# Bio-control of the soil-borne pathogen *Rhizoctonia solani* of radish (*Raphanus sativus* L.) by *Trichoderma* species.

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Jaeseung Lee

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Science.

**Bio-control of the soil-borne pathogen**

***Rhizoctonia solani* of radish (*Raphanus sativus* L.)**

**by *Trichoderma* species.**

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The soil-borne fungus *Rhizoctonia solani* is an important pathogen of *Brassicae* crops worldwide, including red vegetable radish (*Raphanus sativus*). However, the impact of *R. solani* on New Zealand radish production has not been evaluated.

This project examined the effects of the pathogen on red radish production, from seedling emergence and establishment through to final crop yield. *R. solani* infections occurred on different plant parts, causing seed rot, brownish lesions on foliage, damping off, stunted stems and root rot. The damage caused by *R. solani* caused reduction in seedling emergence and plant growth.

The potential of New Zealand strains of different species of the bio-control fungus *Trichoderma* for controlling *R. solani* was investigated, both in *vitro* and *in vivo*. Strains of *Trichoderma* (*T. atroviride, T. asperellum*, *T. hamatum, T. harzianum*, *T. spirale*, *T. polysporum*, *T. viride* and *T. virens*) were obtained from the Bio-Protection Research Centre collection and tested against the pathogen in glasshouse studies. The four most effective *Trichoderma* strains (LU132: *T. atroviride*, LU785: *T. hamatum*, LU1358: *T. polysporum* and LU1347: *T. harzianum*) were formulated into seed coatings. The disease control performance of the seed coatings was compared with that of thiram fungicide seed treatment, currently used for radish, using soil naturally infected with *R. solani*.

The four seed-treatment applications of *Trichoderma* strains in glasshouse experiments significantly increased the fresh shoot weights and total leaf areas per pot, as compared to the *R. solani* control. In the field experiment, the radish yield from the untreated control was 3.9

t/ha for Red Round and 4.6 t/ha for Fresh Breakfast, around half the yield of 7 – 10 t/ha normally obtained commercially. Yield for the thiram seed treatment did not differ from the control. However, compared to the untreated control, the LU785 seed treatment increased yield by 96% for both varieties, while LU132 and LU1358 also significantly increased yield (by 83% and 60% respectively) in French Breakfast. *Trichoderma* seed treatment would be beneficial for radish production in New Zealand.

**Keywords:** Radish, *Trichoderma* species, *Rhizoctonia solani*, seed coating

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# Chapter 1
# General Introduction

## 1.1 Introduction

Radish (*Raphanus sativus* L.) is an important root vegetable crop both in the temperate and tropical regions of the world. It originated in the Mediterranean region and was an edible vegetable in ancient Egypt (approx. 2,700 - 2,200 BC). Radish has been cultivated in Europe from the thirteenth century (Kole, 2007). Today there are different varieties and types such as *Daikon* (e.g. Asia), *Mooli* (e.g. India and South Asia) and red radish (e.g. Europe) which are widely grown. Vegetable radish is an important food worldwide and is commonly consumed as a fresh food (e.g. in a salad, or as sprouting radish), in pickles or in processed food.

The demand for edible radish is high, particularly in Asia. According to Gin and Lee (2006) radish is the one of major vegetables in South Korea, accounting for 24% of total vegetable production. People consume radish at a rate of 20-40 kg/capita/year. Also, edible radish is consumed as a traditional food in India, Nepal and Bhutan and demands for the supply of radish seed worldwide are constantly increasing (FAO, n.d.-a).

New Zealand radish seed production has developed significantly over recent years because of the opportunity provided by contra-seasonal multiplication of seed for Northern Hemisphere based seed companies (Millner & Roskruge, 2013) and the combination of a suitable climate (e.g. low humidity, warm days/cold nights) and grower expertise (Hampton, Rolston, Pyke, & Green, 2012) .

New Zealand produces radish seed for domestic use and for the export market (Millner & Roskruge, 2013). The total value of radish seed was $27.56 million in 2013 (Sanderson & Twaddle, 2014), with 252 tonnes of seed mostly exported to the Asia zone (Millner & Roskruge, 2013) and 2,806 tonnes of seed exported worldwide in 2015 (Sanderson & Fareti, 2016).

There are approximately fifteen varieties of red radish available in New Zealand; e.g. Enza Zaden Ltd (n.d.): *Bravo, Celesta, Lucia, Rudi, Tarzan, Vienna*; South Pacific Seeds Ltd (n.d.): *Gloriette, Radar*; Kings Seeds Ltd. (2014): *German giant, Gourmet blend, Pink beauty, Red cherry*; Egmont Seeds Ltd (n.d.): *Cabernet, Champion, French Breakfast*; Yates Seeds Ltd (n.d.): *Cherry Belle, Red Rubin*). Seed is packed both for garden use (e.g. 300 to 1,000 seeds per pack) and for commercial uses as a bulk product (e.g. 1 kg seed).

In New Zealand, plant diseases threaten both seed and vegetable *Brassica* crops. Common leaf diseases of *Brassica* (Table 1.1) are *Alternaria* leaf spot (*Alternaria brassicicola*), grey leaf spot (*Alternaria brassicae*), Anthracnose leaf spot (*Colletotrichum higginsianum*), bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*), leaf spot (*Alternaria raphani syn. A. matthiolae* and *Xanthomonas campestris* pv. *raphani*), black rot (*Xanthomonas campestris* pv *campestris*), and downy mildew (*Peronospora parasitica*). Root diseases are black rot (*Aphanomyces raphani* and *Gibberella avenacea*), Black leg (*Leptosphaeria maculans*), club root (*Plasmodiophora brassicae*), *Fusarium* yellows (*Fusarium oxysporum* f. sp. *raphani*) and damping off (*Rhizoctonia solani*).

Table 1.1 The main seed-borne and soil-borne pathogens of *R. sativus* L. (Agriseeds, 2017a; Cucuzza et al., n.d.; Sherf & MacNab, 1986).

| Pathogens | Common names |
|---|---|
| *Alternaria brassicae* | Grey leaf spot |
| *Alternaria brassicicola* | Black leaf spot |
| *Alternaria raphani syn. A. matthiolae* | Leaf spot |
| *Aphanomyces raphani* | Black rot |
| *Colletotrichum higginsianum* | Anthracnose, leaf spot |
| *Fusarium oxysporum* f. sp. *raphani* | *Fusarium* yellow |
| *Gibberella avenacea* | Root and stem rot |
| *Leptosphaeria maculans* | Black leg |
| *Peronospora parasitica* | Downy mildew |
| *Plasmodiophora brassicae* | Club root |
| *Rhizoctonia solani* | Damping-off, canker |
| *Xanthomonas campestris* pv. *raphani* | Bacterial spot |
| *Xanthomonas oryzae* pv. *oryzae* | Bacterial leaf blight |

The soil-borne pathogen *Rhizoctonia solani* has damaged radish production both in New Zealand and worldwide (Cabi, 2016; Du Toit & Pelter, 2003; Kareem & Hassan, 2015). *R. solani* is a disease of cool-season crops including many *Brassica* species and other hosts (e.g. grasses, vegetables, rice and sugar beet) (Singleton, Mihail, & Rush, 1992). In particular *R. solani* has a strong impact and can be devastating on vegetable crops such as potato (Loria, Leiner, & Carting, 1993), and *Brassica* crops worldwide, particularly in China and Vietnam (Hua, Bertier, Soltaninejad, & Höfte, 2014; Yang et al., 2004).

*R. solani* is often the cause of poor, patchy forage *Brassica* establishment in the South Island of New Zealand (Agriseeds, 2017a). *R. solani* causes rotting and cracking of radish roots, (Miller & Baysal-Gurel, 2010), death of seedlings, and brown-red rot on twisted (wire) stems (Cabi, 2016; Miller & Baysal-Gurel, 2010). In Northern Alberta, Canada, brown girdling root rot of *Brassica rapa* caused by *R. solani* resulted in a yield loss of up to 30 % (Rimmer,

Shattuck, & Buchwaldt, 2007). Also, cabbage is susceptible to *R. solani* throughout the growing season when the outer leaves come into contact with infected soil in moist conditions. It has been reported that in New Zealand, the seeds and seedlings of pasture species are also damaged by *R. solani* prior to emergence (Kandula et al., 2015).

Furthermore, there is likely to be an increasing problem of soil-borne diseases for radish crop production due to curtailed rotations which encourage build-up of soil-borne inoculum (Hampton et al., 2012).

Chemical seed treatment of radish is currently used to protect against soil-borne diseases in New Zealand. Seed treatment is the application of a compound including chemicals, nutrients, biological product or an energy form (e.g. heat, radiation, electricity or magnetism) (Hampton, personal communication, Feb 2017). Fungicide seed treatments use different chemical groups. South Pacific Seeds Ltd (n.d.) state that most vegetable seed is sold with fungicide treatments such as thiram, ipriodione and metalaxyl-M. The product 'Agricote' by Agriseeds (2017a) is used on forage *Brassicas* to provide protection against insects and fungal disease, and also supplies molybdenum. However, seed treatment is for specific pathogens on specified crops and will not necessarily control all pathogens. Moreover, phytotoxicity may be a problem in that the high concentrations of chemicals can damage the tender tissues of germinating seeds and seedlings (Paulsrud et al., 2001). Potential risk of fungicide resistance build-up is a significant issue as well (MAF, n.d.). Therefore, currently, there is an increasing trend to search for alternatives to the use of synthetic pesticides.

The use of biological control may offer an alternative method to overcome seed treatment problems. A number of commercial products (e.g. microbial inoculants) are available to improve plant growth and provide seed protection. Microorganisms such as *Bacillus* spp, *Pseudomonas* spp., *Penicillum* spp., and *Trichoderma* spp. are well known as biocontrol agents. Products based on these microbes are produced by commercial companies and can be applied as a dry or wettable powder. *Trichoderma* species are not known to attack insects (Lester, 2010), but they can effectively control and induce resistance against many pathogens. Their mode of action includes antibiosis, antagonism, competition and mycoparasitism (Mukherjee, Horwitz, & Kenerley, 2012). Moreover, they are able to grow in different environments (Schuster & Schmoll, 2010; Singh et al., 2014). Some *Trichoderma* species promote plant growth and have been reported to increase seed germination (Gupta & Ebrary, 2014). In the presence of *R. solani* isolates of *Trichoderma atroviride*, *T. virens* and *T. hamatum* were reported to increase perennial ryegrass (*Lolium perenne* L.) emergence by 60-150% (Kandula et al., 2015b). *T. atroviride* gave the strongest biocontrol activity against *R.*

*solani* in perennial ryegrass (Kandula et al., 2010). *T. harzianum* isolates have been reported to protect radish seedlings from *R. solani*, and also increase radish germination (Henis, Ghaffar, & Baker, 1978).

In New Zealand, Agrimm Technologies Ltd is the only manufacturer of *Trichoderma* fertilizer and crop protection products using solid and liquid fermentation techniques (Kumar, Thakur, & Rani, 2014). They have commercial products available in different forms; *Trichoderma* dry (*Trichoderma* flake bio-inoculant), flow/spray (foliar spray) and prills (soil conditioner) based on propagules of *Trichoderma harzianum* and *T. atroviride* (Agrimm Technologies, 2014; Young, 2013).

The effects of the soil-borne pathogen *R. solani* on radish production in New Zealand are unknown. The pathogen can be effectively controlled by chemical seed treatment (Buter, personal communication, March 2017) using products such as Monceren FS (Bayer Crop Sciene, 2015) and Maxim (Syngenta, 2016), and chemical foliar spray such as Monceren DS (Bayer Crop Sciene, 2015) and Amistar (Syngenta, 2016). The active compounds are pencycuron, imazalil, fludioxonil, azoxystrobin and chlorothalonil in the form of a suspension concentrate or wettable powder (Young, 2013).

However according to MAF (n.d.), undefined *R. solani* strains were shown to grow in the presence of fungicides. MAF (n.d.) considered that the inability of the fungicides to totally inhibit growth of the fungus indicated the possibility of resistance could be building-up.

New Zealand vegetable radish and seed radish production is now an important part of the domestic and export market. Minimizing the damage by prevention of pathogen attack is necessary but continued chemical use may lead to resistance developing. Moreover, there is increasing demand for non-chemical control methods and more sustainable farming. The development of bio-control methods based on *Trichoderma* spp. could provide an alternative method of control for this pathogen (Schuster & Schmoll, 2010).

## Objectives:

- To determine the effect of *Rhizoctonia solani* on vegetable radish production.
- To identify *Trichoderma* spp. strains able to provide biocontrol of *Rhizoctonia solani*.
- To formulate a *Trichoderma* seed coating and compare its ability to control the pathogen against that of the current fungicide seed treatment product in a soil naturally infested with *Rhizoctonia solani*.

## Hypotheses:

1. Soil-borne *Rhizoctonia solani* will decrease radish production by negatively affecting radish seedling emergence and plant growth.
2. *Trichoderma* application will promote radish growth and effectively control the pathogen.

# Chapter 2
# Literature review

## 2.1 Radish

### 2.1.1 Origin and distribution

Radish, *Raphanus sativus* L., is an annual broadleaf vegetable crop that belongs to the family *Brassicae* (George, 2009). It originated from the Mediterranean region and it is presumed that *R. sativus* L., were derived by natural or artificial crossing from the wild radish (*R. raphanistrum* L.) (Figure 2.1) with hybridization between *R. landra, R. rostras* and *R. maritimus* (Cabi, 2016; Kole, 2007).

The wild types from the Mediterranean region were thought to be spread as contaminated seeds in cereal crops (e.g. wheat and oat) (Kole, 2007). Radish has been cultivated in Europe since the thirteenth century and developed in Asia and America from the twentieth century (Kole, 2007). The wild varieties, so called Nora-daikon and Hama-daikon were introduced to Asia (e.g. East Asia, China, Japan and Korea) from the Mediterranean region. In China, radish is usually classified into four ecotypes; South Chinese, Middle Chinese, North Chinese and West Plateau types. These types are red skinned and have a white root or a small greenish root (Kole, 2007).

Figure 2.1 Wild radish (*Raphanus raphanistrum*) - Seedling (top-left), root (top-right), flower (bottom-left) and seed pod (bottom-right) (Anonymous, 2017a).

Wild types have evolved in different places worldwide. Today, there are many *Raphanus* species available such as edible cherry radish (*R.sativus* L. var. *radicola pers*), oil radish (*R. sativus* L. var. *oleifera*), feed radish (*R. sativus* L. var. *caudatus*), black radish (*R.sativus* L. var. *niger*) and large root radish (*Daikon*: *R. sativus* L. var. *longipinnatus bailey*). People consume radish as a food and it can also be used as a biofuel crop, thereby increasing demand for the development of new cultivars (Chammoun, 2007; Hazera, 2015).

### 2.1.2 Radish development

- **Phenology**

Radish is a dicotyledonous annual or biennial brassica plant (Zaki et al., 2012). In terms of phenological growth, there are eight different key identification stages (Meier, 2001). These are: 1. Germination; 2. Leaf development; 3. Development of harvestable vegetative parts; 4. Inflorescence emergence; 5. Flowering; 6. Development of root; 7. Ripening of fruit and seed; 8. Senescence (Figure 2.2). However, Malik (2009) used only five different radish growth stages: 1. Cotyledon; 2. '6 to 8' leaf; 3. Bolting; 4. 50% flowering; 5. 50% silique format.

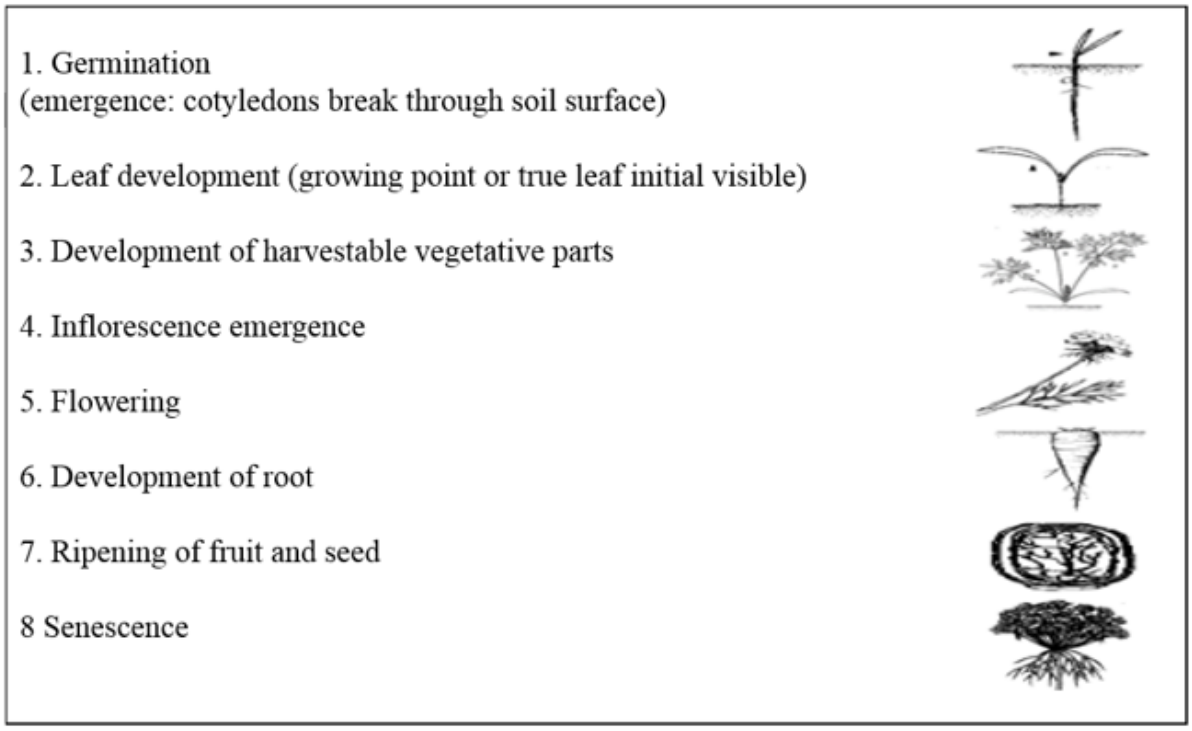


Figure 2.2 Eight different identification key stages (Meier, 2001).

Plants emerge within 5 to 7 days from sowing. Radish produces hairless obcordate cotyledons followed by the first true leaves formed in a basal rosette. They mature as erect branches with

alternate simple leaves (Malik, 2009; Sivan & Chet, 1986). To produce harvestable vegetable radish takes approximately 4 to 5 weeks.

Red radish root types (e.g. long-type, round-type and thin-type) are varied depending on cultivars. The types are distinguished by sub-group identifications such as root thickness (thin, medium or thick), root width, root shape (transverse elliptic, circular, elliptic, obvate, broad/narrow rectangular and iciclical), crown shape (concave and plane), base shape (acute and obtuse), skin colour (one or bi-coloured), and cortex thickness (George, 2009).

A radish root consists of two different anatomical regions (Figure 2.3); hypocotyl (upper parts) and a taproot (lower parts) (Zaki et al., 2012). Radish root has a secondary xylem tissue and a secondary phloem bi-cambial zone. The outer layer has a periderm, which is a secondary cortex from the pericycle (Figure 2.4) (Zaki et al., 2012).

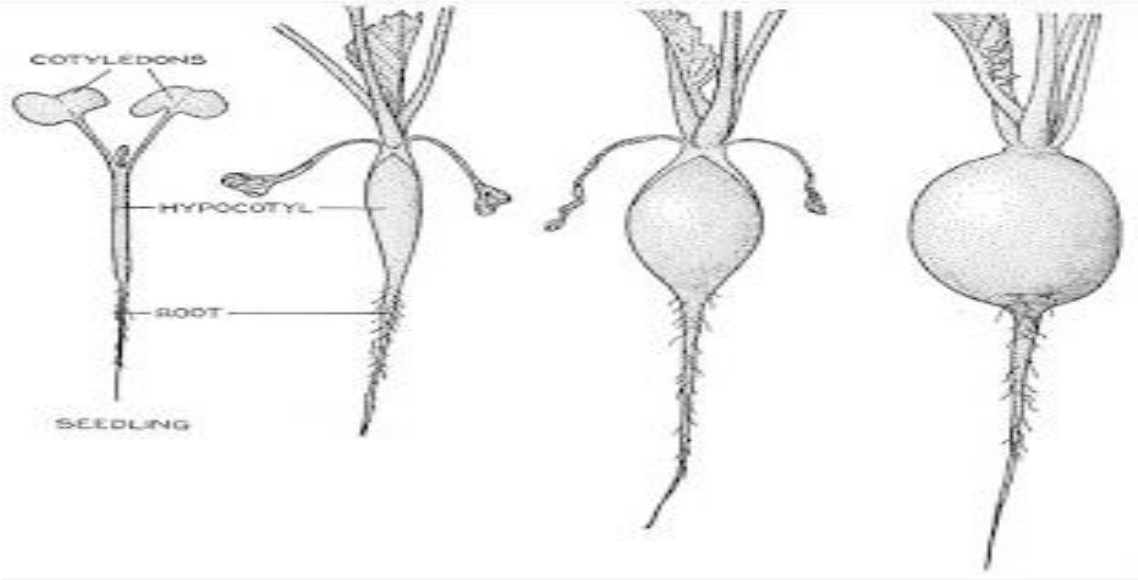


Figure 2.3 *R. sativus* L. seedling stage and development of cotyledons, hypocotyl and root (Essig, 2012).

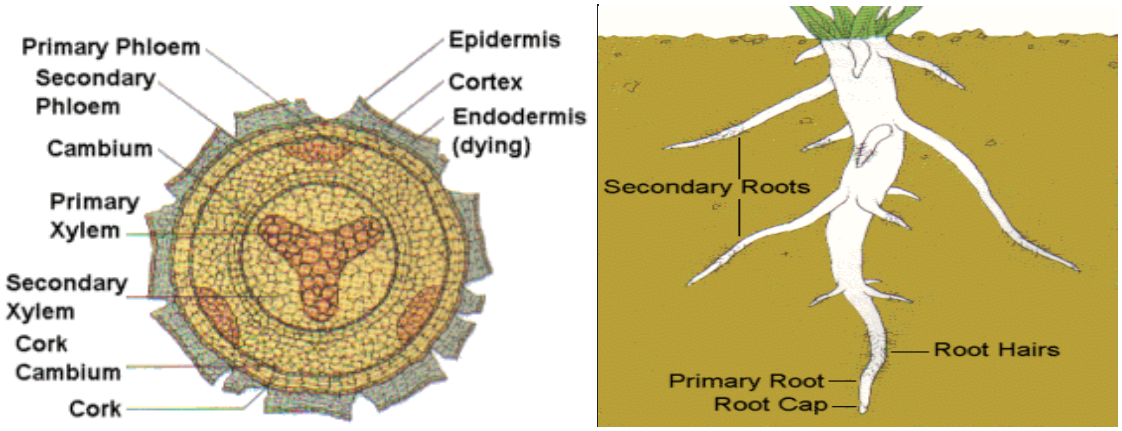


Figure 2.4 Root system of radish in cross section (left) and tap root systems (right) (Anonymous, 2017b).

The hypocotyl increases with the width of root within three weeks after sowing, and the taproot elongates by four weeks (Zaki et al., 2012). The alteration of root shape is associated with cell division and cell enlargement (Figure 2.3).

Vegetable radish cultivars developed for fresh market production are annuals. However for seed production, the radish is biennial and requires a vernalisation period with a specific day length requirement (Cabi, 2016). The juvenile period is for 7 to 9 weeks, and flowering (or bolting) occurs after vernalisation (at approx. 9 weeks) at temperatures ranging from 5°C to 10°C (Engelen-Eigles & Erwin, 1997).

Flower colour is pinky-red or white depending on cultivar. Pollination is required by bees or other insects (Cabi, 2016). The pollinated flowers elongate to form 2-5cm pods within a silique. Seeds can be harvested when they are yellowish-brown in colour and the seed moisture content is around 9% (Hampton & Young, 1988; Nery, Carvalho, Oliveira, Pereira, & Nery, 2014).

### 2.1.3 Crop production systems

Vegetable radish can be grown year-round in New Zealand, either indoors or in open fields in a range of temperatures from 8°C to 25°C (Kole, 2007). Radish production requires a light deep soil, a pH between 5.5 and 6.8, and good drainage (FAO, n.d.-b; George, 2009). The general N: P: K fertilizer application during site preparation is in the ratio 1:3:4.

Phenological development of *Raphanus* spp is dependent on both temperature and day length. Radish requires a minimum 10 hours day length but can grow in a range of temperatures from

5ºC to 35ºC respectively (Malik, 2009). Germination temperatures are between 5 to 15ºC. Radish also requires red light. Light is perceived by photoreceptors (phytochromes) that induce photomorphogenic responses (phytochromic effect). A protein called 'billin chromophore' identifies colour or light for the photoreceptor. Also billin chromophore is responsible for regulating the time of germination of seeds (photoblasty), elongation of seedlings and flowering based on the photoperiodism (the length of day/night) (Taiz & Zeiger, 2010).

Planting methods are either by transplanting seedlings or sowing seed using precision drills. Vegetable radish sowing rate depends on the production method (e.g. indoor or open fields) and cultivar types. Vegetable radish is direct drilled at 10 – 30 kg seed/ha with a row spacing of 10 – 25 cm and 2 – 4 cm between plants in the rows (e.g. Daikon: 10 – 16 kg/ha; Red radish: 10 - 30 kg/ha) (Cabi, 2016; Masabni, n.d.).

Seedling emergence occurs after 4 to 10 days, and the root harvest available period is from 4 to 6 weeks post seedling emergence, depending on varieties (Egmont Seeds, 2017; Kings Seeds, 2014). Approximately 7 - 10 t/ha of red radish can be achieved depending on cultivar.

'Root to seed' and 'seed to seed' systems are widely used for seed production. 'Root to seed' is used for the biennial types in Europe. Roots harvested in autumn are re-planted next spring after a selection process. 'Seed to seed' is common for final seed multiplication stages (George, 2009). Seed radish production sowing rates depend on radish variety and hybrid production method (in situ or transplanting). Hybrid seed production requires a female to male parent row ratio of approx. 1:1 or 1:2. For $F_1$ Cytoplasmic male sterility (CMS) based production the sowing rate is 1,440 g female/ha and 480 g male/ha, and for $F_1$ self-incompatibility (SIC) based is production 1,200 g male and 1,200 g female for SIC x SIC hybrid (Singh, Tripathi, & Somani, 2001). George (2009) reported sowing rates of up to 6 kg per hectare in rows spaced from 50 cm to 90 cm apart and with approximately 5 to 15 cm between seedlings in the rows. The sowing season for a seed crop in NZ is from September to October and the harvesting season is from March to April. Seed yield is usually between 1,000 kg to 2,000kg/ha and the 1,000 seed weight of radish is approximately 10g (George, 2009).

## 2.2 *Rhizoctonia solani*

### 2.2.1 Pathogen biology

- **Identification**

*Rhizoctonia solani* Kühn is a soil-borne plant pathogenic fungus with a wide host range. The fungus binomial name is derived from ancient Greek: *Rhiza*- root, *ktonos*- murder. *Rhizoctonia solani* was first identified on potato in 1858 (Ceresini, 1999) and it now can be found worldwide.

