Plant-mediated effects of *Trichoderma* spp. and *Beauveria bassiana* isolates on insect and pathogen resistance

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A Thesis
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Wesis Pus

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Abstract of a Thesis submitted in partial fulfilment of the
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Brassica oleracea var. *capitata* is grown worldwide under temperate to tropical climate conditions. However, cabbage is attacked by a wide range of insect pests and plant diseases. The phloem-feeding green peach aphid, *Myzus persicae* is a major brassica pest while *Sclerotinia sclerotiorum* and *Leptsophaeria maculans* are two important plant fungi causing white mold and blackleg or phoma stem canker respectively in cabbage and other brassica vegetables. Control of these pests and diseases is largely dependent on chemical pesticides. Due to the many negative effects associated with chemical pesticides and their decreasing availability, biological control options involving endophytic fungi are currrently being explored for pest and disease management. Some entomopathogenic fungi have been reported to be endophytes, living asymptomatically in plant tissues and protecting the host from insect pests and plant diseases.

In this study, six fungal isolates (*Trichoderma atroviride* LU132, *T. hamatum* LU593, *T. virens* LU556; and *Beauveria bassiana* BG11, FRh2 and J18) were tested in a glasshouse for their ability to affect insect performance and disease severity on cabbage. Seven day old cabbage seedlings were inoculated with the biocontrol fungi as root drench and challenged with the insect pests and plant diseases 14-days after inoculation. The results showed that plants inoculated with *T. hamatum* LU593 and *T. virens* LU556 delayed the time taken for aphids to produce the first offspring compared to the control treatment. Total aphid reproduction was significantly reduced when fed on fungal endophyte treated plants compared to control treatment except *B. bassiana* J18 which did not show any significant effect. Aphids fed on plants inoculated with *T. hamatum* LU593, and *B. bassiana* isolates FRh2 and BG 11, had reduced longevity compared to aphids fed on uninoculated control plants. For the effect of the fungal endophytes on *Sclerotinia sclerotiorum*, the strongest effects were observed on plants inoculated with *B. bassiana* isolates J18 and BG 11 and *T. hamatum* LU593, with less leaf lesion area (mm^2) compared to the uninoculated control treatment. No fungal endophytes

showed any significant effect against *Leptosphaeria maculans* infection. All fungal endophyte inoculated plants except *B. bassiana* J18 promoted plant root growth, but there was no significant effect in the number of leaves and shoot growth across all treatment when compared to the untreated controls, indicating that the fungal endophytes likely established and colonised the root rhizospheres.

Fungi recovered from endophytic colonisation showed colonies characteristics of *Trichoderma* species and *B. bassiana*. High recovery rates from surface sterilized root tissue segments plated were observed in *T. atroviride* LU132 (67%), *B. bassiana* BG11 (58%) and *B. bassiana* FRh2 (57%), whereas the other fungal endophytes showed less than 50% colonisation effect. Colonies characteristic of *B. bassiana* isolates FRh2 (17%) and BG11 (8%) were recovered from surface sterilized leaf tissues plated. The present study demonstrates that root drench inoculation of cabbage seedlings with *Trichoderma* spp. and *B. bassiana* can contribute to crop protection by enhancing the resistance of cabbage towards aphids and foliar diseases.

Keywords: *Beauveria bassiana*, biological control, *Brassica oleracea*, fungal endophytes, *Leptosphaeria maculans*, *Myzus persicae*, *Sclerotinia sclerotiorum*, *Trichoderma* spp.

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1 Introduction

1.1 Background

1.1.1 Brassica

Cabbage is cultivated worldwide under temperate to tropical climate conditions and consumed widely around the globe (Chiang et al., 1993). The estimated area of cultivation is more than two million hectares, with an average yield of 27.8 tonnes per hectare (FAOSTAT, 2010). Because of the wide spread with small to large area under cabbage production, there exists a complexity of insect pests and plant pathogens. The attack from insect pests such as *Myzus persicae* and their susceptibility to plant pathogens, including *Sclerotinia sclerotiorum* and *Leptosphaeria maculans,* has led to a diversity of pest management issues.

Worldwide, attempts to manage these pest problems on cabbage crops have largely been through use of chemical pesticides. There is a long history of pesticide use dating back to the 1960s (Suckling et al., 2003), however more recently there has been attempts to reduce their use due to their perceived negative effects on the environment and concerns regarding chemical residues on food and problems associated with pesticide resistance by pests and plant pathogens.

In many developing countries, cabbage is grown for home consumption and to supply local markets. However, for developed countries such as New Zealand, the horticultural industry is export-driven and highly focused on export to distant international markets so reduced use of pesticides to meet the trading partners requirements is needed (Suckling et al., 2003).

1.1.2 Pests and diseases of Brassicas

Green peach aphid

Green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae) is a cosmopolitan pest species (Kusnierczyk et al., 2008) infesting a wide range of host plants from over 50 plant families (Khan et al., 2012). Singh (2012) reported that *M. persicae* can infest over 100 families of economically important plants including brassicas.

The green peach aphid has a simple life cycle on brassicas with adult females parthenogenetically giving birth throughout the year to live offspring (viviparie). Green peach

aphids are generally found on the young foliage and underside of leaves. Both the adults and nymphs are highly specialized in their mode of feeding. They feed exclusively on phloem sieve elements by inserting their stylets into the plant tissue which presents a stress on the plant (Barahona, 2010) and may eventually lead to plant mortality when aphids are in high population densities (Lee et al., 2012). The phloem-sucking mode of feeding, causes leaf chlorosis, stunting, and deformation and provide additional challenges to plants as they deplete photosynthates (sucrose and amino acids) produced in the leaf mesophyll cells that are normally transported to other parts of the plant (Barahona, 2010).

Apart from their constant removal of plant nutrients, aphids probing of intracellular epidermis can also transmit persistent viruses (Gabrys et al., 2015) and introduce chemical and/or protein effectors that alter plant defence signalling, infestation symptoms, and plant development (Barahona, 2010) or by secretion of honeydew which may lead to secondary fungal infections (Edwards et al., 2008).

Among the wide range of plants that *M. persicae* feed on, cabbage (*Brassicae oleracea* var. *capitata*) and other members of the Brassicaceae family have been reported as a host to the pest (Barahona, 2010; Khan et al., 2012; Kusnierczyk et al., 2008; Lee et al., 2012; Singh, 2012).

Diseases of cabbage

Cabbage and other Brassicaceae are also known to be susceptible to many plant pathogens including *Sclerotinia sclerotiorum* (Lib.) de Bary (Helotiales: Sclerotiniaceae) (Bolton et al., 2006) and *Leptosphaeria maculans* (Desm.) Ces. & de Not. (anamorph *Phoma lingam* Tode ex. Fr.) (Pleosporales: Leptosphaeria) (Shoemaker & Brun, 2001). *Sclerotinia sclerotiorum* is a highly destructive necrotrophic pathogen with a wide host range and occurs in many countries worldwide. In cabbage, infection occurs after the head formation (Jones et al., 2014) under cool, wet weather which provides conditions conducive to ascospore release, infection, and subsequent disease development but the pathogen can also infect under other conditions (Hudyncia et al., 2000).

Sclerotinia sclerotiorum can infect to cause stem rot, crown rot, cottony rot, white rot or watery soft rot however, the key feature of the pathogen is its ability to produce black resting

structures “sclerotia” and white fuzzy growth of mycelium on the plant it infects (Warmington & Clarkson, 2016).

The sclerotia are the main survival structure enabling the pathogen to survive for at least three years in soil between crops (Jones et al., 2014; Warmington & Clarkson, 2016). As described by Jones et al. (2014), the sclerotia are able to germinate myceliogenically, to directly infect the crop, or carpogenically, producing windborne ascospores which infect the above ground parts of the crop.

Leptosphaeria maculans, the causal agent of blackleg or phoma stem canker, is a ubiquitous, worldwide pathogen of brassicas, especially on oilseed rape and canola (*Brassica napus* L., *Brassica rapa* L., respectively) (Sivasithamparam et al., 2005; West et al., 2001). This necrotrophic pathogen causes cotyledon and leaf lesions in the early growth stage and stem canker in the later phase of the plant which lead to plant mortality or reduction in yield.

The pathogen survives as a saprophyte, reproducing sexually and releasing ascospores in the spring that serve as the primary inoculum (Hwang et al., 2016). Symptoms of infection by *L. maculans* on plants are greyish-green lesions bearing tiny black spots (pycnidia). The long phase of *L. maculans* is where the fungus develops endophytic growth within plant tissues from the leaves to the stem base before eventually killing the plant (Hwang et al., 2016).

The epidemiology and severity of phoma stem canker differs due to differences in the population structure, brassica species or cultivars grown, climatic conditions and agricultural practices (West et al., 2001). It has been reported that there are two forms, a group A which contained highly virulent isolates which were found to produce a non-host specific phytotoxin, sirodesmin PL (Tox^{+}) and group B which were weakly virulent and did not produce sirodesmin PL (Tox^{0}) (Fitt et al., 2006). Based on these observations and other distinct morphological differences, Shoemaker and Brun (2001) reclassified the group B isolates as a new species, *L. biglobosa* whilst retaining *L. maculans* for the group A isolates. In Australia, the epidemics of *L. maculans* has shown to be severe mainly affecting canola (West et al., 2001) while in New Zealand, the disease is also important on forage brassicas such as swede, turnip, rape and kale as well as oilseed rape (Lob et al., 2013).

1.2 Endophytic fungi

The term endophyte was first coined by the German scientist Heinrich Anton De Bary (1884) to define fungi or bacteria that occur inside plant tissues without causing any apparent disease symptoms in the host (Wilson, 1995). Different genera of fungi known as insect pathogens, for example *Acremonium*, *Beauveria*, *Cladosporium*, *Clonostachys*, and *Paecilomyces*, have been isolated from many plants and can be endophytic at times (Vega, 2008). The most prominent fungal endophytes are dominated by Ascomycota, with *Neotyphodium* and *Epichloe* species as the most extensively studied fungal endophytes of grasses (Akello, 2012; Vega et al., 2008).

Reports on performance tests in control conditions showed that *Neotyphodium* endophytes impaired growth and survival of invertebrate herbivores through the production of specific alkaloids (Saikkonen et al., 2010). However, the role of unspecialized fungal endophytes in mediating plant-insect interactions has not been extensively investigated compared to Clavicipitaceous fungal endophytes.

Mutualistic endophytes, bacteria or fungi, are microbes that live within tissues of living plants throughout or at some stage of their life cycle without causing any apparent disease symptoms (Saikkonen et al., 1999; Saikkonen et al., 2010) and in some cases have been shown to be able to reduce insect herbivory and infection by phytopathogens in colonised plants. For example, mutualistic endophytes‟ presence in many plants have been shown to protect plants from insects with different feeding guilds (Jallow et al., 2004; Vega et al., 2012; Vega et al., 2008).

To date, studies have been conducted globally focusing on plant-mediated interactions between endophytic fungi against herbivorous insects and plant pathogens primarily due to their broader spectrum abilities (Verma et al., 2007) although the most studied fungal endophytes are in the genus *Neotyphodium* that are grass specific (Vega, 2008).

Further research has shown that fungal endophytes, both specialized and non-specialized, and of other genera, including *Acremonium*, *Beauveria*, and *Trichoderma*, are able to protect their host plants against insect pests (Akello & Sikora, 2012; Lopez & Sword, 2015). Some of these microorganisms can aid plant growth by various means. Upon establishment and on colonisation, these microorganisms can promote plant growth through suppression of plant pathogens, via mechanisms such as mycoparasitism, competition, production of antibiotics (secondary metabolites), and by directly promoting plant growth (Dicke & Hilker, 2003; Druzhinina et al., 2011; Gurulingappa et al., 2011; Hartley & Gange, 2009; Howell, 2003).

1.2.1 *Trichoderma* spp. as an endophyte

The genus *Trichoderma* was first described in 1794 by Persoon and to date more than 100 species have been characterized at the molecular level, for many of which the sexual stage is unknown and thus are considered as fungi imperfecti (Mukherjee et al., 2013). *Trichoderma* spp. are ubiquitous, free-living soilborne fungi, which thrive well in all soils from temperate to tropical, and are relatively easy to isolate and culture (Mukherjee et al., 2013). Historically, *Trichoderma* species were typically considered as common soil saprophytes until research over the last two decades found that they were capable of more intimate associations with plant root systems, forming opportunistic avirulent symbiotic relationships (Druzhinina et al., 2011).

Trichoderma spp. comprises a great number of fungal strains however, more than 80 described species act as biological control agents against many soil-borne phytopathogens (Bailey et al., 2008; Howell, 2003), and foliar pathogens including *Sclerotinia sclerotiorum* (Castillo et al., 2011; Elad, 2000). *Trichoderma* species, compared to other fungi grow quickly on many substrates, produce secondary metabolites, and may be mycoparasitic against many pathogens (Grondona et al., 1997; Howell, 2003; Mukherjee et al., 2013). The antagonistic properties of *Trichoderma* spp. are based on the activation of multiple mechanisms including antibiosis, mycoparasitism, competition, plant growth promotion and induced systemic resistance (Bailey et al., 2008; Howell, 2003). Grondona et al. (1997) reported that mycoparasitism and antibiotic production were first demonstrated in *Trichoderma* by Weindling in the early 1930s and many modern biotechnological applications of these fungi as biocontrol agents are derived from these early works. For example, the application of commercial strain T39 of *Trichoderma harzianum* to the leaves of cucumber induced systemic resistance against *S. sclerotiorum* (Elad, 2000).

The critical characteristic of this association is the penetration of the plant‟s root system by *Trichoderma* and the persistent survival of the fungus within living plant tissues (Cripps-Guazzone, 2014; Cripps-Guazzone et al., 2016; Hohmann et al., 2011, 2012). Recent studies have demonstrated, that apart from roots, *Trichoderma* species can also colonize the leaves, in

Theobroma cacao (Bailey et al., 2009) and cabbage (Zhang, 2014). Inoculation of *B. napus* with *T. atroviride* LU132 was shown to significantly increase the root and shoot biomass (Maag et al., 2013) and suggested that the inoculated fungi had endophytically colonised the plant. Furthermore, the application of *T. hamatum* LU593 and *T. virens* LU556 as maizemeal-perlite (MP) soil incorporations was shown to reduce *S. sclerotiorum* infection of cabbage (Jones et al., 2014) and suggested that it was rhizosphere competence and therefore induced resistance.

In another study, pre-inoculation of *Trichoderma* spp. on cotton against *Aphis gossypii* resulted in all leaves being colonized by the fungus (Gurulingappa et al., 2010) and enhanced protection.

Geraldine et al. (2013) described that inoculating common beans with *Trichoderma* spp. against *S. sclerotiorum* resulted in reduction of apothecia density and disease severity, subsequently increasing the number of pods per plant and yields up to 40% compared to controls. Apart from other mechanisms of biocontrol by the fungus, the ability of *Trichoderma* spp. to effectively colonize the plant rhizospheres can result in protection of the host from both biotic and abiotic stresses including protection against phytopathogens.

1.2.2 *Beauveria bassiana* as an endophyte

Beauveria bassiana is a fungal entomopathogen that was discovered by Agostino Bassi de Lodi in 1835 reducing silkworm populations. It was only in 1991 the potential of *B. bassiana* as an endophytic biocontrol agent was recognized by Bing and Lewis (1991) who found that the foliar application of conidial suspension to the whorl-stage of corn plants (*Zea mays* L.) reduced *Ostrinia nubilalis* populations and persisted to provide season-long suppression of the insect indicating the successful establishment of *B. bassiana* as an endophyte (Bing & Lewis, 1991).

Beauveria bassiana are known to infect a diversity of insect pests and plant diseases worldwide. These genera of entomopathogenic fungi are known to have the ability to antagonise and kill insects which places them as an efficient biocontrol agent. In other studies, inoculation of tissue cultured banana (*Musa* sp.) plants with endophytic *B. bassiana* strains affected larval development and reduced damage caused by the banana weevil, *Cosmopolites sordidus* (Akello et al., 2009). Endophytic *B. bassiana* strains were also effective at reducing the damage caused by the coffee berry borer, *Hypothenemus hampei* Ferrari (Vega et al.,

2008), millet stem borer, *Chilo partellus* Swinhoe (Reddy et al., 2009), and cotton bollworm, *Helicoverpa zea* (Lopez & Sword, 2015) on their respective host plants.

As an endophytic fungus, *B. bassiana* has also been reported to affect aphid populations, offspring performance and fecundity in faba beans (*Vicia faba*) when seeds were inoculated with the fungus (Akello & Sikora, 2012). Gurulingappa et al. (2010) also found that the reproduction of *Aphis gossypii* feeding on cotton leaves treated with *B. bassiana* slowed, and the growth of *Chortoicetes terminifera* nymphs slowed when fed on *B. bassiana* treated wheat leaves. Another study using spray inoculation on artichoke (*Cynara scolymus*) plant leaves showed that *B. bassiana* was re-isolated from 56% of newly emerged leaves after 10 days (Guesmi-Jouini et al., 2014). The results suggest that *B. bassiana* colonized the plant endophytically. In a greenhouse experiment, *B. bassiana* strain RSB applied to broccoli foliage significantly reduced the adult and larval populations of western flower thrips, *Frankliniella occidentalis* leading to enhanced plant defence against the pest (Gao et al., 2012), however the endophytic colonisation of the host plant was not considered. The effective colonisation of their host plant by endophytic *B. bassiana* can promote plant growth, improve resistance to abiotic stresses, and protect the host from damage by insects, phytopathogens, and nematodes (Vega et al., 2008).

1.3 Endophyte-mediated induced systemic resistance

Plants are attacked by a wide range of herbivorous insects and pathogens. Plants have developed an array of structural, chemical, and protein based defences designed to detect invading organisms and stop them before they are able to cause extensive damage. Plants can respond to these attacks by direct or indirect defences that negatively affect the herbivore or pathogen (Dicke & Hilker, 2003).

Herbivore damaged or pathogen infection of plants elicit a defence responses via a complex chain of events, from introduction of herbivore- or pathogen- specific elicitors into the wounds at the infection/feeding or oviposition sites, their recognition by the plant, and activation of several signalling cascades that trigger defence responses thereby increasing resistance (Dicke & Hilker, 2003; Halitschke & Baldwin, 2005; van Poecke & Dicke, 2003). Different herbivores or pathogens can evoke different plant responses due to elicitors or wounding.

There are wide range of mechanisms involved however, the two main ones are; systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Shoresh et al., 2010). According to Shoresh et al. (2010), SAR is usually triggered by local infection, provides long-term resistance to subsequent attacks, correlates to the activation of pathogenesis related (PR) genes to invading pathogen, and requires the involvement of the signalling molecule salicylic acid (SA). ISR is known to be activated through colonisation of the roots by certain non-pathogenic rhizosphere microorganisms and is not SA-dependent, but requires components of the jasmonic acid (JA) pathway (Shoresh et al., 2010). These plant hormones (SA and JA) alter their primary and secondary metabolism or change the concentrations of constitutively present chemicals during attack (Halitschke & Baldwin, 2005). Induced defence is also connected with the release of protein inhibitors and polyphenols, which inhibit the insect digestive enzymes or by altering the nutritive value which make it unsuitable for the insect to feed or pathogen to develop (Barahona, 2010; Dicke & Van Poecke, 2002; Gabrys et al., 2015; Gao et al., 2010). For instance, Alizadeh et al. (2013) has shown that with *Trichoderma harzianum* isolate Tr6 inoculation of cucumber and *Arabidopsis thaliana* against *Fusarium oxysporum* f. sp. *radicis cucumerinum* and *Botrytis cinerea*, respectively, induced significantly higher levels of resistance and this was associated with primed expression of defence related genes upon challenge with the respective plant pathogens. In other studies, *T. atroviride* isolate R33 induced systemic resistance to stem inoculation of radiata pine seedlings against *Diplodia pinea,* (Regliński et al., 2012); induction of systemic resistance by *B. bassiana* against aphid species in feeding bioassays (Gurulingappa et al., 2010); and cotton seedling inoculated with *B. bassiana* induced systemic resistance against a bacterial pathogen on foliage (Ownley et al., 2009).

Several strains of *B. bassiana* were shown to be able to colonize their host plant and provide defence against insect herbivores through various mechanisms (Akello, 2012; Greenfield et al., 2016; Guesmi-Jouini et al., 2014; Gurulingappa et al., 2011). It is likely that there is more than one mode of action in suppressing insect pests and plant diseases by *B. bassiana* however, isolates of this fungus are known to produce numerous secondary metabolites (e.g. beauvericin, beauverolides, bassianolides, oosporein, oxalic acid) with antibacterial, antifungal, cytotoxic, and insecticidal activities (McKinnon et al., 2016; Ownley et al., 2009; Vega, 2008). Although antibiosis remains the main mode of action, studies have shown that *B. bassiana* can protect plants from insect herbivores through induced systemic resistance (McKinnon et al., 2016; Ownley et al., 2009).

