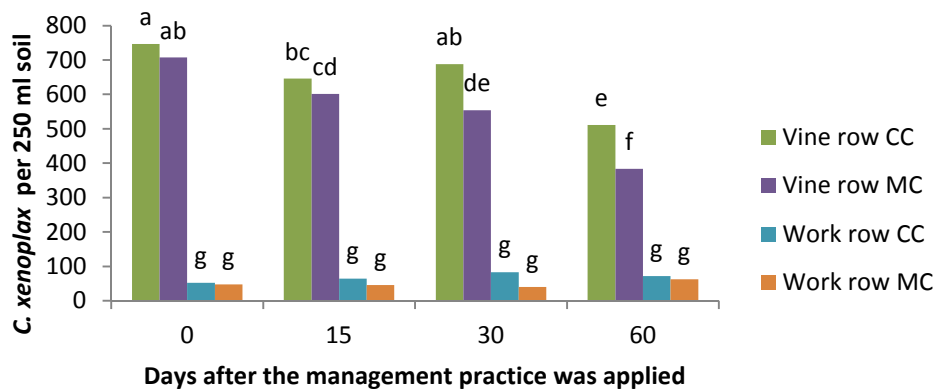


Fig. 4.7. The effect of the management practice on the *Criconemoides xenoplax* numbers in the vine and work row, for the different sampling periods indicated. The results shown are for the data for the three years combined (Interaction Time x Position x Practice). T-test (95% confidence interval) LSD = 12.73.

Effect of management practice on the Meloidogyne sp. in the vine and work row

The impact of the management practice on the *Meloidogyne* sp. numbers in the vine row did not show any significant trend, except for the 15 days after the management practice period (Figure 4.8). Significantly less *Meloidogyne* sp. numbers were present in the vine row, where the MC was applied, compared to the CC. The same trend as for the *C. xenoplax* shows when the 0 days (CC and MC) and 60 days (CC and MC) are compared, with a significantly lower number of *Meloidogyne* sp. present at the 60 day period.

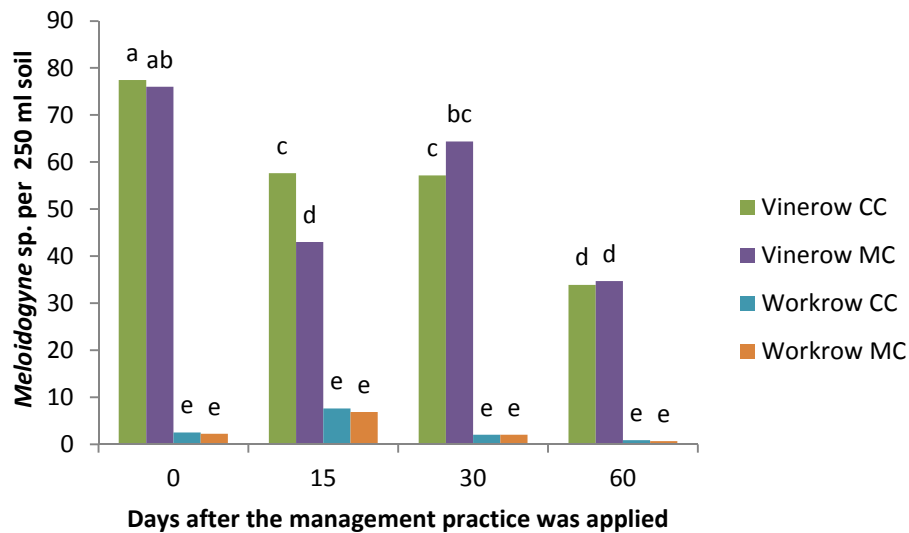


Fig. 4.8. The effect of management practices on the *Meloidogyne* sp. in the vine and work row, for the different sampling periods combined (Interaction Time x Position x Practice). The results are for the data of the three years combined. The interaction was not significant ($p = 0.44$) (95% confidence level) LSD = 12.73.

Conclusion

Cover crops have an essential role to play in the production of grapes in South Africa as part of an integrated approach to nematode and weed management. Certain cover crops can have a suppressing effect on plant-parasitic nematode populations in the following ways: 1) host status for the resident nematode numbers; 2) biofumigation potential; and 3) the secondary effect of green manure. Different cover crops have variable host status for nematodes; such as *C. xenoplax* and *Meloidogyne* sp. and by understanding and identifying cover crops with a poor to non-host status for these nematode species, there can be a long-term decreasing effect, as opposed to a build-up of destructive nematodes in vineyards.