Approximately 100 species have been reported under the genus. There are two life cycles of *Rhizoctonia* species; anamorph (asexual state) and teleomorph (sexual state) (Singleton et al., 1992). *R. solani* is known to be dispersed by basidiospores (sexual spores) and does not produce asexual spores (Bienkowski, 2012). However, *R. solani* can also exist as vegetative mycelium (hyphal growth form) and sclerotia (dense asexual hyphal resting structures) (Ceresini, 1999; Keijer, 1996; Paulsrud et al., 2001).

*Rhizoctonia* is classified into one of the three genera; *Basidiomycotina* (also known as '*Thanatephorus cucumeris*'), *Ceratobasidium* and *Waitea* (Singleton et al., 1992). *Rhizoctonia solani* Kühn belongs to the *Basidiomycotina* (Bienkowski, 2012; Singleton et al., 1992; Tredway & Burpee, 2001). The sexual stage of *R. solani* Kühn (*Thanatephorus cucumeris*) occasionally produces a fruiting body and basidiospores (teleomorph).

The host crop range of *R. solani* includes bean, buckwheat, cabbage, carrot, rice, corn, crimson clover, ginger, lettuce, onion, pea, potatoes, pine, radish, sorghum, soybean, sugar beet, grasses and tomato (Ceresini, 1999; Loria et al., 1993). Late root rot, damping off and black rot caused by *R. solani*, particularly in root crops (e.g. carrot, radish and sugar beet) result in severe damage to plant growth (Henis et al., 1978; Japan-KWS, n.d.; USDA, 2013). However the damage in root crops caused by *R. solani* has not been evaluated for NZ cropping systems (MAF, n.d.). Therefore, the effect of *R. solani* in vegetable radish production needs to be investigated.

### 2.2.2 Hyphal anastomosis

Hyphal anastomosis is a term of hyphal fusion of interconnected fungal hyphae of different or the same colony genotypes which results in a form of hyphal network (Chagnon, 2014; Read & Roca, 2006). Hyphal anastomosis is used to classify *Rhizoctonia solani* worldwide, based on characteristics such as host range, virulence, molecular/biochemical interaction and morphology group (Carling, Kuninaga, & Brainard, 2002).

There are eleven distinct groups of *R. solani* based on anastomosis; AG-1, AG-2, AG-3, AG-4, AG-5, AG-6, AG-BI, AG-7, AG-8, AG-9 and AG-10 (Ceresini, 1999; Singleton et al., 1992), involving 21 sub-groups designated; AGs 1-IA, 1-1B, 1-1C, 1-1D. 2-1, 2-2-IIIB, 2-2-IV, 2-2-LD, 2-3, 2-4,2-BI, 3-IIA, 3-IIB, 3-IIC, 3-TB, 4-HG-I, 4-HG-II, 6-GV, 6-HG-I, 9-TX and 9-TP (Samules & Hebbar, 2015). Moreover 19 bi-nucleate '*Rhizoctonai*' anastomosis groups were recognized (Singleton et al., 1992). Paring of the same AG isolate types or same genotype can occasionally result in hyphal fusion. Bienkowski (2012) and Farrokhi-Nejad et al. (2007) state that radish is susceptible to the bi-nucleate type (AG-3) of *R. solani*.

The classification of hyphal anastomosis groups is poorly documented, particularly the bi-nucleate types (Singleton et al., 1992). However, *R. solani* hyphal anastomosis groups are known to cause root/stem rot or damping off in different plant species.

### 2.2.3 Symptoms and life cycle

Soil-borne *Rhizoctonia solani* can cause damage at different crop growth stages; seed: rot, seedling: damping off, wilting/stunting leaf, and root: rot and cracking (Figure 2.5).

Figure 2.5 *Rhizoctonia solani* infections on root (left: root rot & cracking) (Miller & Baysal-Gurel, 2010), seedling damage (centre: stem rot and wilting) and healthy seed vs infected seed (right: rotten seed).

Brownish lesions, damping off and sprout-nipping of brassica crops including *R. sativus* are caused by *R. solani* (Rimmer et al., 2007). *Solanaceae* species (e.g. potato) are susceptible to *R. solani* (Bienkowski, 2012) which causes black scurf and damping off (Loria et al., 1993). Blight (e.g. rice), damping off and root rot (e.g. strawberry, sugar beet, vegetables species) (Singleton et al., 1992) and brown patch (e.g. bentgrass, fescues and ryegrass) are all caused by *R. solani* (Tredway & Burpee, 2001).

The optimum conditions for growth of *R. solani* are in a range of temperature between 18ºC to 27ºC with high relative humidity (>90% ) (Tredway & Burpee, 2001). Mycelium formation (= bunched hyphae) of *R. solani* initiate in 7-10 days (whitish to colourless) (Figure 2.6).

Figure 2.6 Comparison of *R. solani* growth on at Petri-plate at 7 days (left) and 15 days (right).

Hyphae of *R. solani* have a branching pattern. The hyphae have a septum which is composed of compact cells (= monilioid cells) (Figure 2.7) (Tredway & Burpee, 2001).

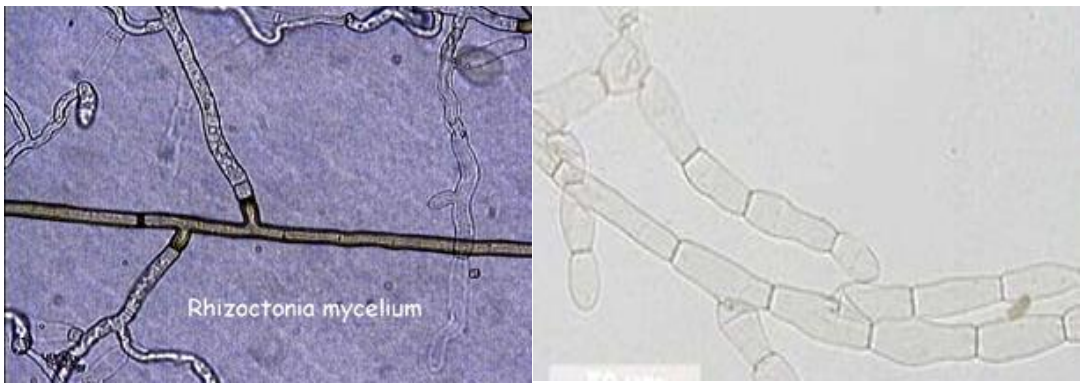


Figure 2.7 *Rhizoctonia solani* mycelium structures. Development of moniloid cells at 400 (left) and 100 µm (right) (Chang & Lee, 2013). Mature hyphae are brown, and septate with a branching construction base (NCCES, 2001).

Mycelium is mature at 10 to 21 days (dark brown) and capable of producing a basidium by hymenia formation (aka 'fungal fruiting body) (Ceresini, 1999; Singleton et al., 1992). The spores produce a penetration peg able to penetrate into plant cell tissue to degrade cellulose, cutin and pectin. Hyphae of *R. solani* grow above the plant epidermal cell junctions/membrane (Figure 2.8) (Brown & Ogle, 1997).

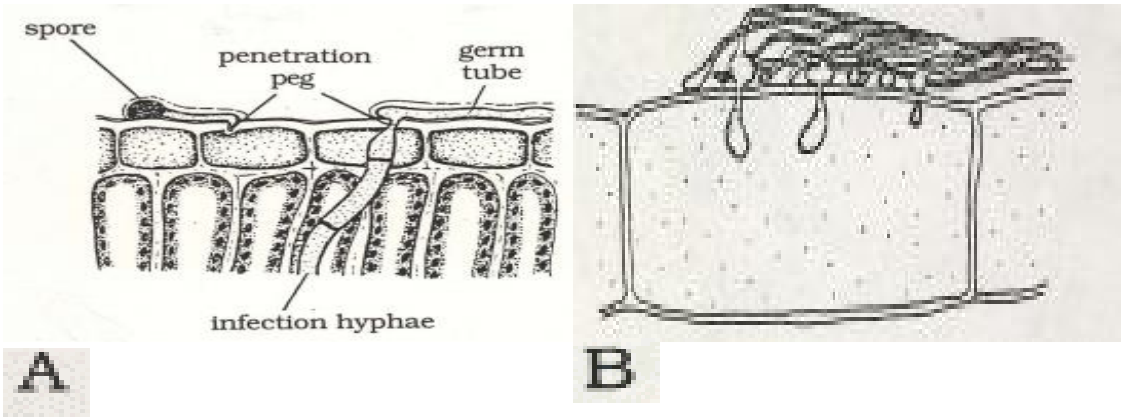


Figure 2.8 The infection process of *Rhizoctonia solani* on a leaf. A: Infection initiation on plant epidermal cell membrane. B: *R. solani* penetration into epidermal cell (Brown & Ogle, 1997).

*R. solani* is able to survive for some time in the absence of living host plants, as a dormant saprophytic form on crop debris, or as resting structures (sclerotia) or mycelium (Tredway & Burpee, 2001).

### 2.2.4 Disease management

*Rhizoctonia solani* attacks underground plant parts (e.g. seeds and roots) and above ground plant parts (e.g. hypocotyls, pods, fruits, leaves and stems). The typical diagnostic symptom for *R. solani* is the presence of root rot and cracking on the root surface (Miller & Baysal-Gurel, 2010). The pathogen has to be controlled by integrated pest management involving cultural, chemical and biological control methods.

- **Cultural control**

*Rhizoctonia solani* is particularly highly pathogenic and grows rapidly in cool-temperate regions (18ºC to 27ºC) with high relative humidity (90%). A consecutive 10 hours of leaf wetness can be problematic because these conditions favour pathogen infection of the wet canopy from decomposing plant residues (Ceresini, 1999). *R. solani* has not been identified as a problem on light soils in the absence of irrigation and prolonged summer drought for sugar beet growing in the UK (Japan-KWS, n.d.). Therefore, plant rows should be spaced sufficiently to let light penetrate and provide good air circulation through the canopy layer to dry up foliage.

Crop rotation is an important method to minimise the fungal inoculum levels. Peters, Sturz, Carter, and Sanderson (2004) found a 3 year crop rotation of barley then red clover and finally potato combined with minimum tillage resulted in the reduction of potato disease (black scurf and silver scurf) caused by *R. solani* and *Helminthosporium solani*. Pathogen colonization was reduced by bio-fumigant crops such as alfalfa, buckwheat corn, rapeseed, red clover, rye, sorghum, oats and wheat (Hansen, 2011; Martinez, 2009; Rothrock, Kirkpatrick, Cochran, Bates, & Cox, 2017).

Soil microorganisms with the addition of organic amendments were able to more aggressively compete with the pathogen to inhibit the pathogen outbreak in the soil (Peters et al., 2004). However, frequent conventional tillage is a potential risk to increasing the disease severity in the soil environment (Japan-KWS, n.d.; Peters et al., 2004; Sturz, Carter, & Johnston, 1997). The pathogen can be spread by unsterilized equipment or machines via inoculated soil particles. Sclerotia have been shown to be relatively resistant to degradation in soil surviving for several years in the absence of crop debris as a saprophyte by colonizing soil organic matter (Loria et al., 1993). Therefore, removal of the potential *R. solani* host plant and crop residues are important cultural methods to decrease the disease threshold.

- **Fungicide**

Fungicides to minimize the pathogen attack and decrease soil-borne inoculum damage are available in New Zealand and worldwide (Table 2.1).

Table 2.1 Fungicide compounds available to control *R. solani* in New Zealand. (Bayer Crop Science, 2015; SS, 2017; Syngenta, 2016; Young, 2013).

| Product | Active ingredient | Disease controlled |
|---|---|---|
| Aliette Super | fosetyl aluminium, thiram, thiabendazole | Damping off |
| Amistar | azoxystrobin | Damping off |
| Apron | metalaxyl-M | Damping off |
| Azoxystrobin | azoxystrobin | Black scurf |
| Captan | captan | Damping-off, root rot |
| Cusol | copper | Leaf spot |
| Liquicop | copper | Leaf spot |
| Mancozeb | dithiocarbamate | Leaf spot |
| Microplus | streptomyces lydicus | Soil borne diseases, Foliar diseases |
| Mogul | prothioconazole, fluoxastrobin | Leaf spot |
| PICASA | metalaxyl | Damping off |
| Previcur N | propamocarb | Damping off |
| Terrazole 35WP | etridiazolee | Damping off |
| Thiram | thiram | Damping off, Leaf spot |

Kataria, Hugelshofer, and Gisi (1991) reported that flusilazole, propiconazole, fenpropimorph and benomyl had strong activity against *Rhizoctonia* species such as *R. cerealis, R. sasakii, R. solani,* and *R. orzae*. The chemical compounds shown in Table 2.1, block fungal activity by mechanisms such as inhibition of the lanosterol (fungal steroids) and ergosterol biosynthesis (= saccharomyces cerevisiae: generation of a fungal plasma membrane) (Kataria et al., 1991; Siegel, 1981).

Fungicides are applied to reduce the disease severity, but phytotoxicity may be a problem because a high concentration of chemical ingredients (e.g. organic acid and surfactants in the formulation) may damage sprouts and shoot growth (Bienkowski, 2012).

- **Biological control**

Biological control is becoming more widely used, and its agents are available as microbial products worldwide. A number of micro-organisms (e.g. *Ampelomyces* spp., *Bacillus* spp., *Candida* spp., *Coniothyrium* spp., *Cryptococuss* spp., *Fusarium* spp., *Gliocladium* spp., *Penicillium* spp., *Phythium* spp., *Phlebiopsis* spp., *Pseudomonas* spp., and *Trichoderma* spp.) have been shown to have bio-activity against diseases (Pal & McSpadden, 2006; Tariq & Leonard, 2000). These microbes have different modes of action including mycoparasitism and production of antibiotic compounds (Samules & Hebbar, 2015).

*Trichoderma* fungi are able to control fungal pathogens, particularly *Rhizoctonia solani* (Tariq & Leonard, 2000). *Trichoderma koningii* inhibited *R. solani* mycelial growth by 79%, *T. harzianum* reduced the viability of sclerotia of *R. solani* by 82% (Soares de Melo & Faull, 2000), and decreased damping-off of radish seedlings caused by *R. solani* (Chet & Baker, 1980).

## 2.3 *Trichoderma*

### 2.3.1 Introduction

Plant diseases caused by fungal and bacterial pathogens damage agricultural crops worldwide. Chemical control is most widely used for disease control and is usually an effective method in the short term. However, there are concerns about continuing chemical usage because of environment pollution and food safety. Pesticides that are toxic to plant pathogens can also damage beneficial microbes. When people ingest the pesticide residue through the food or water, chronic health effects may occur after exposure to pesticides (Toxic Action Center, 2017).

An alternative to the use of chemicals is the use of biological control agents (BCA). Successful biocontrol is based on several factors; 1. The BCA may grow fast and invade a new environment more quickly than the pathogen, 2. BCAs release biosynthesis products to kill host-specific pathogens, and may directly feed on a pathogen, 3. The BCA may induce plant resistance against pathogen attacks and promote plant growth (Schuster & Schmoll, 2010; Stiling & Cornelissen, 2005).

*Trichoderma* species are now known as beneficial BCAs worldwide. *Trichoderma* spp. are soil-borne fungi that are commonly found in soil organic or inorganic matter (Harman, 2017; Kumar et al., 2014; Schuster & Schmoll, 2010).

There are *Trichoderma*-based biocontrol products worldwide in different forms. Unlike the action of most pesticide chemicals, *Trichoderma* protects against various diseases, and enhances plant growth, improving yield and quality of many crops. Also the use of *Trichoderma* does not cause adverse effects to human health and the environment (Agriculture Solutions, n.d.).

### 2.3.2 Morphology

*Trichoderma* morphologies are distinctive and differ with each species (Druzhinina & Kopchinskiy, 2004). Differences in physical appearance of the colonies include colour, radial growth and conidial morphology (e.g. conidiophores, conidial ornamentation, phialides and chlamydospores). The colours of conidia are typically dull yellowish, white, pale colourless or dark greenish depending on *Trichoderma* spp. (Shah, Nasreen, & Sheikh, 2012). Figure 2.9 shows the greenish conidia of *T. virens* after 3 weeks of growth.

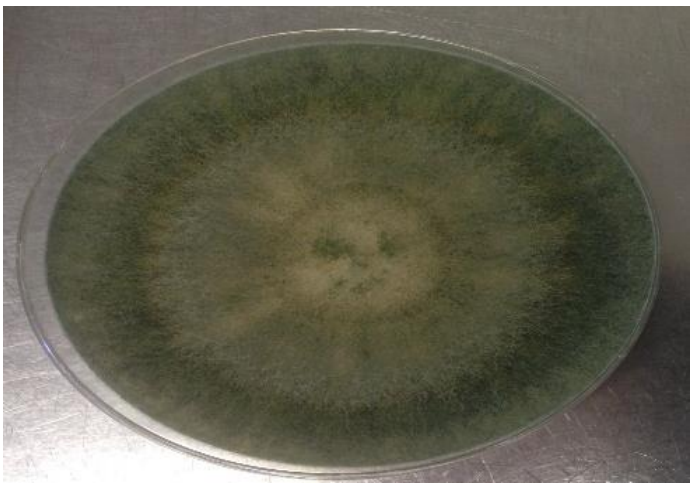

Figure 2.9 An isolate of *Trichoderma virens* (LU540) on a petri-plate after 3 weeks incubation at 25ºC.

Fungal hyphae are able to grow and ramify as mycelium. On a petri-plate containing potato dextrose agar (PDA), 2 to 5mm diameter agar disks of inoculum can produce a mycelium diameter of approximately 10 to 30 mm within 96 hours of incubation at 20ºC (Figure 2.10). Conidial dimensions of most *Trichoderma* spp. are within the range of 2.0 to 3.5µm long and 2.0 to 3.0µm wide (Samules & Hebbar, 2015). Conidia form green or whitish yellow ascomycete spores (conidiospores) which can grow approximately 3 to 5µm within 3 to 5 days.

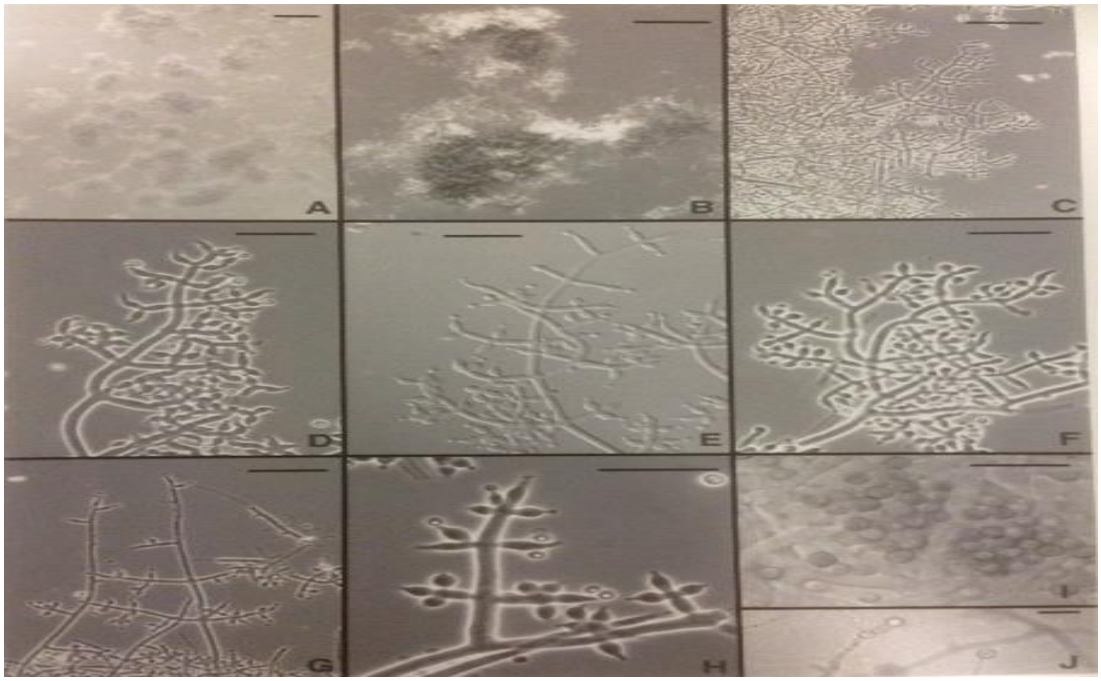


Figure 2.10 *Trichoderma atroviride*. A and B: Conidial pustules., C to H: Conidiophores., I: Conidia., J: Chlamydospores (Samules & Hebbar, 2015).

A conidiophore is an aggregation of branched interwoven hyphae, and phialides. There are four types of pustules recognised in the different *Trichoderma* species (Table 2.2).

Table 2.2 The different types of pustules in *Trichoderma* species (Khalili, Sadravi, Naeimi, & Khosravi, 2012).

| |
|---|
| *T. Gliocladium*: Formation of pustules is discrete, and they reach 1mm in length. |
| *T. Pachybasium*: Pustules are more or less distinct, 0.25- 3mm in diameter. |
| *T. Pyramidal*: Pustules are not well formed and have loosely joined conidiophores. The secondary branches decrease in length with distance from the branching point. |
| *T. Longibrachiatum*: Pustules are less than 0.5mm in diameter and form interwined hyphae, with a dentate or spiky appearance. |

### 2.3.3 Characteristics

*Trichoderma* has two types of reproduction; anamorphic (asexual state: *Trichoderma*) and telemorphic (sexual state: *Hypocrea*). Most *Trichoderma* biocontrol strains are based primarily on asexual sporulation (Harman, 2017).

*Trichoderma* were first classified as sexual states of the ascomycete genus *Hypocrea* in 1794 (Samules & Hebbar, 2015). However the different *Trichoderma* species assigned to the genus were difficult to distinguish morphologically (Schuster & Schmoll, 2010). Therefore, the genera *Hypocrea* and *Trichoderma* were re-classified in 2006. Some 471 different *Hypocrea* species and 165 *Trichoderma* species are currently listed in international culture collections and in GenBank (Samules & Hebbar, 2015).

*Trichoderma* can grow in a wide range of environments (Schuster & Schmoll, 2010). They can be found worldwide. Common species in North/South temperate regions include *T. aggressivum, T. atrobrunneum, T. harzianum T. atroviride, T. polysporum, T. hamatum* and *T. viride* and in tropical regions include *T. asperellum, T. endophyticum, T. ghanense, T. hamatum* and *T. reesei* (Samules & Hebbar, 2015).

Optimum temperature for growth of *Trichoderma* species is 20-25ºC. They can grow in soil pH from 2 to 6 but optimum biomass production occurs at pH 4.6 to 6.8 (Singh et al., 2014). There are some cold tolerant species which can grow at 5ºC (Kredics et al., 2003). However most *Trichoderma* species do not grow at over 35ºC (Samules & Hebbar, 2015).

### 2.3.4 Modes of action of *Trichoderma* species

*Trichoderma* spp. are a rich source of secondary metabolites that involves the biosynthesis of degrading mechanisms such as antibiotics (= antibiosis), mycotoxins (= myco-parasitism) and phytotoxins (= cell wall degradation) (Mukherjee, Horwitz, & Kenerley, 2012). The production of secondary metabolites is a significant mechanism for biocontrol by *Trichoderma*.

- **Antibiotic/Antibiosis metabolites**

Antibiotics produced by *Trichoderma* inhibit or interrupt pathogen growth (Dubey, Tripathi, Dureja, & Grover, 2011). Antifungal substances from *T. lignorum* were first identified in 1936 (Ghisalberti & Sivasithamparam, 1991). *Trichoderma* spp. produce a variety of antibotics (e.g. azaphilones, butenolides, di-ketopiperazines, gliovirin, gliotoxin, hyroxy-lactones, koninginins, nitrogen heterocyclic compounds, pyrenes, peptailbols, viridins, and viridian) against fungal pathogens (Harman, Howell, Viterbo, Chet, & Lorito, 2004; Shi et al., 2012; Vinale et al., 2008).

In particular, peptailbols have been reported as an important antibiotic product of *Trichoderma* spp. (Shi et al., 2012). Peptaibols are produced by *Trichoderma* species such as *T. harzianum*, *T. viride* and *T. longibrachiatum* (Ghisalberti & Sivasithamparam, 1991). Peptailbols have been shown to inhibit fungal development pathways such as mitochondrial ATPase production, oxidative phosphorylation, immunosuppression and platelet aggregation (Shima, Fukushima, Arai, & Terada, 1990).

The antimicrobial peptide 'Trichokonin VI' has shown a strong antifungal activity against soil-borne diseases such as *Botrytis cinerea, Cercospora citrullina, Fusarium oxysporum,* and *Phytohthora parasitica* (Shi et al., 2012). *T. harzianum* and *T. koningii* produce the antibiotics 'octaketide-derived acetal diol' and '3,4-dialkylfuran' to inhibit pathogen growth (Almassi, Ghisalberti, & Narbey, 1991).

Antibiotics produced by *T. harzianum, T. viride* and *T. virens* showed an antagonistic interaction and growth inhibition of *R. solani*, *R. bataticola* and *Fusarium oxysporum* f. sp. *ciceris* (Dubey et al., 2011). *Trichoderma* spp. produce an antibiosis barrier to prevent *Macrophomina phaseolina* development as well (Mendoza et al., 2015). However, the natural

antibiotic compounds are sometimes not produced in laboratory conditions (Vinale et al., 2008) and the characteristic of many compounds are poorly understood (Shi et al., 2012).

- **Mycoparasitism**

The parasitic effect of *Trichoderma* species against plant pathogens (e.g. fungi and nematodes) has been recognized as myco-parasitism (Samules & Hebbar, 2015). *Trichoderma* species such as *T. koningiopsis*, *T. harzianum*, *T. virens, T. hamatum* and *T. viride* are able to control a wide range of pathogens (Ghisalberti & Sivasithamparam, 1991; Mendoza et al., 2015).

*Trichoderma* is able to hyper-parasitise pathogens via appresoria (Mendoza et al., 2015). Appresoria are specialized cells which release enzymes (e.g. glucanases, chitobioses and chitinases) and antibiotics (e.g. viridian, gliotoxin or peptaibols) into the host plants or pathogens (Dubey et al., 2011; Mendoza et al., 2015). The anti-fungal compounds such as chitinases, *B*-glucanases and proteases degrade the cell wall of fungal pathogens.

*Trichoderma* spp. have been shown to produce volatile organic compounds (VOCs) and non-volatile compounds (culture filtrate) (Stoppacher et al., 2010). VOCs include polyketides, pyrenes and terpenes (Schuster & Schmoll, 2010) which inhibit fungal pathogen mycelial growth (e.g. chitin, glucans and proteins enzymes) (Ghisalberti & Sivasithamparam, 1991) as well as the growth of wood decay fungi (Samules & Hebbar, 2015). Non-volatile antibiotic compounds also inhibit pathogen growth. Therefore, *Trichoderma* spp (e.g. *T. reesei*) is used as a cellulose producer to degrade cellulosic waste material in the biofuel industry (Schuster & Schmoll, 2010).

- **Symbiosis**

*Trichoderma* species are able to colonize the roots of plants. Symbiosis between the plant and *Trichoderma* spp. can improve plant health and induce resistance against pathogen attacks (Samules & Hebbar, 2015). *Trichoderma* spp. in the plant rhizosphere increase biomass production and improved the development of plant growth (Henis et al., 1978). Harman (2017) states that *T. harzianum* (strain T-22) enhances root development and the rate of plant growth in field-grown corn and soybean. This is because *Trichoderma* is able to penetrate the

root surface as an avirulent symbiont to provide nutrients to the host-plant. In addition, the plant-derived nutrients (e.g. sucrose and carbohydrates) are provided for *Trichoderma* growth, (Kubicek, Harman, & Ondik, 1998; Vargas, Mandawe, & Kenerley, 2009).