1.4 Aims of the study

Tripartite interactions between soilborne microorganisms, plants, and herbivorous insects or plant pathogens have gained increasing attention in the last two decades. Isolates of *B. bassiana* and *Trichoderma* spp. have been reported to reduce feeding, reproduction and fecundity, and adult longevity in many insects. *Trichoderma* spp. isolates have also been reported to reduce infection of several plant species by many phytopathogens. Although the reduction in feeding and adult populations of herbivorous insects and reduction in plant disease incidences or severity have been significant, little is known about the effect of *B. bassiana* and *Trichoderma* -mediated induced systemic resistance (ISR) against phloem-feeding insects.

Similarly, isolates of some *Trichoderma* species have been shown to suppress many foliar and soil borne pathogens including *S. sclerotiorum* through mycoparasitism and antibiosis (Jones et al., 2014) however, the effect of *B. bassiana*- and *Trichoderma* spp. -mediated responses in host plants against *Sclerotinia sclerotiorum and Leptosphaeria maculans*, has not been widely investigated.

This study aimed to investigate the ability of *Trichoderma* spp. and *B. bassiana* isolates to control the phloem-feeding herbivore *Myzus persicae* and two plant pathogens, *Sclerotinia sclerotiorum* and *Leptosphaeria maculans* of *Brassica oleracea* var. *capitata*. The ability of the isolates to endophytically colonise the plants and effect of the most effective isolates on the production of phytohormones associated with plant defence responses were determined.

2 Materials and Methods

2.1 Plants

Brassica oleracea var. *capitata* „Derby Day" seeds (Egmont Seed Company Ltd, NZ) were used as the test plants for all glasshouse experiments. For each experiment, seeds were placed in an empty tea bag and surface sterilized in 0.01% Triton X-100 (British Drug House (BDH) Chemicals Ltd, England) for 2 mins, followed by 2% sodium hypochlorite (NaOCl), and 70% ethanol for 5 and 1 mins. The seeds were then rinsed five times in sterile distilled water (SDW). Three seeds were sown singly at a depth of 1 cm using a tweezer into each Thermoform square pot (7 x 7 x 8 cm, Pöppelmann, TEKU®, Germany) with a volume of 250 ml filled with 25 g of moist fine vermiculite (Exfoliators, Aust., Pty Ltd). The pots were maintained in a glasshouse at 16-23ºC, 60-70% relative humidity (RH) under 16:8 day/night cycle. The germinating seedlings were inoculated by drenching with spore suspension of either *Trichoderma* spp. or *B. bassiana* isolates seven days after sowing, thinning was done after 10-12 days leaving only one seedling per pot (Figure 2.1). The pots were placed in saucers and watered twice weekly by filling the saucers with tap water. Hydroponic solution (FloraNova 7-4-10, General Hydroponics Inc., USA) was applied to the plantlets at a rate of 1.25 ml/l from the ninth day of sowing and thereafter every two days.

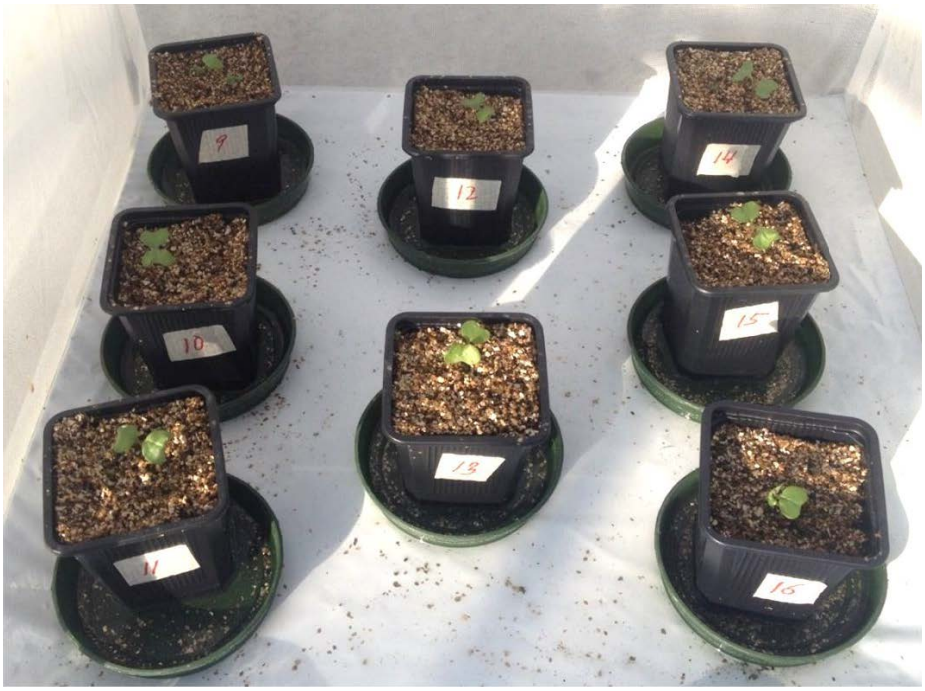

Figure 2.1: Appearance of *Brassica oleracea* var. *capitata* plants 10 days after sowing in vermiculite pots and 3 days post *Trichoderma* spp. or *Beauveria bassiana* inoculation.

2.2 Fungal isolates

Six isolates, three each of *Trichoderma* spp. and *Beauveria bassiana*, were selected for the experiments. These were *Trichoderma atroviride* LU132, *Trichoderma hamatum* LU593, and *Trichoderma virens* LU556. *Beauveria bassiana* isolates were BG11, FRh2, and J18, respectively (Table 2.1).

Selections of *Trichoderma* spp. isolates was based on general knowledge gained through literature of *Trichoderma*"s endophytic ability to induce systemic and localized resistance, promote plant growth, outcompete other microorganisms, effectively colonize the root rhizosphere, and produce antibiotics (Bailey et al., 2008; Muvea et al., 2014).

Trichoderma atroviride LU132 has been shown to promote plant growth when applied as a soil treatment (Maag et al., 2013). *Trichoderma atroviride* LU132 and *T. virens* LU556 have been shown to be rhizosphere competent and endophytic on a range of plant species, including Brassica (Cripps-Guazzone, 2014). Additionally, *T. hamatum* LU593 and *T. virens* LU556 have been shown to reduce infection of cabbage by *Sclerotinia sclerotiorum* with rhizosphere colonisation suggested to induce systemic resistance in cabbage to *S. sclerotiorum* (Jones et al., 2014). *Trichoderma atroviride* LU132 has been shown to promote plant growth but it has not shown to induce systemic resistance in oilseed rape to a chewing insect (Maag et al., 2013). It was tested in this study for its ability to induce systemic resistance to the phloem-feeding green peach aphid, *Myzus persicae,* and the plant pathogens *Sclerotinia sclerotiorum* and *Leptosphaeria maculans*.

The isolates of *B. bassiana* were chosen based on unpublished results of previous pathogenicity and „omics studies conducted by Lincoln University researchers. The six fungal isolates were obtained from the Bio-Protection Research Centre (BPRC) culture collection stored using the ultracold preservation method (-80ºC).

The isolates LU132, LU593, LU556, FRh2, and BG11 were stored as conidial suspensions in glycerol, while J18 conidia was preserved on an agar slope (slant) culture. Aliquots of 0.1 ml from the glycerol suspension of each isolate were pipetted onto the surface of potato dextrose agar (PDA; Difco™, Becton, Dickinson & Company, USA) contained in Petri dishes (90 mm diameter). The suspension was spread over the entire surface using a sterile hockey stick (Disposable cell spreaders, Biologix Group Ltd, China). For the agar slant culture, a sterile

loop was inserted into the test tube removing a loop full of the growth by gently scrapping off spores which were then spread evenly over the surface of the PDA plate.

Petri plates were securely sealed with plastic film (GLAD® Cling Wrap, Chronox, New Zealand Ltd) and labelled accordingly. Four Petri plates per isolate were set up and incubated in a growth chamber at 25ºC under 16:8 photoperiod for seven days. The sporulating culture plates were then placed in a sterile plastic container (Sistema® KLIP IT, NZ) and stored in the fridge at 4ºC until required for further subculturing.

Table 2.1: Details of the genus and species, isolate codes, isolated source, origin, and year isolated of *Trichoderma* spp. and *Beauveria bassiana* isolates used in the study

Genus and Species	**Isolate codes**	**Isolated from**	**Origin**	**Year isolated**
Trichoderma atrovirde	LU132	Soil	Pukekohe	1971
Trichoderma virens	LU556	Soil from onion field	Pukekohe	1986
Trichoderma hamatum	LU593	Soil	Lincoln	1996
Beauveria bassiana	BG11 or (BPRC- F23)	*Bellis perennis*	Christchurch	2012
Beauveria bassiana	FRh2	*Hylastes ater*	Riverheads	2011
Beauveria bassiana	J18	Maize cob	Ashburton	2014

NB: All *Beauveria bassiana* isolates used in the study used the codes of the BPRC *Beauveria* working group. They have not been allocated a Lincoln University isolate code.

2.2.1 Preparation of spore suspensions

For each fungal isolate, one month old cultures growing on PDA were flooded with 10 ml SDW containing 0.01% Triton X-100. The colony surface was gently scrapped off with a disposable cell spreader and the resulting spore suspensions were filtered through sterile cheese cloth and the spore concentrations were adjusted to 1.0 x 10^7 spores/ml for *Trichoderma* spp. isolates and 1.0 x 10^8 spores/ml for *B. bassiana* isolates based on Neubauer haemocytometer counts.

2.2.2 Plant inoculation

Seven day old *B. oleracea* var. *capitata* seedlings were inoculated with either *Trichoderma* spp. or *B. bassiana* isolates by pipetting 1.0 ml aliquots of the spore suspensions as a root drench. Control plants were mock-inoculated with SDW amended with 0.01% Triton X-100. The plants were then placed back in the greenhouse and incubated for 28 days prior to challenging with the insect pest or pathogens.

2.3 Pest insect

A laboratory culture of *M. persicae* was maintained in a climate chamber. The insects were reared on one month old *B. oleracea* var. *capitata* plantlets at a constant temperature of 20°C and under light conditions resembling a 16:8 photoperiod in a growth room. The plants in the growth room were changed monthly or when completely infested.

2.4 Plant pathogens

2.4.1 *Sclerotinia sclerotiorum*

Two isolates of *S. sclerotiorum* were obtained from the BPRC culture collection. The isolates were LU8006 and LU8007, both of which were isolated from oilseed rape (no specific location in New Zealand available). They were sub-cultured on PDA agar and incubated at 19°C under 16:8 photoperiod. A preliminary study was conducted whereby leaves of *B. oleracea* var. *capitata* plants were infected with mycelial plugs (5 mm in diameter) following methods described in Section 2.6. The leaf lesion size and number of plants infected were used to determine their pathogenicity. Of the two isolates tested, isolate LU8007 was more pathogenic than LU8006, thus the former isolate was used for assessing resistance of *Trichoderma* spp. and *B. bassiana* inoculated plants to *S. sclerotiorum* (details in Section 2.6).

2.4.2 *Leptosphaeria maculans*

Three isolates of *L. maculans* were selected (Table 2.2) based on their known pathogenicity to oilseed rape and swede and the production of conidia (Lob, 2014). These isolates were stored

in glycerol at -80°C and obtained from the Lincoln University Plant Pathology culture collection.

The isolates were subcultured onto PDA by placing a mycelial colonised agar disc in the centre of fresh PDA plates and incubated in the light bank at 20ºC under 8:16 photoperiod for 19 days to induce conidial production.

For plant infection, a mixed isolate conidial suspension consisting of equal concentration of the three isolates as described in Section 2.7 was used. A mixed isolate inoculum was used as the pathogenicity of the isolates to cabbage was not known and using more than one isolate of different mating types and virulence groups enhances the likelihood of achieving infection.

Table 2.2: Details of the origin and the mating type and avirulence group of the *Leptosphaeria maculans* isolates used in the study (Source: personal communication, Eirian Jones, 2016)

Lincoln Uni Isolate code	Mating type and avirulence group	Plant (symptom) isolated from	Origin
LUPP2369	MAT1, Avr1, Avr6, Avr4-7	Swede (dry rot)	Puketitiri
LUPP2376	MAT1, Avr1, Avr6	Swede (dry rot)	Gore
LUPP2403	MAT2, Avr6	Oilseed rape (base lesion)	Lincoln

2.5 Experiment 1: Effect of *Trichoderma* spp. or *Beauveria bassiana* inoculation on *Myzus persicae*

Twenty-one day old *B. oleracea* var. *capitata* seedlings, inoculated with 1.0 ml per pot plant with the concentrated suspension (*Trichoderma* spp. at 1.0 x 10^7 spores/ml or *B. bassiana* at 1.0 x 10^8 spores/ml), were challenged with *M. persicae* following the procedures described by Mitchell et al. (2009).

Teneral adults and nymphs of unknown age were carefully picked from the primary colony using a fine camel-hair brush and three nymphs were gently transferred to each clip cage (20 mm diameter). Aphids were placed on the abaxial side of the second youngest true leaf of each plant by carefully clipping the clip cages onto the leaf (Figure 2.2). After 24 hours, all newly emerged nymphs from both the clip cages and leaf surface were removed.

After a further 24 hours, the adults and all nymphs were removed, leaving only three new nymphs (Figure 2.3). At this stage the aphid nymphs had settled on the leaves, and clip cages removed. At 24 hour intervals, new nymphs were removed until all adults had died or on termination of the experiment on the 25th day of observation.

The number of days from birth of the three initial nymphs to their first reproduction (pre-reproductive period), the total number of nymphs produced during 25 days (total reproduction), and the number of days the adults were alive (longevity), were recorded for each replicate. Some adult aphids remained alive beyond the 25 day observation period and, in such cases, maximum adult longevity was arbitrarily set at 30 days, an approximate lifespan of an aphid (Chun et al., 2010).

Figure 2.2: Clip cages containing three teneral *Myzus persicae* nymphs placed on the second youngest true leaf on *Brassica oleracea* var. *capitata* plants

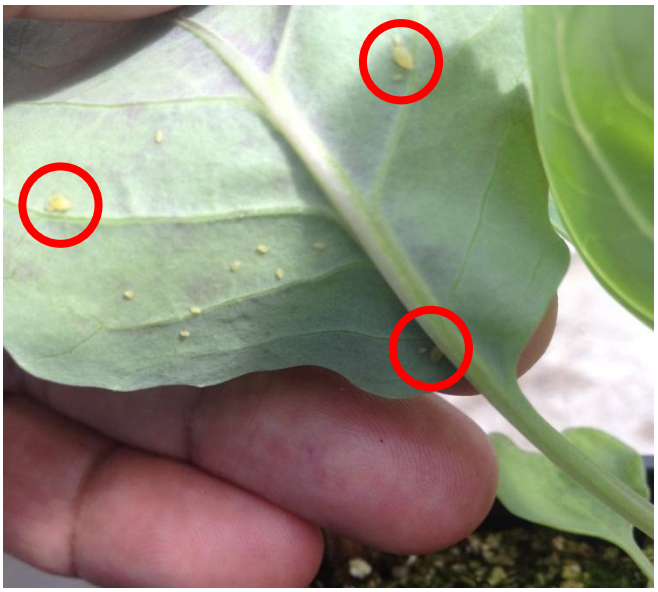

Figure 2.3: *Myzus persicae* nymphs on *Brassica oleracea* var. *capitata* plant leaves, where all new nymphs were removed leaving only three (red circles) at the start and throughout experiment until adult mortality.

2.6 Experiment 2: Effect of *Trichoderma* spp. or *Beauveria bassiana* inoculation on *Sclerotinia sclerotiorum* infection

Cabbage „Derby Day" seedlings inoculated with one of the *Trichoderma* spp. isolates or *B. bassiana* isolates were challenged with *Sclerotinia sclerotiorum*. Cabbage plants were inoculated with discs (5 mm diameter) of actively growing mycelium of *S. sclerotiorum* isolate LU8007 cut from the edge of a colony growing on PDA.

The colonised agar plugs were placed mycelial face down on the leaf surface of the third youngest true leaf of 28 days old treated and control (mock-inoculated) plants. The plugs were then gently covered with plastic foil and the whole plant was placed inside a sterile plastic bag (230 mm x 305 mm) with the bottom end fastened against the plant pot by rubber bands.

Prior to bagging each plant, the interior surface of the plastic bags were moist with a fine mist of SDW. The plastic bags were gently misted daily to maintain high relative humidity for six days. The plants were watered every two days by filling the saucers with tap water.

Seven days after infecting the inoculated cabbage plants, the size of the lesions on the *S. sclerotiorum* inoculated leaves was measured (Figure 2.4). The leaf lesion area was measured by placing a clear transparency film over the infected area and tracing the edge of the lesion onto the film. The traced images were scanned as Tagged Image File Format (TIFF) image files with 300 dpi resolution and then converted into bitmap image files using Paint 6.1 (Windows 7). The bitmap images were then imported into Surface.exe software (written by Carsten Thiemann for Michael Rostas) and the lesion area was calculated.

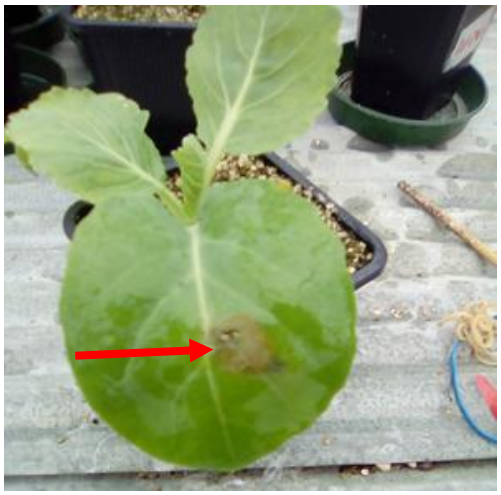

Figure 2.4: *Sclerotinia sclerotiorum* lesion (red arrow) which developed on a *Brassica oleracea* var. *capitata* leaf 5-days post infection.

2.7 Experiment 3: Effect of *Trichoderma* spp. or *Beauveria bassiana* inoculation on *Leptosphaeria maculans* infection

Cabbage „Derby Day" inoculated with *Trichoderma* spp. or *B. bassiana* isolates as outlined in Section 2.2.2 were challenged with *L. maculans*. Sporulating cultures of *L. maculans* isolates LUPP2369, LUPP2376, and LUPP2403 (Section 2.4.2) were flooded with 10 ml SDW containing 0.01% Triton X-100 and the surface rubbed gently with a sterile cell spreader.

The resulting conidial suspensions from each isolate were strained into a glass beaker through Mira cloth to remove hyphal fragments. The beaker containing the mixed isolate conidial suspensions was placed on the mechanical shaker for 5 mins to homogenize and adjusted to 1.0×10^6 conidia/ml based on haemocytometer counts.

Thirty-five day old *Trichoderma* spp. or *B. bassiana* inoculated cabbage seedlings were inoculated with *L. maculans* following the method of Lob (2014) whereby a 10 µl droplet of the mixed isolate conidial suspension was placed onto a freshly wounded second or third youngest leaf (Figure 2.5A). The leaves were wounded by pricking with a Birchwood toothpick (Sara Lee, New Zealand Ltd) just before inoculation. After the inoculum had dried (ca. 1.5 hours), each plant was placed inside a sterile plastic bag (230 mm x 305 mm), which was sprayed with a fine mist of SDW on the inside prior to bagging (Figure 2.5B). The bottom (open end) of the plastic bag was affixed to the pot with rubber bands and the plants watered every two days by filling the saucers with tap water.

Figure 2.5: **A)** Inoculation of 35 days old *Brassica oleracea* var. *capitata* seedlings with *Leptosphaeria maculans* with inoculation point indicated by red arrow; **B)** inoculated plants in misted plastic bags in a cage (cage had 8 treatment plants).

After 10 days, the inoculated leaves were detached and the area of the lesions were traced following procedures described in Section 2.6.

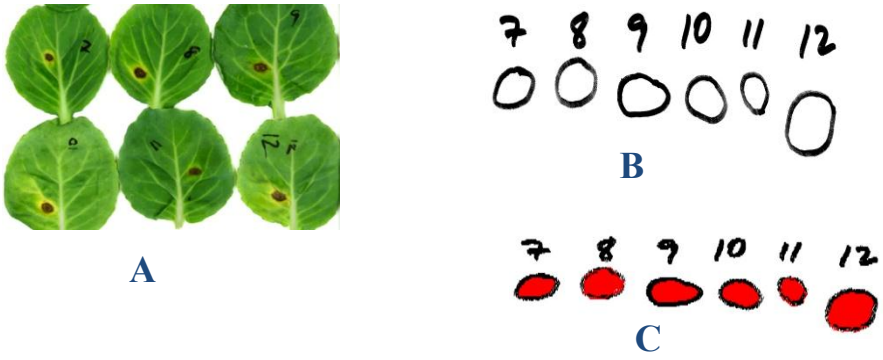

Figure 2.6: Process for determining leaf lesion area; **A)** Leaf lesions which developed on *Brassica oleracea* var. *capitata* at the inoculation point, **B)** Scanned TIFF image of the lesions, and **C)** bitmap image for lesion area calculation with Surface exe.