Some of the cover crops, used in this study, showed potential as biofumigation crops and were selected to determine the biofumigation effect under field conditions. For the biofumigation reaction to be optimal, a wide range of conditions should be met; including biomass production, soil moisture, the physiological stage of the crop, the maceration process, the GSL concentration, the specific ITC released during the biofumigation process and the incorporation process. In this field trial, none of the above-mentioned factors could be performed optimally, due to the limitation in the

irrigation system and the potential negative impact of excessive cover crop fertilisation on the wine quality. The conditions, under which the trials were conducted, however, represent a large percentage of grape cultivation practices in South Africa and, even under the suboptimal conditions described for the biofumigation process, very promising results were obtained with regard to nematode suppression.

The two cover crops, Canola and Caliente 199, showed the best potential for the suppression of *C. xenoplax*. Caliente 199 is considered to be a good biofumigation cover crop and has been successfully applied for this purpose in a wide range of cropping systems internationally. Most research in the field of biofumigation, however, focuses on the suppressing effect on root-knot nematodes, which was also illustrated for *M. javanica* in the bioassays conducted in Chapter 2. To utilise the biofumigation concept fully under vineyard conditions, certain management practices will have to be considered to support the concept fully. Such practices include the irrigation system, the maceration process and the incorporation process.

The results obtained in the current study showed that Canola (CC) and Caliente 199 (CC) have the best potential to be applied as cover crops for the suppression of *C. xenoplax*. It is, therefore, recommended that the cover crops concerned be implemented as part of an integrated approach for total plant-parasitic nematode suppression, with specific focus on *C. xenoplax* management. The results obtained in this study can mainly be ascribed to the nematode host status of the two crops involved and the treatment would be more effective if the biofumigation concept could be further developed under more ideal field conditions and cultural practices. Future research is needed to allow for the refining of the biofumigation concept under field conditions; as it shows promise, demonstrated in previous research, on a range of nematodes, diseases and weeds where green manure crops were implemented.

The impact of the green manure on the structure and function of nematode soil communities and indirectly on 'soil health' is another topic that would be of importance in understanding the impact of different cropping systems on the numbers of plant-parasitic and free living nematode numbers in the soil.

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CHAPTER 5

Conclusion

The overall aim of this study was to determine the effect of certain cover crops in vineyards, specifically brassica crops; known for their biofumigation potential in the suppression of plant-parasitic nematodes. The cover crops were evaluated for their biofumigation potential and also for their host status, to identify crops that can be included as part of an integrated approach to nematode management. Cover crops play a crucial role in vineyards and are a standard practice in South Africa for their role in water run-off management, weed management and prevention of soil erosion. With virgin agricultural land becoming scarce in South Africa, it brings fourth the challenge of replanting crops on the same soil. The challenges that are involved with replanting crops are well known and plant-parasitic nematodes are an important part of the so called replanting complex. With this in mind; the pressure on crop protection solutions is ever increasing, both from an efficacy and from an environmental perspective. Therefore, the need for alternative options, such as cover crops, to be utilized as part of a responsible crop protection programme for the management of plant-parasitic nematodes in vineyards and other crops.

Biofumigation is the term that is used when crops have a biocidal effect when applied as green manure to the soil, because of the release of secondary metabolites after rupturing the plant cells. This reaction is very prominent in most brassica crops, but is not restricted to the Brassicaceae family. The reaction is based on the active ingredient glucosinolate (GSL), present in the plant vacuole, which comes into contact with an enzyme myrosinase (MYR) that is present in the plant cell cytoplasm, to form the active compounds isothiocyanate (ITC) and other secondary metabolites. It is the ITC that is believed to have the biocidal impact on soil borne diseases. The biofumigation reaction is a complex reaction and numerous factors play a role in ensuring effective biofumigation. The main factors include the type and concentration of GSL present in the plant cells; the plant maceration process; soil climatic conditions; plant physiological growth stage; the incorporation process; and the presence of water after incorporation. Biofumigation is successfully applied as part of crop rotation systems in crops like potatoes and onions, as a pre-plant application and is successful in the suppression of a wide range of soil-borne challenges.