- **Induced systemic resistance**

Induced systemic resistance is a response from *Trichoderma* against pathogenic attacks on host plants. *Trichoderma* spp. are enhanced by a hydrolytic protein, that stimulates plant suppression against pathogen damage (Samules & Hebbar, 2015). *Trichoderma* produce or release a variety of compounds (e.g. organic acids, amino acids and sugar) to induce localized or systemic resistance responses in the plant. In particular, the organic acid 'terpenoid' effectively induces resistance in the host against pathogen attacks (Harman et al., 2004). *T. hazianum* has been identified as being able to induce plant resistance against *Alternaria alternata* and *Rhizoctonia solani* (Harman, 2017).

Furthermore, the effects of peptide antibiotics produced by the fungal cells have been identified as being responsible for the systemic activation of the defence mechanisms in leaves (Vargas et al., 2009). Seedling diseases of cotton caused by *R. solani* were reduced by up to 78%, by the application of *T. virens* (strains: G-6, G-6.5 and G-11).

*Trichoderma* can increase the rate of plant growth and development. However, the molecular mechanisms and biochemical pathways between *Trichoderma* and its hosts are still unknown and not well understood (Vargas et al., 2009).

## 2.4 Seed treatment

Seed treatment is the application of chemical ingredients/products or biological agents to improve the establishments of crops, effectively control pathogens and promote plant growth (American Seed Trade Association, 2017; International Seed Fedration, 2015). Seed treatment plays an important role in protecting seedlings against seed-borne and soil-borne pathogens.

Seed treatments are divided into seed coating (e.g. coating with chemical protectant, film coating), pelleting, seed priming (e.g. osmopriming, halo priming, solid matrix priming) and physical seed treatment (solar, dry heat treatment, hot water treatment, aerated steam treatment) (Sharma, 2016). The formulations of seed treatments can differ (Table 2.3).

Table 2.3 Chemical formulations used as seed treatment (Sharma, 2016).

| | |
|---|---|
| DS | Powder for dry seed treatment. It contains the active ingredient with additives to avoid cohesion of particles to seed surface |
| WP | Wettable powders. The wetting agent is for use in a slurry |
| WS | Water dispersible fine powders for dispersion in high concentration |
| LS | Fungicides in non-aqueous solution for direct application to seeds |
| FS | Flowable concentration for direct use on seeds after dilution |

There are ten to eleven seed treatment and seed coated products available in New Zealand for different crops. In addition to fungicide seed treatments, there are also insecticides and biological additives (Hampton, 2016). For example, the current NZ chemical seed coated products are; Agricote grass (insecticide/fungicide), Agricote oversow (fungicide), Cropcoat (insecticide/fungicide) and Prillcote (insecticide/fungicide). Seed treatment chemical products are; Gaucho (insecticide), Pest go (insecticide), Poncho (insecticide), SF force field (insecticide/fungicide), SF force field plus (insecticide/fungicide) and Superstrike grass (insecticide/fungicide) (Agriseeds, 2017a; PggWrightson, 2016; Spec Seed, 2017). Moreover, there are specific biological additive seed treatment products available worldwide (Table 2.4).

Table 2.4 *Trichoderma*-based agricultural products worldwide (Samules & Hebbar, 2015).

| Product | Reported microbe/strain | Reported function | Country |
|---|---|---|---|
| Bio-Cure-F | *T. viride* | General mycoparasite applied typically, as seed dressing and soil drenching | India |
| Bioderma | *T. viride* | Wide-spectrum mycoparasite against root and leaf pathogens; stimulate seed germination | India |
| Bioderma-H | *T. harzianum* | Wide-spectrum mycoparasite against root and leaf pathogens; stimulate seed germination | India |
| BioHealth TH WSG | *Trichoderma sp. T-50* | Soil-borne pathogens; promote plant growth; seed and foliar treatments | Germany |
| Bio-Humaxin Sen Vang 6SC | *Trichoderma spp.* | Applied as seed treatment and in potting mixtures | Vietnam |
| Rhizoderma | *T. harzianum Th2* | Seed treatment to control *Bipolaris sorokiniana, Drechslera triticirepentis, Fusarium graminearum* | Argentina |
| SabraEx PB and SabraEx HC root inoculant | *Trichoderma spp.* | Stimulate growth; planter box treatment and seed treatment | USA |

Seed treatment fungicides have several advantages; 1. Non-selective treatment for control against seed-borne fungi, 2. Additional protection against some soil-borne pathogens, 3. Protection right from the time of sowing, 4. Reduced application rate, 5. Ease of handling, 6. Continuous flow treating (Sharma, Singh, Sharma, Kumar, & Sarma, 2015; Sharma, 2016), and 7. Low doses of fungicide per hectare compared with spray applications.

Seed treatments are superior to control and reduce disease incidences at seedling stages. A treated commercial brassica seed (e.g. Agricote brassica) protects against soil-borne pathogens and insect attack. It also assists seedling establishment by supplying molybdenum (Agriseeds, 2017a). A product ‘Prillcote grass’ has a lime-based coat on the seed surface

which provides a pH correction around the seedling. Moreover, coated seed ensures that seed can reach the target soil surface when aerially applied.

The disadvantages of fungicide seed treatments are; 1. Not effective against bacteria, 2. Laborious work because of soaking seeds for more than 24 hours, seeds need to be dried for 6-12 hours, 3. Phytotoxicity, 4. Potential risk of pathogen fungicide resistance build-up, 5. Sometimes chemical wastage (Sharma et al., 2015; Sharma, 2016).

Seed treatments often contain a combination of growth enhancement additives (e.g. trace elements) with fungicide and insecticide. However the combined applications with a chemical and a biological additives (e.g. *Trichoderma*) are expected to be one of the faster growing seed treatment sectors in the near future (Sharma et al., 2015).

### 2.4.1 *Trichoderma* seed treatment

Seed treatment with beneficial micro-organisms (e.g. *Trichoderma* spp.) can promote seed germination and plant growth, and enhance plant health (Rakholiya & Jadeja, 2010; Sharma et al., 2015). *Trichoderma* spp. are applied as seed treatment at different rates depending on crops (Table 2.5).

Table 2.5 *Trichoderma* seed treatment formulations of food crops (Sharma, 2016).

| | Crop | Pest and disease | Seed treatment |
|---|---|---|---|
| **Food crop** | Rice | Root rot disease | *Trichdoderma* spp 5-10g per kg seed |
| | Maize | Soil & seed-borne disease | *T. viride* and *T. harzianum* 4g per kg seed |
| | Pearl millets | Soil-borne disease | *T. viride* and *T. harzianum* 4g per kg seed |
| | Sorghum | | *T. viride* and *T. harzianum* 4g per kg seed |
| **Vegetables** | Chillies | *Antbracnose* spp. *Pseudomonas* spp | *T. viride* and *T. harzianum* 4g per kg seed |
| | Pea | Root rot, White rot | *T. harzianium* 4g/kg |
| | Onion | Smut | *T. viride* 2g per kg seed |
| | Tomato | Early blight, damping off | *T. viride* and *T. harzianum* 2g per kg seed |
| | Cucurbits | Soil-borne disease | *T. viride* and *T. harzianium* 4g per kg seed |
| | Leguminous vegetables | Soil-borne disease | *T. viride* 2g per kg seed |

Seed treatments with beneficial microbes are becoming more important because of the potential for control of seed-borne diseases and soil-borne diseases. As demand is increasing for safe food production and sustainable farming, there is an increasing trend for introducing bio control agents. However, the use of *Trichoderma* as a bio-inoculant should be effective, reliable and economic.

## 2.5 Discussion

Edible radish production is an important industry in New Zealand. However, soil-borne diseases are threatening crop production, causing damage such as wilting, girdling (wire) stem rot and damping off. *R. solani* is a common soil-borne pathogen in moist and cool temperature regions, and therefore the damage caused by *R. solani* can be severe all around New Zealand. However, seed growers and farmers are concerned more about other diseases such as *Erwinia* spp, *Pseudomonas* spp, club root (*Plasmodiophora brassicae*) and *Alternaria* spp than *R. solani* (Buter & South Pacific Seeds, personal communication, March 2017). Chemical seed treatment is able to provide protection against the pathogen *R. solani*. However chemical seed treatment cannot prevent all the seed-borne and soil-borne diseases. Moreover, seed treatment can have a potential risk of phytotoxicity, and can cause environmental issues when it is excessively used. As pressure increases for more sustainable farming and safe food production, the development of biological control agents is becoming more important.

# Chapter 3: Experiment 1

# Determining *R. solani* inoculum rate

## 3.1 Introduction

Radish (*Raphanus sativus* L.; 2n = 2x=18: diploid or tetraploid) is a vegetable *Brassica* crop that is consumed as a fresh food. Radish seed is a major component of New Zealand's vegetable seed industry. The soil-borne pathogen *R. solani* has been reported worldwide as causing damage to *Brassicae*, *Solanaceae* and *Poaceae* crops. Typical damage includes damping off of seedlings, stem wilting of seedlings, brown red rot of the stem (wire stem) and dark vascular discoloration of roots (Miller & Baysal-Gurel, 2010).

When evaluating the ability of biocontrol agents to protect seedlings from *R. solani* in glasshouse studies, a decision is required as to the amount of inoculum of the pathogen to add to the growing medium. Kandula, Jones, Stewart, McLean, and Hampton (2015) suggested that an inoculum rate which resulted in 50% diseased seedlings was appropriate for this purpose. This experiment was therefore designed to determine how much of the pathogen inoculum needed to be added to the growing medium to achieve 50% of radish seedlings damaged by *R. solani*.

## 3.2 Materials and methods

### 3.2.1 Preparation of pathogen inoculum

- **PDA**

Potato Dextrose Agar (PDA) was made to culture a colony of *R. solani*. 39g of PDA and 1 litre of distilled water were mixed in a 1 litre Schott bottle using a magnetic mixer. PDA was used because it is cost effective and has relatively rich nutrients for growing *R. solani* isolates (General Mycology, n.d.).

The medium was autoclaved (wet cycle) for 15 mins at 121$^{o}$C, 15 Psi, then poured into sterile petri-plates on a flat surface in a Laminar flow cabinet using an electronic dispenser which delivered 18 mL of PDA to each petri-plate. The poured PDA petri-plates were allowed to solidify for 25-30 minutes in the Laminar flow cabinet, then the lids were placed on and the plates stored in plastic bags at -8$^{o}$C until use.

- **Culturing *Rhizoctonia solani***

A colony of *R. solani* (strain RS73/LU8003) was sourced from the Lincoln University microbial culture collection. The isolate of *R. solani* had been stored at -5 ºC for approx. 2 months, and it was re-cultured on new PDA for 7 days. A colonized agar block (5mm circle per block) cut from the isolate using a sterilized cork borer, needle and scalpel was placed onto the centre of a new PDA plate (Figure 3.1) and the plates sealed with para-film were then placed in an incubator in a 12 hour light and dark cycle at 25 ºC, for 7 days.

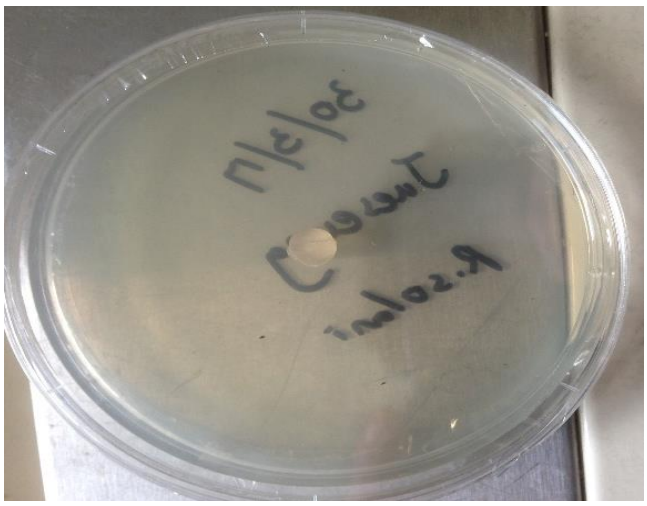

Figure 3.1 Colonized *R. solani* agar block on a petri-plate.

- **Inoculation of *R. solani* into wheat-bran & peat mix**

Wheat-bran and peat were provided from the Horticulture Research Centre, Lincoln University. Two litres of dried wheat-bran and peat were mixed with 2 litres of distilled water (ratio Wheat-bran: Peat: Water = 1:1:1) (Chai, personal communication, March 2017). The mixed medium was autoclaved for 15 mins at 121ºC, 15 Psi. After autoclaving, 200g of the medium was placed into each of three different sterilized plastic containers.

Six colonized agar blocks (5mm circle per block) cut from the 7 day old *R. solani* plate were mixed into each sterile wheat bran container to allow *R. solani* to multiply for 13 days at 23ºC (Figure 3.2).

Figure 3.2 Inoculating wheat-bran & peat mix with *R. solani*. Left: *R. solani* agar blocks in the container. Right: Inoculated wheat-bran sealed in plastic zip lock bags to prevent moisture loss.

### 3.2.2 Germination and conductivity test

A germination and a conductivity vigour test were conducted to assess the quality of seeds of the two radish varieties (Open-pollinated: French Breakfast and Hybrid: Red Round) used in the experiment. The test methods followed the International Seed Testing Association rules (ISTA, 2017) and personal advice from the Bio Protection Research Centre (Kakhiki & Khan, personal communication, March 2017).

- **Germination test**

The between paper (BP) method was used (ISTA, 2017). Four replicates of 100 seeds per variety were tested. The germination paper was soaked in water and drained to remove excess water. One sheet of paper was placed flat on the bench and two rows of 25 seeds placed near to the top edge (Figure 3.3). The paper was then folded up from the bottom and rolled to form a tube. The rolls were enclosed in zip lock plastic bags and placed vertically in the incubator at 20ºC with 12 cycles of light/dark for 10 days (Figure 3.3).

The tests were evaluated after 10 days and the number of normal seedlings, abnormal seedlings and un-germinated seed (fresh seeds and dead seeds) were counted (ISTA, 2017; Kakhiki & Khan personal communication, March 2017). These are determined by ISTA (2017) as follows;

- Normal seedlings: Seedling with all of the essential structures (e.g. root system = primary and secondary roots; shoot axis = hypocotyl and epicotyl; cotyledons and coleoptile) well developed, complete and healthy (Figure 3.4).

- Abnormal seedlings: Seedlings do not show the potential to develop into a normal plant. Seedlings missing one or more of the essential seedling structures or deformed, unbalanced or decayed. (Figure 3.5).

- Un-germinated seed: Seeds which have not germinated by the end of the test period under a favourable environment (Figure 3.6).

  * Fresh seed: Seed remaining clean and firm because of dormancy; have failed to germinate under the conditions of the germination test, but still have the potential to develop into a normal seedling.

  * Dead seeds: Seeds are not fresh or have produced any part of a seedling. Seeds absorb water but show no sign of seedling development.

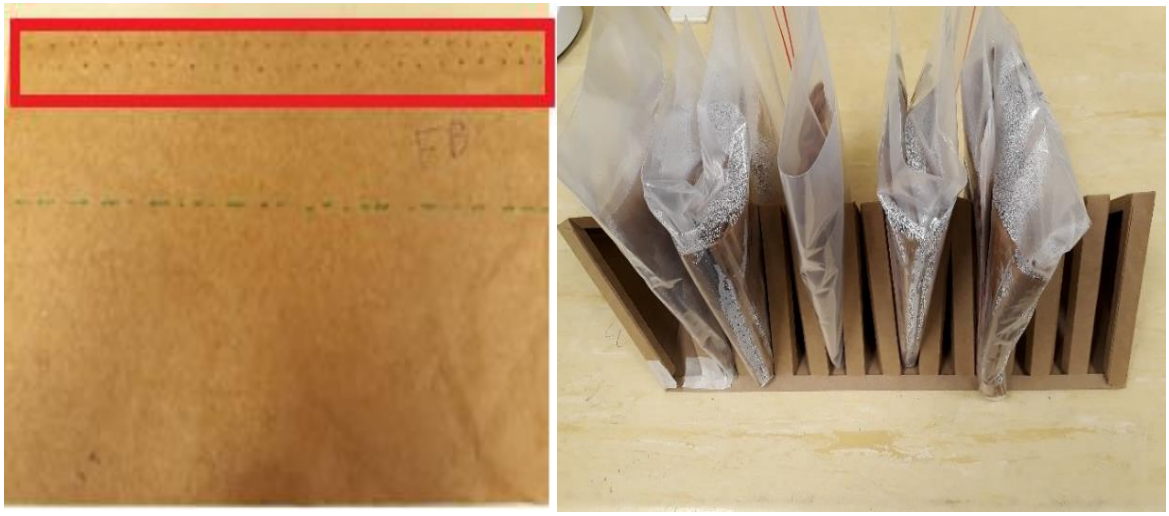

Figure 3.3 Two rows of 25 seeds placed on the top edge of the germination paper (left). The folded rolls were enclosed in zip lock plastic bags before placing in the incubator (right).

Figure 3.4 Normal seedlings; French Breakfast (left) and Red Round (right) after 10 days at 20ºC with 12h cycles of light/dark.

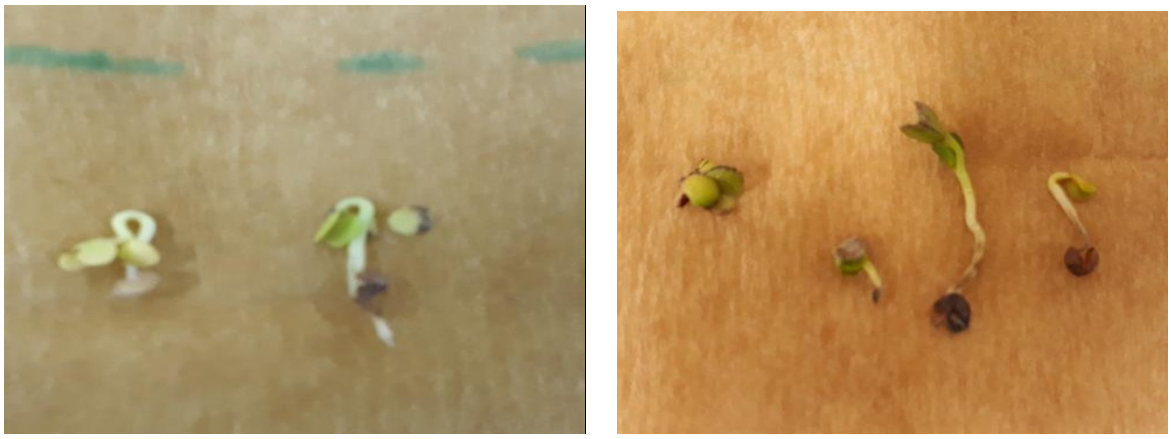

Figure 3.5 Abnormal seedlings; French Breakfast (left) and Red Round (right) after 10 days at 20ºC with 12h cycles of light/dark.

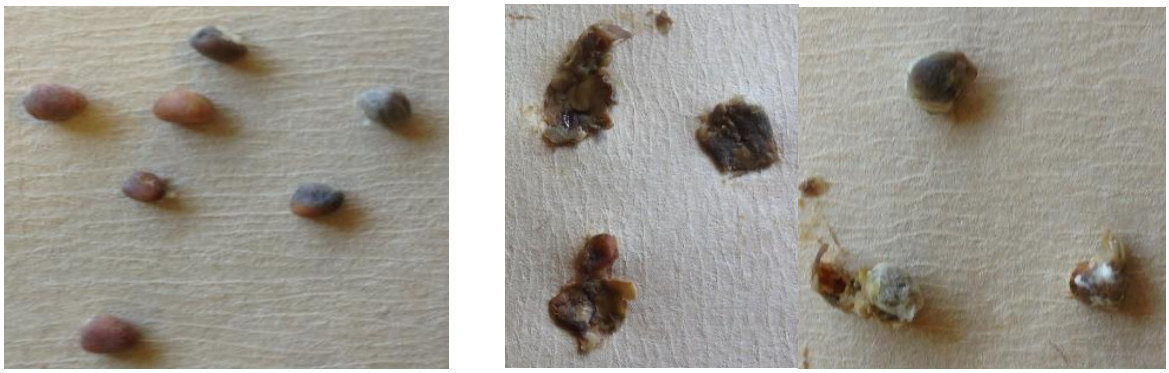

Figure 3.6 Fresh seed (left); because of dormancy have failed to germinate under the test conditions, but remain clean and are able to imbibe water and have the potential to develop into normal seedlings. Dead seed (right); seeds have imbibed water but are soft or mouldy and no sign of seedling development.

- **Conductivity test**

The conductivity test was used to assess seed vigour (ISTA, 2017). Requirements for the conductivity meter, containers (e.g. flasks or beakers), water and germinator (or incubator) are presented in Table 3.1.

Table 3.1 Apparatus of conductivity test (ISTA, 2017).

| | |
|---|---|
| Conductivity meter | A constant temperature of 20ºC and a conductivity range of 0 to 1999 µs/cm. |
| Containers (flasks or beakers) | Approximately 500 mL with a base diameter of 80 mm to contain deionised water. |
| Water (background reading) | Distilled water at a conductivity range of up to 5 µs/cm at 20ºC. |
| Germinator (or incubator) | A constant temperature of 20ºC is required. |
| Calibration of the dip cell | Conductivity meter should be calibrated to read conductivity between 0 and 1,900 µs/cm. To adjust EC rate between 1,273 and 1,278 µs/cm, 0.745g potassium chloride can be dissolved with 1 litre of deionised water. |

After the preparation of the conductivity test apparatus four replicates of 50 seeds of each variety were weighed to two decimal places. Each lot of 50 seeds were placed in a sterilised empty plastic container with 50mL of distilled water (Kakhiki, personal communication, March 2017). Each container with seed was gently swirled for 15 seconds to ensure mixing of the leachate. The containers were covered with aluminium foil to prevent contamination, then the containers were placed in an incubator at 20ºC for 24 hours. After 24 hours, each sample was tested for conductivity. Care was taken to avoid placing the conductivity cell rod directly onto the seed. After each reading, the conductivity cell rod was rinsed using distilled water and dried with paper towels. To calculate conductivity ($\mu S\ cm^{-1}\ g^{-1}$) results for each sample, the following equation was used;

{Conductivity reading ($\mu S\ cm^{-1}$) – background reading}
÷ weight (g) of seed / replicate = Conductivity ($\mu S\ cm^{-1}\ g^{-1}$).

Note: Background reading is the distilled water conductivity, which should be less than 5µs/cm at 20ºC. For this experiment, the background reading was 4.29µs/cm.

### 3.2.3 Preliminary glasshouse experiements

- **Potting mix**

Sieved commercial potting mix (3 - 4 months old mix: 500L) was provided from the Horticulture Research Centre, Lincoln University. The composition of ingredients was composted bark (400L), pumice (100L), mineral fertilizers (Osmocote: 1,500g; N: P: K, 16 - 35 - 10) and horticulture lime (500g). Trays (size 41.5cm x 30cm x 6.5cm) were bleached (ratio bleach: water = 1: 9) for 15 mins and required a total of 3.5 kg potting mix per tray to fill. The first 2.5 kg potting mix (approx. 2 litre) was placed into a tray. Next the *R. solani* inoculated potting mix was prepared. The different *R. solani* inoculum rates (0.25g, 0.5g and 1g per 100g of potting mix) and nil control (no pathogen) were added to each 1,000g potting mix as follows:

* 0.25g of *R. solani* inoculated wheat-bran per 100g of potting mix = 2.5g of inoculated wheat-bran per 1,000g of potting mix.

* 0.5g of *R. solani* inoculated wheat-bran per 100g of potting mix = 5g of inoculated wheat-bran per 1,000g of potting mix.

*1.0g of *R. solani* inoculated wheat-bran per 100g of potting mix = 10g of inoculated wheat-bran per 1,000g of potting mix.

After completely mixing, the *R. solani* inoculated potting mix was applied on the top layer of different trays. A total of 48 trays were used: two varieties x four treatments x six replicates. The trays per variety were laid out in a completely randomized block design.

- **Seed sowing**

Two varieties ‘Red Round’ (Hybrid) and ‘French Breakfast’ (Open-pollinated) were used. The hybrid ‘Red Round’ was provided from the Bio Protection Research Centre (Khan, personal communication, March 2017). Its parentage was a female line ‘Radish Cherry’ and unknown (open-pollinated) male parent line (Khan, personal communication, March 2017). The open pollinated variety ‘French Breakfast’ was purchased from Egmont Seeds Ltd.

30 seeds were sown by hand in each tray at a depth of 1-2 cm in rows spaced from 3 to 4 cm apart and with approximately 2 to 3 cm between seeds in the row (Figure 3.7).

Figure 3.7 Radish seedlings in trays in the Horticulture Research Glasshouse, Lincoln University in April 2017.

The sown trays were placed in a glasshouse with a temperature range of 10.3 to 25ºC (average: 17.7ºC) with a relative humidity of >40%. Watering was done using a water spray gun when soils appeared dry.

### 3.2.4 Data collection at 20 and 33 days after sowing

The numbers of emerged seedlings, diseased seedlings and dead seedlings were counted daily for 33 days after sowing. The number of seedlings that died post emergence were counted daily from maximum emergence until 33 days later.

**i) At 20 DAS**

At 20 days after sowing, one of the six replicates was assessed in order to observe the plant development and *R. solani* damage threshold at the seedling stage (leaf size approx. 5cm x 5cm). First of all, the numbers of live seedlings and dead seedlings per tray were counted. The live seedlings were carefully extracted from the trays without removing their fibrous roots, and washed using a water-spray gun to remove potting mix from the root surface. The number of diseased seedlings were counted, and the disease score for each was recorded using three different categorises (Figure 3.8, 3.9 and 3.10). The shoot and root length (cm) were measured with a digital calliper (mm), and the fresh weights were determined by using an electronic scale (g) which weighed up to two decimal points. Next, each seedling was enclosed in a

paper bag (size 90 x 50 x 205 mm) and dried in an oven at 65ºC for three consecutive days before recording their dry weights.

After the assessment at 20 DAS, the numbers of seedlings, diseased seedlings and dead seedlings for five of the six replicates were counted continuously for a further 13 days.

### ii) At 33 DAS

At 33 DAS the identical measurements and observations from 'the assessment at 20 DAS' were conducted. The final harvest at 33 DAS was also used to measure the pathogen impact on harvestable vegetative plant parts. Plant numbers (surviving plants, diseased plants and dead plants) and plant growth parameters (length and weight) were measured at 33 days after sowing. In addition, the numbers of seedlings that died post-emergence were counted and accumulated daily. Data were analysed by analysis of variance (ANOVA) using GenStat version 18.

Figure 3.8 Disease score '1': Symptoms of infections (small lesions) on leaf, hypocotyl and root. Seedlings at 9 days (left), 20 days (centre) and 33 days after sowing (right).

Figure 3.9 Disease score ‘2’: Some damage and necrosis on root and leaf. Seedlings at 9 days (left), 20 days (centre) and 33 days after sowing (right).

Figure 3.10 Disease score ‘3’: Severe damage on tap root and leaf. Seedlings at 9 days (left), 20 days (centre) and 33 days after sowing (right).

## 3.3 Results

### 3.3.1 Germination and conductivity

The germination of French Breakfast (FB) was 91% which was significantly higher than Red Round (RR) (86%) ($P<0.01$) (Table 3.2). Abnormal seedling percentage was significantly higher in RR than FB ($P<0.01$). Fresh seed and dead seed percentage did not differ significantly between the two varieties.