2.8 Plant performance in response to *Trichoderma* spp. or *Beauveria bassiana* inoculation

To determine whether inoculation with *Trichoderma* spp. isolates or *B. bassiana* isolates had any effect on plant performance after herbivore challenge, the total number of leaves, shoot and root lengths from all treatment plants were assessed on completion of the aphid experiment.

For leaf counts, old leaves that had fallen off the plants were excluded if the plant from which they had fallen off was not known. Shoot lengths were measured from the stem-vermiculite interface level using a 30 cm ruler to the nearest millimetre.

To measure the root lengths, the vermiculite was cleaned off the roots in running tap water and the length of the primary root was measured to the nearest mm. Although dry weights provide accurate measures of plant growth, time limitations could not allow this to be undertaken.

2.9 Endophyte colonisation

Trichoderma spp. and *B. bassiana* were reisolated from surface-sterilized leaf and root sections. On completion of the aphid and *L. maculans* experiments, three randomly selected sample plants from each treatment were taken. The random selection was performed by writing the names of the treatment and the block on pieces of paper, folded individually to protect identity of names written on them and were placed in a plastic bag. They were

thoroughly mixed by shaking manually and one piece of the paper was drawn at a time without replacement.

The treatment with its block name that was drawn first, second, and third were sampled and continued through for the rest of the treatments. The leaf adjacent to the one which the aphids were clipped onto or inoculated with *L. maculans* detached.

The roots were washed under running tap water to remove vermiculite. Both leaf and root samples were wrapped in moist filter paper and placed in sealed plastic bags, and all samples were processed the same day.

Lateral roots were removed leaving only the primary roots which were cut into approximately 1 cm root segments using a sterile scalpel blade. The leaves were cut into 5-10 equally sized pieces using a sterile scalpel blade. These root and leaf segments were then surface sterilized in a laminar flow hood by immersing in 0.01% Triton X-100 for 2 mins, followed by 2% NaOCl, and 70% ethanol for, 5 and 2 mins, respectively. The tissue samples were then rinsed three times with SDW and dried on sterile filter paper in a sterile airstream in the laminar flow hood for 5 mins. The sterilized leaf and root segments from *Trichoderma* spp. and *B. bassiana* treated plants were plated on *Trichoderma* selective media (TSM) [(McLean et al., 2005), Appendix A.2] and *Beauveria* selective media (BSM, BPRC, 2012, Appendix A.3), respectively. Leaf and root sections of six control treatment plants were separated equally and plated on TSM and BSM respectively.

After incubating in darkness at 20°C for seven days, the number of root or leaf segments from which colonies characteristic of the inoculated fungus were observed growing from the plated tissue segments onto the agar and the total number of segments plated were recorded (Figure 2.7). These were used to calculate percentage colonisation.

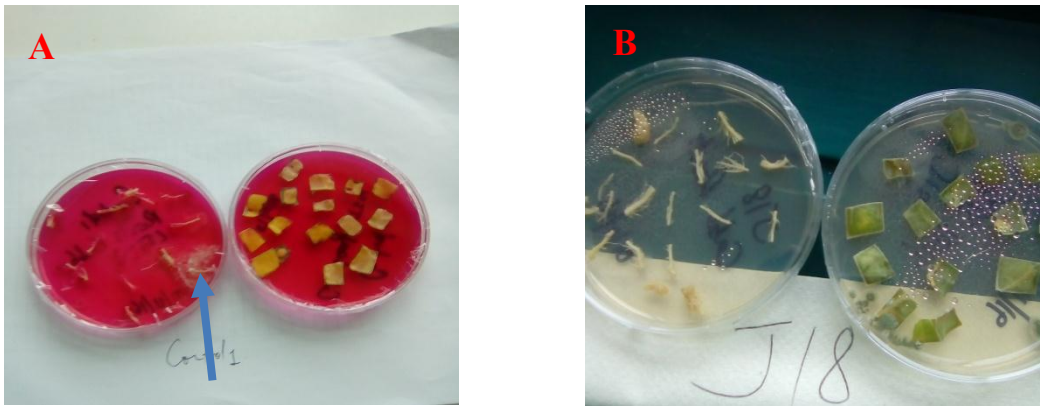

Figure 2.7: Surface sterilised leaf and root tissue segments plated on A) *Trichoderma* selective media with blue arrow indicating colonies characteristic of *Trichoderma* spp.; B) and *Beauveria* selective media

2.10 Phytohormone analysis

All cabbage leaves directly challenged by *Myzus persicae* were harvested from the treated *Brassica oleracea* var. *capitata* plants (88 samples). Each sample was immediately wrapped in aluminium foil, labelled accordingly, frozen in liquid nitrogen and immediately stored at -80ºC for 1 month prior to phytohormone analysis.

Phytohormone analysis was conducted with six replicate samples from *T. atroviride* LU132, *B. bassiana* BG11, and the control treatments respectively. These were selected for phytohormone analysis as these showed the largest positive effect on *Myzus persicae*. Jasmonic acid (JA) and salicylic acid (SA) were extracted using the vapour phase extraction (VPE) protocol and levels subsequently analyzed by gas chromatography mass spectrometry (GCMS) as described by Schmelz et al. (2004).

2.10.1 Vapour Phase Extraction (VPE)

Frozen leaf tissues were ground to fine powder by addition of liquid nitrogen and further ground by crushing between the aluminium foil. Approximately 150 mg were weighed into reaction tubes and finely pulverized and homogenized using a tissue homogenizer (Spex® Sample Prep LLC, 1600 MiniG™, Metuchen NJ 08840, USA) with pre-cooled holders at a frequency of 30 Hz for 1 min.

To each sample 600 µl of pre-heated (70ºC) extraction buffer (water:1-propanole:$HCl_{conc.}$ = 1:2:0.005) was added and the tissues were vigorously agitated to homogenize them. After addition of 20 µl of the internal standard (10 µg/ml dihydrojasmonic acid in methanol) and 1.0 ml of methylene chloride, samples were centrifuged for 1 min at 10,000 *g* to obtain phase separation.

The lower organic phase was transferred to 4 ml glass vials and dried over Na_2SO_4. To increase the volatility of the phytohormones and enable separation by gas chromatography, the samples were derivatized with 2 µl of 2 M trimethylsilyldiazomethane (TMS) in hexane (Sigma-Aldrich, USA) for 5 min at room temperature. TMS is a methylating agent that converts carboxylic acids into methyl esters. The methylation reaction was stopped by adding 2 µl of 2 M acetic acid in hexane.

The samples were then subjected to vapour phase extraction. The volatile collection filters contained Super Q absorbent (Altech, IL, USA), a highly stable divinylbenzene polymer tolerant to H_2O vapour and sensitive only to temperatures above 300ºC. The method involved two evaporation steps, the first one at 100ºC and the second at 200ºC in order to make use of the Super Q adsorbent properties and increase the range of analytes recovered.

First the 4 ml vial was sealed using high temperature septum (Schott, Germany), a Super Q filter was inserted through the septum followed by a needle that supplied a gentle stream of nitrogen (flow rate 0.8 l min^{-1}). The connected vial was then placed in a dry block heater adjusted to 100ºC to expedite the evaporation of the derivatized extract.

After the solvent has evaporated (2-3 mins), the vial was transferred to a second heating block at 200ºC for approximately 2 mins. This step was required to collect compounds of lower volatility. When this was completed, the samples were eluted from the filters with 1 ml methylene chloride into the reaction vials.

Finally, the sample volume was reduced to 40 µl and transferred into microinserts and stored at -20ºC for three days then under -80ºC for 39 days until GCMS analysis was carried out.

2.10.2 Gas chromatography - mass spectrometry (GC-MS)

The samples for methyl jasmonate and methyl salicylate analysis were supplied in the solvent dichloromethane (DCM) (Applied Biosystems, Foster City, Canada). They were analysed using a Shimadzu GCMS-QP2010 Ultra (Shimadzu, Japan) gas chromatograph mass spectrometer fitted with a Restek Rxi-1ms fused silica capillary column (30.0 m x 0.25 mm i.d. x 0.25 µm, Bellefonte, PA, USA).

A CTC-Combi PAL auto-sampler (PAL LHX-xt, CTC analytics AG, Switzerland) was used to inject 1.5 µl of the sample into the GC injection port, operating in high pressure injection splitless mode at 220ºC and with a pulse of 241 kPa for 40 seconds. After injection, the column oven was held at 50ºC for 3 mins, then heated to 320ºC at 8ºC min^{-1}, and held at this temperature for 8 mins. Helium was used as the carrier gas with the constant linear velocity set at 44.4 cm sec^{-1} in split mode (1.5 ml min^{-1}).

The mass spectrometer (MS) was operated in single ion monitoring mode with selected masses used to identify methyl salicylate (target ion m/z 120, confirming ions-m/z 92 and m/z 152), methyl jasmonate (target ion m/z 151, confirming ions-m/z 193 and m/z 224), and

internal standard dihydro-methyl-jasmonate (target ion m/z 156, confirming ions-m/z 153 and m/z 195).

The temperature of the capillary interface was 320ºC, with the MS source temperature set at 230ºC. Initial confirmation of the retention times for the two compounds of interest was performed by injecting the individual standards and matching their mass spectra with the spectra of reference compounds in the NIST EPA/NIH Mass Spectral Library database (National Institute of Standards and Technology, NIST11). The peaks of the quantifying ions were calculated according to the following formula:

$$\textit{Leaf content (ng g}^{-1}\textit{ FW)} = \frac{\textit{area}_{m/z}\textit{ (compound of interest). 200}}{\textit{area}_{m/z}\textit{ (internal standard)}}$$

2.11 General design of the experiments

All experiments were set up at the Lincoln University nursery in a glasshouse with temperature range of 16-23°C and 60-70% relative humidity and under normal day/night (16L:8D) conditions. Randomized complete block design (RCBD) with eleven blocks and eight treatments (seven treatments but double controls) was used. Eleven collapsible insect cages (47.5 x 47.5 x 47.5 cm, BugDorm, Taiwan) each equating to a statistical block contained eight plants (a plant of each treatment), $n = 88$.

The insect cages were placed in two rows on a flat bench of five and six cages respectively and numbered from 1-11. The treatments were; (i) *Trichoderma atroviride* LU132, (ii) *T. virens* LU556, (iii) *T. hamatum* LU593, (iv) *Beauveria bassiana* FRh2, (v) *B. bassiana* BG11, (vi) *B. bassiana* J18, (vii) $Control_1$, and (viii) $Control_2$. From a total experimental unit of 88 potted cabbage seedlings, 66 were inoculated with the respective *Trichoderma* spp. or *B. bassiana* isolates while 22 were mock-inoculated with SDW amended with 0.01% Triton X-100 as controls.

Positions of each individual treatment plants was assigned using the "alternative to random allocation" method whereby treatment names were written on pieces of paper, folded individually to protect identity of names written on them and were placed in a shopping plastic bag. They were thoroughly mixed by shaking manually and one piece of

the paper was drawn at a time without replacement. The treatment that was drawn first was allocated to the first plot in a cage, second treatment drawn to plot 2, and third treatment drawn to plot 3, and continued through until the last treatment was positioned to plot number 8.
The same procedure was used for the remainder of the insect cages/blocks until all treatment were allocated randomly across all 11 blocks.

After random allocation of treatments, the plant pots were numbered from 1 through to 88 in ascending order of the insect cages. Recordings were made by pot number (blind) rather than by treatments to avoid biasness. Double the number of controls were used in the experiments to enable the determination of whether the fungal isolate treatments differed significantly from the controls. This is because statistically, there should be 2.45 (i. e. $\sqrt{6}$ treatments) replicates for the control for each of the six treatments.

2.12 Statistical Analysis

All experimental data were analysed using analysis of variance (ANOVA). Lesion area and aphid data were square-root or log10 transformed to improve homogeneity of variance before analysis by ANOVA using Genstat version 18.

All means were compared using Tukeys" post hoc test at $P<0.05$ and presented as the mean of back transformed data. For calculating the average adult longevity of an aphid, a numeral 30, assumed as the approximate adult lifespan, was assigned to aphids that lived beyond 25 days (experiment completion). No transformation was carried out for plant performance data (number of leaves, shoot and root length) in response to *Trichoderma* and *B. bassiana* inoculation treatments.

Trichoderma and *B. bassiana* colonisation data were calculated as a percentage of segments positive for *Trichoderma* or *B. bassiana* from the total number of segments plated.

For the phytohormone analysis, comparison of the two hormones of interest (JA and SA), due to aphid feeding on *Trichoderma* spp. or *B. bassiana* inoculated plants, showed that they were not normally distributed and they were therefore analysed using the Kruskal-Wallis general analysis of variance. Graphs were generated using Microsoft Excel 2016 (Microsoft Corporation, USA).

3 Results

3.1 Experiment 1: Effects of *Trichoderma* spp. or *Beauveria bassiana* inoculation of plants on *Myzus persicae*

3.1.1 Pre-reproductive period

Forty-eight hours after removing new nymphs and adults, three nymphs per treatment plant were monitored for aphid performance. The number of days from birth to the first reproduction of oviviparous females were observed daily. Results of square-root transformed data showed a significant effect ($F_{6,85}$ = 3.66, P=0.003) between treatments (Appendix C.1, means and standard deviations; and C.2, ANOVA output). The onset of reproduction was delayed among aphids that fed on *Trichoderma* spp. or *Beauveria bassiana* inoculated plants compared to those fed on control plants. On average, reproduction started on the 6th day post infestation (dpi) on the control plants in comparison to *Trichoderma* or *B. bassiana* inoculated plants which started on the 8th dpi (Figure 3.1).

Among treatments, the only significant effect was observed in plants inoculated with *T. hamatum* LU593 (8.6 days ± 0.6) and *T. virens* LU556 (8.8 days ± 0.4) treated plants in comparison to controls (6.3 days ± 0.4) (Figure 3.1). Mean number of days to first birth for endophyte treated cabbage plants were in the range of 7.9 - 8.1 (± 0.3 - 0.7) compared to control (6.3 ± 0.4). Treatments *T. atroviride* LU132, *B. bassiana* isolates J18, BG11, and FRh2 were not significantly different to the untreated control or *T. hamatum* LU593 and *T. virens* LU556. There was no significant effect between blocks (P=0.099; Appendix C.2).

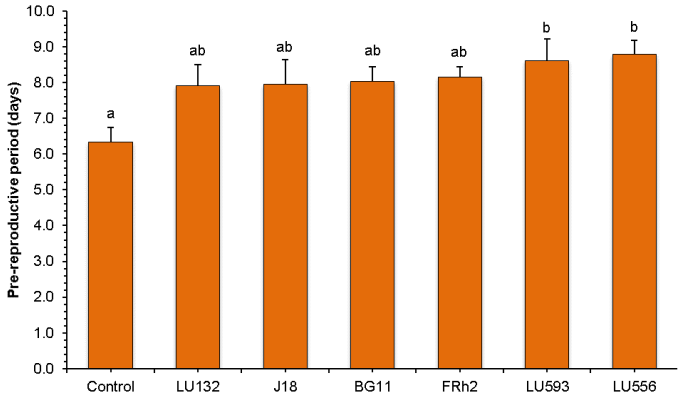

Figure 3.1: Effect of *Trichoderma* spp. (LU132, LU593, and LU556) or *Beauveria bassiana* (J18, BG11, and FRh2) inoculation of cabbage on pre-reproductive period of *Myzus persicae*. Error bars represent the standard error of the actual means. Bars followed by the same letters are not significantly different at P<0.05 (Tukey"s post hoc test, n = 11).

3.1.2 Total reproduction of *Myzus persicae* nymphs in a lifetime

The total number of green peach aphid nymphs produced during the observation period was significantly different between treatments ($F_{6,86}$ = 7.05, P<0.001; Appendix C.3). Over a 25 day period, the total number of offspring produced by aphids that fed on *B. oleracea* var. *capitata* plants inoculated with *T. hamatum* LU593 (22.9 ± 5.2), *T. virens* LU556 (28.9 ± 3.2), *T. atroviride* LU132 (30.8 ± 4.3) or *B. bassiana* isolates BG11 (26.5 ± 3.3) and FRh2 (23.3 ± 4.4) was significantly lower (P<0.05) than by aphids that fed on the control treatment (58.0 ± 4.9) (Figure 3.2). Aphids that fed on *B. bassiana* J18 inoculated plants produced on average 34.8 ± 5.2 nymphs which was not significantly different compared with the control treatment (58.0 ± 4.9) or any of the other *Trichoderma* spp. or *B. bassiana* treatments. There was no significant effect of blocks (insect cages) ($F_{6,10}$ = 1.22, P=0.292).

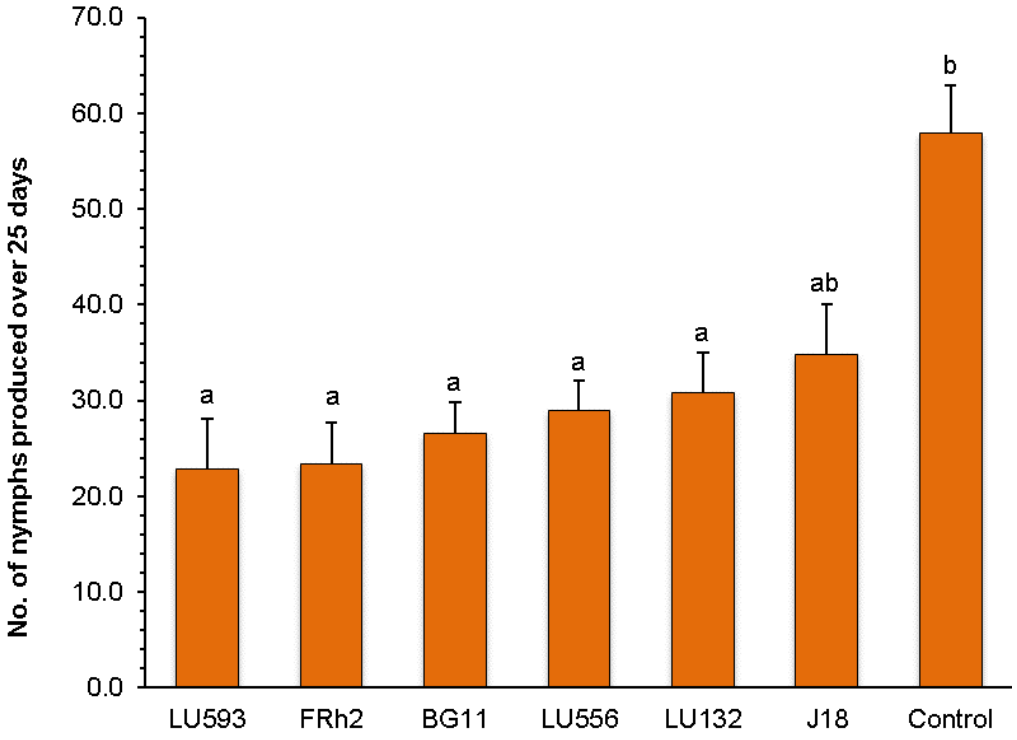

Figure 3.2: Effect of *Trichoderma* spp. (LU593, LU556, and LU132) or *Beauveria bassiana* (FRh2, BG11, and J18) inoculation of *Brassica oleracea* var. *capitata* plants on the reproduction of *Myzus persicae* offspring. Error bars represent standard error of back-transformed means. Bars followed by the same letters are not significantly different at P<0.05 (Tukey's post hoc test $F_{6, 86}$ = 7.05, P<0.001).

3.1.3 *Myzus persicae* adult longevity

Aphid lifespan was monitored from first birth until mortality or on completion of the experiment. There was a significant effect of treatment ($F_{6,86}$ =5.60, P<0.001; Appendix C.4) on adult longevity. Aphids fed on *T. hamatum* LU593 (19.7 ± 1.3) and *B. bassiana* FRh2 (20.4 ± 2.5) and BG11 (20.7 ± 1.7) inoculated cabbage plants had significantly reduced longevity (P<0.05) compared with the untreated controls (Figure 3.3). In *T. atroviride* LU132, *T. virens* LU556, and *B. bassiana* J18 inoculated plants, adult longevity did not differ significantly (P<0.05) compared with the untreated control or *Trichoderma* spp. or *B. bassiana* treatments (Figure 3.3).