Five cover crops, namely: Oats (*Avena sativa* cv. Pallinup), White mustard (*Sinapis alba* cv. Braco), Canola (*Brassica napus* cv. AV Jade), Caliente 199 (*Brassica juncea* cv. Caliente 199) and Nemat (*Eruca sativa* cv. Nemat) were used in laboratory bioassays to determine the biofumigation effect on *Meloidogyne javanica* and *Criconemoides xenoplax*. The crop biomass was applied as chopped up green manure to sterilized soil, inoculated with either *M. javanica* or *C. xenoplax*. The results indicated that White mustard, Caliente 199 and Nemat, all well-known biofumigation species, had a suppressing effect on *M. javanica*, measured according to the gall index. There was, however, no significant impact of the biofumigation action on *C. xenoplax*, and further research is needed to determine the effect of higher biomass applications and therefore higher ITC concentrations in the suppression of *C. xenoplax*.

Crops can be classified as good hosts, maintenance or poor hosts for specific nematode species. It is important to know the host status of a cover crop; as this can have a significant increasing or decreasing impact on the economic important plant-parasitic nematode population. When planting crops that are good host for *M. javanica*, for example, it can stimulate the population build-up of nematodes during the growing season and at the end put pressure on the management practices needed to keep the population below damaging levels. The same is true for *C. xenoplax*, which is a very challenging nematode to manage and can cause significant economic losses, when present in high numbers on crops like grape, plum and peach, grafted on susceptible rootstocks or where replanting of these crops occur on infested soil. On the other hand, the host status of a crop can be a powerful tool in managing the nematode population when a poor host is applied as part of a rotation program or cover crop system. During this study five different crops were planted and inoculated with either *M. javanica* or *C. xenoplax*, to determine their host status for these nematode species. From the results obtained, it was found that Nemat acts as a poor host for *M. javanica* and could play a role in suppressing population build-up, when applied as a rotation crop or cover crop. These results correlate with previous research; indicating the role of Nemat as a trap crop for certain root-knot nematodes. In the *C. xenoplax* crop host trials, the Canola treatment showed a very promising trend and although it was not significantly lower than the other crops, except for Nemat, this trend gave a positive perspective on *C. xenoplax* population management for future research.

The final objective of this study was to determine whether the selected cover crops, will have a suppressing effect on the plant-parasitic nematode population when applied as cover crops in a vineyard. The three year field study investigated the role of different cover crops and management practices of the cover crops (mechanical control or chemical control) on the nematode population in the work row and the vine row. There were numerous interactions involved in this study, as well as different nematode species identified, but only the effect of the *Meloidogyne* sp. and *C. xenoplax* in the work row and the vine row was found to be of significance. Over the three years, the impact of the biofumigation potential of the cover crops was not clear and did not show definite trends. This can be attributed to many factors that could not be optimally performed at the field trial site, due to practical limitations for optimal biofumigation to take place.

Previous research, as well as the results obtained during this study, on *Meloidogyne* sp. suppression, however, encourages further research in this regard; as there is a definite suppression of *Meloidogyne* species expected when biofumigation is performed optimally. The results from this field study have a practical implication for the grape industry. In accordance with the crop host status, the Canola treatment, as well as the Caliente 199 treatments showed a significant decrease in the *C. xenoplax* population in the vine row in the long term (2009 to 2011). The most prominent of these results was the chemical control management practice of these cover crops, conducted just before bud break in the vineyard. This correlates well with the results obtained from the crop host trials were Canola and Caliente 199 gave promising results in suppressing *C. xenoplax*.

Based on the results obtained in this study, as well as the extensive research conducted on biofumigation worldwide, it can be concluded that biofumigation crops have the potential to be utilized as part of a cover crop rotation system in vineyards for the purpose of plant-parasitic nematode suppression. The results of the field trial focussed on the crop host status and the role this plays in suppressing nematodes and are supported by the biofumigation results obtained in the bioassay trials. It is, however, important to remember that biofumigation is a very dynamic topic, with many variables and that all factors, including other control options and long term objectives, must be considered to ensure a long term solution in vineyards.