Table 3.2 Germination percentage of the two radish varieties.

| Varieties | Normal Seedlings (%) | Abnormal Seedlings (%) | Fresh seed (%) | Dead seed (%) |
|---|---|---|---|---|
| French Breakfast | 91 | 5 | 3 | 1 |
| Red Round | 86 | 10 | 2 | 2 |
| LSD (5%) | 3 | 2 | 1 | 2 |
| Significance of difference | ** | ** | NS | NS |

The three variates in the conductivity test differed significantly between the two varieties ($P<0.001$) (Table 3.3). The weight of 50 seeds (g) for RR (0.614g) was significantly higher than for FB (0.452g). The conductivity reading ($\mu S^{-1}$ $cm^{-1}$) and electrical conductivity ($\mu S^{-1}$ $cm^{-1}$ $g^{-1}$), also differed significantly between the two varieties ($P<0.001$). FB had lower seed vigour than RR.

Table 3.3 Vigour of the two radish varieties as determined by the conductivity test.

| Varieties | Weight (g) per 50 seeds | Conductivity reading ($\mu S^{-1}$ cm) | Electrical conductivity ($\mu S^{-1}$ $cm^{-1}$ $g^{-1}$) |
|---|---|---|---|
| French Breakfast | 0.452 | 58.1 | 119.3 |
| Red Round | 0.614 | 41.5 | 60.7 |
| LSD (5%) | 0.029 | 6.2 | 14.8 |
| Significance of difference | *** | *** | *** |

### 3.3.2 Assessment at 20 DAS (3 weeks)

Only one of the six replicates was assessed at 20 days after sowing (DAS) and so data could not be analysed.

The numbers of seedlings at 20 DAS differed between the different *R. solani* inoculum rates and the two varieties (Figure 3.11). As the pathogen inoculum rate increased, the number of seedlings to emerge decreased (Figure 3.11 and Table 3.4), while the numbers of diseased and dead plants increased as did the disease score (Figure 3.11, Table 3.4 and 3.5). The three *R. solani* inoculum rates (0.25g, 0.5g and 1g) had a lower number of seedlings than the nil control at 20 DAS (Figure 3.11).

For French Breakfast, the 0.5g *R. solani* inoculum rate had a lower number of seedlings than the other two inoculum rates from 15 to 20 DAS. For Red Round, the 1g inoculum rate had a lower number of seedlings than the other pathogen inoculum rates from 5 to 20 DAS.

Number of seedlings for the 0.25g *R. solani* inoculum rate in Red Round was similar to that of the nil control between 1 and 10 DAS but decreased after 10 days. Nil control of the two varieties had a greater number of seedlings than the three *R. solani* inoculum rates at 20 days after sowing (Figure 3.11).

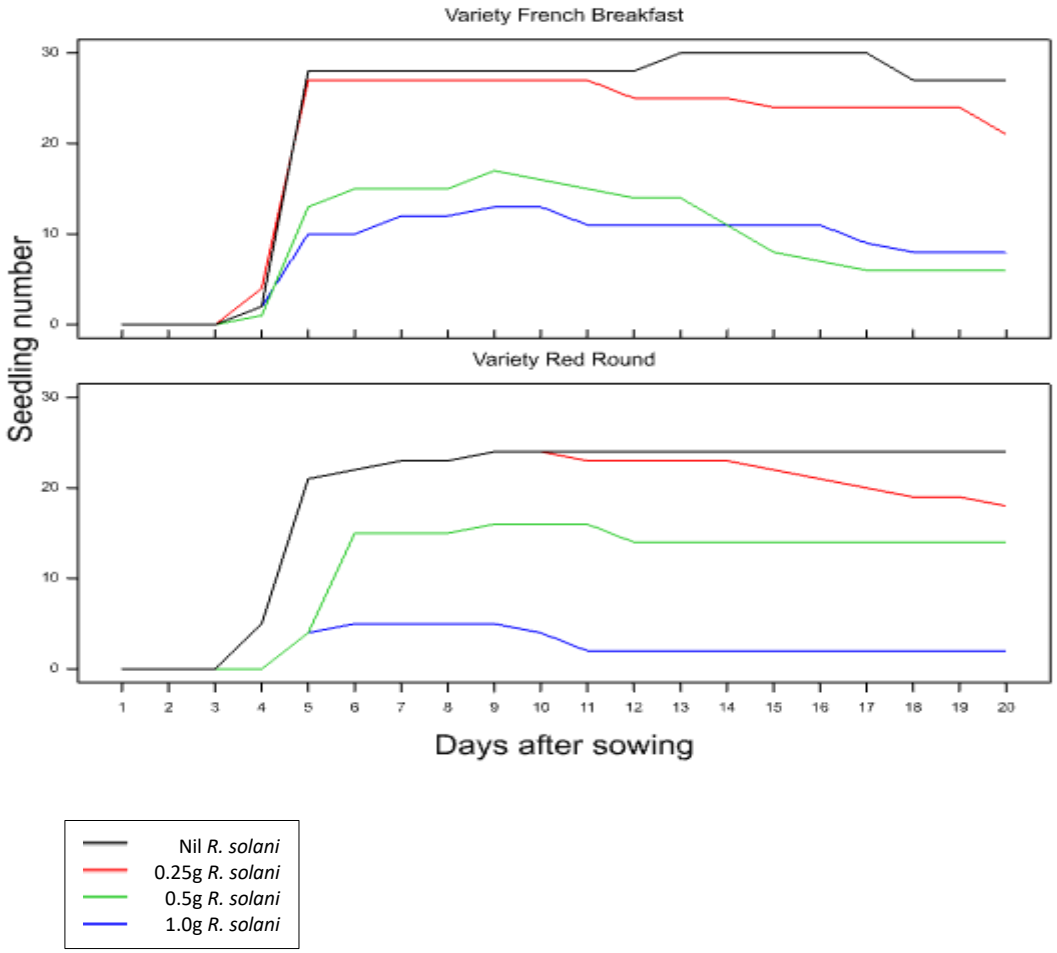


Figure 3.11 Effects of *R. solani* inoculum rates on seedling numbers (out of 30 seeds sown) per tray for the two varieties between 1 and 20 DAS in the Horticulture Research Glasshouse, Lincoln University in April 2017.

Maximum numbers of seedlings for the two varieties emerged between 5 and 13 DAS (Table 3.4). The nil control for both varieties had a higher number of seedlings than all the inoculum rates, both at maximum emergence and 20 DAS (Table 3.4 and 3.5). There were no diseased or dead seedlings at the date of maximum emergence (Table 3.4).

For French Breakfast at the 1g inoculum rate, only 13 seedlings had emerged at the maximum date (Table 3.4). The number of diseased seedlings was higher than for the other inoculum rates (0.25g and 0.5g), but there were no dead seedlings. At 20 DAS, the 0.5g inoculum rate had fewer surviving seedlings than the other pathogen inoculum rates (Table 3.5). In addition, a higher number of seedlings died post-emergence than for the other pathogen inoculum rates.

The 1g inoculum rate of Red Round had fewer seedlings than the other inoculum rates at both maximum emergence and 20 DAS (Table 3.4 and Table 3.5). The inoculum rate at 0.5g had

more diseased seedlings at maximum emergence and 20 DAS. Moreover, inoculum rates at 0.5g and 1g had a higher disease score than the other pathogen inoculum rate (Table 3.5). There were no dead seedlings, but at 20 DAS the 0.25g inoculum rate had a higher number of dead seedlings post-emergence than the other two inoculum rates (Table 3.5).

Table 3.4 Effect of *R. solani* inoculum rates on maximum number of emerged seedlings and number of days after sowing when maximum emergence was recorded.

| *R. solani* inoculum concentrations (g) | Variety | Maximum number of emerged seedlings | Days after sowing to achieve maximum emergence | Number of diseased seedlings at time of maximum emergence | Number of dead seedlings at time of maximum emergence |
|---|---|---|---|---|---|
| 0 | FB | 30 | 13 | 0 | 0 |
| 0.25 | FB | 27 | 5 | 0 | 0 |
| 0.5 | FB | 17 | 9 | 7 | 0 |
| 1 | FB | 13 | 9 | 8 | 0 |
| 0 | RR | 24 | 9 | 0 | 0 |
| 0.25 | RR | 24 | 9 | 4 | 0 |
| 0.5 | RR | 16 | 9 | 6 | 0 |
| 1 | RR | 5 | 6 | 0 | 0 |

Note: Data were not statistically analysed because only one replicate was measured.

Table 3.5 Effect of *R. solani* on the number of surviving seedlings, number of diseased seedlings, disease score and number of seedlings which had died post emergence at 20 DAS.

| | | Assessment at 20 DAS | | | |
|---|---|---|---|---|---|
| *R. solani* inoculum concentrations (g) | Variety | Number of surviving seedlings | Number of diseased seedlings | Disease score | Number of seedlings which died post emergence |
| 0 | FB | 27 | 0 | 0 | 3 |
| 0.25 | FB | 21 | 6 | 1 | 6 |
| 0.5 | FB | 6 | 6 | 2 | 11 |
| 1 | FB | 8 | 8 | 2 | 5 |
| 0 | RR | 24 | 0 | 0 | 0 |
| 0.25 | RR | 18 | 5 | 1 | 6 |
| 0.5 | RR | 14 | 6 | 3 | 2 |
| 1 | RR | 2 | 1 | 3 | 3 |

Note: Data were not statistically analysed because only one replicate was measured. Disease score (DS ‘0’ = no symptoms or no lesions; DS ‘1’ = symptoms of infection, small lesions on stem, root and leaf; DS ‘2’ = lightly damaged and necrosis occurred on root and leaf; DS ‘3’: completely damaged on tap root and leaf).

At 20 DAS, the FB control had 90% healthy seedlings which the RR control had 80% (Figure 3.12). The presence of the pathogen resulted in large reductions of healthy seedlings percentages, with the highest rate (1g) rate resulting in 0% healthy seedlings for FB and 7% for RR (Figure 3.12).

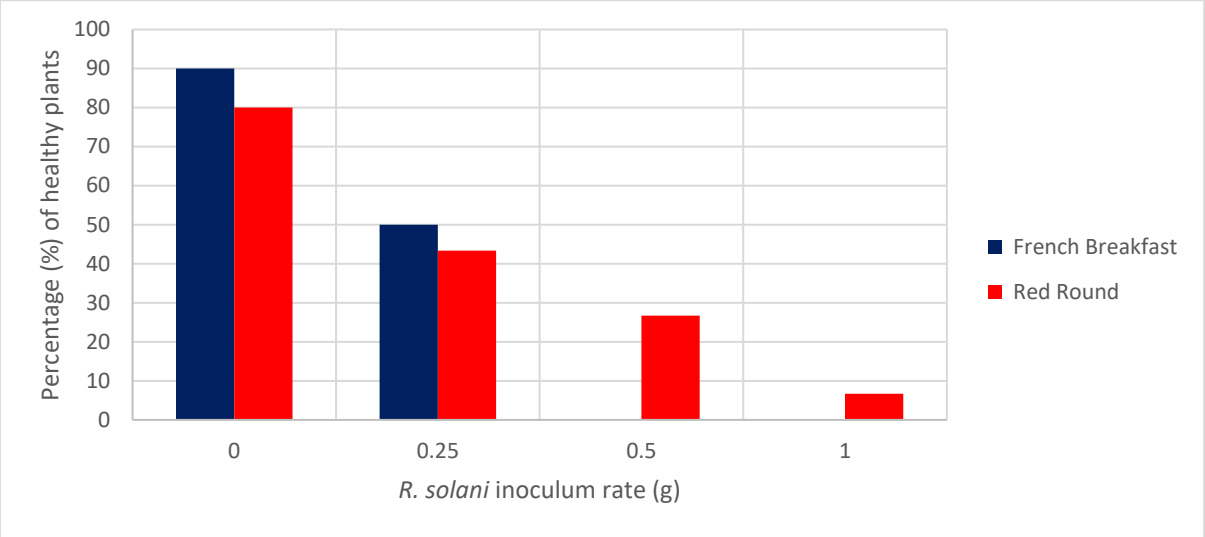


Figure 3.12 Effects of *R. solani* inoculum rate on the percentages of healthy seedlings of the two varieties at 20 DAS in the Horticulture Research Glasshouse, Lincoln University in April, 2017 (one replicate only).

The shoot and root lengths of French Breakfast were higher than for RR for all treatments (Figure 3.13 and Figure 3.14). Shoot lengths of the nil control averaged 14cm for FB and 9cm for RR, and root length of FB (6cm) was higher than for RR (4cm).

Shoot lengths of the two varieties were longer than the root lengths (Figure 3.13 and 3.14). Both lengths for the two varieties decreased with the increasing pathogen inoculum rates. The 1g *R. solani* inoculum rate of each variety had lower shoot (6cm for FB and 2cm for RR) and root (3cm for FB and 1cm for RR) lengths than the other two pathogen inoculum rates (0.25g and 0.5g). However, the *R. solani* inoculum rate at 0.25g for RR had the same shoot length (9cm) as the nil control.

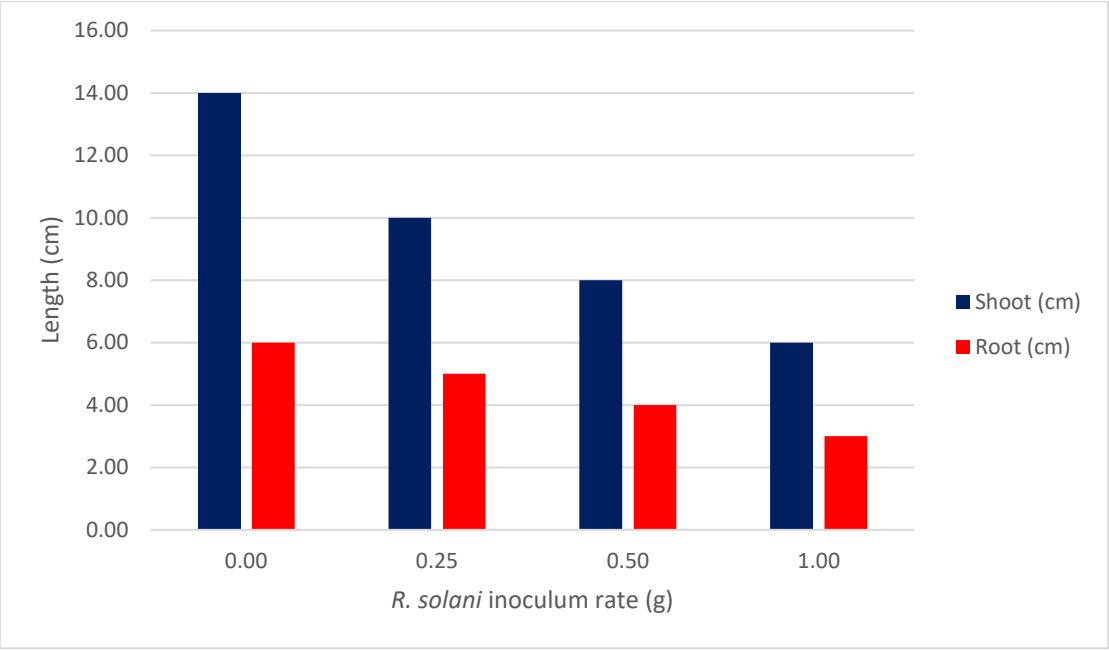


Figure 3.13 Effects of *R. solani* inoculum rate on shoot and root lengths of French Breakfast at 20 DAS in the Horticulture Research Glasshouse, Lincoln University in April 2017 (one replicate only).

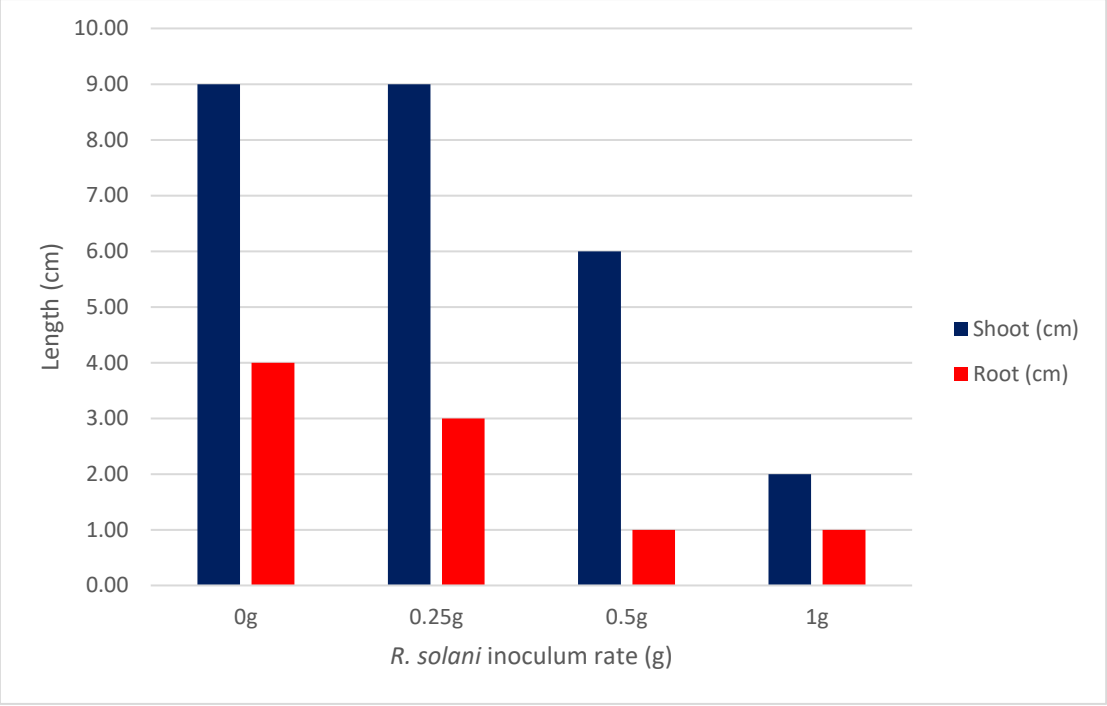


Figure 3.14 Effect of *R. solani* inoculum rate on shoot and root lengths of Red Round at 20 DAS in the Horticulture Research Glasshouse, Lincoln University in April 2017 (one replicate only).

For both cultivars fresh and dry weights per tray decreased as the *R. solani* inoculum concentrations increased. For each variety, the fresh and dry weights per tray of the nil control (0g) were heavier than the other inoculum rates (0.25g, 0.5g and 1g) (Table 3.6).

However, the fresh and dry weights per plant of the two varieties did not decrease at the increased pathogen inoculum rates. Interestingly, the fresh weight per plant for *R. solani* inoculum rate at 1g was 2.92g for RR and 2.09g for FB, which were heavier than those of the other inoculum rates. The dry weight per plant at 1g inoculum rate for RR was heavier than other inoculum rates (Table 3.6).

Table 3.6 Effects of *R. solani* inoculum rate on plant fresh and dry weights at 20 DAS.

| | | Fresh weight | | Dry weight | |
|---|---|---|---|---|---|
| *R. solani* inoculum concentration (g/tray) | Variety | g/tray | g/plant | g/tray | g/plant |
| 0 | FB | 39.80 | 1.47 | 2.25 | 0.08 |
| 0.25 | FB | 28.80 | 1.37 | 1.52 | 0.10 |
| 0.5 | FB | 12.53 | 1.43 | 0.59 | 0.07 |
| 1 | FB | 11.45 | 2.09 | 0.45 | 0.06 |
| 0 | RR | 35.79 | 1.49 | 1.85 | 0.08 |
| 0.25 | RR | 32.14 | 1.79 | 1.69 | 0.13 |
| 0.5 | RR | 19.74 | 1.41 | 1.10 | 0.14 |
| 1 | RR | 5.83 | 2.92 | 0.32 | 0.16 |

Note: Data were not statistically analysed because only one block was measured.

### 3.3.3 Assessment at 33 DAS (5 weeks)

All *R. solani* inoculum rates had significantly reduced the number of surviving seedlings for both varieties by 33 DAS (LSD 5% for FB: 1.534 and for RR: 1.403) (Figure 3.15 and 3.16). At 33 days, the 1g inoculum rate had the lowest number of seedlings, while the nil control had the highest number of seedlings.

Maximum seedling emergence of the two varieties occurred between 6 and 9 DAS (Table 3.7). As the pathogen inoculum rate increased, the number of seedlings to emerge decreased (Figure 3.15 and 3.16), and the number of diseased and dead seedlings increased (Table 3.8).

The nil control for both varieties had a significantly higher number of survived seedling than all the pathogen inoculum rates (0.25g, 0.5g and 1g) (Table 3.8).

The number of diseased seedlings at maximum emergence and at 33 DAS differed significantly among the inoculum rates (Table 3.7 and 3.8). At maximum emergence, the three inoculum rates had a significantly higher number of diseased seedlings than the nil control for both varieties. At 33 DAS, they also had a significantly higher number of diseased plants than the nil control (Table 3.8). The disease score increased as pathogen inoculum rate increased. Inoculum rates for 0.5g for RR, and 1g for FB and RR had the highest disease score ‘category 3’ (= completely damaged tap root and leaf) (Table 3.8). However, the nil control had no diseased plants at either maximum emergence or 33 DAS.

There was only one dead seedling for the inoculum rates at 0.25g and 1g in Red Round at maximum emergence (Table 3.7). However, the numbers of seedlings which died post-emergence of the two varieties had increased by 33 DAS (Table 3.8), and these differed significantly between the inoculum rates ($P<0.05$). In particular, the inoculum rate at 0.5g had a significantly higher number of dead seedlings of both varieties than the other treatments (Table 3.8).

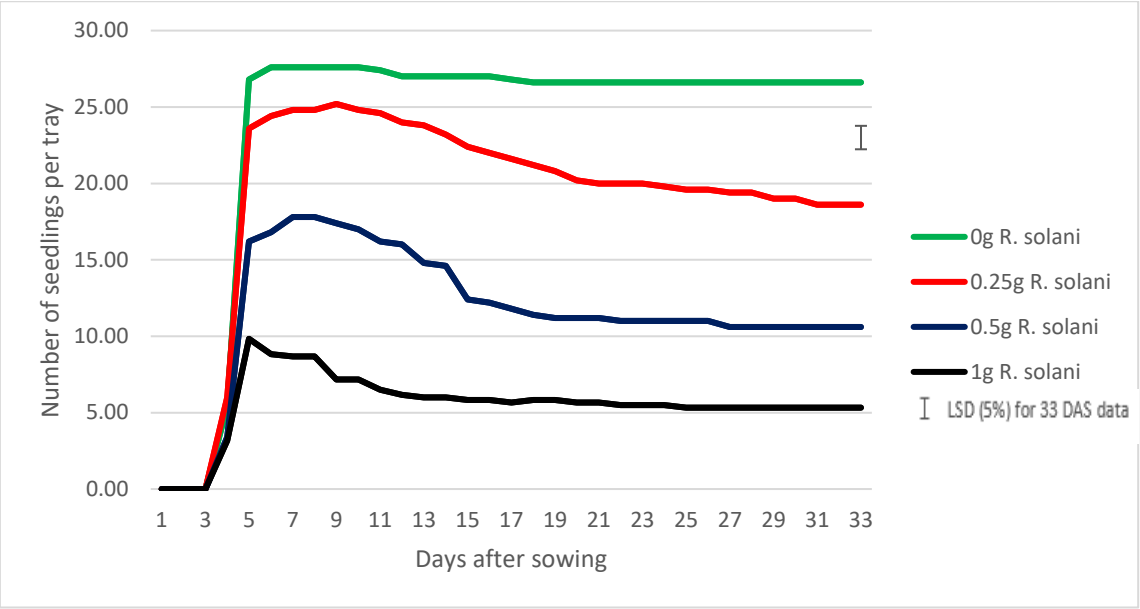


Figure 3.15 Effects of *R. solani* inoculum rate on seedling number per tray of French Breakfast up to 33 DAS in the Horticulture Research Glasshouse, Lincoln University, in April to May 2017. The vertical bar is the LSD (5%) for 33 DAS.

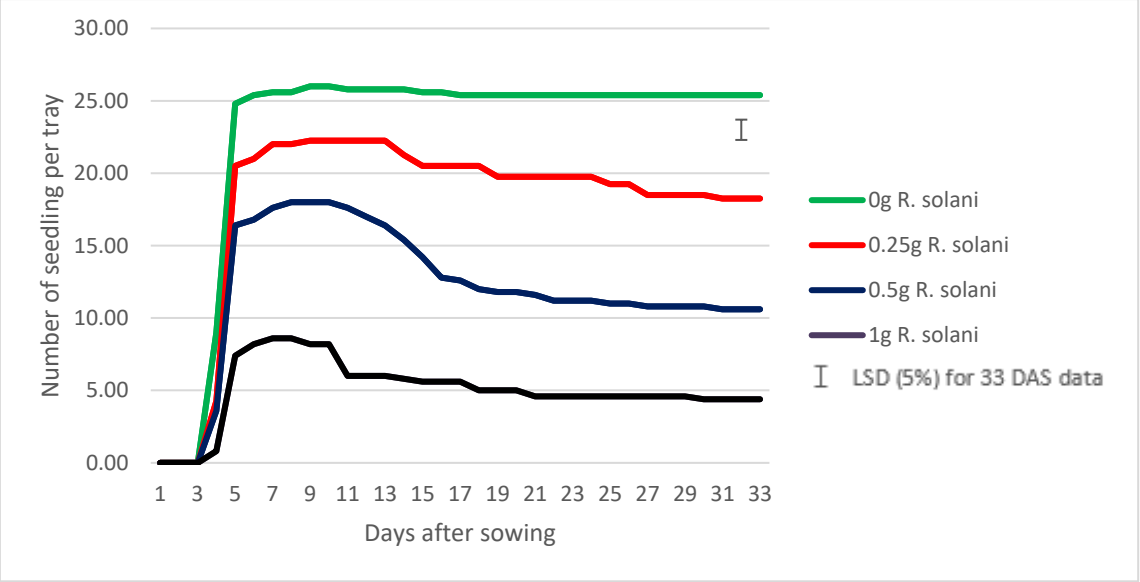


Figure 3.16 Effects of *R. solani* inoculum rate on seedling number per tray of Red Round up to 33 DAS in the Horticulture Research Glasshouse, Lincoln University, in April to May 2017. The vertical bar is the LSD (5%) for 33 DAS.