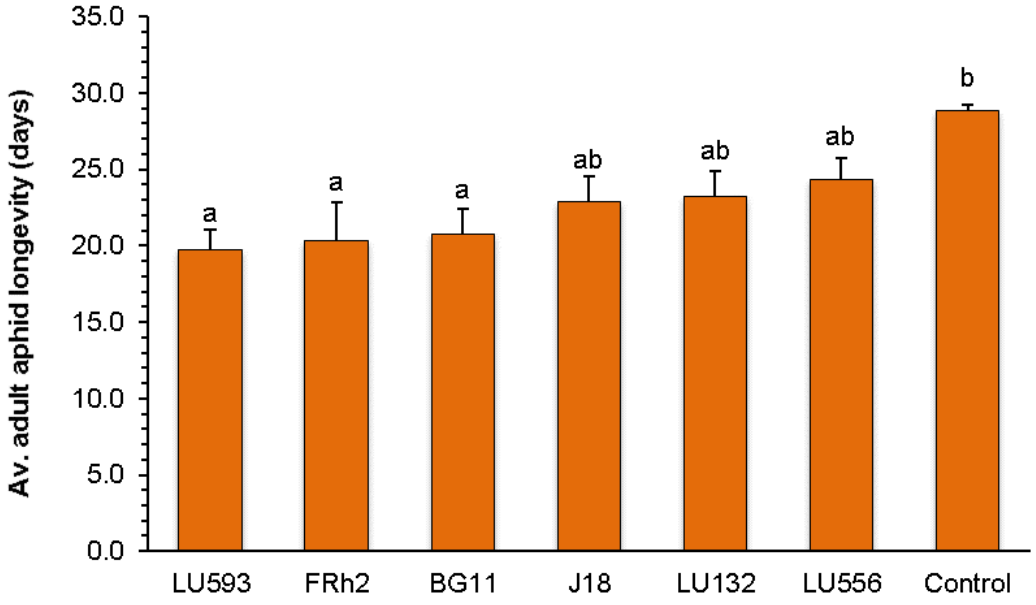

Figure 3.3: Average lifespan (days) of an adult *Myzus persicae* after feeding on *Trichoderma* spp. (LU593, LU132, and LU556) or *Beauveria bassiana* (FRh2, BG11, and J18) inoculated *Brassica oleracea* var. *capitata* plants. Error bars represent standard error of the back transformed data, with mean comparisons based on ANOVA of square-root transformed data. Different letters above columns indicate significant differences at P<0.05 (Tukey's post hoc test, $F_{6,86}$ =5.60, P<0.001).

3.2 Experiment 2: Effects of *Trichoderma* spp. or *Beauveria bassiana* inoculation of *Brassica oleracea* var. *capitata* on *Sclerotinia sclerotiorum* infection

Results from measurements of diseased leaf surface area showed that there were significant differences among treatments ($F_{6,87}$ = 2.98, P=0.012) (Appendix C. 5). The mean leaf lesion (infected) area in the control was three times higher than that of the *Trichoderma* or *B. bassiana* inoculated plants (Figure 3.4).

Beauveria bassiana J18 (1.8 ± 1.3) and BG11 (2.4 ± 2.5) and *T. hamatum* LU593 (3.1 ± 1.7) inoculated plants had significantly lower mean leaf lesion area (mm^2) compared with the control plants (15.6 ± 4.2). Treatment with *B. bassiana* FRh2 (5.0 ± 2.2) and *T. atroviride* LU132 (5.1 ± 1.8) and *T. virens* LU556 (3.9 ± 5.1) did not show any significant difference ($P<0.05$) to the untreated control or any other treatment (Figure 3.4).

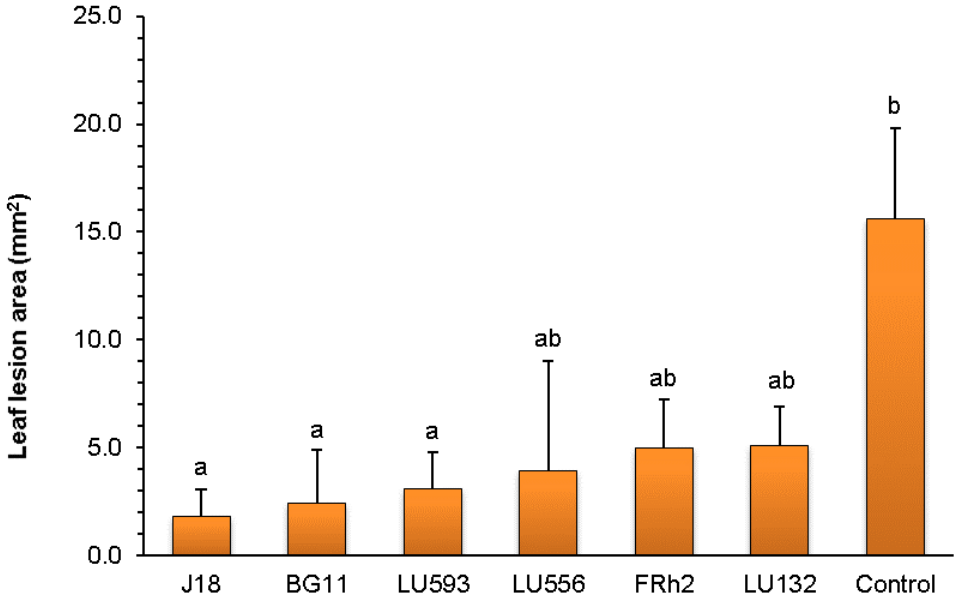

Figure 3.4: Effect of *Trichoderma* spp. (LU593, LU556, and LU132) or *Beauveria bassiana* (J18, BG11, and FRh2) inoculation of *Brassica oleracea* var. *capitata* plants on the area of leaf lesion (mm^2) following inoculation with *Sclerotinia sclerotiorum*. Error bars represent standard error of the back-transformed means. The mean comparisons are based on ANOVA of back-transformed data followed by Tukey's test ($P=0.012$, $n = 11$). Bars followed by the same letters are not significantly different at $P<0.05$.

3.3 Experiment 3: Effect of *Trichoderma* spp. or *Beauveria bassiana* inoculation of *Brassica oleracea* var. *capitata* on *Leptosphaeria maculans* infection

Results on the effect of *Trichoderma* spp. or *B. bassiana* inoculation on *B. oleracea* var. *capitata* against *L. maculans* infection showed that there was no significant treatment effect ($F_{6,87}$ = 1.35, P=0.247, Appendix C.6). Leaf lesions for plants inoculated with *T. atroviride* LU132 were 3.5 mm^2 ± 0.5, *B. bassiana* BG11 were 3.6 mm^2 ± 0.5, *B. bassiana* FRh2 were 4.1 mm^2 ± 0.5, *T. hamatum* LU593 were 4.1 mm^2 ± 0.6, *T. virens* LU556 were 5.3 mm^2 ± 0.7, and *B. bassiana* J18 were 5.7 mm^2 ± 0.6 compared with the control which were 4.0 mm^2 ± 0.4 (Figure 3.5).

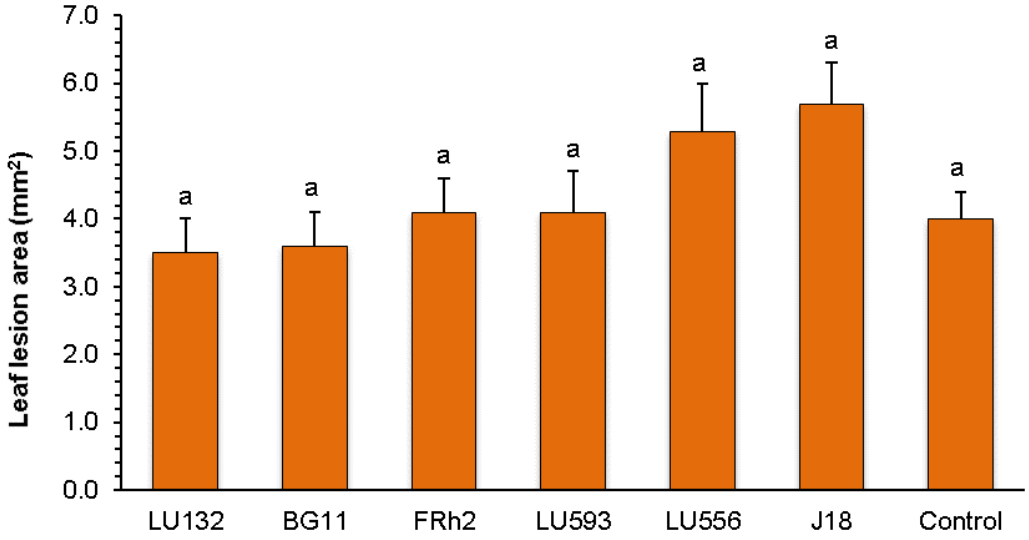

Figure 3.5: Effect of *Trichoderma* spp. (LU132, LU593, and LU556) or *Beauveria bassiana* (BG11, FRh2, and J18) inoculation of *Brassica oleracea* var. *capitata* plants on the area of leaf lesions (mm^2) following inoculation with *Leptosphaeria maculans*. Error bars represent means of back transformed data. Letters separating means are based on ANOVA followed by Tukeys test of square-root transformed data. Bars with the same letters are not significantly different at P< 0.05, n = 11.

3.4 Plant growth performance in response to *Trichoderma* spp. or *Beauveria bassiana* inoculation

The root drench application of *Trichoderma* spp. or *B. bassiana* isolates to *Brassica oleracea* var. *capitata* did not significantly affect the total number of true leaves ($F_{6,86}$ = 1.72, P= 0.129) and shoot lengths ($F_{6,86}$ = 1.40, P= 0.227), however there was a significant effect on the root lengths (F6,86 = 5.53, P<0.001) (Table 3.1). All *Trichoderma* spp. and *B. bassiana* treatments, apart from *B. bassiana* J18, significantly (P< 0.05) increased root length compared with the untreated control.

Table 3.1: Growth performance of *Brassica oleracea* var. *capitata* plants in response to *Trichoderma* spp. or *Beauveria bassiana* inoculations after 30 days

Treatment	Parameters measured		
	No. of true leaves	**Shoot length (cm)**	**Root length (cm)**
T. atroviride LU132	10.1 ± 0.3 a	5.3 ± 0.2 a	9.5 ± 0.5 b
T. hamatum LU593	9.9 ± 0.1 a	5.1 ± 0.5 a	9.3 ± 0.7 b
T. virens LU556	9.9 ± 0.2 a	5.0 ± 0.2 a	9.0 ± 0.7 b
B. bassiana BG11	9.8 ± 0.3 a	5.3 ± 0.1 a	9.4 ± 0.7 b
B. bassiana FRh2	10.2 ± 0.2 a	5.5 ± 0.2 a	10.0 ± 0.9 b
B. bassiana J18	10.2 ± 0.3 a	5.1 ± 0.2 a	8.5 ± 0.6 ab
Control	9.5 ± 0.2 a	4.9 ± 0.2 a	6.5 ± 0.3 a
F ratio (df)	1.72 (6,86)	1.40 (6,86)	5.53 (6,86)
F probability	0.129	0.227	<0.001

Data within a column followed by the same letters are not significantly different based on ANOVA followed by Tukey‟s test at P< 0.05. Each parameter was compared separately against treatment.

3.5 Endophyte colonisation

In general, the colony morphology of the isolates which grew from the plated tissues were characteristic of *Trichoderma* spp. and *B. bassiana*, however confirmation of the identity of the reisolated strains as the inoculated strains was not carried out.

Colonies characteristic of *Trichoderma* spp. and *B. bassiana* were observed to grow from the leaf and root segments (pooled data of aphid and *L. maculans* study) from the inoculated *B. oleracea* var. *capitata* plants (Figure 3.6). Over 39% of *Trichoderma* spp. and *B. bassiana* were recovered from roots of inoculated cabbage plants. The highest recovery was in *T. atroviride* LU132 inoculated plants (67%), followed by *B. bassiana* BG11 (58%), and FRh2 (57%) inoculated plants respectively. *Trichoderma virens* LU556 (43%), *B. bassiana* J18 (40%), and *T. hamatum* LU593 (39%) exhibited the least percentage colonisation observed from the root segments. Surface sterilized root segments plated from control treatment had

13% colonisation by *Trichoderma* species (isolate/species was not confirmed) but there was no recovery of *B. bassiana*.

From the leaf segments, colonies characteristics of *B. bassiana* were only recovered from plants inoculated with *B. bassiana* isolates FRh2 (17%) and BG11 (8%). No colonies characteristic of *Trichoderma* or *B. bassiana* were observed to grow from the leaf segments plated from the control treatment plants.

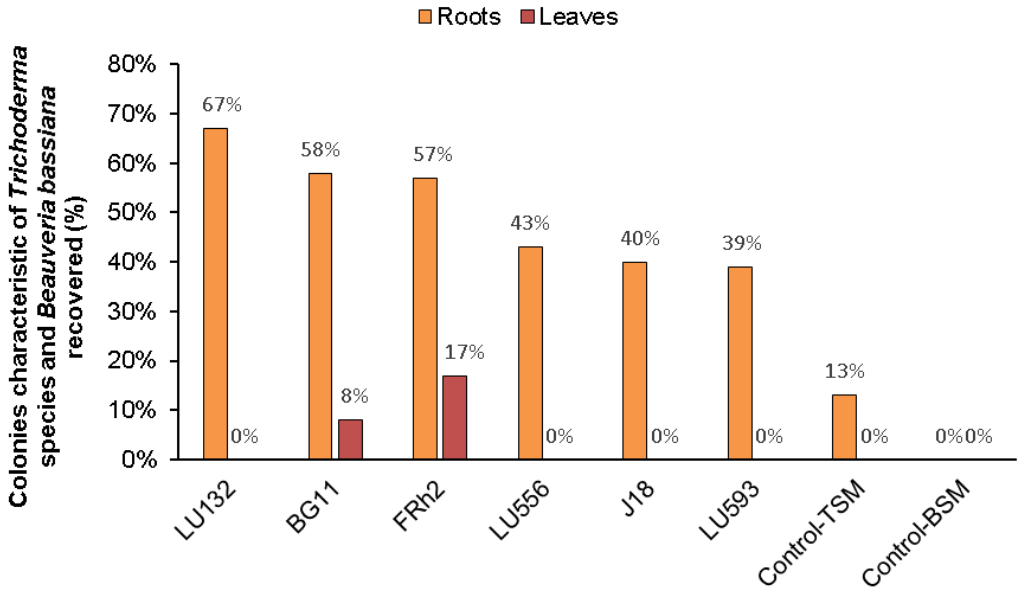

Figure 3.6: The percentage of root and leaf segments (pooled data of aphid and *Leptosphaeria maculans* study) on which colonies characteristic of *Trichoderma* spp. (LU132, LU556, and LU593) or *Beauveria bassiana* (BG11, FRh2, and J18) were reisolated from pre-inoculated *Brassicae oleracea* var. *capitata* plants. Segments from *Trichoderma* spp. treated plants were plated on *Trichoderma* selective media (TSM) while segments from *Beauveria bassiana* treated plants were plated on *Beauveria* selective media (BSM). Surface sterilized segments from six control treatment plants were plated equally on either TSM or BSM. N = 30 and 25 from roots and leaves, respectively

3.6 Plant hormone analysis

The levels of the two phytohormones of interest, jasmonic acid (JA) and salicylic acid were analysed in leaf tissues that had been challenged with *M. persicae* for 25 days. The results showed that there were no significant differences (Kruskal-Wallis test) for both phytohormones between treatments. There was no significant effect of treatment on either salicylic acid ($F_{13,2}$ = 1.19, P=0.341, Figure 3.7, Appendix C.11) or JA contents ($F_{8,2}$ = 1.28, P=0.344, Figure 3.8, Appendix C.12).

Although there was no significant difference in SA levels between treatments, there was a trend for lower SA content for plants inoculated with *T. atroviride* LU132 (165.2 ± 45.3 ng g^{-1} FW, n = 4) and *B. bassiana* BG11 (141.6 ± 35.9 ng g^{-1} FW, n = 4), whereas the control plants showed high quantities (1134.6 ± 713.1 ng g^{-1} FW, n = 6).

There was variation in the JA data however, there was a trend for lower JA levels for plants inoculated with *B. bassiana* BG11 (28.9 ± 6.9 nanograms per gram of fresh weight, n = 3) and *Trichoderma* LU132 (29.1 ± 20.6 ng g^{-1} FW, n = 2) compared with the control treatment plants (190.6 ± 132 ng g^{-1} FW, n = 4) after being challenged with aphids for 25 days.

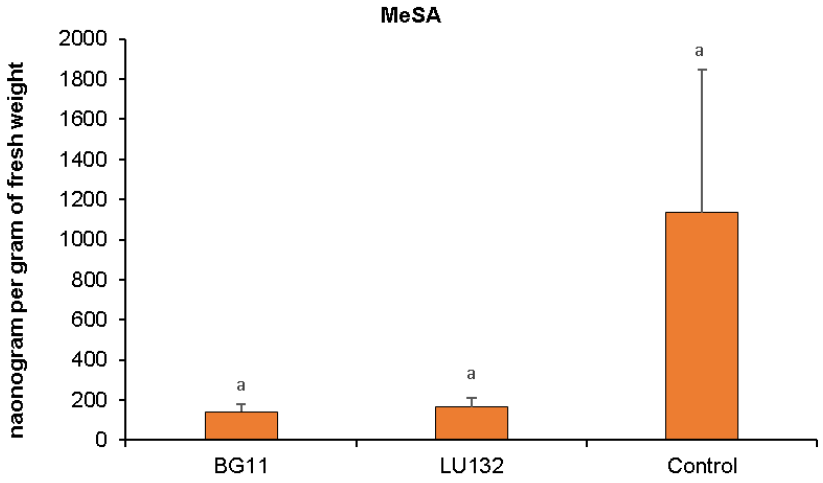

Figure 3.7: Salicylic acid concentrations (ng g^{-1} FW) measured in *Beauveria bassiana* BG11 and *Trichoderma atroviride* LU132 inoculated *Brassica oleracea* var. *capitata* leaf tissues challenged with *Myzus persicae* for 25 days. Error bars represent means of actual data. Mean separation is based on ANOVA followed by Tukey‟s test. Bars with same letters are not significantly different at P<0.05.

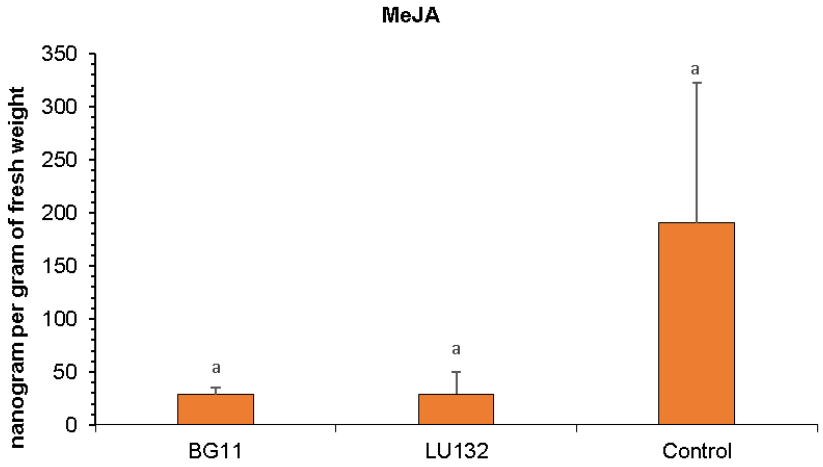

Figure 3.8: Jasmonic acid concentrations (ng g^{-1} FW) measured in *Beauveria bassiana* BG11 and *Trichoderma atroviride* LU132 inoculated *Brassica oleracea* var. *capitata* leaf tissues challenged with *Myzus persicae* for 25 days. Error bars represent means of actual data. Mean separation is based on ANOVA followed by Tukey‟s test. Bars with same letters are not significantly different at P<0.05.

4 Discussion

4.1 Effect of *Trichoderma* spp. or *Beauveria bassiana* inoculation on *Myzus persicae*

This research suggests that some of the tested isolates of *Trichoderma* spp. and *B. bassiana* have the potential to provide *B. oleracea* var. *capitata* plants with protection against a plant phloem-feeding insect. Aphid development, reproduction, and longevity were reduced when fed on plants pre-inoculated with *T. hamatum* LU593 and two *B. bassiana* isolates FRh2 and BG11. The observed effects are consistent with previous studies which demonstrated that *Trichoderma* spp. and *B. bassiana* are able to provide resistance against phloem-sap sucking insects in other crops. For example, the population of *M. persicae* on *T. atroviride* LU132 treated *Brassica napus* „Ability" plants was significantly lower than on the control treatments after feeding for five days (Maag, 2011). Similar results were observed in faba bean seed treated with *T. asperellum* M2RT4 and *B. bassiana* strains G1LU3 and S4SU1, where slow offspring development and fecundity of *Acyrthosiphon pisum* and *Aphis fabae* was found (Akello & Sikora, 2012). Another study provided evidence that exposing *Aphis gossypii* to cotton leaves colonised by *B. bassiana* reduced aphid reproduction (Gurulingappa et al., 2010). Although the exact mechanism involved in reducing aphid performance was not studied here, it has been suggested that the presence of *Trichoderma* spp. or *B. bassiana* within the root rhizospheres or in the plant tissues may have played a role through altering the nutritional content of the plants providing benefit against the phloem-sucking aphids (Gurulingappa et al., 2010; Vidal & Jaber, 2015; Zhang, 2014).