Table 3.7 Effects of *R. solani* inoculum rate on maximum seedling emergence, days after sowing for maximum emergence and numbers of diseased seedlings and dead seedlings at maximum emergence.

| *R. solani* inoculum concentration (g) | Variety | Maximum seedling emergence | Days after sowing to achieve maximum emergence | Number of diseased seedlings at time of maximum emergence | Number of dead seedlings at time of maximum emergence |
|---|---|---|---|---|---|
| 0 | FB | 27.6 | 6 | 0.0 | 0.0 |
| 0.25 | FB | 24.8 | 7 | 2.2 | 0.0 |
| 0.5 | FB | 17.8 | 7 | 3.8 | 0.0 |
| 1 | FB | 9.8 | 5 | 0.0 | 0.0 |
| 0 | RR | 26.0 | 9 | 0.0 | 0.0 |
| 0.25 | RR | 22.3 | 9 | 4.3 | 0.3 |
| 0.5 | RR | 18.0 | 9 | 4.2 | 0.0 |
| 1 | RR | 8.6 | 7 | 3.6 | 0.2 |
| LSD (5%) Inoculum | | 4.56 | - | 1.3 | - |
| Significant difference | | *** | - | *** | - |

Table 3.8 Effects of *R. solani* inoculum rates on number of surviving seedlings, number of diseased seedlings, disease score and number of dead seedlings post-emergence at 33 DAS.

| | | At 33 DAS | | | |
|---|---|---|---|---|---|
| *R. solani* inoculum concentration (g) | Variety | Number of surviving seedlings | Number of diseased seedlings | Disease score | Number of seedlings which died post emergence |
| 0 | FB | 26.80 | 0.00 | 0 | 1.6 |
| 0.25 | FB | 21.00 | 4.80 | 1 | 6.2 |
| 0.5 | FB | 12.60 | 4.40 | 2 | 7.2 |
| 1 | FB | 3.20 | 2.60 | 3 | 4.5 |
| 0 | RR | 25.40 | 0.00 | 0 | 0.6 |
| 0.25 | RR | 21.00 | 6.60 | 1 | 4.0 |
| 0.5 | RR | 11.00 | 6.60 | 3 | 7.4 |
| 1 | RR | 4.80 | 4.40 | 3 | 4.2 |
| LSD (5%) Inoculum | | 3.57 | 2.42 | - | 0.1 |
| Significant difference | | *** | *** | - | * |

Note: Disease score (DS ‘0’ = no symptoms or no lesions; DS ‘1’ = symptoms of infection, small lesions on stem, root and leaf; DS ‘2’ = lightly damaged and necrosis occurred on root and leaf; DS ‘3’: completely damaged tap root and leaf).

Healthy plant percentages of the two varieties were significantly reduced when *R. solani* inoculum rates were increased (Figure 3.17) (LSD 5%: 11.90 and P<0.001). The 1g *R. solani* inoculum rate of each variety had the lowest healthy plant percentage (11% for FB and 16% for RR), while the nil control had the highest healthy plant percentage (89% for FB and 85% for RR).

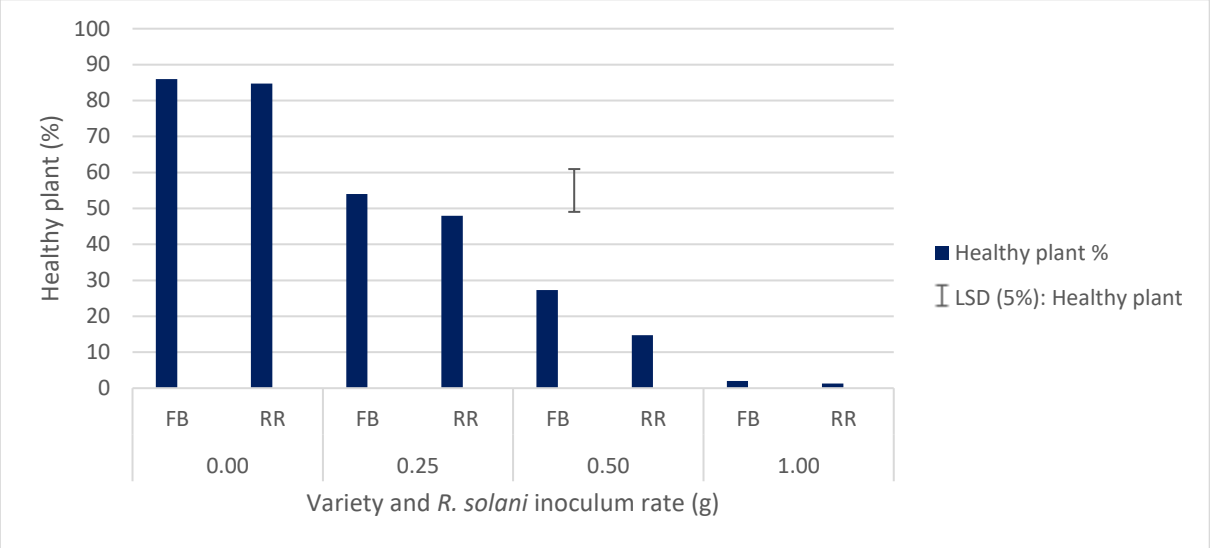


Figure 3.17 Effects of variety and *R. solani* inoculum rate on healthy plant percentage at 33 DAS. Vertical bar is LSD (5%).

Shoot and root length of the two varieties differed significantly among the inoculum rates (shoot length: LSD 5%: 3.83 and P<0.01) (root length: P<0.05 and LSD 5%: 2.31). The shoot length of each variety was longer than the root length.

The shoot length of French Breakfast was significantly reduced as the *R. solani* inoculum rates increased. Shoot length of the nil control (15.98cm) was significantly higher than for the three *R. solani* inoculum rates (Figure 3.18). The shoot length of Red Round decreased significantly as *R. solani* inoculum concentrations increased (Figure 3.18). The 0.5g inoculum rate had a significantly lower shoot length (6.24cm) than the nil control (11.65cm) and 0.25g (12.26cm).

Root length of the two varieties decreased significantly with the increase in *R. solani* inoculum rate (Figure 3.19). In particular, the nil control (7.3cm) for FB produced a significantly higher root length than the inoculum rate at 1g (3.46cm).

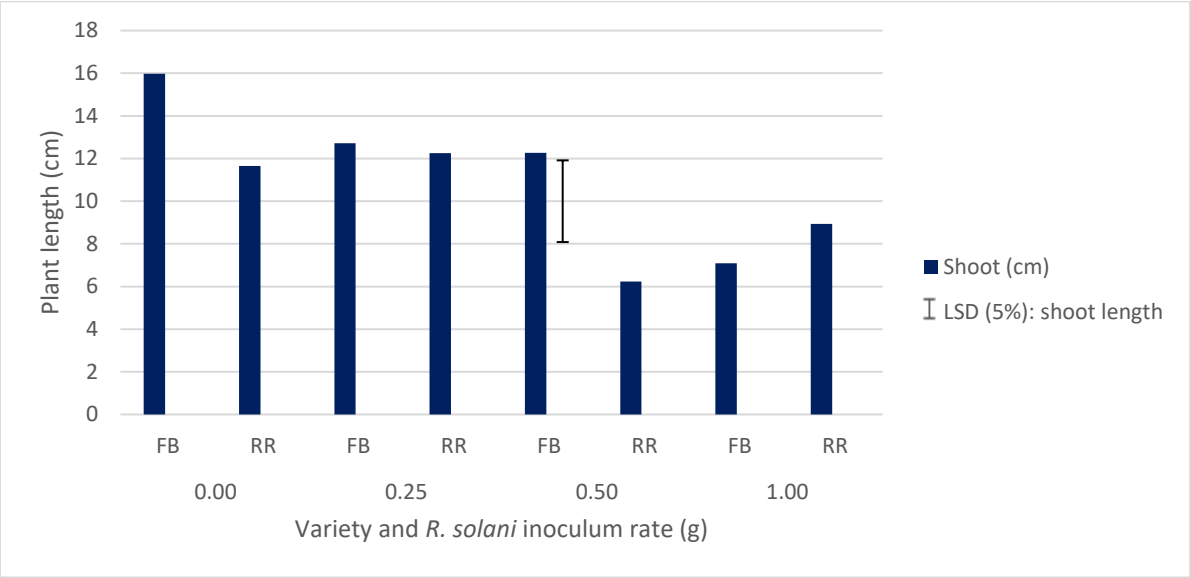


Figure 3.18 Effects of variety and *R. solani* inoculum rate on shoot length at 33 DAS. Vertical bar is LSD (5%).

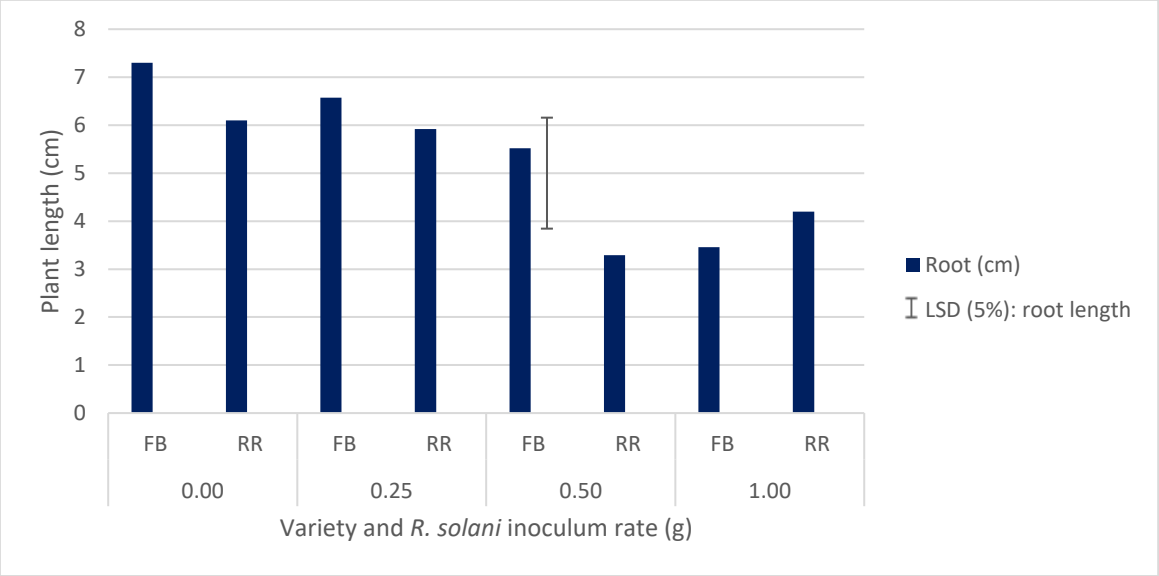


Figure 3.19 Effects of variety and *R. solani* inoculum rate on root length at 33 DAS. Vertical bar is LSD (5%).

Fresh weight and dry weight (g) per tray differed significantly among the inoculum rates ($P<0.001$) (Table 3.9). The nil control of the two varieties had the highest fresh weight (170.2g) and dry weight (13.78g) per tray. The fresh and dry weights per tray for both varieties were significantly reduced with the increase in *R. solani* inoculum rate ($P<0.001$) (Table 3.9). *R. solani* inoculum rate at 1g had lower fresh and dry weights per tray than the other treatments.

Fresh weight per plant did not differ significantly (P>0.05) among the two varieties and *R. solani* inoculum rates (*R. solani* linear x cultivar). However, the dry weight per plant for the two varieties differed significantly among the inoculum rates (*R. solani* rate linear) (P<0.01). The 0.5g inoculum rate for RR produced a significantly heavier dry weight per plant (0.702g) than the other inoculum rates (Table 3.9).

Table 3.9 Effects of *R. solani* inoculum rate on percentage plant survival and plant fresh and dry weight, at 33 DAS.

| | | | Fresh weight | | Dry weight | |
|---|---|---|---|---|---|---|
| Variety | *R. solani* inoculum concentration (g) | Plant survival (%) | g/tray | g/plant | g/tray | g/plant |
| French Breakfast | 0 | 89.3 | 170.2 | 6.21 | 13.78 | 0.509 |
| | 0.25 | 70.0 | 143.3 | 6.89 | 12.91 | 0.418 |
| | 0.5 | 42.0 | 73.3 | 6.32 | 5.01 | 0.323 |
| | 1 | 10.7 | 17.8 | 4.85 | 0.93 | 0.237 |
| Red Round | 0 | 84.7 | 242.3 | 5.75 | 14.93 | 0.355 |
| | 0.25 | 70.0 | 162.1 | 4.48 | 10.36 | 0.272 |
| | 0.5 | 36.7 | 50.5 | 9.25 | 2.62 | 0.702 |
| | 1 | 16.0 | 9.2 | 5.58 | 0.45 | 0.375 |
| LSD (5%) | | 16.83 | 56.4 | 4.39 | 4.74 | 0.293 |
| Significance of contrasts | | | | | | |
| *R. solani* rate (linear) | | *** | *** | ns | *** | * |
| Cultivar | | ns | ns | ns | ns | ns |
| *R. solani* (linear) x Cultivar | | ns | ns | ns | ns | ns |

Ns = Not significantly different
* = 5% significant difference (evidence of difference)
** = 1% significant difference (strong evidence of a difference)
*** = 0.1% significant difference (very strong evidence of a difference)

## 3.4 Discussion

The soil-borne pathogen *Rhizoctonia solani* has been reported to have damaged *Brassica* crops worldwide (Cabi, 2016; Hua et al., 2014). However, its effects on radish production are unknown in New Zealand. This experiment determined the effects of different *R. solani* inoculum rates on radish seedling emergence and growth. In addition, it determined the pathogen inoculum rate to be added to the medium in order to achieve around 50% damaged seedlings for Experiment 2.

Prior to sowing, the germination and vigour of seeds of the two radish varieties (French Breakfast and Red Round) were tested to assess the seed quality. FB had a greater germination (91%) than RR (86%), because it had a lower percentage of abnormal seedlings (5%) than RR (10%). This is possibly because the RR seeds were 2 - 3 years old (Khan, personal communication, March 2017). However, FB had a lower seed weight and lower seed vigour than RR.

Seeds of the two varieties were sown in April and plants harvested at 33 DAS. All *R. solani* inoculum concentrations had reduced the number of seedlings by 33 DAS as the number of diseased seedlings and dead plants had increased.

The number of seedlings differed significantly between the *R. solani* inoculum rates. In particular, the 1g inoculum rate reduced seedling numbers of the two varieties. The 0.25g inoculum rate resulted in an over 70% survival of seedlings (21 seedlings for FB and RR) at 33 DAS. Disease scores were higher as the inoculum rates increased. In particular, *R. solani* at 1g had a higher disease score (category 3: completely damaged tap root and leaf) than the other treatments. Therefore, the healthy plant percentages of the two varieties differed significantly between the different pathogen inoculum rates.

The nil control of the two varieties produced a higher shoot length and root length than any of the pathogen inoculum rates. The radish shoots can grow to a mature height of 15cm for French Breakfast and 12cm for Red Round. In addition, the harvestable root is 8 to 9cm for French Breakfast and 5-6cm for Red Round (Burpee, 2017). However by 33 DAS root rot caused by *R. solani* had interrupted and damaged plant parts, causing cracked roots and stunted leaves as described by Neher & Gallian (2011). Moreover, the tray depth (size 41.5 x 30 x 6.5cm) was shallow, which caused longitudinal pressure on the radish roots. This barrier resulted in several curvatures of the fibrous root (Figure 3.20) (Brayton, 1996).

Figure 3.20 French Breakfast's curvatures of fibrous root at 33 DAS.

The nil control of the two varieties produced higher fresh & dry weights per tray than the *R. solani* inoculum rates. However, the fresh weight and dry weights per plant did not decrease as the *R. solani* inoculum rates increased. For example, the *R. solani* inoculum rate at 0.5g for RR had higher fresh (9.25g) and dry (0.702g) weight per plant than the nil control and 0.25g inoculum rate. This outcome is due to the plant response to changes in plant population and interplant competition. The inoculum rate at 0.5g for RR was able to reach maximum light interception in the low plant communities, because the low interplant competition between the plant spacing allowed a higher fresh and dry weight per plant (Mellendorf, 2011). However, 1g *R. solani* inoculum rate for FB produced lower fresh and dry weights than the other treatments. This was because the seedlings which survived post-emergence had already been severely damaged by *R. solani*.

In conclusion, the damage caused to radish production by *R. solani* was evident in the glasshouse experiment, a result previous reported by Kandula et al. (2015) for forage species. The different *R. solani* inoculum rates resulted in a reduction of plant growth. The inoculum rate of 0.25g resulted in around 50% damaged seedlings, and this was therefore the rate chosen to be used in Experiment 2.

# Chapter 4: Experiment 2

# To identify *Trichoderma* spp. capable of providing biocontrol of *R. solani*

## 4.1 Introduction

*Rhizoctonia solani* Kuhn is a pathogenic fungus causing damping-off and root rot diseases of vegetable crops. There are fungicides available (e.g. chlorothalonil, thyophanate methyl and iprodione) to control the pathogen. However often chemical control is not effective because *R. solani* can spread so rapidly from plant to plant (Soares de Melo & Faull, 2000), and inoculum can survive in the soil.

Biological control of plant pathogens by the addition of microorganisms to soil is a potential alternative (Elad, Chet, & Katan, 1980). *Trichoderma* is known worldwide as a beneficial soil-borne fungus that is capable of hyper-parasitizing pathogenic fungi (Elad et al., 1980; Khalili et al., 2012; Naeimi et al., 2010).

The application of *Trichoderma* spp. has significantly reduced the disease occurrence caused by pathogens such as *R. solani* and *Sclerotium rolfsii*, and promoted seedling growth (e.g. ryegrass; Kandula et al., 2015) and yield (e.g. beans; Elad et al., 1980). Henis et al. (1978) reported that *T. harzianum* effectively controlled the damping off in radish caused by soil-borne *R. solani* by the mechanism of antagonism.

Investigating biocontrol by *Trichoderma* spp. against *R. solani* as a pathogen of radish has not been previously undertaken. This chapter reports screening of *Trichoderma* strains from the Bio-Protection Research Centre collection for bioactivity against *R. solani* using dual culture assays, and then in a glasshouse pot trial.

## 4.2 Screening of *Trichoderma* isolates; production and application of *Trichoderma* innoculum

### 4.2.1 Production of *Trichoderma* inoculum

Isolates of 21 strains of eight *Trichoderma* species (Table 4.1) were provided from the Bio-Protection Research Centre's *Trichoderma* collection. They were selected based on their biocontrol capability data 'APP *Trichoderma* paper supplement lists' from the Bio-Protection Research Centre' and advice from Dr. Kandula (personal communication, March 2017). The isolates were placed onto the centre of a new PDA plate using the method described in 'Experiment 1: 3.2.1 Preparation of pathogen inoculum'. After that, the plates were sealed with para-film and placed in an incubator in a 12 hour light and dark cycle at 25ºC for 11 days.

Table 4.1 Strains used in this study.

| Species | Strains | Species | Strains |
|---|---|---|---|
| *T. asperellum* | LU512 | *T. polysporum* | LU809 |
| | LU691 | | LU1358 |
| *T. atroviride* | LU132 | | LU1361 |
| | LU521 | *T. spirale* | LU811 |
| | LU1514 | | LU827 |
| *T. hamatum* | LU592 | *T. virens* | LU540 |
| | LU785 | | LU547 |
| *T. harzianum* | LU819 | *T. viride* | LU620 |
| | LU514 | | LU616 |
| | LU1315 | | LU618 |
| | LU1347 | | |

- **Dual culture**

A colony of *R. solani* was sourced from the isolate used in Experiment 1. Two day old cultured *Trichoderma* strains (mycelium only) were used. This was because sporulation may occur in 3 day old *Trichoderma* isolates, which can result in a risk of spreading spores in dual culture assays that may cause non-uniform dual culture growth (Kandula, personal communication, March 2017).

Agar blocks from each *Trichoderma* isolate, and *R. solani* colony agar blocks (5mm) cut from PDA plates were placed on each side of a petri-plate with a space of 3 mm between the agar block and edge of the petri plate using a sterilized needle and scalpel (Figure 4.1).

A total of 63 petri-plates (= three replications per treatment) was used for the dual culture assays.

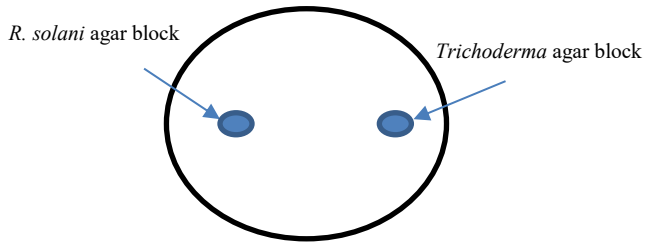


Figure 4.1 Dual culture assay between *R. solani* and *Trichoderma* spp. on a petri-plate.

After placing the agar blocks, the plates were sealed with para-film and were incubated for 9 days at 25°C under a regime of 12 hours white fluorescent light and 12 hours dark. Colony radial growth was measured daily using a digital caliper (mm) for 9 days.

- **Screening process**

Dual culture assays were assessed at day 9 to determine the interaction types of *Trichoderma* isolates. Two interaction types (B and D types) were classified as described below (Figure 4.2 and 4.3) (Kandula et al., 2015).

1. *'B'* type interaction: Growing margins of the two colonies meet. Pathogen growth inhibited and/or overgrown by the *Trichoderma* isolate (Figure 4.2).

2. *'D'* type interaction: Pathogen inhibited at a distance leaving a zone of inhibition (Figure 4.3).

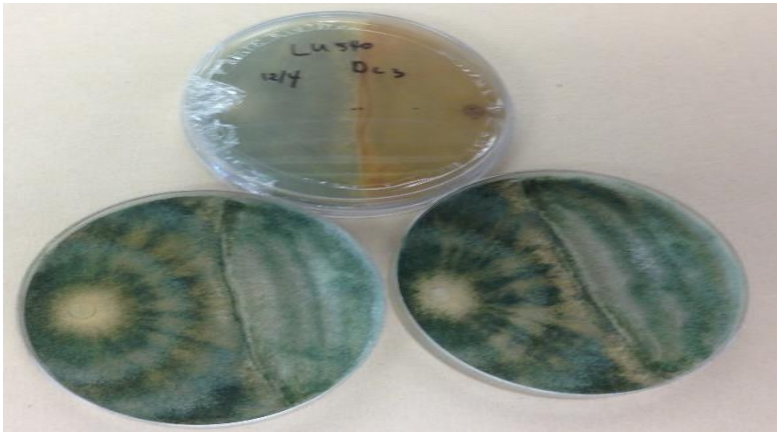

Figure 4.2 Dual culture assay ('*B*' type) between *T. virens* (LU540) and *R. solani* (RS73) at 9 days.

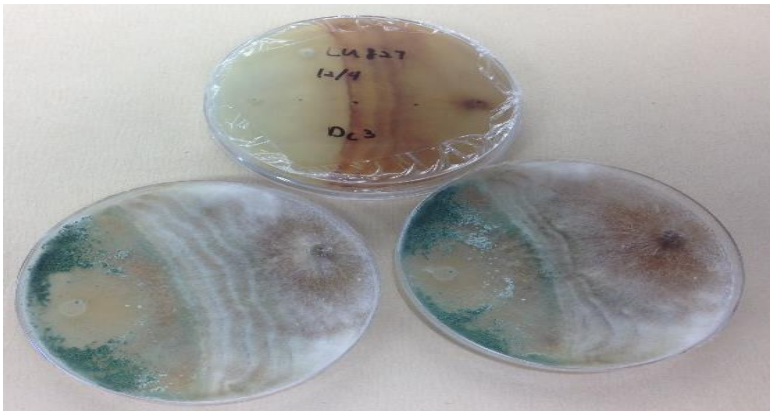

Figure 4.3 Dual culture assay ('*D*' type) between *T. spirale* (LU540) and *R. solani* (RS73) at 9 days.

- **Inoculation of *Trichoderma* strains and *R. solani* into wheat-bran and peat mix**

*R. solani* and each *Trichoderma* strain were inoculated into a wheat-bran and peat mix. *R. solani* was inoculated into three different sterilized plastic containers using the method described in Experiment 1 (3.2.1: Preparation of pathogen inoculum). However, *Trichoderma* spp. inoculation into the wheat-bran medium used potato dextrose broth (PDB) for rapid *Trichoderma* spore sporulation. PDB provides nutrients for subsequent growth of the fungus in a liquid formulation.

7ml of autoclaved (half strength) PDB (ratio 10g PDB per 1L of water) were poured using a pipette on the PDA plates of two weeks old *Trichoderma* isolates. Spores of the isolate were

scraped with a sterilized rod (aka 'hockey stick') and collected into a separate sterile universal bottle (=10mL). The rod was sterilized after every time the different *Trichoderma* isolate was scraped. The scraped spores (7mL) in the bottle were evenly poured and distributed using the rod into the separate wheat-bran medium in plastic containers. After that, the plastic containers were sealed within zip lock bags and kept at room temperature (21 – 25ºC) for 2 weeks.

### 4.2.2 Glasshouse experiment

Potting mix (500L; Peat: 70%, Pumice: 30% and Osmocote: 16 – 35 – 10) was provided as the plant growth medium from the Horticultural Research Centre, Lincoln University. The pumice (size 5mm to 10mm) was sieved prior to mixing.

Each pot (size 13cm x 14cm: 1.5L) was bleached for 15 mins and initially filled with l.25L of potting mix. *R. solani* was added to additional potting mix at a rate of 0.25g per 100g potting mix (see Experiment 1: 3.2.3) and *Trichoderma* isolates at 0.5g per 100g potting mix. There was a total of 364 pots (two radish varieties x 26 treatments x seven replications). The 26 treatments were 21 different *Trichoderma* isolates with *R. solani*; four *R. solani* controls (+ ve controls) and 1 nil control (- ve control).

Seeds of the two varieties were sown by hand in each pot. A total of 10 seeds were sown in rows spaced from 3cm to 3.5cm apart and 2 to 3 cm between seedlings in the rows. The pots were placed in the Horticulture Research Glasshouse in a completely randomized block design (seven replications) for 56 days (Figure 4.4). The glasshouse had a temperature range of 15.2 to 19.2ºC (average: 16.7ºC) with a relative humidity of >40%. Watering was done by water spray gun when the soil appeared dry.


Figure 4.4 Seedlings at 10 days after sowing (left), and randomized block design (right) in the Horticulture Research Glasshouse, Lincoln University in June 2017.

After sowing, the identical observations and measurements from ‘Experiment 1: glasshouse experiment’ were conducted at 20 DAS and 56 DAS (7 weeks). All data were analysed by ANOVA and GenStat version 18.

## 4.3 Results

### 4.3.1 Dual culture

The radial growth of *Trichoderma* isolates differed significantly between the isolates and days ($P<0.001$) (Figure 4.5). At day 2, the colony diameter of isolates LU1315 (16.92mm) and LU1347 (16.71mm) was significantly higher than that of the other *Trichoderma* isolates ($P<0.001$) (Figure 4.5). However, isolates LU521 (66.37mm) and LU132 (65.70mm) had significantly larger colony diameters than that of the other isolates from days 3 to 8.

The colony diameter of *R. solani* from day 2 decreased significantly with the increasing growth of the '*B*' type interaction isolates (LU132, LU512, LU521, LU540, LU547, LU691, LU514, LU819, LU811 and LU809) (Figure 4.5). However, the growth of *R. solani* did not decrease from day 3 in the presence of the '*D*' type *Trichoderma* isolates (LU592, LU616, LU1514, LU618, LU1347, LU1315, LU785, LU827, LU1361, LU620 and LU1358).