Beauveria bassiana has also been reported to be effective against nymphs of phloem-feeding *Bemisia tabaci* on cotton, tomato, and capsicum applied at the rate of 2.4 $x10^7$ spores/ml or at 2.0 x 10^8 spores/ml (Zafar et al., 2016). Mortality of up to 88% of nymphs was obtained. Consistent with the results of the studies by Zafar et al. (2016) and Gurulingappa et al. (2011), it was anticipated that the observed effects were probably related to the root drenching method of inoculation, where the *Trichoderma* spp. or *B. bassiana* had better chance to establish and colonise the developing roots and hypocotyl effectively. Muvea et al. (2014) found for another sap-sucking pest *Thrips tabaci*, that onion plants inoculated either as seed or seedling with *T. atroviride* ICIPE 710, *T. asperellum* M2RT4, and *T. harzianum* 709 had reduced the thrips population compared with the control treatments, which suggests that the

Trichoderma spp. and *B. bassiana* inoculation in this study had colonized the cabbage plants and provided protection against green peach aphid.

The reduced performance of *M. persicae* in terms of development, reproduction, fecundity, and longevity may have been attributed to changes in the chemical properties of the *Trichoderma* spp. or *B. bassiana* inoculated cabbage plants. For example, the hypersensitive (HR) response, in which cells immediately surrounding the stylet insertion site may rapidly die and fill with antimicrobial compounds to prevent spread of the aphid‟s watery saliva within the sieve elements, is an effective defence mechanism against phloem-feeding aphids (Kessler & Baldwin, 2002). Reduced performances of aphids on *Trichoderma* spp. or *B. bassiana* isolate inoculated plants can also be triggered by many other factors. For instance, Jallow et al. (2004) hypothesized that alterations in phytosterol composition mediated by an unspecialized root endophyte (*Acremonium strictum*) of tomato may explain the reduced larval performance of the caterpillar *Helicoverpa armigera* on inoculated plants. Furthermore, Barahona (2010) demonstrated that tomato, squash, melon, and pepper seedlings colonized by *Fusarium oxysporum* strain 162 (Fo162) had altered concentrations of unspecified metabolites in the presence of three phloem-feeding insects (*Trialeurodes vaporariorum*, *A. gossypii* and *M. persicae*). It has also been reported that terpenoids of plant origin can strongly affect the behaviour of aphids and prevent them from feeding and settling (Gabrys et al., 2015).

Cabbage plants are also known to produce a complex of volatiles induced by insect herbivory; these are known as herbivore-induced plant volatiles (HIPVs). HIPVs are chemical cues that help predatory insects find food, but little is known as to whether entomopathogenic fungi are recruited. The HIPVs may be released as the aphid punctures the leaf surface or as the stylet goes around the plant cell unit before reaching the phloem. Glucosinolates and their secondary products are known to defend plants against attack by harmful organisms (Bohinc et al., 2012). Furthermore, it is possible that the *Trichoderma* spp. and *B. bassiana* isolates used in this study may have altered the chemical compositions of the inoculated cabbage plants. It has been shown that root- and shoot-feeding herbivores have such an effect on lucerne aphids (Ryall et al., 2016). Studies have revealed that the presence of the endophyte changes the amino acid composition of the plants that subquently has an impact on the herbivores (Akello, 2012; Barahona, 2010; Cory & Hoover, 2006; Dicke & Van Poecke, 2002; Gunatilaka, 2006).

The presence of endophytes has been shown to frequently increase carbohydrate concentrations thereby altering the C:N ratio of the leaves and making them a less favorable

food source for the herbivores while the plant uses the nitrogen to form N-based secondary metabolites such as alkaloids (Barahona, 2010; Hamada & Jonsson, 2013). The symbiotic relationship of the inoculated *Trichoderma* spp. or *B. bassiana* may have changed the nutrient content of the cabbage plants making them less likely to suffer damage or alternatively had a direct impact on the aphids making them less productive. *Trichoderma* spp. and *B. bassiana* have been reported to produce various types of secondary metabolites which are believed to be crucial in the antagonistic activities against insect pests and plant pathogens (Akello, 2012).

Trichoderma spp. are known to secrete an array of secondary metabolites including harzianolide, harzianic acid, 6-*n*-pentyl-6H-pyran-2-one, 1-hydroxy-3-methl-anthraquinone, e.t.c., which are potent toxins with insecticidal, antifeedant, antimicrobial or deterrent properties (Vinale et al., 2008). *Beauveria bassiana* produce oosporein, beauvericin, bassianolides and beauveriolides as some of the metabolies (Akello, 2012; Bailey et al., 2008; Verma et al., 2007). It is possible that the isolates of *Trichoderma* and *B. bassiana* used in the present study released such toxic metabolites that reduced the aphid performance. However, studies showing that these are excreted in planta are lacking so far.

4.2 Effect of *Trichoderma* spp. or *Beauveria bassiana* inoculation of *Brassica oleracea* var. *capitata* on *Sclerotinia sclerotiorum* infection

The experiments showed a reduction in the total infected leaf lesion area on *B. oleracea* var. *capitata* plants inoculated with *Trichoderma hamatum* LU593 or *B. bassiana* J18 and BG11 compared to the control treatments. *Trichoderma hamatum* LU593 and *T. virens* LU556 have previously been reported to reduce infection of cabbage through mycoparasitism of the *S. sclerotiorum* hyphae and reduction in the sclerotial viability respectively (Jones et al., 2014). Similar results were described where the growth of *S. sclerotiorum* was reduced by 85-93% by coiling and formation of penetration structures against the hyphae of the pathogenic fungi by *T. atroviride* PTCC5220 (Matroudi et al., 2009). The reduced leaf infection area is thought to be associated with induced resistance mediated by the *Trichoderma* spp. or *B. bassiana* isolates that have endophytically colonised inoculated cabbage.

In field experiments, *T. hamatum* LU593 has been shown to be effective in reducing infection of cabbage plants when applied as maizemeal-perlite (MP) inoculum or as a transplant incorporation (Jones et al., 2014). *Trichoderma hamatum* LU593 and *T. virens* LU556 have

been reported to be sclerotial parasites, thereby reducing sclerotial viability and subsequently reduction in inoculum production and thereby disease incidence (Jones et al., 2014). However, in the present study, the reduction in the leaf lesion area on *Trichoderma* spp. or *B. bassiana* isolates inoculated plants is not due to this mode of action as plants were directly inoculated with mycelial discs.

In bean plants inoculated as root drench with three *Trichoderma* spp. (*T. viride*, *T. aureoviride* and *T. harzianum*) and inoculating with *S. sclerotiorum* as mycelial suspension, all isolates showed antagonistic potential (measured through classes of antagonism of Bell et al. (1982)) against *S. sclerotiorum*, with *T. harzianum* performing best (de Figueirêdo et al., 2010). Although the plants and *Trichoderma* spp. used in the current study were not the same as the above reports, there is evidence that *Trichoderma* spp. can play an antagonistic role against *S. sclerotiorum* to reduce the leaf lesion area. Such antagonistic ability of *Trichoderma* may also be related to the production of secondary metabolites and cell-wall degrading enzymes (CWDEs) which may have reduced the mycelial growth. *Trichoderma asperellum* inoculation in bean plants was shown to be effective in the reduction of *S. sclerotiorum* apothecia density and disease severity and the production of CWDEs NAGase and β-1,3-glucanase degraded the sclerotia (Geraldine et al., 2013), but there was no mycoparasitism or direct antagonistic effect involved in this study.

Trichoderma spp. and *B. bassiana* have also been reported to be capable of inducing systemic resistance against a wide range of plant pathogenic fungi including *S. sclerotiorum* (Alizadeh et al., 2013; Castillo et al., 2011; Olson & Benson, 2007). There have been interesting results over the last decade in the use of beneficial fungi including *Trichoderma* spp. and *B. bassiana* strains on a wide range of plant pathogenic fungi in relation to induced systemic resistance. For example, *Cucumis sativus* and *Arabidopsis thaliana* inoculated with *T. harzianum* isolate Tr6 showed induced systemic resistance against *Fusarium oxysporum* f. sp. *radicis cucumerinum* and *Botrytis cinerea*, respectively (Alizadeh et al., 2013). It is possible that *Trichoderma* spp. or *B. bassiana* may have colonised the root rhizosphere or endophytically colonised the root and induced the systemic pathway resulting in enhanced host defence response to *S. sclerotiorum* infection.

4.3 Effects of *Trichoderma* spp. or *Beauveria bassiana* inoculation of *Brassica oleracea* var. *capitata* on *Leptosphaeria maculans* infection

The results of *Trichoderma* spp. or *B. bassiana* inoculation to *B. oleracea* var. *capitata* plants challenged with the phytopathogenic fungi, *Leptosphaeria maculans* showed no significant

effect. In a previous study, it was found that foliar application of conidial suspensions of *T. atroviride* and *T. hamatum* on oilseed rape plants reduced the incidence and severity of phoma leaf spots, but this was not due to endophytic colonisation of the plants by the *Trichoderma* isolates (Dawidziuk et al., 2016).

According to Chen and Fernando (2006), isolates of *L. maculans* are highly virulent and are assigned to pathogenicity groups PG-2, PG-3, and PG-4 while group one (PG-1) are assigned to *L. biglobosa*. Inoculation of cotyledons with the highly virulent groups alone increased leaf lesion area whereas pre- or co- inoculation with weakly virulent group had smaller leaf lesion areas (Chen & Fernando, 2006). However, pre-inoculation with a highly virulent group followed by inoculation with a weakly virulent group on the same plant, had increased leaf lesion area. In the current study, pre-inoculation of plants with *Trichoderma* spp. or *B. bassiana* followed by wound inoculation with three isolates combined conidial suspension did not show any significant effect. The phenomenon involved is quite complex however, it might be due to the three isolates combined conidial suspension of *L. maculans* being more virulent than either *Trichoderma* spp. or *B. bassiana*. The pre-inoculation of plants with weakly virulent groups induced resistance against highly virulent groups (Chen & Fernando, 2006) but it might be possible that *Trichoderma* spp. or *B. bassiana* did not induce resistance because the plants defence responses may have been weakened by *L. maculans*.

4.4 Plant performance in response to *Trichoderma* spp. or *Beauveria bassiana* inoculation

Many studies conducted over the last decade have reported that *Trichoderma* spp. and *B. bassiana* isolates are able to live asymptomatically within its host tissues and aid in plant health probably through rhizospheric activity by improving water and nutrient absorption (Barelli et al., 2016; Gao et al., 2010; Greenfield et al., 2016; Howell, 2003; Lopez & Sword, 2015). An increase in plant growth can prevent a range of abiotic and biotic stresses, reflected in plant vigor or persistence and considered as a potential protection against invading attackers (Gao et al., 2010). Many studies showed that plants inoculated with endophytes obtain growth promotion (Gao et al., 2010; Lopez & Sword, 2015; Maag, 2011) and tolerance to soils within pH range common to New Zealand soils (Cripps-Guazzone et al., 2016).

The current study also assessed whether the selected *Trichoderma* spp. and *B. bassiana* isolates promote growth of *B. oleracea* var. *capitata* plants. The results showed that statistically there were no differences in the total number of leaves and the shoot lengths

between *Trichoderma* spp. or *B. bassiana* inoculated plants and uninoculated controls. This study measured the lengths of the primary root as a proxy for root biomass. The root lengths of plants inoculated with *Trichoderma* spp. or *B. bassiana* was significantly increased compared to control plants. Plants inoculated with *B. bassiana* FRh2 and *T. atroviride* LU132 were the best performing isolates suggesting that their rhizosphere competence ability promotes root biomass thereby increasing the surface area for absorption of water and nutrients uptake (Cripps-Guazzone et al., 2016).

A similar study found that *T. atroviride* LU132 significantly enhanced growth of *B. napus* „Ability" plants by increasing the root and shoot biomass which demonstrates the beneficial effects of this isolate on plant performance (Maag et al., 2013). Promotion of plant growth by *Trichoderma* spp. and *B. bassiana* have been attributed to enhancement of root biomass as well as increased nutrient mobilization and uptake in radiata pine seedlings (Regliński et al., 2012). Cotton seeds inoculated with *B. bassiana* enhanced plant growth and increased the plant dry biomass, number of nodes, and number of reproductive tissues than the control plants (Lopez & Sword, 2015). Although the root biomass was not measured in the present study, the longer root lengths could be associated with extended activity beyond the rhizosphere zone (Hohmann et al., 2012) and root penetration for water absorption and nutrient uptake.

According to Howell (2003), seed inoculation of corn plants in low nitrogen soil with *T. harzianum* (T22) had increased stem diameter and yield of grain and silage which could be associated increased root biomass or increased root length for absorption of water and nutrients below the root zone. However, inoculation of banana with *B. bassiana* against *Cosmopolites sordidus* (banana weevil) showed that plant growth was not affected even if applications were made at higher dosage rates (Akello et al., 2009).

Although this study was conducted under controlled environmental conditions, with a shallow rooted crop, it was reported that drenching of soil around a cassava stem cutting (deep rooted crop) with *B. bassiana* increased plant growth (Greenfield et al., 2016). Few studies have shown positive effects on plant growth following application of conidial suspension of *Trichoderma* spp. or *B. bassiana* on root biomass, shoot growth, number of leaves produced, and stem diameter (Lee et al., 2012; Lopez & Sword, 2015; Maag et al., 2013; Ownley et al., 2009; Vega et al., 2008; Vidal & Jaber, 2015).

The actual mechanisms by which *Trichoderma* spp. or *B. bassiana* isolates promote plant growth is not known. However, it could be due to the promotion in the uptake of soil

nutrients, production of growth regulating metabolites by the inoculated fungal isolates, or alteration of the host plant growth hormones (Lopez & Sword, 2015; Maag, 2011; Vinale et al., 2008). The enhancement of plant growth may be influenced by phytohormones produced by the inoculated fungal endophytes. Vinale et al. (2008) reported that gibberellin phytohormones produced by some strains of *Trichoderma* were found to elicit a variety of response in higher plants including shoot elongation, fruit development, and seed germination. The current study did not investigate whether these isolates produced plant growth hormones and warrants further investigation, however it is reasonable to believe that enhanced plant growth can be induced by fungal endophytes subsequently providing indirect protection to insect pests.

4.5 Endophyte colonisation

Results from surface sterilised plant tissues showed that *Trichoderma* spp. or *B. bassiana* had endophytically colonised the inoculated *B. oleracea* var. *capitata* roots with a 40% colonisation. Reports have shown that reisolation could reach more than 70% colonisation on a diversity of plants (Gurulingappa et al., 2010; Hohmann et al., 2012; Maag, 2011; Vega et al., 2008) but the maximum percentage recovery of *Trichoderma* spp. or *B. bassiana* in the current study did not exceed 60% and this could be related to the sterilization protocol that may have killed the fungal mycelium.

Irrespective of the sampling pools (from *M. persicae* and *L. maculans* studies), root tissue was colonized by *Trichoderma* spp. or *B. bassiana* isolates after inoculation. However, there was variation among the fungal isolates with greater colonisation occurring in *T. atroviride* LU132 in the roots but this strain was not recovered in the leaf segments. The findings reflect those of Akello (2012) who found that recovery in the roots was 68.3 % while in the pseudostem was 40.9% in banana one month after inoculation with *B. bassiana.* In addition, the days to sampling post-inoculation could be a factor that could have reduced the recovery percentage as it has been shown by Greenfield et al. (2016) that the colonisation levels of *B. bassiana* were higher when plants were sampled at 7-9 dpi (84%) compared to 47-49 dpi (40%). In the current study, the samples used to analyse for colonisation were taken at 46 dpi and the results showed similar trends to these previous studies suggesting that the days to sampling or the plant part selected is fundamentally important. It has also been reported in a dissertation report that *B. bassiana* has shown to be capable of colonizing cabbage plants in the early growth stages though colonisation rates varied among treatments (Zhang, 2014). Also in a

dissertation report, cauliflower was the most receptive plant to *Trichoderma* species (Cripps-Guazzone, 2014).

The percentage root colonisation of plants inoculated with *T. virens* LU556 and *T. hamatum* LU593 were 43% and 39%, respectively, compared to the other treatments. Although the colonization percentage is low, they have shown to be able to effectively delay onset of reproduction, reduction in aphid populations and shortened aphid adult longevity.

Recovery of the inoculated fungal isolates from the leaf samples were only found in the *B. bassiana* isolates FRh2 and BG11 inoculated plants while the samples from *Trichoderma* spp. had no fungal outgrowth. This indicates that *B. bassiana* colonises the plant systemically (roots and aboveground parts), while *Trichoderma* spp. are mostly root colonising fungi. As demonstrated by Cripps-Guazzone et al. (2016), *T. atroviride* LU132 inoculated on sweet corn and ryegrass colonised the rhizosphere and roots of both plants but were only recovered from the upper parts of sweet corn roots.

For *B. bassiana*, it has been revealed that they are effective colonizers of many plants and this was revealed in maize where the plant was colonized at whorl stage, moved within the plant, and persisted to provide season long suppression of corn borer (Bing & Lewis, 1991). In cotton seedlings, all leaves from inoculated plants were colonised after challenge with *A. gossipii* and the colonisation maintained over time in actively growing seedlings (Gurulingappa et al., 2010). In cocoa, colonisation rates in roots were higher than those in stems and leaves(Vega et al., 2008), with a similar effect where two *B. bassiana* isolates colonizing the roots had higher percentage than in leaves.

This study observed that surface sterilized root segments from uninoculated control plants that were plated on TSM had outgrowth of colonies characteristic to *Trichoderma* spp. The reasons behind this is not known however, it could be assumed to be experimental error, cross contamination within the treatment blocks, from rainwater splash through leaked roof, or infection during surface sterilization. Druzhinina et al. (2011) stated that, *Trichoderma* spp. are frequently found in soil and growing on wood, bark, other fungi, and innumerable other substrates, demonstrating their high opportunistic potential and their adaptability to various ecological conditions. On the other hand, the colonisation of plants by *B. bassiana* may have evolved as way of survival in the soils in the absence of their insect host. Moreover, fungal inoculation at seed stage could have had more advantage of colonizing both the seed radicle and plumule which are more close to one another in the seed than the root drench application (Muvea et al., 2014).

4.6 Phytohormone analysis

In the current study the JA and SA contents were low in *B. oleracea* var. *capitata* leaves after 25 days of continuous feeding by *M. persicae*. This was because the variability in hormone levels was high and the number of replicates was low to be able to detect them. Changes in chemical properties of plants in response to herbivory are generally mediated by phytohormone signalling (Kutyniok & Muller, 2012). The three signalling molecules regulated in response towards aphid attack are JA, SA, and ethylene (ET), with the exact behaviour of these phytohormones after aphid attack varying between different plants species (Kutyniok & Muller, 2012; Maag, 2011; Morkunas et al., 2011; Thompson & Gogginn, 2006). Other studies have demonstrated changes in JA- and SA -related gene expression levels due to the various induction events (Maag, 2011; Moran-Diez et al., 2012; Morkunas et al., 2011; Thompson & Gogginn, 2006; van Poecke & Dicke, 2003). However, the current study is one of the few studies where the phytohormone concentrations was measured after aphid feeding (Kutyniok & Muller, 2012; Maag, 2011). It has been reported that the local JA contents were not affected by aphid infestation in *A. thaliana* (Thompson & Gogginn, 2006). Similarly, it has been revealed that the JA biosynthesis pathway was only moderately more activated than the SA pathway in *A. thaliana* plants within the first 24 hours of infestation by the aphid *Brevicoryne brassicae* (Kusnierczyk et al., 2008).

5 Conclusion and future prospects

The results presented in this study suggest *Trichoderma* spp. or *Beauveria bassiana* can elicit a moderate resistance effect against the phloem-feeding aphid, *M. persicae* and the plant pathogen, *S. sclerotiorum*. The study elucidated that *T. hamatum* LU593 and *B. bassiana* FRh2 inoculation of plant roots reduced aphid fecundity, reproduction, and longevity. Two *B. bassiana* isolates (J18 and BG11) and *T. hamatum* (LU593) reduced the disease incidences of *S. sclerotiorum* in *B. oleracea* var. *capitata* plants. However, none of the fungal isolates inoculated to *B. oleracea* var. *capitata* showed a significant reduction in disease incidence of *L. maculans*.

Plant growth in response to the presence of endophytic fungi did not show any significant difference in terms of total number of leaves and shoot length. However, root elongation was significantly affected by all fungal isolates, except *B. bassiana* J18, compared to the control treatment. The elongation of the plant roots is probably related to the rhizosphere competence ability of the fungal endophytes to aid in water absorption and nutrient uptake. Endophytic colonisation was found in over 40% of the plated root tissue segments. However, in surface sterilised leaf segments, only two isolates (*B. bassiana*, FRh2 and BG11) showed 17% and 8% endophytic colonization of the aboveground plant tissues, respectively.

Phytohormone analysis results showed low levels of JA or SA levels in local *B. oleracea* var. *capitata* leaves after 25 days of feeding by *M. persicae*. However, the variability in hormone levels was high and the number of replicates were low, therefore no clear conclusions whether or not the endophytes influenced phytohormone levels can be drawn at this stage.

In conclusion, this study suggests that there is potential for several of the tested fungal endophytes to be utilized as biological control agents. However further work is required and may include the following:

1. The study was conducted under controlled conditions. Therefore field studies are needed to determine if similar results can be obtained in a natural environment.