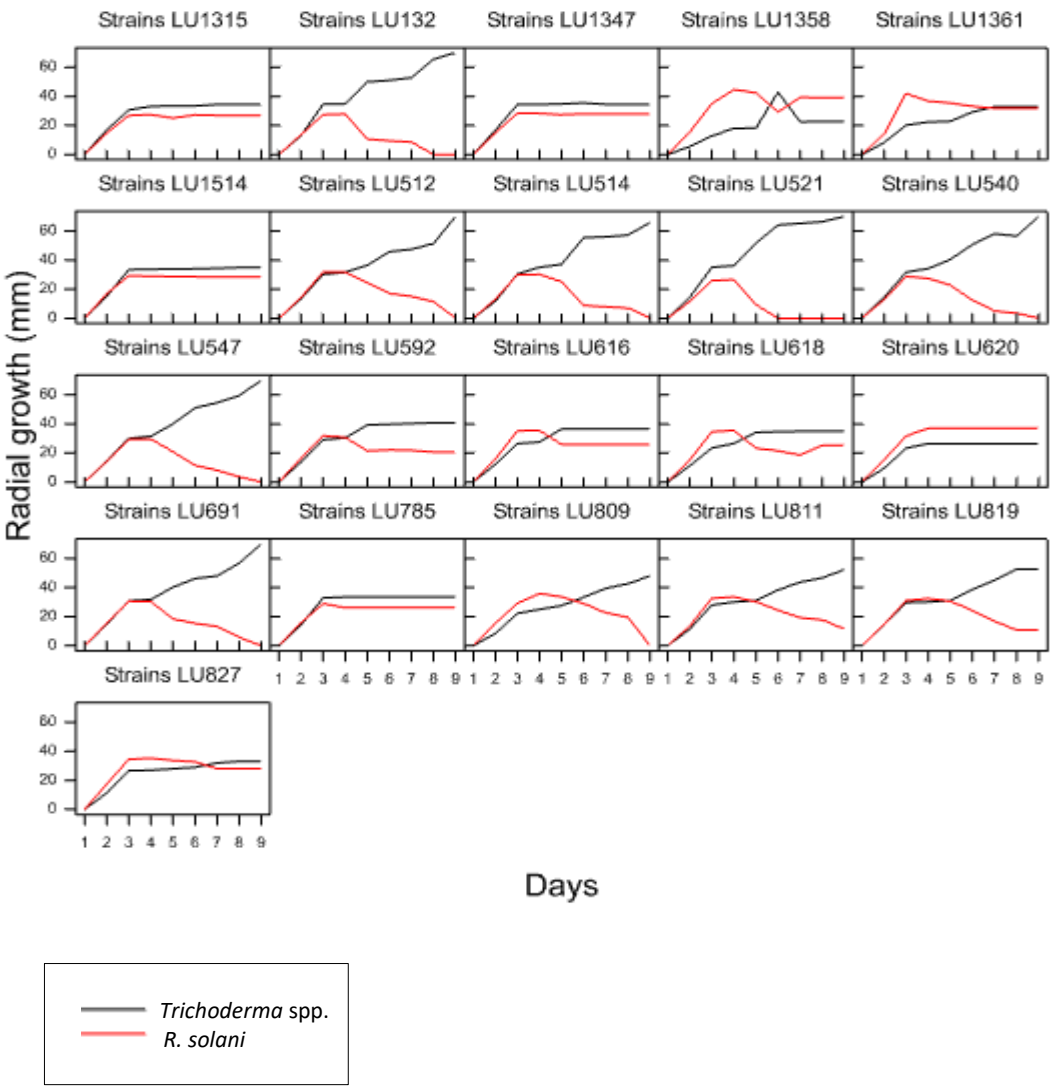


Figure 4.5 Radial growth of *Trichoderma* isolates and *R. solani* in dual culture assays for 9 days at an incubator at a 12 hour light and dark cycle at 25ºC (LSD 5% for *Trichoderma* isolates growth: 1.26; LSD 5% for *R. solani* growth: 2.26).

The two types of *Trichoderma* interaction ('*B*' and '*D*' types) in the dual culture assays were measured at 9 days (Table 4.2 and 4.3). There were significant differences in growth among the *Trichoderma* isolates, as well as among *R. solani* ($P<0.001$).

For the '*B*' type interaction', eight *Trichoderma* isolates; LU132, LU512, LU521, LU540, LU547, LU691, LU514 and LU809 completely inhibited and dominated the *R. solani* growth (Table 4.2). The radial growth of the first seven isolates (65.82mm to 70mm) at day 9 was significantly higher than that of the other isolates (LU819, LU811 and LU809).

For the '*D*' type interaction, only isolate LU592 had a colony radius that was significantly higher than the other isolates ($P<0.001$) (Table 4.3). *R. solani* colony diameter was reduced by

*Trichoderma* isolates LU592, LU616, LU1514, LU618, LU1347, LU1315, LU785, LU827 and LU1361 (Table 4.3).

Table 4.2 '*B*' type interaction at 9 days, dual culture. *Trichoderma* spp. vs *R. solani*.

| Species | Isolate | Interaction type | *Trichoderma* growth (mm) | *R. solani* growth (mm) |
|---|---|---|---|---|
| *T. atroviride* | LU132 | B | 70.00* | 0 |
| *T. asperellum* | LU512 | B | 70.00* | 0 |
| *T. atroviride* | LU521 | B | 70.00* | 0 |
| *T. virens* | LU540 | B | 70.00* | 0 |
| *T. virens* | LU547 | B | 70.00* | 0 |
| *T. asperellum* | LU691 | B | 70.00* | 0 |
| *T. harzianum* | LU514 | B | 65.82* | 0 |
| *T. harzianum* | LU819 | B | 52.64 | 10.75* |
| *T. spirale* | LU811 | B | 52.27 | 11.53* |
| *T. polysporum* | LU809 | B | 47.90 | 0 |
| LSD (5%) | - | - | 7.92 | 0.93 |
| Significance of differences | - | - | *** | *** |

Note: Mean value of three replications followed by significant differences according to Fisher's unprotected LSD test. '*' indicates isolates that differ significantly among the growth of *Trichoderma* isolates (P<0.05).

Table 4.3 '*D*' type interaction at 9 days, dual culture. *Trichoderma* spp. vs *R. solani*.

| Species | Isolate | Interaction type | *Trichoderma* growth (mm) | *R. solani* growth (mm) |
|---|---|---|---|---|
| *T. hamatum* | LU592 | D | 40.67* | 20.55 |
| *T. viride* | LU616 | D | 36.50 | 25.84 |
| *T. atroviride* | LU1514 | D | 34.88 | 28.37 |
| *T. viride* | LU618 | D | 34.82 | 25.25 |
| *T. harzianum* | LU1347 | D | 34.49 | 27.87 |
| *T. harzianum* | LU1315 | D | 34.33 | 26.87 |
| *T. hamatum* | LU785 | D | 33.50 | 26.15 |
| *T. spirale* | LU827 | D | 33.04 | 28.09 |
| *T. polysporum* | LU1361 | D | 32.84 | 31.83 |
| *T. viride* | LU620 | D | 26.38 | 36.88* |
| *T. polysporum* | LU1358 | D | 22.73 | 39.22* |
| LSD (5%) | - | - | 3.77 | 4.50 |
| Significance of difference | - | - | *** | *** |

Note: Mean value of three replications followed by significant differences according to Fisher's unprotected LSD test. '*' indicates isolates that differ significantly among the growth of *Trichoderma* isolates (P<0.05).

### 4.3.2 Assessment at 20 DAS (3 weeks)

The numbers of seedlings per pot up to 20 DAS did not differ significantly among the isolates or between the two varieties (LSD 5%: 1.765) (Figure 4.6). At 5, 10, 15 and 20 DAS, there were no interactions between variety or isolate (Table 4.4).

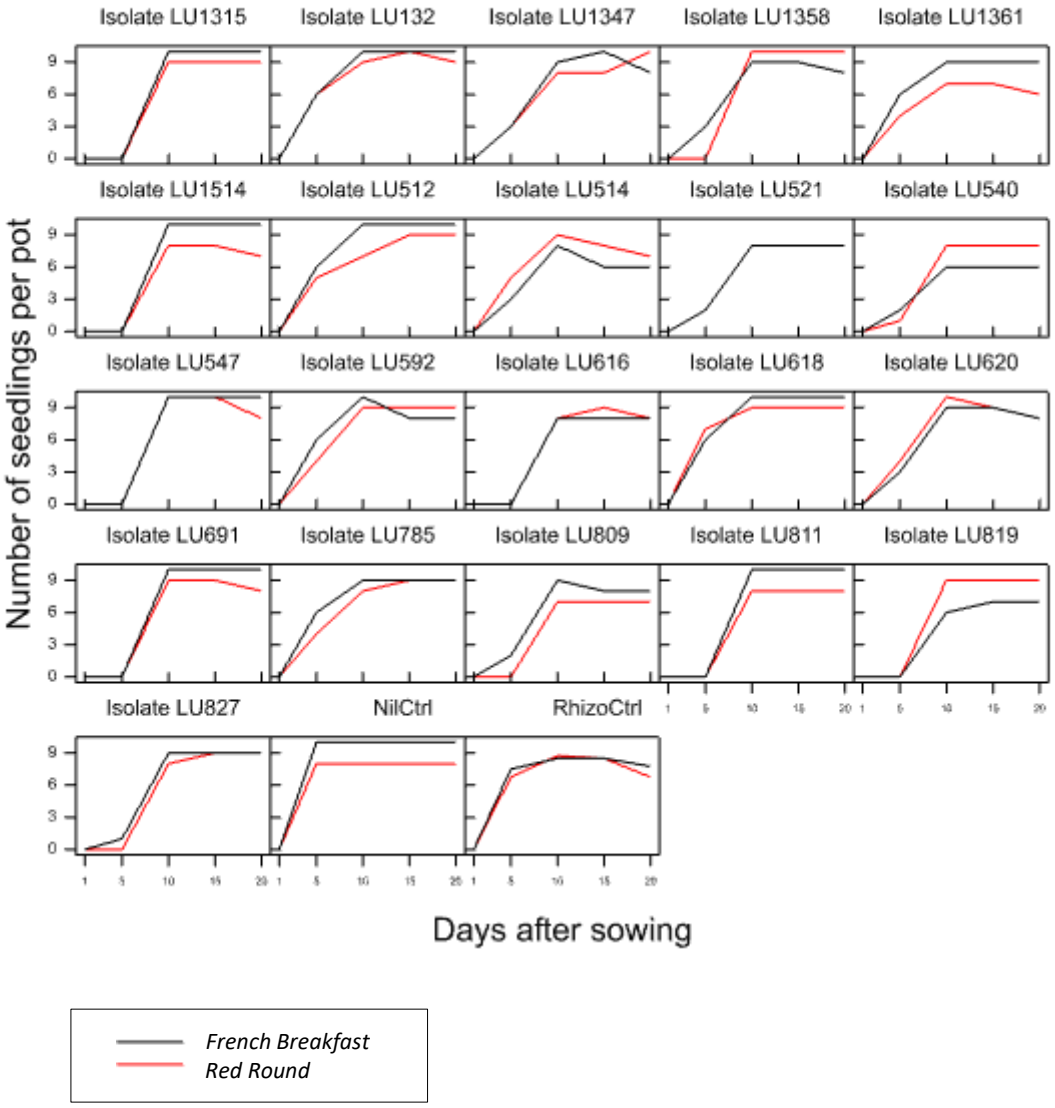


Figure 4.6 Effect of *Trichoderma* isolate and variety on number of seedlings per pot from 1 to 20 DAS in the Horticulture Research Glasshouse, Lincoln University, from May to June 2017.

Table 4.4 Least significant difference and significance of difference for the main effects of variety, isolate, and interaction on seedling numbers per pot at 5, 10, 15 and 20 DAS.

| FB vs RR | Day 5 | Day 10 | Day 15 | Day 20 |
|---|---|---|---|---|
| Main effect of variety | 1.610 | 1.100 | 1.239 | 1.505 |
| Main effect of isolate | 4.588 | 3.134 | 3.532 | 4.289 |
| Variety x isolate interaction | 6.488 | 4.432 | 4.995 | 6.066 |
| Significance of main effect of variety | ns | ns | ns | ns |
| Significance of main effect of isolate | ns | ns | ns | ns |
| Significance of interaction | ns | ns | ns | ns |
| ns = Not significantly different | | | | |

Maximum numbers of seedlings for the two varieties emerged between 5 and 15 DAS (Table 4.5 and 4.6). At the maximum emergence date, neither the numbers of seedlings to emerge nor the number of diseased seedlings, differed significantly among the isolates for either variety.

By 20 DAS, the number of seedlings per pot for the two varieties had begun to decline, while the numbers of diseased seedlings increased. Isolates LU521 and LU512 for French Breakfast, and isolates LU620 and LU819 for Red Round, had the highest numbers of diseased seedlings at 20 DAS (Table 4.5 and 4.6).

For French Breakfast isolate LU592 had the same disease score as the *R. solani* control (Table 4.5). For Red Round isolates LU1347, LU1358, LU540, LU691 and LU809 had the same disease score as the *R. solani* control (Table 4.6). There were no dead seedlings at the maximum emergence date. From then onwards, the numbers of seedlings that died post-emergence increased for both varieties (Table 4.5 and 4.6). However, the numbers of surviving seedlings, diseased seedlings, and seedlings that died post-emergence did not differ from the *R. solani* control at 20 days after sowing (Table 4.5).

Table 4.5 Comparisons of the variates (number of seedlings, diseased seedlings, disease score and seedlings that died post-emergence) at 20 DAS, cv French Breakfast.

| **French Breakfast** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Isolate | Maximum number of seedlings emerged | Days after sowing for maximum emergence | Number of diseased seedlings after maximum emergence | Number of surviving seedlings at 20 DAS | Number of diseased seedlings at 20 DAS | Disease score at 20 DAS | Number of seedlings which had died post emergence at 20 DAS |
| LU1315 | 10 | 10 | 0.0 | 10.0 | 1.0 | 1 | 0.0 |
| LU132 | 10 | 10 | 0.0 | 10.0 | 3.0 | 1 | 0.0 |
| LU1347 | 10 | 15 | 0.0 | 8.0 | 2.0 | 1 | 2.0 |
| LU1358 | 9 | 10 | 1.0 | 8.0 | 3.0 | 1 | 1.0 |
| LU1361 | 9 | 10 | 0.0 | 9.0 | 0.0 | 0 | 0.0 |
| LU1514 | 10 | 10 | 0.0 | 10.0 | 0.0 | 0 | 0.0 |
| LU512 | 10 | 10 | 0.0 | 10.0 | 4.0 | 1 | 0.0 |
| LU514 | 8 | 10 | 1.0 | 6.0 | 1.0 | 1 | 2.0 |
| LU521 | 8 | 10 | 0.0 | 8.0 | 4.0 | 1 | 0.0 |
| LU540 | 6 | 10 | 0.0 | 6.0 | 0.0 | 0 | 0.0 |
| LU547 | 10 | 10 | 0.0 | 10.0 | 0.0 | 0 | 0.0 |
| LU592 | 10 | 10 | 0.0 | 8.0 | 2.0 | 2 | 2.0 |
| LU616 | 8 | 10 | 0.0 | 8.0 | 0.0 | 0 | 0.0 |
| LU618 | 10 | 10 | 0.0 | 10.0 | 0.0 | 0 | 0.0 |
| LU620 | 9 | 10 | 0.0 | 8.0 | 3.0 | 1 | 1.0 |
| LU691 | 10 | 10 | 0.0 | 10.0 | 1.0 | 1 | 0.0 |
| LU785 | 9 | 10 | 0.0 | 9.0 | 1.0 | 1 | 0.0 |
| LU809 | 9 | 10 | 0.0 | 8.0 | 1.0 | 1 | 1.0 |
| LU811 | 10 | 10 | 0.0 | 10.0 | 3.0 | 1 | 0.0 |
| LU819 | 7 | 15 | 0.0 | 7.0 | 1.0 | 1 | 0.0 |
| LU827 | 9 | 10 | 0.0 | 9.0 | 1.0 | 1 | 0.0 |
| Nil control | 10 | 5 | 0.0 | 10.0 | 0.0 | 0 | 0.0 |
| *R. solani* control | 9 | 10 | 0.8 | 7.8 | 3.8 | 2 | 1.2 |
| LSD 5% | 4.2 | - | 2.1 | 7.9 | 3.4 | - | 1.8 |
| Significant difference | ns | - | ns | ns | ns | - | ns |

Table 4.6 Comparisons of the variates (number of seedlings, diseased seedlings, disease score and seedlings that died post-emergence) at 20 DAS, cv Red Round.

| **Red Round** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Isolate | Maximum number of seedlings emerged | Days after sowing for maximum emergence | Number of diseased seedlings at maximum emergence | Number of surviving seedlings at 20 DAS | Number of diseased seedlings at 20 DAS | Disease score at 20 DAS | Number of seedlings which had died post emergence at 20 DAS |
| LU1315 | 9 | 10 | 0 | 9.0 | 0.0 | 0 | 0.0 |
| LU132 | 10 | 15 | 0 | 9.0 | 2.0 | 1 | 1.0 |
| LU1347 | 10 | 10 | 0 | 10.0 | 4.0 | 2 | 0.0 |
| LU1358 | 10 | 10 | 0 | 10.0 | 2.0 | 2 | 0.0 |
| LU1361 | 7 | 10 | 0 | 6.0 | 0.0 | 0 | 1.0 |
| LU1514 | 8 | 10 | 0 | 7.0 | 0.0 | 0 | 1.0 |
| LU512 | 9 | 15 | 0 | 9.0 | 3.0 | 1 | 0.0 |
| LU514 | 8 | 10 | 1 | 7.0 | 2.0 | 0 | 2.0 |
| LU521 | 8 | 10 | 0 | 8.0 | 3.0 | 1 | 0.0 |
| LU540 | 8 | 10 | 0 | 8.0 | 2.0 | 2 | 0.0 |
| LU547 | 10 | 10 | 0 | 8.0 | 1.0 | 1 | 2.0 |
| LU592 | 9 | 10 | 0 | 9.0 | 2.0 | 1 | 0.0 |
| LU616 | 9 | 15 | 1 | 8.0 | 1.0 | 1 | 1.0 |
| LU618 | 9 | 10 | 0 | 9.0 | 4.0 | 1 | 0.0 |
| LU620 | 10 | 10 | 0 | 8.0 | 5.0 | 1 | 2.0 |
| LU691 | 9 | 10 | 1 | 8.0 | 3.0 | 2 | 1.0 |
| LU785 | 9 | 15 | 0 | 9.0 | 2.0 | 1 | 0.0 |
| LU809 | 7 | 10 | 0 | 7.0 | 1.0 | 2 | 0.0 |
| LU811 | 8 | 10 | 0 | 8.0 | 1.0 | 1 | 0.0 |
| LU819 | 9 | 10 | 0 | 9.0 | 5.0 | 1 | 0.0 |
| LU827 | 9 | 15 | 3 | 9.0 | 4.0 | 1 | 0.0 |
| Nil control | 8 | 10 | 0 | 8.0 | 0.0 | 0 | 0.0 |
| *R. solani* control | 9 | 10 | 1 | 6.8 | 4.5 | 2 | 3.0 |
| LSD 5% | 6 | - | 3 | 7.9 | 4.6 | - | 7.7 |
| Significant difference | ns | - | ns | ns | ns | - | ns |

### 4.3.3 Assessments at 56 DAS (9 weeks)

Seedling numbers per pot for the two varieties from 5 to 56 DAS are shown in Figure 4.7. Seedling numbers differed significantly between the isolates and the two varieties ($P<0.05$ and LSD 5%: 0.7436) (Figure 4.7). At 56 DAS, isolates LU132, LU785, LU1347 and LU1361 had significantly higher plant numbers for both varieties than the *R. solani* control (Figure 4.7). Only isolate LU514 had significantly fewer plants than the *R. solani* control.

Maximum numbers of seedling for the two varieties emerged by 15 DAS (Figure 4.7). The numbers of seedlings present for the two varieties were reduced after maximum seedling emergence. After 15 DAS, the number of dead plants of the two varieties increased, which resulted in the reduction of seedling numbers at 56 DAS.

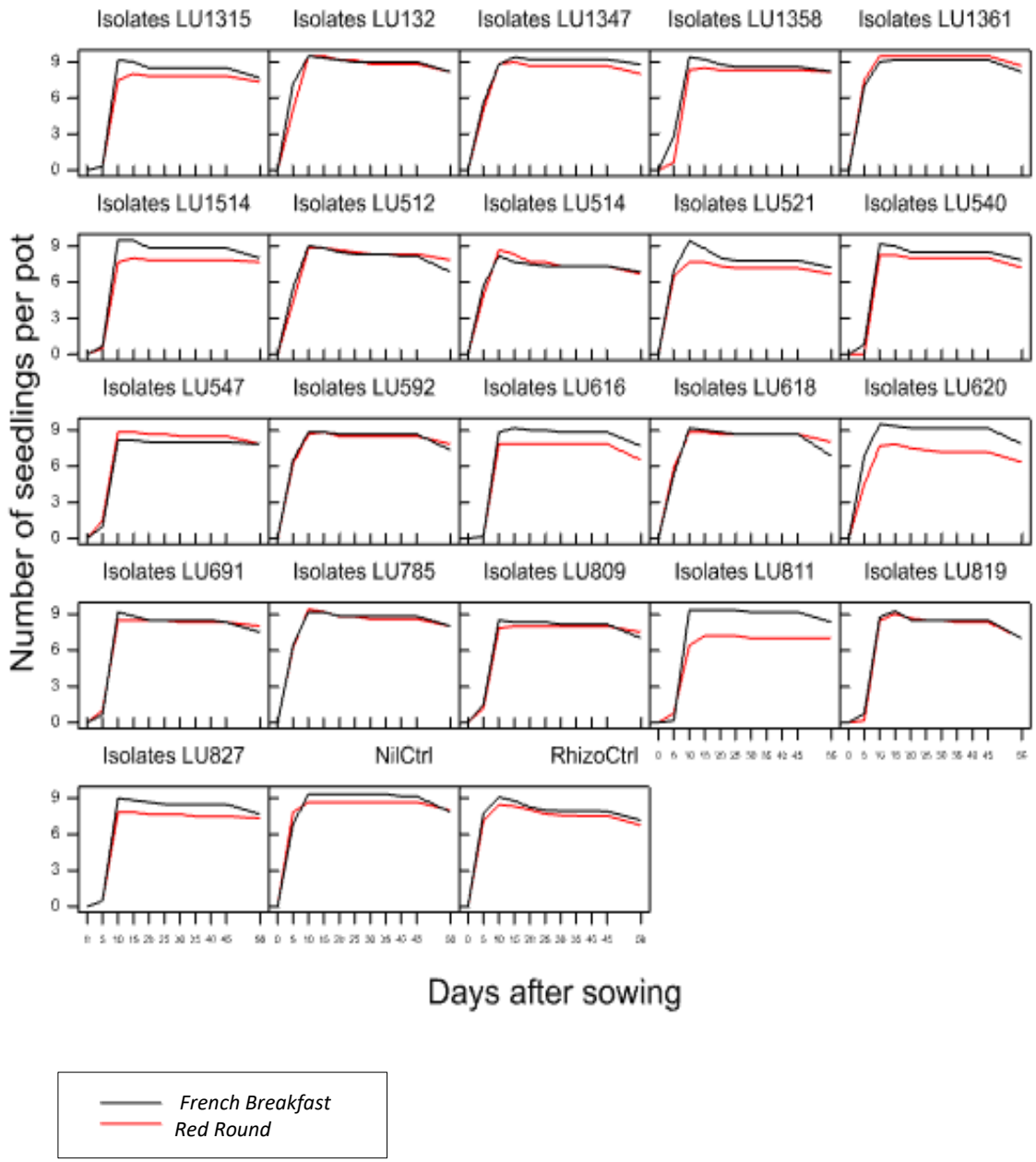


Figure 4.7 Effect of *Trichoderma* isolates on seedlings per pot of the two varieties for up to 56 DAS in the Horticulture glasshouse, Lincoln University in May to July 2017.

At 5 DAS, French Breakfast seedling emergence was significantly delayed by 15 of the isolates, as compared to the nil and *R. solani* control (Table 4.7). Only LU132, LU1361, LU521, LU592, LU620 and LU785 did not delay emergence. However, by 10 DAS, emergence of the isolates did not differ from the nil and *R. solani* controls, with the exception of LU514, which had a significantly lower emergence ($P<0.05$). At this time, all isolates had diseased seedlings, but only the number of diseased seedlings for LU616 did not differ from

that of the *R. solani* control. The number of dead seedlings did not differ among the treatments except for LU514 which was higher than all other treatments (Table 4.7).

At 15 DAS, the seedling numbers for French Breakfast did not differ among the treatments, with the exception of isolate LU514, which had a significantly lower number of seedlings than the *R. solani* control (Table 4.8). Diseased seedling numbers for the *R. solani* control were significantly higher than the *Trichoderma* isolates, except for isolate LU521 (Table 4.8). Numbers of dead seedlings at 15 DAS did not differ significantly among the treatments.

At 56 DAS, *Trichoderma* isolates LU1347 and LU811 had significantly higher numbers of surviving plants than the *R. solani* control (Table 4.8). Diseased plant numbers for the *Trichoderma* isolates were significantly lower than the *R. solani* control, apart from isolates LU691, LU616 and LU1347. The *R. solani* control had the highest disease score of 2, while all isolates had a disease score of 1.

The number of seedlings that died post-emergence did not differ significantly between the *Trichoderma* isolates and *R. solani* control (Table 4.8), apart from isolates LU1347 and LU547, which had significantly lower dead seedling numbers post-emergence than the *R. solani* control.

Table 4.7 Effect of *Trichoderma* isolates on the number of seedlings for French Breakfast at DAS and 10 DAS, and the number of diseased and dead seedlings at 10 DAS.

| **French Breakfast** | | | | |
|---|---|---|---|---|
| Isolate | Number of seedlings emerged at 5 DAS | Number of seedlings emerged at 10 DAS | Number of diseased seedlings at 10 DAS | Number of dead seedlings at 10 DAS |
| LU1315 | 0.33* | 9.17 | 0.00* | 0.83 |
| LU132 | 7.17 | 9.50 | 0.33* | 0.50 |
| LU1347 | 5.56* | 8.80 | 0.38* | 1.21 |
| LU1358 | 2.94* | 9.40 | 0.22* | 0.60 |
| LU1361 | 7.00 | 9.00 | 0.33* | 1.00 |
| LU1514 | 0.67* | 9.50 | 0.00* | 0.50 |
| LU512 | 5.50* | 9.00 | 0.17* | 1.00 |
| LU514 | 5.67* | 8.17* | 0.18* | 1.83* |
| LU521 | 6.89 | 9.43 | 0.60* | 0.57 |
| LU540 | 0.83* | 9.17 | 0.33* | 0.83 |
| LU547 | 1.00* | 8.17 | 0.17* | 1.83 |
| LU592 | 6.50 | 8.83 | 0.33* | 1.17 |
| LU616 | 0.17* | 8.83 | 0.83 | 1.17 |
| LU618 | 5.33* | 9.17 | 0.17* | 0.83 |
| LU620 | 6.83 | 9.50 | 0.17* | 0.50 |
| LU691 | 0.67* | 9.17 | 0.33* | 0.83 |
| LU785 | 6.33 | 9.17 | 0.17* | 0.83 |
| LU809 | 1.50* | 8.50 | 0.00* | 1.50 |
| LU811 | 0.17* | 9.33 | 0.50* | 0.67 |
| LU819 | 0.76* | 8.76 | 0.01* | 1.24 |
| LU827 | 0.50* | 9.00 | 0.00* | 1.00 |
| Nil control | 6.83 | 9.33 | 0.00* | 0.67 |
| *R. solani* control | 7.75 | 9.08 | 1.25 | 0.92 |
| LSD (5%) | 1.46 | 0.85 | 0.50 | 0.85 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$). At 5 DAS, there were no dead seedlings and only one diseased seedling in the *R. solani* control.

Table 4.8 Effect of *Trichoderma* isolates on seedling performance of French Breakfast at 15 DAS and 56 DAS.

| **French Breakfast** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Isolate | Number of surviving seedlings at 15 DAS | Number of diseased seedlings at 15 DAS | Number of dead seedlings at 15 DAS | Number of surviving plants at 56 DAS | Number of diseased plants at 56 DAS | Disease score at 56 DAS | Number of seedlings which had died post emergence at 56 DAS |
| LU1315 | 9.00 | 1.50* | 1.00 | 7.67 | 1.67* | 1 | 1.50 |
| LU132 | 9.33 | 1.00* | 0.67 | 8.17 | 0.83* | 1 | 1.33 |
| LU1347 | 9.37 | 1.22* | 0.63 | 8.77* | 1.78 | 1 | 0.63* |
| LU1358 | 9.22 | 1.09* | 0.78 | 8.23 | 1.12* | 1 | 1.17 |
| LU1361 | 9.17 | 1.33* | 0.83 | 8.17 | 1.67* | 1 | 1.00 |
| LU1514 | 9.50 | 1.00* | 0.50 | 8.00 | 1.33* | 1 | 1.50 |
| LU512 | 8.83 | 1.17* | 1.17 | 6.83 | 1.67* | 1 | 2.17 |
| LU514 | 7.67* | 2.00* | 2.33 | 6.83 | 1.00* | 1 | 1.33 |
| LU521 | 8.84 | 2.15 | 1.16 | 7.23 | 1.07* | 1 | 2.21 |
| LU540 | 9.00 | 2.00* | 1.00 | 7.83 | 1.17* | 1 | 1.33 |
| LU547 | 8.17 | 0.67* | 1.83 | 7.83 | 1.33* | 1 | 0.66* |
| LU592 | 8.83 | 1.33* | 1.17 | 7.33 | 1.67* | 1 | 1.67 |
| LU616 | 9.17 | 1.33* | 0.83 | 7.67 | 1.83 | 1 | 0.97 |
| LU618 | 9.00 | 1.83* | 1.00 | 6.83 | 1.33* | 1 | 2.33 |
| LU620 | 9.33 | 1.17* | 0.67 | 7.83 | 1.50* | 1 | 1.67 |
| LU691 | 8.83 | 1.33* | 1.17 | 7.50 | 2.00 | 1 | 1.83 |
| LU785 | 9.17 | 1.50* | 0.83 | 8.00 | 1.50* | 1 | 1.33 |
| LU809 | 8.33 | 1.50* | 1.67 | 7.00 | 1.17* | 1 | 1.50 |
| LU811 | 9.33 | 1.50* | 0.67 | 8.33* | 1.67* | 1 | 1.00 |
| LU819 | 9.20 | 1.84* | 0.80 | 6.90 | 1.17* | 1 | 2.37 |
| LU827 | 8.83 | 0.83* | 1.17 | 7.67 | 1.33* | 1 | 1.33 |
| Nil control | 9.33 | 0.00* | 0.67 | 7.83 | 0.00* | 0 | 1.50 |
| *R. solani* control | 8.79 | 2.96 | 1.21 | 7.17 | 2.42 | 2 | 2.00 |
| LSD (5%) | 0.89 | 0.96 | 0.89 | 1.08 | 0.74 | - | 1.03 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$).

For Red Round at 5 DAS, 16 of the *Trichoderma* isolates (LU1315, LU132, LU1347, LU1358, LU1514, LU512, LU514, LU540, LU547, LU616, LU620, LU691, LU809, LU811, LU819 and LU827) had a significantly ($P<0.001$) delayed seedling emergence compared with the nil and *R. solani* control (Table 4.9). There were no dead seedlings, and only one diseased seedling in the *R. solani* control.

At 10 DAS, seedling numbers did not differ significantly from the *R. solani* control, apart from isolates LU132, LU1361 and LU811. Two of the *Trichoderma* isolates (LU132 and

LU1361) had significantly higher numbers of seedlings than that the *R. solani* control (Table 4.9). All the isolates had diseased seedlings, with the exception of LU1358, LU1361, LU785, LU1514, LU540 and the nil control. Thirteen of the isolates (LU1315, LU1358, LU1361, LU1514, LU540, LU547, LU616, LU618, LU691, LU785, LU809, LU811 and the nil control) had significantly ($P<0.05$) lower numbers of diseased seedlings than the *R. solani* control. *Trichoderma* isolates LU132 and LU1361 had significantly lower numbers of dead seedlings than the *R. solani* control, but LU811 had a significantly higher number of dead seedlings than the nil and *R. solani* control.

At 15 DAS, isolates LU132 and LU1361 had significantly higher seedling numbers than the *R. solani* control (Table 4.10). However, LU811 had significantly fewer seedlings than the *R. solani* control. Diseased seedling numbers for the *R. solani* control were significantly ($P<0.05$) higher than the *Trichoderma* isolates, apart from isolates LU514, LU521 and LU592 (Table 4.10). For the number of dead seedlings, isolates LU132 and LU1361 were significantly lower than the *R. solani* control, but isolate LU811 had a significantly higher number of dead seedlings than the *R. solani* control.

At 56 DAS, *Trichoderma* isolates LU1315, LU132, LU1347, LU1358, LU1361, LU512, LU547, LU592, LU618, LU691 and LU785 had significantly higher numbers of surviving plants than the *R. solani* control (Table 4.10). All of the isolates had a significantly lower number of diseased plants than the *R. solani* control, with the exception of LU620, LU691 and LU785, which did not differ significantly from the *R. solani* control. All *Trichoderma* isolates had a disease score of 1, and the highest disease score was 2 for the *R. solani* control.

The number of seedlings to die post-emergence differed significantly among the isolates. By 56 DAS, isolates LU1315, LU1358, LU1514, LU592, LU618, LU691, LU809, LU811 and the nil control had significantly ($P<0.05$) lower numbers of dead seedlings than the *R. solani* control.

Table 4.9 Effect of *Trichoderma* isolates on the number of seedlings of Red Round at 5 DAS and 10 DAS, and the number of diseased and dead seedlings at 10 DAS.

| **Red Round** | | | | |
|---|---|---|---|---|
| Isolate | Number of seedlings emerged at 5 DAS | Number of seedlings emerged at 10 DAS | Number of diseased plants at 10 DAS | Number of dead plants at 10 DAS |
| LU1315 | 0.33* | 7.50 | 0.17* | 2.50 |
| LU132 | 5.00* | 9.50* | 0.83 | 0.50* |
| LU1347 | 5.00* | 8.83 | 0.33 | 1.17 |
| LU1358 | 0.67* | 8.33 | 0.00* | 1.67 |
| LU1361 | 7.50 | 9.50* | 0.00* | 0.50* |
| LU1514 | 0.50* | 7.67 | 0.00* | 2.33 |
| LU512 | 4.33* | 8.83 | 0.50 | 1.17 |
| LU514 | 4.83* | 8.67 | 0.33 | 1.33 |
| LU521 | 6.50 | 7.67 | 0.83 | 2.33 |
| LU540 | 0.13* | 8.19 | 0.00* | 1.81 |
| LU547 | 1.50* | 8.83 | 0.17* | 1.17 |
| LU592 | 6.17 | 8.67 | 0.33 | 1.33 |
| LU616 | 0.17* | 7.83 | 0.17* | 2.17 |
| LU618 | 5.83 | 8.83 | 0.17* | 1.17 |
| LU620 | 4.50* | 7.67 | 0.33 | 2.33 |
| LU691 | 1.00* | 8.50 | 0.17* | 1.50 |
| LU785 | 6.31 | 9.40 | 0.00* | 0.60 |
| LU809 | 1.17* | 7.83 | 0.17* | 2.17 |
| LU811 | 0.91* | 6.40* | 0.18* | 3.60* |
| LU819 | 0.17* | 8.50 | 0.67 | 1.50 |
| LU827 | 0.50* | 7.83 | 0.33 | 2.17 |
| Nil control | 7.83 | 8.67 | 0.00* | 1.33 |
| *R. solani* control | 7.13 | 8.46 | 0.67 | 1.54 |
| LSD (5%) | 1.44 | 0.97 | 0.46 | 0.97 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$).

Table 4.10 Effect of *Trichoderma* isolates on seedling performance of Red Round at 15 DAS and 56 DAS.

| **Red Round** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Isolate | Number of surviving seedlings at 15 DAS | Number of diseased plants at 15 DAS | Number of dead plants at 15 DAS | Number of surviving plants at 56 DAS | Number of diseased plants at 56 DAS | Disease score at 56 DAS | Number of seedling which had died post emergence at 56 DAS |
| LU1315 | 8.00 | 0.67* | 2.00 | 7.33* | 1.00* | 1 | 0.83* |
| LU132 | 9.50* | 1.33* | 0.50* | 8.17* | 0.17* | 1 | 1.33 |
| LU1347 | 9.00 | 1.33* | 1.00 | 8.00* | 1.00* | 1 | 1.00 |
| LU1358 | 8.50 | 0.33* | 1.50 | 8.17* | 0.67* | 1 | 0.67* |
| LU1361 | 9.50* | 0.50* | 0.50* | 8.67* | 1.67 | 1 | 1.00 |
| LU1514 | 8.00 | 0.50* | 2.00 | 7.67 | 1.17* | 1 | 0.33* |
| LU512 | 8.83 | 1.33* | 1.17 | 7.83* | 1.33* | 1 | 1.00 |
| LU514 | 8.33 | 1.83 | 1.67 | 6.67 | 1.17* | 1 | 2.00 |
| LU521 | 7.67 | 1.83 | 2.33 | 6.67 | 1.33* | 1 | 1.17 |
| LU540 | 8.16 | 0.99* | 1.84 | 7.20 | 1.25* | 1 | 0.98 |
| LU547 | 8.83 | 0.67* | 1.17 | 7.83* | 1.33* | 1 | 1.17 |
| LU592 | 8.83 | 1.83 | 1.17 | 7.83* | 1.17* | 1 | 0.83* |
| LU616 | 7.83 | 0.17* | 2.17 | 6.50 | 1.17* | 1 | 1.33 |
| LU618 | 8.83 | 1.17* | 1.17 | 8.00* | 1.33* | 1 | 0.83* |
| LU620 | 7.83 | 1.50* | 2.17 | 6.33 | 1.67 | 1 | 1.50 |
| LU691 | 8.50 | 0.50* | 1.50 | 8.00* | 1.67 | 1 | 0.67* |
| LU785 | 9.15 | 0.38* | 0.85 | 7.93* | 1.74 | 1 | 1.45 |
| LU809 | 8.00 | 0.33* | 2.00 | 7.50 | 1.00* | 1 | 0.50* |
| LU811 | 7.15* | 1.18* | 2.85* | 6.93 | 0.94* | 1 | 0.25* |
| LU819 | 9.00 | 1.50* | 1.00 | 7.00 | 1.17* | 1 | 2.00 |
| LU827 | 7.83 | 0.83* | 2.17 | 7.33 | 1.17* | 1 | 1.00 |
| Nil control | 8.67 | 0.17* | 1.33 | 8.00* | 0.00* | 0 | 0.67* |
| *R.solani* control | 8.33 | 2.54 | 1.67 | 6.75 | 2.04 | 2 | 1.79 |
| LSD (5%) | 0.90 | 0.78 | 0.9 | 1.01 | 0.57 | - | 0.89 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Healthy plant percentages for the two varieties differed significantly among the isolates (P<0.001) (Table 4.11). For French Breakfast, *Trichoderma* isolates had a significantly higher healthy plant percentage than the *R. solani* control, with the exception of LU512, LU592, LU618, LU691 and LU819.

For Red Round, healthy plant percentages also differed significantly between the isolates (P<0.001) (Table 4.11). All *Trichoderma* isolates had significantly higher percentages than the *R. solani* control, apart from the four isolates LU514, LU521, LU616 and LU620, which did not differ significantly from *R. solani*. However, none of the *Trichoderma* isolates for the two varieties had lower healthy plant percentages than the *R. solani* control.

Table 4.11 Effect of *Trichoderma* isolates on the healthy plant percentage of the two varieties at 56 DAS.

| Isolate | French Breakfast healthy plant (%) at 56 DAS | Red Round healthy plant (%) at 56 DAS |
|---|---|---|
| LU1315 | 60.00* | 63.33* |
| LU132 | 73.34* | 80.00* |
| LU1347 | 69.92* | 70.00* |
| LU1358 | 71.08* | 75.00* |
| LU1361 | 65.00* | 70.00* |
| LU1514 | 66.67* | 65.00* |
| LU512 | 51.66 | 65.00* |
| LU514 | 58.33* | 55.00 |
| LU521 | 61.56* | 53.33 |
| LU540 | 66.66* | 59.81* |
| LU547 | 65.00* | 65.00* |
| LU592 | 56.66 | 66.67* |
| LU616 | 58.34* | 53.33 |
| LU618 | 55.00 | 66.67* |
| LU620 | 63.33* | 46.67 |
| LU691 | 55.00 | 63.33* |
| LU785 | 65.00* | 61.90* |
| LU809 | 58.33* | 65.00* |
| LU811 | 66.66* | 59.90* |
| LU819 | 57.31 | 58.33* |
| LU827 | 63.34* | 61.67* |
| Nil control | 78.33* | 80.00* |
| *R. solani* control | 47.50 | 47.08 |
| LSD (5%) | 9.96 | 8.70 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$).

The shoot lengths of French Breakfast did not differ significantly among the isolates (Table 4.12). However, two of the *Trichoderma* isolates (LU1514 and LU811) produced significantly higher root lengths than the *R. solani* control (Table 4.12).

For Red Round, three of the isolates (LU132, LU1361 and LU827) had significantly higher shoot lengths than the *R. solani* control ($P<0.05$) (Table 4.13). However, none of the isolate root lengths differed significantly from the *R. solani* control (Table 4.13).

Table 4.12 Shoot length and root length of French Breakfast at 56 DAS in the Horticulture Research Glasshouse, Lincoln University, in July 2017.

| Isolate | Shoot length (cm) | Root length (cm) |
|---|---|---|
| LU1315 | 13.52 | 11.98 |
| LU132 | 13.96 | 12.20 |
| LU1347 | 12.84 | 10.92 |
| LU1358 | 13.35 | 11.91 |
| LU1361 | 13.82 | 11.62 |
| LU1514 | 13.22 | 12.58* |
| LU512 | 13.43 | 11.42 |
| LU514 | 12.77 | 11.57 |
| LU521 | 13.01 | 11.51 |
| LU540 | 13.35 | 11.72 |
| LU547 | 13.05 | 11.40 |
| LU592 | 13.93 | 12.02 |
| LU616 | 13.53 | 12.00 |
| LU618 | 13.25 | 11.65 |
| LU620 | 13.77 | 11.72 |
| LU691 | 12.80 | 12.00 |
| LU785 | 13.13 | 11.85 |
| LU809 | 13.60 | 11.73 |
| LU811 | 13.80 | 12.28* |
| LU819 | 13.36 | 11.26 |
| LU827 | 12.85 | 11.03 |
| Nil control | 13.12 | 11.07 |
| *R. solani* control | 13.03 | 11.04 |
| LSD (5%) | 1.22 | 1.23 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$).

Table 4.13 Shoot length and root length of Red Round at 56 DAS in the Horticulture Research Glasshouse, Lincoln University, in July 2017.

| Isolate | Shoot length (cm) | Root length (cm) |
|---|---|---|
| LU1315 | 12.08 | 10.03 |
| LU132 | 13.02* | 10.72 |
| LU1347 | 10.82 | 9.43 |
| LU1358 | 11.90 | 10.48 |
| LU1361 | 13.00* | 10.25 |
| LU1514 | 11.85 | 10.35 |
| LU512 | 11.75 | 9.95 |
| LU514 | 11.85 | 9.25 |
| LU521 | 11.20 | 10.32 |
| LU540 | 12.34 | 9.32 |
| LU547 | 12.23 | 10.38 |
| LU592 | 12.05 | 10.48 |
| LU616 | 11.88 | 9.48 |
| LU618 | 11.32 | 10.03 |
| LU620 | 11.42 | 9.72 |
| LU691 | 11.77 | 9.78 |
| LU785 | 12.42 | 10.76 |
| LU809 | 11.53 | 10.15 |
| LU811 | 11.44 | 9.64 |
| LU819 | 12.15 | 9.90 |
| LU827 | 13.08* | 10.30 |
| Nil control | 12.63 | 10.83 |
| *R. solani* control | 11.69 | 9.93 |
| LSD (5%) | 1.14 | 1.23 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$).

The fresh weight (g) per pot for French Breakfast differed significantly among the isolates (Table 4.14). Three of the *Trichoderma* isolates (LU1315, LU540 and LU616) produced significantly greater fresh shoot weights than the *R. solani* control. In particular, LU1315 and LU616 had significantly higher fresh shoot weights per pot than the nil control. However, the fresh weights per plant did not differ significantly from the *R. solani* control.

Both the dry weights per pot (g) and plant (g) differed significantly from the *R. solani* control. *Trichoderma* isolates LU1315, LU132, LU540, LU616 and LU620 had significantly heavier dry weights per pot than the *R. solani* control. Isolates LU540 and LU616 produced significantly higher dry weights per plant than the *R. solani* control.

Table 4.14 Effects of *Trichoderma* isolate on fresh and dry weights for French Breakfast.

| French Breakfast | Fresh weight | | Dry weight | |
|---|---|---|---|---|
| *Trichoderma* isolates | g/pot | g/plant | g/pot | g/plant |
| LU1315 | 74.26* | 9.73 | 3.863* | 0.505 |
| LU132 | 65.80 | 8.15 | 3.959* | 0.490 |
| LU1347 | 58.51 | 6.83 | 3.421 | 0.401 |
| LU1358 | 64.81 | 7.97 | 3.399 | 0.418 |
| LU1361 | 66.11 | 8.45 | 3.590 | 0.447 |
| LU1514 | 60.12 | 7.61 | 3.070 | 0.386 |
| LU512 | 65.16 | 9.59 | 3.303 | 0.492 |
| LU514 | 54.92 | 8.37 | 2.889 | 0.439 |
| LU521 | 66.52 | 9.29 | 3.683 | 0.510 |
| LU540 | 72.33* | 9.46 | 4.240* | 0.554* |
| LU547 | 57.04 | 7.37 | 3.249 | 0.415 |
| LU592 | 61.31 | 8.67 | 3.510 | 0.493 |
| LU616 | 74.28* | 9.94 | 4.105* | 0.553* |
| LU618 | 55.09 | 8.32 | 3.073 | 0.462 |
| LU620 | 65.81 | 8.55 | 3.903* | 0.505 |
| LU691 | 56.77 | 7.78 | 3.040 | 0.418 |
| LU785 | 69.86 | 8.94 | 3.737 | 0.482 |
| LU809 | 61.38 | 9.04 | 3.238 | 0.471 |
| LU811 | 55.74 | 6.64 | 3.342 | 0.397 |
| LU819 | 67.92 | 9.67 | 3.797 | 0.534 |
| LU827 | 60.63 | 7.99 | 3.250 | 0.429 |
| Nil control | 63.22 | 8.12 | 3.708 | 0.476 |
| *R. solani* control | 60.77 | 8.58 | 3.135 | 0.443 |
| LSD (5%) | 11.23 | 1.65 | 0.674 | 0.095 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$).

The fresh weights per pot and plant for Red Round differed significantly from the *R. solani* control (Table 4.15). *Trichoderma* isolates LU592 and LU785 produced significantly higher fresh weights per pot than the *R. solani* control. However, three of the isolates (LU1347, LU1358 and LU691) had significantly lower fresh weights per plant than the *R. solani* control.

Five of the *Trichoderma* isolates (LU132, LU1361, LU592, LU785 and LU809) had significantly higher dry weights per pot than the *R. solani* control (Table 4.15). Two of the *Trichoderma* isolates (LU1347 and LU1358) had significantly lower dry weights per plant than the *R. solani* control.

Table 4.15 Effects of *Trichoderma* isolate on fresh and dry weights for Red Round.

| Red Round | Fresh weight | | Dry weight | |
|---|---|---|---|---|
| *Trichoderma* isolates | g/pot | g/plant | g/pot | g/plant |
| LU1315 | 81.47 | 11.29 | 3.085 | 0.430 |
| LU132 | 79.36 | 9.95 | 3.705* | 0.462 |
| LU1347 | 76.59 | 9.46* | 3.163 | 0.392* |
| LU1358 | 72.21 | 8.91* | 3.038 | 0.375* |
| LU1361 | 88.64 | 10.29 | 3.818* | 0.443 |
| LU1514 | 81.70 | 10.87 | 3.338 | 0.445 |
| LU512 | 80.47 | 10.21 | 3.474 | 0.441 |
| LU514 | 70.55 | 10.63 | 2.925 | 0.439 |
| LU521 | 72.81 | 11.22 | 3.013 | 0.469 |
| LU540 | 71.96 | 10.04 | 3.217 | 0.448 |
| LU547 | 90.94 | 11.72 | 3.626 | 0.463 |
| LU592 | 100.03* | 12.95 | 3.879* | 0.498 |
| LU616 | 84.86 | 13.24 | 3.283 | 0.514 |
| LU618 | 81.17 | 10.20 | 3.558 | 0.448 |
| LU620 | 71.74 | 11.18 | 2.989 | 0.466 |
| LU691 | 76.67 | 9.45* | 3.200 | 0.393 |
| LU785 | 96.92* | 12.25 | 4.166* | 0.527 |
| LU809 | 84.43 | 11.18 | 3.669* | 0.484 |
| LU811 | 77.66 | 11.18 | 2.964 | 0.426 |
| LU819 | 69.72 | 10.12 | 3.138 | 0.457 |
| LU827 | 79.09 | 11.12 | 3.371 | 0.471 |
| Nil control | 86.57 | 10.95 | 3.525 | 0.446 |
| *R. solani* control | 77.21 | 11.98 | 3.135 | 0.482 |
| LSD (5%) | 13.93 | 2.06 | 0.553 | 0.072 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$).

## 4.4 Discussion

Results from the dual culture assays differed significantly among the isolates. The eight *Trichoderma* strains which had '*B*' type interactions significantly inhibited and grew over the *R. solani* pathogen. The eleven strains with '*D*' type interactions inhibited the pathogen at a distance, leaving an inhibition zone. Strains of *T. asperellum*, *T. atroviride*, *T. virens* and *T. harzianum* completely prevented the growth of *R. solani*, but all the *Trichoderma* spp. antagonized the pathogen growth to varying degrees. Similar variable responses have been reported against other pathogens (Henis, Adams, Lewis, & Papavizas, 1983; Rahman, Begum, & Alam, 2009).

Pot experiments were conducted to determine the ability of the *Trichoderma* strains to provide biocontrol against *R. solani* in glasshouse conditions. Maximum seedling emergence did not differ among treatments, but some *Trichoderma* isolates slightly delayed maximum seedling emergence, compared to the nil control. According to Junges, Muniz, Bastos, and Oruoski (2015), the bio-priming of bean seeds with *Trichoderma* spp. under water restricted conditions resulted in delayed seedling emergence. Because *Trichoderma* spp. can use the seed as a nutrition source, as saprophytic fungi they may cause seed death. However, where soil moisture is limiting (Mukhtar, 2008), or where nutrients compounds in the soil are limiting, *Trichoderma* can increase emergence (Okoth, Otadoh, & Ochanda, 2011), primarily because of more rapid root growth. The reasons for the delaying seedling emergence were not determined in this study, but may have been a random effect, as the three isolates which delayed emergence of French Breakfast did not do so for Red Round, and vice versa.

Between 15 and 56 DAS, the numbers of seedlings of the two varieties decreased, because the number of seedling killed by *R. solani* increased, a result also reported by Kandula et al. (2015). By 56 DAS, only two isolates for French Breakfast but eleven isolates for Red Round had more surviving plants than the *R. solani* control.

The numbers of diseased plants and seedlings that had died post-emergence for the two varieties at 56 DAS differed significantly among the isolates. Most of the *Trichoderma* isolates had significantly fewer diseased plants of the two varieties at 56 DAS than the *R. solani* control. *Trichoderma* can induce localized and systemic resistance by releasing bioactive molecules that colonize the root epidermis (Harman, 2005; Mghalu, Tsuji, Kubo, Kubota, & Hyakumachi, 2007). For the *R. solani* control, around 22% of the seedlings which emerged had died by 56 DAS, while for the most effective *Trichoderma* treatments, this was reduced to between 4 and 7%.

For French Breakfast isolates LU1347 and LU547 had significantly lower ($P<0.05$) numbers of dead seedlings than the *R. solani* control. For Red Round isolates LU1315, LU1358, LU1514, LU592, LU618, LU691, LU809 and LU811 also had significantly lower numbers of dead seedlings. *Trichoderma* spp. can produce antibiotics which interrupt and inhibit fungal pathogen mycelial growth (Dubey et al., 2011; Ghisalberti & Sivasithamparam, 1991), but whether this was the mechanism for the above results was not determined.

For French Breakfast isolates LU1315, LU132, LU540, LU616 and LU620 and for Red Round isolates LU132, LU1361, LU592, LU785 and LU809 produced significantly heavier dry weights per pot than the *R. solani* control. In French Breakfast isolates LU540 and LU616 produced significantly greater dry weight per plant than the *R. solani* control, but this did not occur in Red Round. However, the results expressed per pot may not reflect the results for each individual plant, because if as a result of disease attack there are fewer seedlings per pot, this may allow the production of bigger plants, as there will be less competition for resources (Kandula, et al., 2015).

In the absence of a pathogen, stimulation of plant growth induced by *Trichoderma* fungi is widely established (Harman 2005; Inbar et al., 1994; Kandula et al., 2015; Nieto-Jacobo et al., 2017). However, with one exception, this response was not recorded in this experiment when plant growth parameters for the *Trichoderma* isolates were compared to that of the nil control. Only LU785 significantly increased dry weight per pot and per plant for Red Round, but not for French Breakfast. Plant growth stimulation has been attributed to enhanced root growth, allowing increased uptake and use of nutrients (Kandula et al., 2015; Harman et al., 2004; Henis et al., 1978), but in this experiment root length was not increased. However because dry weight was determined on a whole plant basis, any possible increases in root dry weight were not detected.

In conclusion, there was no strong relationship between dual culture assay and pot trial results, as previously reported for other potential biocontrol agents (Ghazalibiglar, Hampton, van Zijildejong, & Holyoake, 2016; Schreiber, Gregory, Krause, & Ichida, 1998; Wulff, Mguni, Mansfeld-Giese, Fels, Lubeck, & Hockenhull, 2002), and for *Trichoderma* by Montealegre, Ochoa, Besoain, Herrera, and Perez (2014). *Trichoderma* spp. may have given different results in the dual culture assays and glasshouse experiments because of their differing control mechanisms (e.g. antibiosis, mycoparatism, symbiosis and induced systemic resistance) (Mghalu et al., 2007). While antibiosis can be detected in a dual cultures assay, other mechanisms can not (Ghazalibiglar et al., 2016). The selection of potential biocontrol

agents based solely on dual culture assays may lead to poor and contradictory results in in *vitro* studies (Ghazalibiglar et al., 2016).

*Trichoderma* spp. can suppress *R. solani* (Mghalu et al., 2007), and lead to a decline in severity of disease compared to the *R. solani* control. Moreover, some *Trichoderma* isolates increased the healthy plant percentage and plant growth parameters compared to the *R. solani* control. Therefore, the addition of *Trichoderma* spp. to the potting mix suppressed radish seedling damping-off caused by *R. solani*, a result also reported by Henis et al. (1978), Mghalu et al. (2007) and Yobo, Laing, & Hunter (2011).

On the basis of their performance in the pot trials, four *Trichoderma* isolates, LU132, LU785, LU1347 and LU1358 were selected for further experiments. These isolates represented four species; *T. atroviride* (LU132), *T. hamatum* (LU785), *T. harzianum* (LU1347) and *T. polysporum* (LU1358) known to be successful biocontrol agents worldwide (Henis et al., 1978; Samules & Hebbar, 2015; Schuster & Schmoll, 2010). In particular, strain LU132 of *T. atroviride* has been proven to be a beneficial BCA in the New Zealand agriculture and horticulture industry (Agrimm Technologies, 2014). Therefore, the selected strains were formulated into seed treatments in the Experiment 3 pot trials and Experiment 4 field trials.