2. The observed effects mediated by *Trichoderma* spp. or *B. bassiana* need to be further explored to determine the mode of action of the fungal isolates on phloem-feeding insects and plant pathogens.

6 References

Akello, J. (2012). Biodiversity of fungal endophytes associated with maize, sorghum and Napier grass and the influence of biopriming on resistance to leaf mining, stem boring and sap sucking insect pests. *Ecology and Development Series, 86*, 148.

Akello, J., Dubois, T., Coyne, D., & Kyamanywa, S. (2009). The effects of *Beauveria bassiana* dose and exposure duration on colonization and growth of tissue cultured banana (*Musa* sp.) plants. *Biological Control, 49*(1), 6-10. doi:10.1016/j.biocontrol.2008.06.002

Akello, J., & Sikora, R. (2012). Systemic acropedal influence of endophyte seed treatment on *Acyrthosiphon pisum* and *Aphis fabae* offspring development and reproductive fitness. *Biological Control, 61*, 215-221.

Alizadeh, H., Behboudi, K., Ahmadzadeh, M., Javan-Nikkhah, M., Zamioudis, C., Pieterse, C. M. J., & Bakker, P. A. H. M. (2013). Induced systemic resistance in cucumber and *Arabidopsis thaliana* by the combination of *Trichoderma harzianum* Tr6 and *Pseudomonas* sp. Ps14. *Biological Control, 65*(1), 14-23. doi:10.1016/j.biocontrol.2013.01.009

Bailey, B. A., Bae, H., Strem, M. D., Crozier, J., Thomas, S. E., Samuels, G. J., . . . Holmes, K. A. (2008). Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. *Biological Control, 46*(1), 24-35. doi:10.1016/j.biocontrol.2008.01.003

Bailey, B. A., Strem, M. D., & Wood, D. (2009). *Trichoderma* species form endophytic associations within *Theobroma cacao* trichomes. *Mycol Res, 113*(Pt 12), 1365-1376. doi:10.1016/j.mycres.2009.09.004

Barahona, R. D. M. (2010). The systemic activity of mutualistic endophytic fungi in Solanaceae and Cucurbitaceae plants on the behaviour of the phloem-feeding insects *Trialeurodes hvaporariorum*, *Aphis gossypii* and *Myzus persicae.*

Barelli, L., Moonjely, S., Behie, S. W., & Bidochka, M. J. (2016). Fungi with multifunctional lifestyles: endophytic insect pathogenic fungi. *Plant Mol Biol, 90*(6), 657-664. doi:10.1007/s11103-015-0413-z

Bell, D., K, Wells, H., D, & Markham, C., R. (1982). In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology, 72*(4), 379-382.

Bing, L., A, & Lewis, L., C. (1991). Suppression of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. *Environmental Entomology, 20*(4), 1207-1211. doi:10.1093/ee/20.4.1207

Bohinc, T., Ban, G., Ban, D., & Trdan, S. (2012). Glucosinolates in plant protection strategies: a review. *Archives of Biological Sciences, 64*(3), 821-828. doi:10.2298/abs1203821b

Bolton, M., D, Thomas, B., P, & Nelson, B., D. (2006). *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. *Molecular Plant Pathology, 7*(1), 1-16. doi:10.1111/j.1364-3703.2005.00316.x.

Castillo, F. D. H., Padilla, A. M. B., Morales, G. G., Siller, M. C., Herrera, R. R., Gonzales, C. N. A., & Reyes, F. C. (2011). In vitro antagonist action of *Trichoderma* strains against *Sclerotinia sclerotiorum* and *Sclerotium cepivorum*. *American Journal of Agricultural and Biological Sciences, 6*(3), 410-417.

Chen, Y., & Fernando, W., G., D. (2006). Induced resistance to blackleg (*Leptosphaeria maculans*) disease of canola (*Brassica napus*) caused by a weakly virulent isolate of *Leptosphaeria biglobosa*. *Plant Disease, 90*(8), 1059-1064. doi:10.1094/pd-90-1059

Chiang, M., S, Chang, C., Landry, B., S, & Crete, R. (1993). Cabbage. In G. Kalloo, B.O. Berg (Eds.), Genetic Improvement of Vegetable Crops. *Agricultural Sciences in China*, 100-152.

Chun, Y., S, Na, J., H, Shin, J., H, Nam, Y., & Ryoo, M., I. (2010). Effects of CMV resistant gene introduced pepperine on the life span of green peach aphid. *Korean Journal of Horticulture Science and Technology, 28*(2), 265-269.

Cory, S., Jenny, & Hoover, K. (2006). Plant-mediated effects in insect-pathogen interactions. *TRENDS in Ecology and Evolution, 21*(5), 277-286. doi:10.1016/j.tree.2006.02.005

Cripps-Guazzone, N. (2014). *Rhizosphere competence of selected Trichoderma species.* (PhD), Lincoln University, Unpublished.

Cripps-Guazzone, N., Jones, E. E., Condron, L., M, McLean, K., L, Stewart, A., & Ridgway, H., J. (2016). Rhizosphere and endophytic colonisation of ryegrass and sweet corn roots by the isolate *Trichoderma atroviride* LU132 at different soil pHs. *New Zealand Plant Protection, 69*, 78-85.

Dawidziuk, A., Popiel, D., Kaczmarek, J., Strakowska, J., & Jedryczka, M. (2016). Optimal *Trichoderma* strains for control of stem canker of brassicas: molecular basis of biocontrol properties and azole resistance. *BioControl, 61*(6), 755-768. doi:10.1007/s10526-016-9743-2

de Figueirêdo, G. S., de Figueirêdo, L. C., Cavalcanti, F. C. N., dos Santas, A. C., da Costa, A. F., & de Oliveira, N. T. (2010). Biological and chemical control of *Sclerotinia sclerotiorum* using *Trichoderma* spp. and *Ulocladium atrum* and pathogenicity to bean plants. *Brazilian Archives of Biology and Technology, An International Journal, 53*(1), 1-9.

Dicke, M., & Hilker, M. (2003). Induced plant defences: from molecular biology to evolutionary ecology. *Basic and Applied Ecology, 4*(1), 3-14. doi:10.1078/1439-1791-00129

Dicke, M., & Van Poecke, R., M., P. (2002). Signaling in plant-insect interactions: signal transduction in direct and indirect plant defence. In: D. Scheel & C. Wasternack (eds.) Plant Signal Transduction. . 289-316.

Druzhinina, I. S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B. A., Kenerley, C. M., Monte, E., . . . Kubicek, C. P. (2011). *Trichoderma*: the genomics of opportunistic success. *Nat Rev Microbiol, 9*(10), 749-759. doi:10.1038/nrmicro2637

Edwards, O., R, Franzmann, B., Thackray, D., & Micic, S. (2008). Insecticide resistance and implications for future aphid management in Australian grains and pastures: A Review. *Australian Journal of Experimental Agriculture, 48*(12), 1523-1530.

Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection, 19*, 709-714.

FAOSTAT (Producer). (2010, January 12, 2017). Food and Agriculture Commodities Production. Retrieved from http://faostat.fao.org/

Fitt, B. D. L., Brun, H., Barbetti, M. J., & Rimmer, S. R. (2006). World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). *European Journal of Plant Pathology, 114*(1), 3-15. doi:10.1007/s10658-005-2233-5

Gabrys, B., Dancewicz, K., Gliszczyn'ska, A., Kordan, B., & Wawrzen'czyk, C. (2015). Systemic deterrence of aphid probing and feeding by novel β-damascone analogues. *Journal of Pest Science, 88*, 507-516.

Gao, F., Dai, C., & Liu, X. (2010). Mechanisms of fungal endophytes in plant protection against pathogens. *African Journal of Microbiology Research, 4*(13), 1346-1351.

Gao, Y., Reitz, S. R., Wang, J., Xu, X., & Lei, Z. (2012). Potential of a strain of the entomopathogenic fungus *Beauveria bassiana* (Hypocreales: Cordycipitaceae) as a biological control agent against western flower thrips,*Frankliniella occidentalis* (Thysanoptera: Thripidae). *Biocontrol Science and Technology, 22*(4), 491-495. doi:10.1080/09583157.2012.662478

Geraldine, A. M., Lopes, F. A. C., Carvalho, D. D. C., Barbosa, E. T., Rodrigues, A. R., Brandão, R. S., . . . Lobo Junior, M. (2013). Cell wall-degrading enzymes and parasitism of sclerotia are key factors on field biocontrol of white mold by *Trichoderma* spp. *Biological Control, 67*(3), 308-316. doi:10.1016/j.biocontrol.2013.09.013

Greenfield, M., Gómez-Jiménez, M. I., Ortiz, V., Vega, F. E., Kramer, M., & Parsa, S. (2016). *Beauveria bassiana* and *Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation. *Biological Control, 95*, 40-48. doi:10.1016/j.biocontrol.2016.01.002

Grondona, I., Hermosa, R., Tejada, M., Gomis, M., D., Mateos, P., F., Bridge, P., D., . . . Garcia-Acha, I. (1997). Physiological and biochemical characterization of *Trichoderma harzianum*, a biological control agent against soilborne fungal plant pathogens. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 63*(8), 3189-3198.

Guesmi-Jouini, J., Garrido-Jurado, I., Lopez-Diaz, C., Ben Halima-Kamel, M., & Quesada-Moraga, E. (2014). Establishment of fungal entomopathogens *Beauveria bassiana* and *Bionectria ochroleuca* (Ascomycota: Hypocreales) as endophytes on artichoke *Cynara scolymus*. *J Invertebr Pathol, 119*, 1-4. doi:10.1016/j.jip.2014.03.004

Gunatilaka, L., A., A. (2006). Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. *J Nat Prod, 69*(3), 509-526. doi:10.1021/np058128n

Gurulingappa, P., McGee, P. A., & Sword, G. (2011). Endophytic *Lecanicillium lecanii* and *Beauveria bassiana* reduce the survival and fecundity of *Aphis gossypii* following contact with conidia and secondary metabolites. *Crop Protection, 30*(3), 349-353. doi:10.1016/j.cropro.2010.11.017

Gurulingappa, P., Sword, G. A., Murdoch, G., & McGee, P. A. (2010). Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when in planta. *Biological Control, 55*(1), 34-41. doi:10.1016/j.biocontrol.2010.06.011

Halitschke, R., & Baldwin, T. (2005). Jasmonates and related compounds in plant-insect interactions. *J Plant Growth Regul., 23*, 238-245. doi:10.1007/s00344-004-0037-z

Hamada, A. M., & Jonsson, L. M. (2013). Thiamine treatments alleviate aphid infestations in barley and pea. *Phytochemistry, 94*, 135-141. doi:10.1016/j.phytochem.2013.05.012

Hartley, S. E., & Gange, A. C. (2009). Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. *Annu Rev Entomol, 54*, 323-342. doi:10.1146/annurev.ento.54.110807.090614

Hohmann, P., Jones, E. E., Hill, R. A., & Stewart, A. (2011). Understanding Trichoderma in the root system of Pinus radiata: associations between rhizosphere colonisation and growth promotion for commercially grown seedlings. *Fungal Biol, 115*(8), 759-767. doi:10.1016/j.funbio.2011.05.010

Hohmann, P., Jones, E. E., Hill, R. A., & Stewart, A. (2012). Ecological studies of the bio-inoculant *Trichoderma hamatum* LU592 in the root system of *Pinus radiata*. *FEMS Microbiol Ecol, 80*(3), 709-721. doi:10.1111/j.1574-6941.2012.01340.x

Howell, C., R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. *Plant Disease, 87*(1), 4-10.

Hudyncia, H., Shew, H., D, Cody, B., R, & Cubeta, M., A. (2000). Evaluation of wounds as a factor to infection of cabbage by ascospores of *Sclerotinia sclerotiorum*. *The American Phytopathological Society, 84*(3), 316-320.

Hwang, S. F., Strelkov, S. E., Peng, G., Ahmed, H., Zhou, Q., & Turnbull, G. (2016). Blackleg (*Leptosphaeria maculans*) severity and yield loss in canola in Alberta, Canada. *Plants (Basel), 5*(3). doi:10.3390/plants5030031

Jallow, M. F. A., Dugassa-Gobena, D., & Vidal, S. (2004). Indirect interaction between an unspecialized endophytic fungus and a polyphagous moth. *Basic and Applied Ecology, 5*(2), 183-191. doi:10.1078/1439-1791-00224

Jones, E., Eirian, Rabeendran, N., & Stewart, A. (2014). Biocontrol of *Sclerotinia sclerotiorum* infection of cabbage by *Coniothyrium minitans* and *Trichoderma* spp. *Biocontrol Science and Technology, 24*(12), 1363-1382. doi:10.1080/09583157.2014.940847

Kessler, A., & Baldwin, T., Ian. (2002). Plant response to insect herbivory: the emerging molecular analysis. *Annual Review of Plant Biology., 53*, 299-328. doi:10.1146/annurev.arplant.53.100301.135207

Khan, S., Guo, L., Shi, H., Mijit, M., & Qiu, D. (2012). Bioassay and enzymatic comparison of six entomopathogenic fungal isolates for virulence or toxicity against green peach aphids *Myzus persicae. African Journal of Biotechnology, 11*(77). doi:10.5897/ajb12.1592

Kusnierczyk, A., Winge, P., Jorstad, T., S, Troczynska, J., Rossiter, J., T, & Bones, A., M. (2008). Towards global understanding of plant defence against aphids-timing and dynamics of early *Arabidopsis* defence responses to cabbage aphid (*Brevicoryne brassicae*) attack. *Plant, Cell and Environment, 31*(8), 1097-1115.

Kutyniok, M., & Muller, C. (2012). Crosstalk between above- and belowground herbivores is mediated by minute metabolic responses of the host *Arabidopsis thaliana. Journal of Experimental Botany, 63*(17), 6199-6120. doi:10.1093/jxb/ers274

Lee, B., Lee, S., & Ryu, C. (2012). Foliar aphid feeding recruits rhizosphere bacteria and primes plant immunity against pathogenic and non-pathogenic bacteria in pepper. *Annals of Botany, 110*, 281-290. doi:10.1093/aob/mcs055

Lob, S. (2014). *Leptosphaeria diseases of oilseed rape and swede: Identification and Epidemiology.* (PhD), Lincoln University, New Zealand.

Lob, S., Jaspers, M., V, Ridgway, H., J, & Jones, E. E. (2013). *Leptosphaeria maculans*/*L. biglobosa* disease progression in oilseed rape and timing of ascospore release under New Zealand conditions. *New Zealand Plant Protection, 66*, 214-222.

Lopez, D. C., & Sword, G. A. (2015). The endophytic fungal entomopathogens *Beauveria bassiana* and *Purpureocillium lilacinum* enhance the growth of cultivated cotton (*Gossypium hirsutum*) and negatively affect survival of the cotton bollworm (*Helicoverpa zea*). *Biological Control, 89*, 53-60. doi:10.1016/j.biocontrol.2015.03.010

Maag, D. (2011). *Induced herbivore defence responses triggered by fungal biocontrol agents of the genus Trichoderma.* (Diploma), Lincoln University, New Zealand.

Maag, D., Kandula, D. R. W., Müller, C., Mendoza-Mendoza, A., Wratten, S. D., Stewart, A., & Rostás, M. (2013). *Trichoderma atroviride* LU132 promotes plant growth but not induced systemic resistance to *Plutella xylostella* in oilseed rape. *BioControl, 59*(2), 241-252. doi:10.1007/s10526-013-9554-7

Matroudi, S., Zamani, M., & Motallebi, M. (2009). Antagonistic effects of three species of *Trichoderma* sp. on *Sclerotinia sclerotiorum*, the causal agent of canola stemrot. *Egyptian Journal of Biology, 11*, 37-44.

McKinnon, A. C., Saari, S., Moran-Diez, M. E., Meyling, N. V., Raad, M., & Glare, T. R. (2016). *Beauveria bassiana* as an endophyte: a critical review on associated methodology and biocontrol potential. *BioControl, 62*, 1-17. doi:10.1007/s10526-016-9769-5

McLean, K., L, Harper, G., E, Frampton, C., M, & Stewart, A. (2005). Dormancy of *Sclerotium cepivorum* sclerotia in New Zealnd soils. *New Zealand Plant Protection Society, 58*, 245-250.

Mitchell, C., Johnson, S. N., Gordon, S. C., Birch, A. N. E., & Hubbard, S. F. (2009). Combining plant resistance and a natural enemy to control *Amphorophora idaei*. *BioControl, 55*(3), 321-327. doi:10.1007/s10526-009-9257-2

Moran-Diez, E., Rubio, B., Dominguez, S., Hermosa, R., Monte, E., & Nicolas, C. (2012). Transcriptomic response of *Arabidopsis thaliana* after 24 h incubation with the biocontrol fungus *Trichoderma harzianum. J Plant Physiol, 169*(6), 614-620. doi:10.1016/j.jplph.2011.12.016

Morkunas, I., Mai, V. C. M., & Gabrys, B. (2011). Phytohormonal signaling in plant responses to aphid feeding. *Acta Physiol Plant, 33*, 2057-2073. doi:10.1007/s11738-011-0751-7

Mukherjee, P., K, Horwitz, B., A, Singh, H., S, Mukherjee, M., & Schmoll, M. (Eds.). (2013). *Trichoderma: Biology and Applications*. Croydon, United Kingdom: CPI Group (UK) Ltd.

Muvea, A. M., Meyhofer, R., Subramanian, S., Poehling, H. M., Ekesi, S., & Maniania, N. K. (2014). Colonization of onions by endophytic fungi and their impacts on the biology of *Thrips tabaci. PLoS One, 9*(9), e108242. doi:10.1371/journal.pone.0108242

Olson, H. A., & Benson, D. M. (2007). Induced systemic resistance and the role of binucleate *Rhizoctonia* and *Trichoderma hamatum* 382 in biocontrol of *Botrytis* blight in geranium. *Biological Control, 42*(2), 233-241. doi:10.1016/j.biocontrol.2007.05.009

Ownley, B. H., Gwinn, K. D., & Vega, F. E. (2009). Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. *BioControl, 55*(1), 113-128. doi:10.1007/s10526-009-9241-x

Reddy, N. P., Ali Khan, A. P., Devi, U. K., Sharma, H. C., & Reineke, A. (2009). Treatment of millet crop plant (*Sorghum bicolor*) with the entomopathogenic fungus (*Beauveria bassiana*) to combat infestation by the stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae). *Journal of Asia-Pacific Entomology, 12*(4), 221-226. doi:10.1016/j.aspen.2009.06.001

Regliński, T., Rodenburg, N., Taylor, J. T., Northcott, G. L., Ah Chee, A., Spiers, T. M., & Hill, R. A. (2012). *Trichoderma atroviride* promotes growth and enhances systemic resistance to *Diplodia pinea* in radiata pine (*Pinus radiata*) seedlings. *Forest Pathology, 42*(1), 75-78. doi:10.1111/j.1439-0329.2010.00710.x

Ryall, J., M., W, Moore, B., D, Riegler, M., & Johnso, S., N. (2016). Above–belowground herbivore interactions in mixed plant communities are influenced by altered precipitation patterns. *Frontiers in Plant Science*, 1-14. doi:10.3389/fpls.2016.00345

Saikkonen, K., Helander, M., Faeth, S., H, Schiulthess, F., & Wilson, D. (1999). Endophyte-grass-herbivore interactions: the case of Neotyphodium endophytes in *Arizona fescue* populations. *Oecologia, 121*, 411-420.

Saikkonen, K., Saari, S., & Helander, M. (2010). Defensive mutualism between plants and endophytic fungi? *Fungal Diversity, 41*(1), 101-113. doi:10.1007/s13225-010-0023-7

Schmelz, E. A., Engelberth, J., Tumlinson, J. H., Block, A., & Alborn, H. T. (2004). The use of vapor phase extraction in metabolic profiling of phytohormones and other metabolites. *Plant J, 39*(5), 790-808. doi:10.1111/j.1365-313X.2004.02168.x

Shoemaker, R., A, & Brun, H. (2001). The teleomorph of the weakly aggressive segregate of *Leptosphaeria maculans*. *Canadian Journal of Botany, 79*(4), 412-419.

Shoresh, M., Harman, G. E., & Mastouri, F. (2010). Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu Rev Phytopathol, 48*, 21-43. doi:10.1146/annurev-phyto-073009-114450

Singh, V. (2012). *Studies on plant-aphid interactions: a novel role for trehalose metabolism in Arabidopsis defense against green peach aphid.* (Doctor of Philosophy), University of North Texas, Texas, USA.

Sivasithamparam, K., Barbetti, M. J., & Li, H. (2005). Recurring challenges from a necrotrophic fungal plant pathogen: a case study with *Leptosphaeria maculans* (causal agent of blackleg disease in brassicas) in Western Australia. *Ann Bot, 96*(3), 363-377. doi:10.1093/aob/mci194

Suckling, D., M, Meckenna, C., & Walker, J., T, S. (2003). *Integrated Pest Management in New Zealand Horticulture*. Wallingford, Oxon, GBR: CABI Publishing.

Thompson, G., A, & Gogginn, F., L. (2006). Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. *Journal of Experimental Botany, 57*(4), 755-766.

van Poecke, R., M., P, & Dicke, M. (2003). Signal transduction downstream of salicylic and jasmonic acid in herbivory-induced parasitoid attraction by *Arabidopsis* is independent of JAR1 and NPR1. *Plant, Cell and Environment, 26*, 1541-1548.

Vega, F., E. (2008). Insect pathology and fungal endophytes. *J Invertebr Pathol, 98*(3), 277-279. doi:10.1016/j.jip.2008.01.008

Vega, F., E, Meyling, N. V., Luangsa-ard, J. J., & Blackwell, M. (2012). Fungal entomopathogens. 171-220. doi:10.1016/b978-0-12-384984-7.00006-3

Vega, F., E, Posada, F., Catherine Aime, M., Pava-Ripoll, M., Infante, F., & Rehner, S., A. (2008). Entomopathogenic fungal endophytes. *Biological Control, 46*(1), 72-82. doi:10.1016/j.biocontrol.2008.01.008

Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R. Y., & Valéro, J. R. (2007). Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. *Biochemical Engineering Journal, 37*(1), 1-20. doi:10.1016/j.bej.2007.05.012

Vidal, S., & Jaber, R., Lara. (2015). Entomopathogenic fungi as endophytes: plant-endophyte-herbivore interactions and prospects for use in biological control. *Current Science, 109*(1), 46-54.

Vinale, F., Sivasithamparam, K., Ghisalberti, E., L, Marra, R., Barbetti, M., J, Li, H., . . . Lorito, M. (2008). A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiological and Molecular Plant Pathology*(72), 80-86. doi:10.1016/j.pmpp.2008.05.005

Warmington, R., & Clarkson, J. P. (2016). Volatiles from biofumigant plants have a direct effect on carpogenic germination of sclerotia and mycelial growth of *Sclerotinia sclerotiorum*. *Plant and Soil, 401*, 213-229. doi:10.1007/s11104-015-2742-8

West, J., S, Kharbanda, P., D, Barbetti, M., J, & Fitt, B., D. (2001). Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe *Plant Pathology* (21 Dec 2001 ed., Vol. 50, pp. 10-27).

Wilson, D. (1995). Endophyte-the evolution of a term, and clarification of its use and definition. *Oikos, 73*(2), 274-276.

Zafar, J., Freed, S., Khan, B., A , & Farooq, M. (2016). Effectiveness of *Beauveria bassiana* against cotton whitefly, *Bemisia tabaci* Gennadius (Aleyrodidae: Homoptera) on different host plants. *Pakistan Journal of Zoology, 48*(1).

Zhang, L. (2014). *Colonization pattern of crop plants by endophytic fungi.* (PhD), Georg-August-University Göttingen, Germany.

Appendix A: Recipe

A.1 Potato dextrose agar (PDA)

- Potato Starch (for infusion) ……..4.0 g
- Dextrose ……20.0 g
- Agar ……....15.0 g

Suspended 39 g PDA to 1 l tap water, thoroughly mixed and autoclaved at 121ºC for 20 mins.

A.2 *Trichoderma* selective media: malt yeast extract + rose bengal agar (MRB)

- Malt extract ……...6.0 g
- Yeast extract ……...0.6 g
- Terrachlor (quintozene) ……0.12 g
- Rose Bengal (50 mg/ml) ……1.8 ml
- Agar ……12.0 g
- Distilled water ……600 ml

  + 0.6 ml chloramphenicol stock solution (100 mg/ml) before autoclaving.

A.3 *Beauveria* selective media (BSM)

- Potato starch ……39.0 g
- Tap water ……..1,000 ml

After autoclaving, the following were added aseptically:

- Tetracycline chloride ……3.33 ml
  (15 mg/ml stock solution in methanol)
- Streptomycin sulphate ……..3.5 ml
  (100 mg/ml stock solution in dH_2O)
- Cycloheximide ……125 mg
  (in 4 ml methanol, added 4 ml H_2O, once dissolved filter and added 8 ml of 1-56% cycloheximide)

NB: All from Sigma Aldrich, USA.
Tetracycline chloride ($C_{22}H_{24}ClN_1O_8$) → 500 ml stock (7.5 g/500 ml methanol).
Streptomycin sulphate → 500 ml stock (50 g/500 ml H_2O-filtered).

Appendix B: Plant food supplement

B.1 FloraNova Grow 7-4-10 (hydroponic solution)

- Total N ……...……7%
  (0.9% ammonia nitrogen + 6.1% nitrate nitrogen)
- Available phosphate (P_2O_5) ……...4%
- Soluble potash (K_2O) ……10%
- Other micronutrients were less than 1%.

Appendix C: Statistical analyses

C.1 Effect of *Trichoderma* spp. or *Beauveria bassiana* on *Myzus persicae*. The means and standard error are of back transformed data, but the a-bs mean separation are based on ANOVA of square-root transformed data followed by Tukey's post hoc test (P<0.05, n = 11)

Treatment	Parameters measured		
	Pre-reproductive period	Total reproduction	Longevity
1. *B. bassiana* (BG11)	8.0 ± 0.4 ab	26.6 ± 3.3 a	20.7 ± 1.7 a
2. *B. bassiana* (FRh2)	8.1 ± 0.3 ab	23.3 ± 4.4 a	20.4 ± 2.5 a
3. *B. bassiana* (J 18)	7.9 ± 0.7 ab	34.8 ± 5.2 ab	22.9 ± 1.6 ab
4. *T. atroviride* (LU132)	7.9 ± 0.6 ab	30.8 ± 4.3 a	23.2 ± 1.7 ab
5. *T. hamatum* (LU593)	8.6 ± 0.6 b	22.9 ± 5.2 a	19.7 ± 1.3 a
6. *T. virens* (LU556)	8.8 ± 0.4 b	28.9 ± 3.2 a	24.4 ± 1.4 ab
7. Control	6.3 ± 0.4 a	58.0 ± 4.9 b	28.9 ± 0.4 b

C.2 Analysis of variance (ANOVA) on the effect of *Trichoderma* spp. or *Beauveria bassiana* on the days to first birth of *Myzus persicae* (output from square root transformed data)

Source of variation	d.f.	Missing values	Sum of squares	Mean square	Variance	*P*-value
Treatment	6		2.282	0.3803	3.66	0.003
CageNo	10		1.7657	0.1766	1.70	0.099
Residual	69	2	7.1778	0.104		
Total	85	2	11.1194			

C.3 ANOVA on effect of *Trichoderma* spp. or *Beauveria bassiana* inoculation on the reproduction of *Myzus persicae* offspring (output of square root transformed data)

Source of variation	d.f.	Missing values	Sum of squares	Mean square	Variance	*P*-value
Treatment	6		101.196	16.866	7.05	<0.001
CageNo	10		29.231	2.923	1.22	0.292
Residual	70	1	167.426	2.392		
Total	86	1	297.339			

C.4 ANOVA on the effect *Trichoderma* spp. or *Beauveria bassiana* inoculation on *Myzus persicae* adult longevity (output of square root transformed data)

Source of variation	d.f.	Missing values	Sum of squares	Mean square	Variance	*P*-value
Treatment	6		10.2011	1.7002	5.60	<0.001
CageNo	10		4.2761	0.4276	1.41	0.195
Residual	70	1	21.2478	0.3035		
Total	86	1	35.6908			

C.5 ANOVA on the effect of *Trichoderma* spp. or *Beauveria bassiana* inoculation on *Sclerotinia sclerotiorum* infection (leaf lesion area with lesion in mm^2). Log10-transformed data

Source of variation	d.f.	Sum of squares	Mean square	Variance	*P*-value
Treatment	6	9.5471	1.5912	2.98	0.012
Rep	10	7.4701	0.747	1.40	0.199
Residual	71	37.9215	0.5341		
Total	87	54.9387			

C.6 ANOVA on effect of *Trichoderma* spp. or *Beauveria bassiana* inoculation on *Leptosphaeria maculans* infection (leaf surface area with lesion in mm^2)

Source of variation	d.f.	Sum of squares	Mean square	Variance	*P*-value
Treatment	6	1.7149	0.2858	1.35	0.247
Block	10	1.6549	0.1655	0.78	0.646
Residual	71	25.0381	0.2118		
Total	87	18.4078			

C.7 Response of *Trichoderma* spp. or *Beauveria bassiana* on *Leptosphaeria maculans* infection. Means and standard error are of back transformed data, but the letters comparing means are based on ANOVA of square-root transformed data followed by Tukey's post hoc test (P<0.05, n=11)

Treatment	Mean leaf lesion area (mm^2)
T. atroviride LU132	3.5 ± 0.5 a
B. bassiana BG11	3.6 ± 05 a
B. bassiana FRh2	4.1 ± 0.5 a
T. hamatum LU593	4.1 ± 0.6 a
T. virens LU556	5.3 ± 0.7 a
B. bassiana J18	5.7 ± 0.6 a
Control	4.0 ± 0.4 a

C.8 ANOVA on plant performance on the total (mean) number of leaves produced in response to inoculation of *Brassica oleracea* var. *capitata* with *Trichoderma* spp. or *Beauveria bassiana*

Source of variation	d.f.	Missing values	Sum of squares	Mean square	Variance	*P*-value
Treatment	6		7.0026	1.1671	1.72	0.129
Rep	10		16.4306	1.6431	2.42	0.015
Residual	70	1	47.4511	0.6779		
Total	86	1	70.8506			

C.9 ANOVA on plant performance (shoot lengths in cm) in response to plant inoculation with *Trichoderma* spp. or *Beauveria bassiana*

Source of variation	d.f.	Missing values	Sum of squares	Mean square	Variance	*P*-value
Treatment	6		3.4595	0.5766	1.4	0.227
Rep	10		2.7199	0.272	0.66	0.757
Residual	70	1	28.8403	0.412		
Total	86	1	34.8506			

C.10 ANOVA on plant performance (root lengths in cm) in response to plant inoculation with *Trichoderma* spp. or *Beauveria bassiana*

Source of variation	d.f.	Missing values	Sum of squares	Mean square	Variance	*P*-value
Treatment	6		143.485	23.914	5.53	<0.001
Rep	10		47.535	4.753	1.1	0.374
Residual	70	1	302.461	4.321		
Total	86	1	490.316			

C.11 ANOVA of phytohormone analysis of salicylic acid (SA) in response to *Myzus persicae* feeding on *Trichoderma atroviride* LU132 or *Beauveria bassiana* BG11 inoculated *Brassica oleracea* var. *capitata* plants for 25 days

Source of variation	d.f.	Sum of squares	Mean square	Variance	*P*-value
Treatment	2	3302177	1651089	1.19	0.341
Residual	11	15283550	1390323		
Total	13	18595727			

C.12 ANOVA of phytohormone analysis of jasmonic acid (JA) in response to *Myzus persicae* feeding on *Trichoderma atroviride* LU132 or *Beauveria bassiana* BG11 inoculated *Brassica oleracea* var. *capitata* plants for 25 days

Source of variation	d.f.	Missing value	Sum of squares	Mean square	Variance	*P*-value
Treatment	2		89641	44821	1.28	0.344
Residual	6	5	210100	35017		
Total	8	5	268306			

Appendix D: Raw data

D.1 *Myzus persicae* performance on *Trichoderma* spp. or *Beauveria bassiana* inoculated cabbage plants. Treatments with *Trichoderma* spp. have Lincoln University (LU) codes while treatment with *Beauveria bassiana* have no Lincoln University (LU) codes

		Pre-reproductive period (day			No. of nymphs produced over 25 days																
Cage/ Block No.	Treatment	Nymphs @ day zero	1st birth hrs in days	Average (days)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	LU 132	1	6	6.0	0	0	0	0	0	2	3	3	2	1	0	1	6	4	2	1	2
2	LU 132	3	8	2.7	0	0	0	0	0	0	0	6	3	9	6	6	3	11	1	5	11
3	LU 132	3	9	3.0	0	0	0	0	0	0	0	0	2	3	11	0	1	4	4	9	5
4	LU 132	2	10	5.0	0	0	0	0	0	0	0	0	0	1	6	6	7	1	0	3	2
5	LU 132	2	11	5.5	0	0	0	0	0	0	0	0	0	0	6	1	3	6	3	10	3
6	LU 132	2	7	3.5	0	0	0	0	0	0	5	6	2	4	4	6	10	5	6	7	15
7	LU 132	3	10	3.3	0	0	0	0	0	0	0	0	0	3	0	0	*All dead or missing				
8	LU 132	3	8	2.7	0	0	0	0	0	0	0	6	9	6	4	2	5	4	9	9	3
9	LU 132	3	7	2.3	0	0	0	0	0	0	8	9	10	7	9	8	2	5	3	4	6
10	LU 132	3	7	2.3	0	0	0	0	0	0	5	3	1	5	4	3	6	3	1	16	6
11	LU 132	3	5	1.7	0	0	0	0	1	3	9	3	16	5	4	12	4	6	3	7	6
1	LU 556	3	11	3.7	0	0	0	0	0	0	0	0	0	0	1	1	2	0	3	1	0
2	LU 556	3	9	3.0	0	0	0	0	0	0	0	0	1	5	4	4	5	16	4	2	10
3	LU 556	3	10	3.3	0	0	0	0	0	0	0	0	0	4	6	4	5	0	12	4	14
4	LU 556	2	7	3.5	0	0	0	0	0	0	3	2	2	1	2	3	2	3	2	2	2
5	LU 556	3	9	3.0	0	0	0	0	0	0	0	0	3	2	2	4	4	3	1	8	4
6	LU 556	3	9	3.0	0	0	0	0	0	0	0	0	3	5	8	10	9	6	3	10	12
7	LU 556	3	10	3.3	0	0	0	0	0	0	0	0	0	7	10	8	6	6	5	3	4
8	LU 556	2	8	4.0	0	0	0	0	0	0	0	3	4	14	7	6	4	0	1	7	8
9	LU 556	3	8	2.7	0	0	0	0	0	0	0	2	2	1	1	4	2	1	33	9	7
10	LU 556	2	8	4.0	0	0	0	0	0	0	0	2	4	5	4	0	3	0	5	1	3
11	LU 556	2	8	4.0	0	0	0	0	0	0	0	4	6	2	3	3	2	4	1	8	5
1	LU 593	2	13	6.5	0	0	0	0	0	0	0	0	0	0	0	1	2	6	1	1	1
2	LU 593	3	11	3.7	0	0	0	0	0	0	0	0	0	0	1	5	7	1	6	9	6
3	LU 593	3	8	2.7	0	0	0	0	0	0	0	8	5	13	10	3	3	1	0	7	6
4	LU 593	1	7	7.0	0	0	0	0	0	0	1	0	5	3	1	1	2	0	0	0	0
5	LU 593	3	10	3.3	0	0	0	0	0	0	0	0	0	4	3	0	4	6	2	1	4
6	LU 593	2	8	4.0	0	0	0	0	0	0	0	1	2	2	3	1	2	1	2	8	4
7	LU 593	2	7	3.5	0	0	0	0	0	0	7	3	3	2	7	8	8	1	2	2	12
8	LU 593	3	7	2.3	0	0	0	0	0	0	12	9	8	7	14	8	17	7	2	13	14
9	LU 593	3	9	3.0	0	0	0	0	0	0	0	0	1	0	4	0	0	0	0	2	6
10	LU 593	3	10	3.3	0	0	0	0	0	0	0	0	0	2	5	9	3	2	2	1	1
11	LU 593	2	6	3.0	0	0	0	0	0	2	3	3	1	4	3	3	3	4	0	11	5
1	FRh 2	2	10	5.0	0	0	0	0	0	0	0	0	0	2	2	7	3	6	4	3	0
2	FRh 2	3	0	0.0	0	0	0	0	0	0	0	0	* All aphids dead/missing by 192 hrs								
3	FRh 2	3	8	2.7	0	0	0	0	0	0	0	4	3	6	5	5	2	7	17	8	16
4	FRh 2	2	9	4.5	0	0	0	0	0	0	0	0	1	1	6	5	2	0	0	5	0
5	FRh 2	3	9	3.0	0	0	0	0	0	0	0	0	7	5	5	9	2	10	3	3	3
6	FRh 2	2	9	4.5	0	0	0	0	0	0	0	0	2	3	4	3	2	3	4	7	6
7	FRh 2	2	7	3.5	0	0	0	0	0	0	3	4	3	3	14	6	4	5	7	7	4
8	FRh 2	3	8	2.7	0	0	0	0	0	0	0	2	1	5	6	4	2	3	9	12	8
9	FRh 2	2	7	3.5	0	0	0	0	0	0	3	2	4	6	0	2	3	4	1	15	3
10	FRh 2	3	8	2.7	0	0	0	0	0	0	0	6	2	5	5	5	3	2	0	6	8
11	FRh 2	1	0	0.0	0	0	0	0	*Plant dead (soft stem rot)												

		No. of nymphs produced (days)								Average	Longevity (days)			
Cage/ Block No.	Treatment	18	19	20	21	22	23	24	25	nymphs produced	Aphid 1	Aphid 2	Aphid 3	Av. days alive
1	LU 132	3	11	2	3	0	*Dead			46.0	22			22.0
2	LU 132	11	5	2	4	3	17	7	6	38.7	25	25	30	26.7
3	LU 132	4	4	2	4	10	1	5	0	23.0	18	18	24	20.0
4	LU 132	7	0	3	1	2	4	5	3	25.5	19	25		22.0
5	LU 132	5	6	6	3	4	3	8	1	34.0	24	30		27.0
6	LU 132	5	2	6	3	1	10	11	6	57.0	30	30		30.0
7	LU 132									1.0	6	12	12	10.0
8	LU 132	4	5	2	11	3	4	2	8	32.0	20	22	25	22.3
9	LU 132	8	6	5	2	3	5	16	4	40.0	25	30	30	28.3
10	LU 132	6	4	10	2	6	6	8	8	34.3	25	30	30	28.3
11	LU 132	4	1	0	5	1	7	5	4	35.3	19	24	25	22.7
1	LU 556	7	1	*Aphids dead or missing						5.3	8	13	19	13.3
2	LU 556	11	3	9	8	5	5	13	2	35.7	22	25	25	24.0
3	LU 556	11	6	1	2	17	5	3	0	31.3	22	23	25	23.3
4	LU 556	1	1	4	4	0	1	2	2	19.5	22	30		26.0
5	LU 556	6	10	4	3	12	8	6	3	27.7	25	25	30	26.7
6	LU 556	16	6	4	4	7	6	7	4	40.0	20	25	30	25.0
7	LU 556	9	4	3	3	2	3	4	3	26.7	17	19	25	20.3
8	LU 556	9	12	4	2	0	4	3	2	45.0	24	25		24.5
9	LU 556	9	3	8	2	4	6	2	16	37.3	30	30	30	30.0
10	LU 556	5	2	1	3	9	4	5	7	31.5	30	30		30.0
11	LU 556	1	1	1	6	2	8	5	2	32.0	25	30		27.5
1	LU 593	1	6	0	0	0	*Aphids dead c			9.5	7	23		15.0
2	LU 593	2	1	1	1	2	2	2	1	15.7	18	19	25	20.7
3	LU 593	8	5	4	6	7	0	2	1	29.7	22	23	25	23.3
4	LU 593	*Dead or missing								13.0	17			17.0
5	LU 593	1	1	0	1	1	4	0	0	10.7	5	6	24	11.7
6	LU 593	14	2	3	5	3	6	6	3	34.0	9	30		19.5
7	LU 593	4	2	3	4	2	4	1	3	39.0	22	25		23.5
8	LU 593	10	15	3	5	2	14	6	3	56.3	25	25	30	26.7
9	LU 593	1	1	3	0	5	6	4	3	12.0	13	14	30	19.0
10	LU 593	5	0	0	*Aphids dead or missing					10.0	18	19	20	19.0
11	LU 593	9	2	10	7	21	5	4	0	50.0	24	25		24.5
1	FRh 2	*All aphids dead/missing from 408hrs								13.5	10	16		13.0
2	FRh 2									0.0	2	6	9	5.7
3	FRh 2	7	3	4	2	8	6	12	7	40.7	20	23	30	24.3
4	FRh 2	*All aphids dead/missing by 408hrs								10.0	15	17		16.0
5	FRh 2	2	8	9	5	7	3	6	4	30.3	23	24	25	24.0
6	FRh 2	6	2	2	7	5	8	7	6	38.5	30	30		30.0
7	FRh 2	5	7	3	2	4	3	1	4	44.5	23	25		24.0
8	FRh 2	8	2	5	3	3	9	4	8	31.3	25	30	30	28.3
9	FRh 2	2	8	2	4	0	9	11	7	43.0	25	30		27.5
10	FRh 2	0	3	3	3	3	0	0	*All a	18.0	9	20	24	17.7
11	FRh 2									0.0				0.0

		Pre-reproductive period (day			No. of nymphs produced over 25 days																
Cage/ Block No.	**Treatment**	**Nymphs @ day zero**	**1st birth hrs in days**	**Average (days)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1	BG 11	3	7	2.3	0	0	0	0	0	0	3	1	3	3	7	2	1	5	3	9	18
2	BG 11	2	7	3.5	0	0	0	0	0	0	4	0	8	5	2	5	2	3	9	6	14
3	BG 11	3	10	3.3	0	0	0	0	0	0	0	0	0	7	7	3	4	8	10	5	7
4	BG 11	2	10	5.0	0	0	0	0	0	0	0	0	0	1	4	0	2	3	0	3	3
5	BG 11	2	6	3.0	0	0	0	0	0	1	1	1	0	0	0	*All aphids dead/missing					
6	BG 11	2	10	5.0	0	0	0	0	0	0	0	0	0	6	3	4	1	3	2	2	3
7	BG 11	1	8	8.0	0	0	0	0	0	0	0	2	4	2	0	4	1	4	3	4	1
8	BG 11	3	8	2.7	0	0	0	0	0	0	0	5	8	4	4	11	4	3	4	6	6
9	BG 11	3	9	3.0	0	0	0	0	0	0	0	0	2	5	7	5	3	0	1	15	6
10	BG 11	3	7	2.3	0	0	0	0	0	0	2	3	4	3	5	10	4	12	9	13	0
11	BG 11	2	7	3.5	0	0	0	0	0	0	3	2	3	4	5	5	2	3	0	11	0
1	J 18	2	6	3.0	0	0	0	0	0	2	1	0	1	3	3	4	3	8	1	1	2
2	J 18	3	7	2.3	0	0	0	0	0	0	4	2	9	5	0	5	5	0	0	8	5
3	J 18	3	12	4.0	0	0	0	0	0	0	0	0	0	0	0	3	4	5	4	0	3
4	J 18	3	11	3.7	0	0	0	0	0	0	0	0	0	0	1	3	6	4	6	3	5
5	J 18	2	9	4.5	0	0	0	0	0	0	0	0	3	6	9	8	3	3	8	11	20
6	J 18	2	7	3.5	0	0	0	0	0	0	1	2	4	6	2	2	5	6	6	3	8
7	J 18	3	8	2.7	0	0	0	0	0	0	0	6	9	10	5	4	14	10	10	15	19
8	J 18	3	9	3.0	0	0	0	0	0	0	0	0	4	8	14	3	4	7	8	13	8
9	J 18	2	8	4.0	0	0	0	0	0	0	0	1	2	2	8	11	8	9	5	11	6
10	J 18	2	4	2.0	0	0	0	3	4	2	4	2	20	8	15	5	4	6	11	2	1
11	J 18	3	8	2.7	0	0	0	0	0	0	0	4	6	8	6	13	12	4	3	10	10
1	Control 1	3	4	1.3	0	0	0	2	0	0	4	2	1	1	8	9	8	16	17	6	12
2	Control 1	3	8	2.7	0	0	0	0	0	0	0	4	3	6	3	5	7	8	9	20	15
3	Control 1	2	6	3.0	0	0	0	0	0	1	2	1	0	2	5	13	13	10	28	7	24
4	Control 1	2	9	4.5	0	0	0	0	0	0	0	0	4	2	5	3	6	6	6	7	5
5	Control 1	1	3	3.0	0	0	3	4	3	2	2	3	2	1	2	2	3	7	13	7	6
6	Control 1	3	5	1.7	0	0	0	0	3	5	4	2	0	3	4	2	2	8	11	11	13
7	Control 1	2	7	3.5	0	0	0	0	0	0	4	1	4	4	0	9	14	6	4	7	24
8	Control 1	2	8	4.0	0	0	0	0	0	0	0	3	5	6	8	8	9	22	30	18	16
9	Control 1	3	5	1.7	0	0	0	0	2	0	0	1	1	6	5	5	10	2	3	12	13
10	Control 1	3	5	1.7	0	0	0	0	2	3	3	6	5	14	3	6	5	20	20	10	11
11	Control 1	3	3	1.0	0	0	0	3	5	2	3	6	10	4	6	3	5	10	4	12	7
1	Control 2	3	10	3.3	0	0	0	0	0	0	0	0	0	6	5	3	4	9	8	7	8
2	Control 2	2	8	4.0	0	0	0	0	0	0	0	1	1	4	5	1	5	11	7	30	13
3	Control 2	3	5	1.7	0	0	0	0	1	2	2	2	4	7	8	4	6	10	3	9	9
4	Control 2	2	7	3.5	0	0	0	0	0	0	4	4	2	3	5	5	5	6	5	2	2
5	Control 2	2	7	3.5	0	0	0	0	0	0	2	4	9	6	9	4	3	4	5	11	8
6	Control 2	2	11	5.5	0	0	0	0	0	0	0	0	0	0	7	9	8	5	3	13	9
7	Control 2	2	8	4.0	0	0	0	0	0	0	0	3	2	10	4	3	5	8	14	7	10
8	Control 2	3	6	2.0	0	0	0	0	0	3	0	0	5	17	14	7	13	6	8	8	17
9	Control 2	3	7	2.3	0	0	0	0	0	0	4	3	5	17	11	2	2	9	5	5	4
10	Control 2	3	6	2.0	0	0	0	0	0	3	3	6	8	3	7	6	8	7	1	7	10
11	Control 2	3	5	1.7	0	0	0	0	2	1	5	3	8	15	12	23	11	7	9	3	7

		No. of nymphs produced (days)								Average	Longevity (days)			
Cage/ Block No.	**Treatment**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**nymphs produced**	**Aphid 1**	**Aphid 2**	**Aphid 3**	**Av. days alive**
1	BG 11	10	2	9	5	4	1	2	0	29.3	9	23	25	19.0
2	BG 11	11	1	5	2	3	1	3	0	42.0	23	25		24.0
3	BG 11	5	5	1	5	12	11	9	3	34.0	11	25	30	22.0
4	BG 11	2	4	0	4	3	2	1	1	16.5	12	25		18.5
5	BG 11									1.5	10	10		10.0
6	BG 11	3	4	4	4	3	3	2	4	25.5	16	25		20.5
7	BG 11	2	4	1	0	*Dead or missing				32.0	21			21.0
8	BG 11	3	2	3	8	5	10	6	2	31.3	6	13	25	14.7
9	BG 11	6	10	6	4	1	6	19	5	33.7	22	30	30	27.3
10	BG 11	6	1	3	2	5	6	7	2	32.3	22	24	30	25.3
11	BG 11	5	2	1	5	2	9	4	2	34.0	30	30		30.0
1	J 18	2	4	3	3	4	6	8	6	32.5	19	30		24.5
2	J 18	1	0	3	1	2	3	2	2	19.0	10	11	25	15.3
3	J 18	0	2	1	1	1	2	0	0	8.7	17	18	24	19.7
4	J 18	1	1	4	2	2	3	4	2	15.7	5	19	25	16.3
5	J 18	7	5	5	7	3	7	8	2	57.5	24	30		27.0
6	J 18	2	5	1	2	1	3	7	0	33.0	12	25		18.5
7	J 18	18	8	4	2	4	8	8	5	53.0	20	30	30	26.7
8	J 18	3	8	4	3	5	7	10	6	38.3	30	30	30	30.0
9	J 18	7	4	1	1	2	14	6	9	53.5	30	30		30.0
10	J 18	5	10	3	2	4	1	0	0	56.0	20	25		22.5
11	J 18	4	4	5	6	10	6	8	3	40.7	24	25	25	24.7
1	Control 1	32	14	15	7	10	8	9	33	71.3	25	25	30	26.7
2	Control 1	6	7	10	9	8	8	35	9	57.3	30	30	30	30.0
3	Control 1	18	5	7	18	3	6	11	8	91.0	30	30		30.0
4	Control 1	5	8	4	7	2	5	13	10	49.0	30	30		30.0
5	Control 1	8	2	2	5	15	2	7	5	106.0	30			30.0
6	Control 1	12	6	9	2	6	2	6	3	38.0	25	25	30	26.7
7	Control 1	10	17	8	8	3	13	7	7	75.0	30	30		30.0
8	Control 1	5	2	27	7	13	18	10	31	119.0	25	30		27.5
9	Control 1	8	7	3	0	1	0	19	5	34.3	30	30	30	30.0
10	Control 1	16	13	17	6	18	3	4	27	70.7	30	30	30	30.0
11	Control 1	8	2	8	2	17	8	4	2	43.7	23	24	25	24.0
1	Control 2	5	2	8	5	14	10	13	6	37.7	30	30	30	30.0
2	Control 2	9	6	7	9	8	5	12	5	69.5	24	30		27.0
3	Control 2	11	2	3	3	5	3	4	3	33.7	30	30	30	30.0
4	Control 2	2	2	1	2	3	12	6	7	39.0	30	30		30.0
5	Control 2	7	16	6	9	9	12	4	2	65.0	30	30		30.0
6	Control 2	14	5	5	5	4	5	7	4	51.5	30	30		30.0
7	Control 2	4	6	13	9	7	7	14	11	68.5	30	30		30.0
8	Control 2	15	9	4	7	4	6	21	8	57.3	30	30	30	30.0
9	Control 2	9	10	9	10	4	4	12	25	50.0	25	30	30	28.3
10	Control 2	8	3	9	8	15	13	4	5	44.7	30	30	30	30.0
11	Control 2	3	2	7	6	3	3	2	1	44.3	22	25	30	25.7

D.2 Effect of *Trichoderma* spp. or *Beauveria bassiana* inoculation on *Sclerotinia sclerotiorum* infection

Block	**Treatment**	Leaf lesion area (cm^2)	LLA (mm^2)	Block	Treatment	Leaf lesion area (cm^2)	LLA (mm^2)
1	LU 132	0.04	0.40	1	BG 11	0.06	0.6
2	LU 132	0.00	0.00	2	BG 11	0	0
3	LU 132	0.09	0.90	3	BG 11	0.03	0.3
4	LU 132	0.02	0.20	4	BG 11	0	0
5	LU 132	0.17	1.70	5	BG 11	0.27	2.7
6	LU 132	0.20	2.00	6	BG 11	0.06	0.6
7	LU 132	0.11	1.10	7	BG 11	0.04	0.4
8	LU 132	0.10	1.00	8	BG 11	0.1	1
9	LU 132	0.07	0.70	9	BG 11	0.06	0.6
10	LU 132	0.05	0.50	10	BG 11	0.17	1.7
11	LU 132	0.05	0.50	11	BG 11	0	0
1	LU 556	0.10	1.00	1	J 18	0.04	0.4
2	LU 556	0.60	6.00	2	J 18	0.07	0.7
3	LU 556	0.00	0.00	3	J 18	0	0
4	LU 556	0.17	1.70	4	J 18	0.04	0.4
5	LU 556	0.03	0.30	5	J 18	0	0
6	LU 556	0.07	0.70	6	J 18	0.11	1.1
7	LU 556	0.03	0.30	7	J 18	0.11	1.1
8	LU 556	0.12	1.20	8	J 18	0.03	0.3
9	LU 556	0.12	1.20	9	J 18	0	0
10	LU 556	0.03	0.30	10	J 18	0.07	0.7
11	LU 556	0.00	0.00	11	J 18	10.05	100.5
1	LU 593	0.04	0.40	1	Control 1	0.87	8.7
2	LU 593	0.19	1.90	2	Control 1	0.5	5
3	LU 593	0.07	0.70	3	Control 1	0.15	1.5
4	LU 593	0.12	1.20	4	Control 1	0.22	2.2
5	LU 593	0.04	0.40	5	Control 1	0.2	2
6	LU 593	0.10	1.00	6	Control 1	0.2	2
7	LU 593	0.04	0.40	7	Control 1	0.28	2.8
8	LU 593	0.06	0.60	8	Control 1	0.21	2.1
9	LU 593	0.04	0.40	9	Control 1	0	0
10	LU 593	0.00	0.00	10	Control 1	0.21	2.1
11	LU 593	0.00	0.00	11	Control 1	0.46	4.6
1	FRh 2	0.12	1.20	1	Control 2	0.29	2.9
2	FRh 2	0.21	2.10	2	Control 2	0.25	2.5
3	FRh 2	0.13	1.30	3	Control 2	0.07	0.7
4	FRh 2	0.00	0.00	4	Control 2	0.19	1.9
5	FRh 2	0.01	0.10	5	Control 2	0.1	1
6	FRh 2	0.17	1.70	6	Control 2	0.56	5.6
7	FRh 2	0.11	1.10	7	Control 2	0.11	1.1
8	FRh 2	0.05	0.50	8	Control 2	0.14	1.4
9	FRh 2	0.04	0.40	9	Control 2	0.02	0.2
10	FRh 2	0.02	0.20	10	Control 2	0.14	1.4
11	FRh 2	0.17	1.70	11	Control 2	0.21	2.1

D.3 Effect of *Trichoderma* spp. or *Beauveria bassiana* inoculation on *Leptosphaeria maculans* infection

Block	**Treatment**	Leaf lesion area (cm^2)	LLA (mm^2)	Block	Treatment	Leaf lesion area (cm^2)	LLA (mm^2)
1	LU 132	0.04	0.40	1	BG 11	0.06	0.6
2	LU 132	0.00	0.00	2	BG 11	0	0
3	LU 132	0.09	0.90	3	BG 11	0.03	0.3
4	LU 132	0.02	0.20	4	BG 11	0	0
5	LU 132	0.17	1.70	5	BG 11	0.27	2.7
6	LU 132	0.20	2.00	6	BG 11	0.06	0.6
7	LU 132	0.11	1.10	7	BG 11	0.04	0.4
8	LU 132	0.10	1.00	8	BG 11	0.1	1
9	LU 132	0.07	0.70	9	BG 11	0.06	0.6
10	LU 132	0.05	0.50	10	BG 11	0.17	1.7
11	LU 132	0.05	0.50	11	BG 11	0	0
1	LU 556	0.10	1.00	1	J 18	0.04	0.4
2	LU 556	0.60	6.00	2	J 18	0.07	0.7
3	LU 556	0.00	0.00	3	J 18	0	0
4	LU 556	0.17	1.70	4	J 18	0.04	0.4
5	LU 556	0.03	0.30	5	J 18	0	0
6	LU 556	0.07	0.70	6	J 18	0.11	1.1
7	LU 556	0.03	0.30	7	J 18	0.11	1.1
8	LU 556	0.12	1.20	8	J 18	0.03	0.3
9	LU 556	0.12	1.20	9	J 18	0	0
10	LU 556	0.03	0.30	10	J 18	0.07	0.7
11	LU 556	0.00	0.00	11	J 18	10.05	100.5
1	LU 593	0.04	0.40	1	Control 1	0.87	8.7
2	LU 593	0.19	1.90	2	Control 1	0.5	5
3	LU 593	0.07	0.70	3	Control 1	0.15	1.5
4	LU 593	0.12	1.20	4	Control 1	0.22	2.2
5	LU 593	0.04	0.40	5	Control 1	0.2	2
6	LU 593	0.10	1.00	6	Control 1	0.2	2
7	LU 593	0.04	0.40	7	Control 1	0.28	2.8
8	LU 593	0.06	0.60	8	Control 1	0.21	2.1
9	LU 593	0.04	0.40	9	Control 1	0	0
10	LU 593	0.00	0.00	10	Control 1	0.21	2.1
11	LU 593	0.00	0.00	11	Control 1	0.46	4.6
1	FRh 2	0.12	1.20	1	Control 2	0.29	2.9
2	FRh 2	0.21	2.10	2	Control 2	0.25	2.5
3	FRh 2	0.13	1.30	3	Control 2	0.07	0.7
4	FRh 2	0.00	0.00	4	Control 2	0.19	1.9
5	FRh 2	0.01	0.10	5	Control 2	0.1	1
6	FRh 2	0.17	1.70	6	Control 2	0.56	5.6
7	FRh 2	0.11	1.10	7	Control 2	0.11	1.1
8	FRh 2	0.05	0.50	8	Control 2	0.14	1.4
9	FRh 2	0.04	0.40	9	Control 2	0.02	0.2
10	FRh 2	0.02	0.20	10	Control 2	0.14	1.4
11	FRh 2	0.17	1.70	11	Control 2	0.21	2.1

D.4 Plant performance in response to *Trichoderma* spp. or *Beauveria bassiana* inoculation

Replicate	Treatment	No. of leaves	Root length (mm)	Shoot length (mm)	Replicate	Treatment	No. of leaves	Root length (mm)	Shoot length (mm)
1	LU 132	12	90	60	1	BG 11	11	120	55
2	LU 132	10	80	50	2	BG 11	11	100	60
3	LU 132	10	100	45	3	BG 11	9	50	50
4	LU 132	10	60	45	4	BG 11	11	110	55
5	LU 132	11	100	50	5	BG 11	10	110	50
6	LU 132	10	110	60	6	BG 11	10	120	50
7	LU 132	10	120	55	7	BG 11	10	60	55
8	LU 132	10	90	60	8	BG 11	8	90	55
9	LU 132	9	90	50	9	BG 11	9	100	45
10	LU 132	10	100	55	10	BG 11	10	80	50
11	LU 132	9	100	55	11	BG 11	9	90	55
1	LU 556	10	110	55	1	J 18	11	80	40
2	LU 556	10	120	50	2	J 18	11	50	50
3	LU 556	11	120	55	3	J 18	11	90	45
4	LU 556	10	70	55	4	J 18	11	75	55
5	LU 556	11	90	45	5	J 18	10	75	55
6	LU 556	9	60	40	6	J 18	10	80	45
7	LU 556	10	90	55	7	J 18	11	80	50
8	LU 556	9	80	45	8	J 18	10	70	50
9	LU 556	10	110	55	9	J 18	9	110	50
10	LU 556	10	80	45	10	J 18	8	120	60
11	LU 556	9	60	45	11	J 18	10	110	60
1	LU 593	10	120	60	1	Control 1	10	70	50
2	LU 593	10	50	55	2	Control 1	10	50	45
3	LU 593	10	70	45	3	Control 1	11	70	50
4	LU 593	10	70	50	4	Control 1	8	50	45
5	LU 593	10	110	55	5	Control 1	11	60	45
6	LU 593	10	110	55	6	Control 1	7	50	80
7	LU 593	10	100	50	7	Control 1	9	90	50
8	LU 593	9	80	45	8	Control 1	8	70	45
9	LU 593	10	100	45	9	Control 1	10	80	45
10	LU 593	10	110	45	10	Control 1	10	70	50
11	LU 593	10	100	55	11	Control 1	8	70	45
1	FRh 2	11	75	55	1	Control 2	9	80	50
2	FRh 2	10	60	50	2	Control 2	10	80	40
3	FRh 2	10	80	50	3	Control 2	11	45	40
4	FRh 2	11	100	65	4	Control 2	10	40	40
5	FRh 2	11	80	60	5	Control 2	9	60	50
6	FRh 2	10	150	50	6	Control 2	10	50	50
7	FRh 2	10	80	50	7	Control 2	8	80	55
8	FRh 2	11	140	60	8	Control 2	9	70	55
9	FRh 2	9	120	50	9	Control 2	11	30	50
10	FRh 2	10	110	55	10	Control 2	9	75	50
11	FRh 2	*	*	*	11	Control 2	10	80	45

D.5 Endophytic colonisation

	Aphid experiment			
Treatment	Root Segments Plated	Root segments recovered	Leaf segments plated	Leaf segments recovered
LU 132	19	13	15	0
LU 556	16	11	15	0
LU 593	18	7	15	0
FRh 2	18	13	15	3
BG 11	17	11	17	1
J 18	19	10	16	0
Control-TSM	15	2	16	0
Control-BSM	15	0	16	0
		Leptosphaeria maculans **experiment**		
	Root Segments Plated	Root segments recovered	Leaf segments plated	Leaf segments recovered
LU 132	11	7	10	0
LU 556	14	2	10	0
LU 593	10	4	10	0
FRh 2	12	4	9	1
BG 11	13	3	8	1
J 18	11	2	8	0
Control-TSM	13	1	9	0
Control-BSM	15	0	10	0

D.6 Phytohormone analysis

				Peak Area		
Sample ID			**RT**	**Me SA**	**Me JA**	**I.S dh-MeJA**
	Treatment			m/z 120	m/z 151	m/z 156
Ratio to TIC*		**FW grams**		0.253	0.047	0.043
2	BG 11	0.144	11.182	160,915	34,378	1,944,869
12	BG 11	0.161	11.179	69,562	n.d	898,291
17	BG 11	0.155	11.12	121,227	20,488	628,992
18	BG 11	0.162	11.127	340,430	61,321	3,946,497
4	LU 132	0.163	11.134	9,825	n.d	72,446
6	LU 132	0.153	11.171	139,206	11,870	1,861,794
9	LU 132	0.153	11.188	68,165	n.d	859,365
10	LU 132	0.154	11.106	377,247	63,681	1,672,333
1	Control	0.164	11.17	85,645	13,283	1,303,727
7	Control	0.158	11.15	12,339	n.d	29,077
8	Control	0.157	11.133	49,320	n.d	100,823
13	Control	0.164	11.107	109,645	30,932	626,747
14	Control	0.149	11.117	231,552	36,196	452,137
15	Control	0.144	11.126	2,232,137	278,456	664,351
IS dhJA and MeSA			11.091	26,524,434	10,951	813,423