# Chapter 5: Experiment 3

# *Trichoderma* control of the pathogen in naturally infested soil

## 5.1 Introduction

Integrated pest management (IPM) is an ecosystem-based strategy for reducing pest or disease damage through a variety of techniques (e.g. biological, cultural, chemical, mechanical and physical controls) (Bottrell & Smith, 1982; EPA, 2017; UCIPM, 2017). In many production systems, fungicides are still commonly used because they are effective at minimising pathogen attacks in the short term. However, they may cause phytotoxicity and environmental damage when used excessively. The use of bio-control agents may be an alternative to the use of synthetic chemical compounds.

Henis et al. (1978) reported that the application of 0.04 - 0.15g/kg of *T. harzianum* wheat-bran cultures to the soil protected radish seedlings from damping off, and increased radish emergence. More recently, Kandula et al. (2015) reported that the addition of *T. atroviride* (LU140) and *T. virens* (LU540) increased seedling emergence in soil naturally infected with *R. solani*. In addition, seed treatment or bio-priming by *Trichoderma harzianum* alleviated drought and salinity stress in rice (Rawat, Shah, Shukla, & Kumar, 2012), and wheat (Shukla, Awasthi, Rawat, & Kumar, 2014). Each method of application (wheat-bran and seed coating) was reported to be beneficial to plant growth.

In the previous glasshouse experiments, *R. solani* grown in cultures was added to the potting mix growing medium. For this experiment, the four *Trichoderma* strains (LU132, LU785, LU1347 and LU1358) selected from the results of Experiment 2 were formulated into both a seed coating and wheat-bran inoculum and tested for their biocontrol ability in soil shown by Kandula et al. (2015) to be naturally infested with *R. solani*.

## 5.2 Materials and methods

### 5.2.1 Preparation of 1st glasshouse experiment (Inoculation of *Trichoderma* strains and *R. solani* wheat-bran mix inoculation)

- **PDA**

The four *Trichoderma* isolates were sub-cultured on new PDA plates (60 plates per isolate) for three weeks. A colonized block (5mm circle per block) cut from each isolate was placed in the centre of a new PDA plate using a sterilized cork borer, needle and scalpel (see 3.2.1. Preparation of pathogen inoculum). The plates were then sealed with parafilm and incubated at 25ºC for two weeks in a 12 hour light/dark cycle.

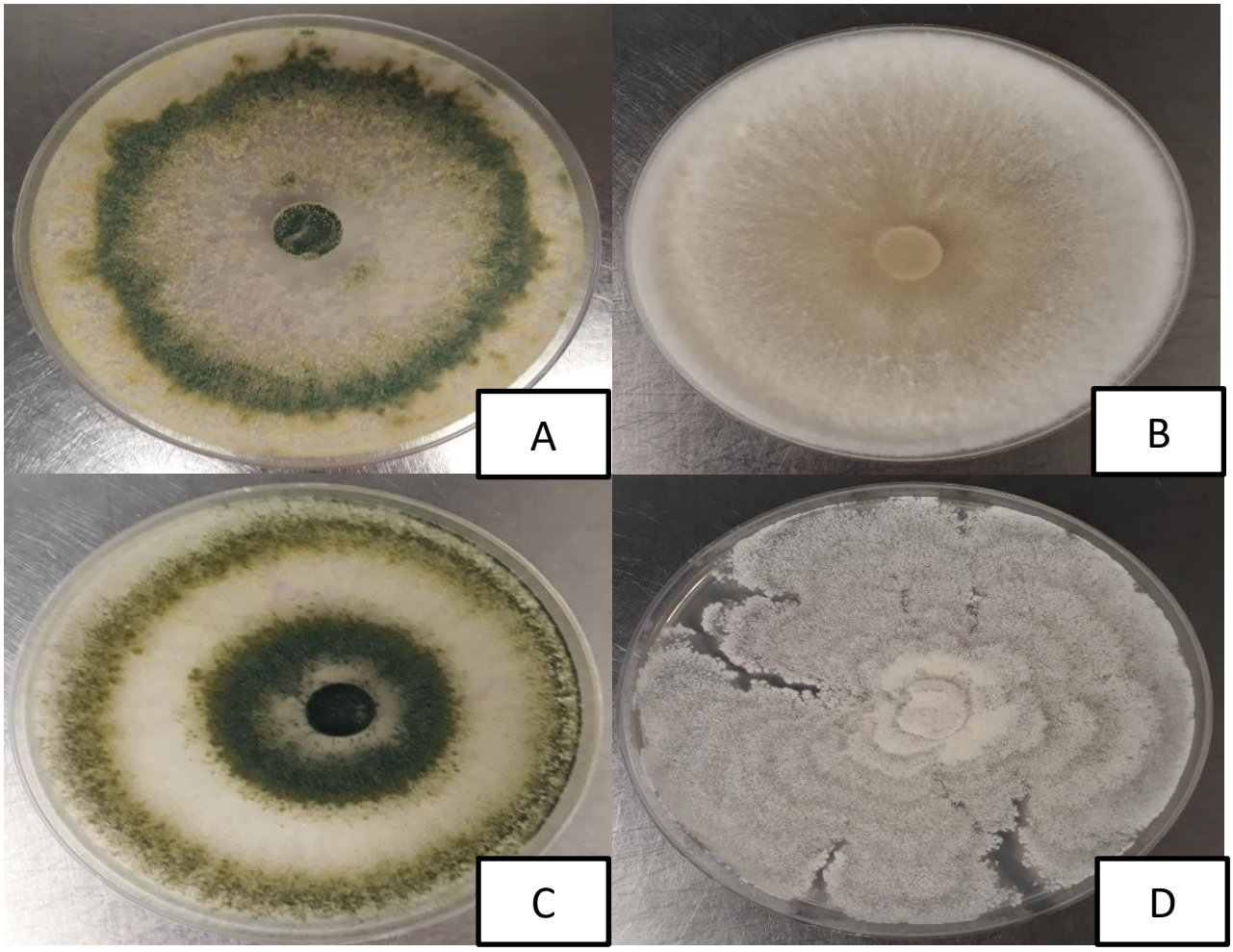


Figure 5.1 *Trichoderma* isolates on PDA after 2 weeks in incubator. A: *T. atroviride* (LU132), B: *T. hamatum* (LU785), C: *T. harzianum* (LU1347) and D: *T. polysporum* (LU1358).

- **Inoculation of *Trichoderma* strains into wheat-bran & peat mix**

4L of wheat-bran mix (ratio wheat-bran to peat to water = 1:1:1) was autoclaved for 15 minutes at 121°C, 15 Psi. *Trichoderma* isolates (strains LU132, LU1347, LU1358 and LU785) were inoculated into two sterilized plastic containers (approx. 200g wheat-bran mix per container) using the method described in 4.2.1 (Production of *Trichoderma* inoculum).

Strain LU1358 in the wheat-bran medium had not sporulated after 10 days, whereas the other *Trichoderma* strains sporulated within 7 days (Figure 5.2). Therefore, a furtherer 7ml LU1358 inoculated PDB was added to the containers, which were placed under blue light at 23°C for 24 hours. Blue light is known to promote *Trichoderma* sporulation, reproduction and secondary metabolite biosynthesis (Alizadeh, personal communication, May 2017).

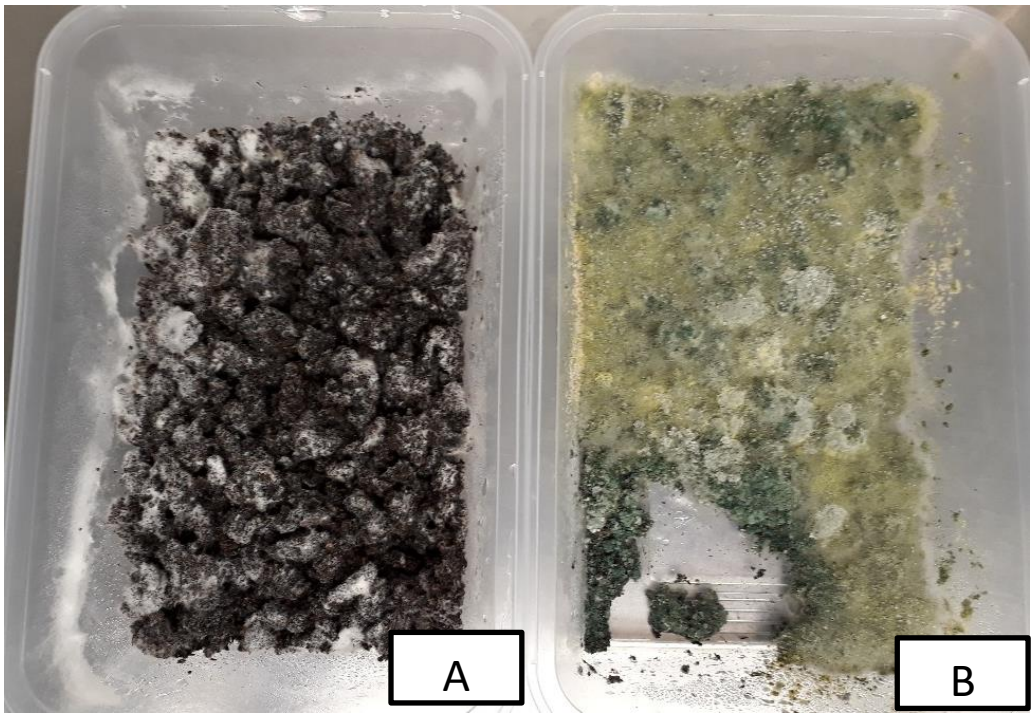


Figure 5.2 LU1358 in wheat-bran (A: whitish mycelium only) & LU785 in wheat-bran (B: greenish spores) 10 days after inoculation.

- **Glasshouse experiment**

Dry *R. solani* infested soil (= Wakanui silt loam: approx. 500L collected 3 months previously) was provided from the Bio-protection Research Centre (Kandula, personal communication, March 2017). Sieved pumice (size 5 - 10mm) was mixed with the infested soil (dilution ratio of *R. solani*-infested soil to pumice = 4:1). Kandula (personal communication, March 2017) recommended a 4:1 dilution because previous emergence showed that it resulted in seedling damping-off caused by *R. solani* of approximately 50%. A total of 80 pots were prepared for the following treatments: control (*R. solani* infested soil), and four *Trichoderma* strains (LU132, LU785, LU1347 and LU1358); two radish varieties x five treatments x eight replications.

Each pot (size 13 x 14cm = 1.5L) was surface sterilised with 10ml of NaOCl per 10L water for 15 minutes, and ambient air dried for two weeks. Next, the pots were filled with 1.25L natural *R. solani*-infested soil. Ten seeds were sown per pot, as described in 'Experiment 2: Glasshouse experiment'. The seeds were placed on top of the soil in rows spaced 3.0 - 3.5cm apart, with 2 - 3cm between each seed (Figure 5.3).

A total of 250mL infested soil (approx. 200g) was mixed with each *Trichoderma* isolate in wheat-bran (0.5g wheat-bran per 100g infested soil) in a 20L plastic bucket, and evenly added to the top layer of the sixteen pots for each *Trichoderma* isolate. Next, all pots were placed in a glasshouse in a randomized block design (Figure 5.4). The glasshouse temperature ranged from 10.4ºC - 27.1ºC (average $16.9^0$C). Pots were regularly watered to avoid moisture stress.


Figure 5.3 Radish seedlings in a pot at the Horticulture Research Glasshouse, Lincoln University.

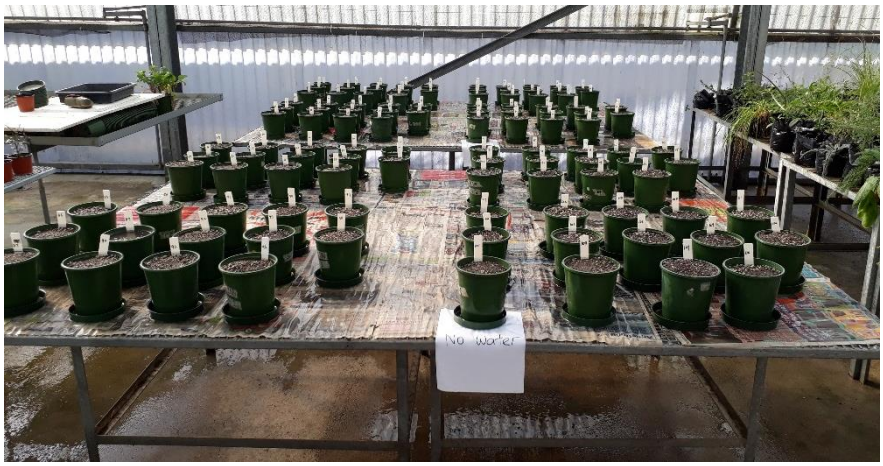


Figure 5.4 The experimental design was a completely randomized block in the Horticulture Research Glasshouse, Lincoln University from August to September 2017.

- **Data collection at 24 DAS and 40 DAS after sowing**

After sowing, the numbers of emerged seedlings, diseased seedlings and dead seedlings were counted every two days up to 40 DAS. In addition, a SPAD (Soil Plant Analysis Development) chlorophyll meter (wavelength 502nm) was used from 24 DAS to measure leaf chlorophyll concentration (Figure 5.5). Ten randomly-chosen healthy leaves per pot were measured (mature leaf size more than 30 x 30mm). If leaf size was less than 30 x 30mm, it could not be analysed by the chlorophyll meter (data shown as 'error').


Figure 5.5 SPAD chlorophyll measurements. Ten randomly-selected leaves were measured in the Horticulture Research Glasshouse, Lincoln University.

At 24 DAS (one replicate) and 40 DAS, the observations and measurements were conducted as described in Experiment 1 'Data collection'. Each plant was carefully extracted from the pot without removing any fibrous roots and washed using a spray-gun to remove soil residue from the root surface. Next, the number of diseased seedlings was counted; and disease score (category 0 to 3), plant parameters, shoot length (growing point to end), root length (fibrous root and hypocotyl) and hypocotyl radial size were measured using a digital caliper (mm) (Figure 5.6). Each plant part (shoot, hypocotyl and fibrous root) was cut separately using scissors and the fresh weight of each plant part was measured using an electronic scale (g) that weighed to two decimal points. Next, the leaf numbers of surviving plants per pot were counted, and the leaf area ($cm^2$) measured using a leaf area meter (Li-3,100C area meter).

After taking these measurements, the plant parts were enclosed in a separate paper bag (size 90 x 50 x 205 mm for shoot and hypocotyl; size 127 x 89mm envelope for fibrous roots) and dried in an oven at $65^0$C for three days. Finally, the dry weights of both shoots and roots

(hypocotyl and fibrous roots) were measured. Data from the 40 DAS assessments were analysed by GenStat version 18 (e.g. ANOVA and Fisher's unprotected LSD test).


Figure 5.6 Isolate LU132 (Red round) (left). Plant parameters (right) were measured using a digital caliper.

### 5.2.2 Colony forming units

*Trichoderma* colony forming units (CFU) were recorded in both the 1st and 2nd (see 5.2.3) glasshouse experiments at 20 DAS.

#### i) Preparation of *Trichoderma* selective media

*Trichoderma* selective media (= TSM, aka 'Rose Bengal streptomycin agar') was prepared. The chemical ingredients, 1g $KH_2PO_4$, 0.5g $MgSO_4$, 5g tryptone peptone, 10g glucose anhydrous, 150 mg rose bengal and 20g PDA were weighed out and dissolved in l L distilled water in a Schott bottle (Figure 5.7). The medium was wet-cycle autoclaved for 15 mins at 121ºC, 15 Psi.

After autoclaving, 3 ml of 1% streptomycin sulphate stock solution with 0.2 ml formaldehyde was added into the medium using a pipette. Streptomycin should not be autoclaved because it is inactivated by 50% at 100ºC within minutes (Applichem, 2017). Approximately 16ml of the medium was poured in a laminar flow cabinet into a sterile petri-plate, and the plates were allowed to solidify for 30 min in the laminar-flow cabinet. The lids were placed onto the plates, and the plates were stored at room temperature (20ºC) before being using (Figure 5.7). A total of 160 petri-plates (= twenty soil samples x two dilution factors x two varieties x two replications) were used for determining CFU.

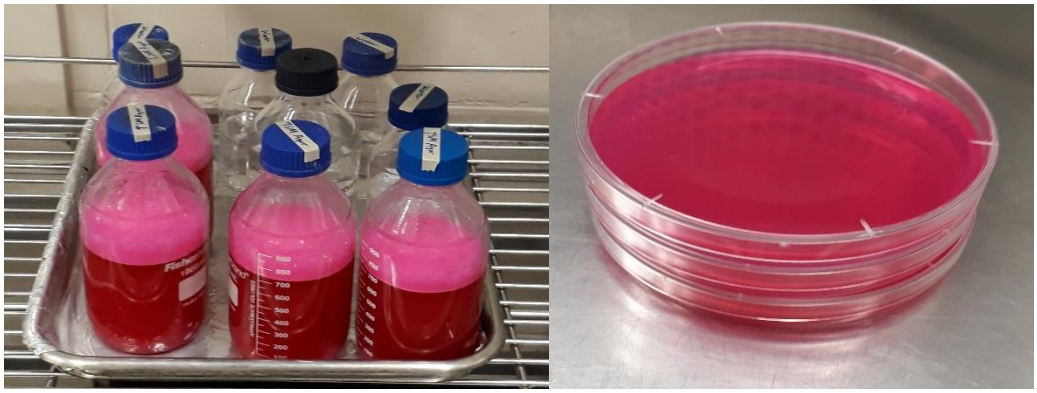

Figure 5.7 *Trichoderma* selective media after autoclaving (left) and poured plates (right).

After the preparation of TSM, 10ml distilled water was poured into each of 48 Schott bottles, and 9ml of distilled water into each of 96 universal bottles using an electronic dispenser. The bottles were wet-cycle autoclaved for 15 minutes at 121ºC, 15 Psi, and then stored at room temperature (21ºC). The Schott bottles were used as dilution factors for $10^{-1}$ (100µL), and the universal bottles used for $10^{-2}$ and $10^{-3}$ dilutions.

### ii) Soil sampling

Four replicates of each isolate were sampled. A spoon was used to extract 10 – 15g soil, from a depth of 5 - 7cm from each pot. Samples were collected in plastic zip-lock bags (size 50 x 75mm) and labelled, then stored at $-8^0$C before being used to determine CFU. Care was taken not to sample pumice, bark or plant residues (e.g. fibrous root).

### iii) Colony forming units and analysing

Soil samples were weighed to 10g using an electric scale and diluted by adding 90 ml water to each $10^{-1}$ Schott bottle, then labelled and mixed using a flask shaker at 300 osc/minute for 10 minutes (Figure 5.8).

After mixing, 1ml of diluted water from a $10^{-1}$ Schott bottle was extracted using a pipette, then transferred into a $10^{-2}$ universal bottle containing 9ml distilled water which was vortexed for 5 seconds. Next, 1ml of diluted water from the $10^{-2}$ universal bottle was transferred into $10^{-3}$ universal bottles and vortexed for 5 seconds. After each transferring, the pipette tip was renewed to prevent contamination.

- *R. solani* control: colony forming units to $10^{-1}$ and $10^{-2}$
- *Trichoderma* isolates: colony forming units to $10^{-1}$, $10^{-2}$ and $10^{-3}$

After dilution, 0.1ml (100µL) of diluted water was extracted from each bottle ($10^{-1}$, $10^{-2}$ and $10^{-3}$) using a pipette and poured into separate TSM, then gently distributed using a sterilized rod. Pouring into TSM was done from the lowest concentration to the highest concentration (i.e. $10^{-3}$ to $10^{-2}$ then to $10^{-1}$).

The lids were then placed on the petri-plates and labelled, sealed with para-film, and stored at room temperature (23ºC) without UV light for 7 days. After 7 days, each colony of *Trichoderma* on the plates were counted by naked eye. Each colony of *Trichoderma* on petri-plate were dull yellowish and dark greenish depending on *Trichoderma* spp.

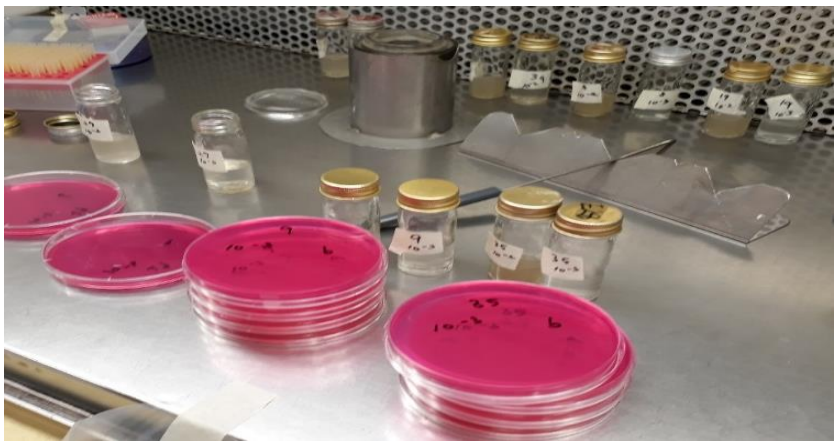

Figure 5.8 *Trichoderma* selective media and universal bottles ($10^{-2}$ and $10^{-3}$).

### 5.2.3 Preparation of 2nd glasshouse experiment

In the second experiment the *Trichoderma* strains were applied in a seed coating only.

- **Seed coating**

Seeds of the two radish varieties were coated with the four selected *Trichoderma* strains (LU132, LU785, LU1347 and LU1358) to use in both the 2nd glasshouse and field experiment. The inoculum rate of *Trichoderma* was 0.3g spores per 20g seed with 0.28µL seed coating polymer (ratio polymer to water = 1:1) (Germains Seed Technology, n.d.).

**i) *Trichoderma* seed coating from PDA**

Each *Trichoderma* isolate was grown on PDA in a 12-hour light/dark cycle at a temperature of $25^0$C for one month (see Experiment 2: 4.2.5 Production of *Trichoderma* inoculum). After one month, sterilized water (approx. 5ml) was poured into each PDA plate using a pipette in a laminar flow cabinet. A sterilized rod was then used to mix spores into the water before the solution was poured into a one-litre beaker. The beakers were covered in aluminium foil to prevent contamination.

### ii) *Trichoderma* seed coating from wheat-bran mix (or bulk spore production)

A wheat-bran mix was made for each *Trichoderma* isolate using the method described in Experiment 1 (3.2.1: Preparation of pathogen inoculum). *Trichoderma* spp. inoculation into wheat-bran mix using PDB was carried out using the method described in Experiment 2 (4.2.1 Production of *Trichoderma* inoculum). Each *Trichoderma*-inoculated wheat-bran mixture was placed in an incubator at 25ºC for 6 weeks. After that, 300ml sterilized water was added to the *Trichoderma*-inoculated wheat-bran medium and mixed in plastic mini-grip bags (size 180 x 255mm). After being completely mixed, the liquid was poured into a separate one-litre beaker and covered with aluminium foil.

### iii) Harvesting spores and seed coating

Harvested spores from the PDA and wheat-bran mix were first poured into a double layer of muslin cloth stretched over a funnel and collected into a sterilized beaker (Figure 5.9). After that, the collected spores in the liquid were filtered again through a double layer of Mira cloth to remove all the slurry (i.e. PDA residues and wheat bran) (Figure 5.9).

Figure 5.9 First filtration (left) through muslin cloth on top of a funnel. Second filtration (right) through Myra cloth.

The filtered spores were carefully poured into 50ml falcon tubes using a funnel. Each 50ml of *Trichoderma* liquid produced approximately 0.3g spores. The falcon tubes were labelled and centrifuged in 'Eppendorf Centrifuge 5810R' at $21^0$C for 10 minutes, 3,500 RPM, in order to separate the water and spores (Figure 5.10).

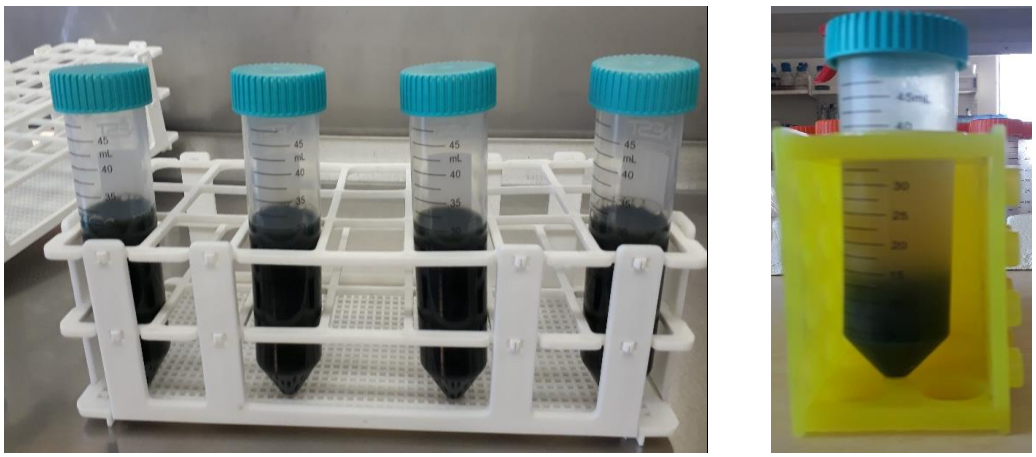

Figure 5.10 *Trichoderma* isolate (LU132) in a falcon tube before centrifuging (left) and after centrifuging (right).

The centrifuged falcon tubes were placed in ice for 20 minutes to steady the spores at the bottom of tubes. Afterwards, the centrifuged water was removed from the tubes. 0.5ml (or 500µL) sterilized water was added to each tube using a pipette, then vortexed for 10 seconds to mix it.

After that, 20g seed of each radish cultivar was mixed into each *Trichoderma* isolate's falcon tube by hand, and then vortexed for one minute. Finally, 0.28 microlitres polymer per 20g seed for each tube was added with a pipette, in order to adhere the spores to the surface of the seeds; they were then immediately mixed using a vortex for one minute, in order to prevent the seeds from forming clumps. After coating, the seeds were dried in a laminar cabinet for two hours (Figure 5.11). The seeds were then placed in a sterilized plastic container and stored at 4ºC before sowing.

Figure 5.11 *Trichoderma* LU132 – coated French breakfast (FB) seed (left), uncoated Red Round (RR) seed (middle) and LU132 – coated RR seed (right).

### iv) Spore concentration by Haemocytometer counts

10 seeds coated with each *Trichoderma* isolate were placed into a universal bottle with 10ml sterilized water. The bottle was mixed using a flask shaker at 300 osc/minute for 10 mins. 1mL throughout (or 1,000µL) diluted water from the universal bottle ($10^{-1}$) was extracted using a pipette and transferred into the universal bottles ($10^{-2}$) with 9ml of sterilized water and mixed using a flask shaker at 300 osc/minute for 10 min.

Next, 1mL (or 1,000µL) of diluted water was extracted from the $10^{-2}$ universal bottles using a pipette and poured into a 5ml cap tube. The spore concentration for each isolate was determined using a haemocytometer as follows;

1. Take 10µL of sample from a cap tube and pipette it onto a Nueman Bayer Haemocytometer.

2. Place a microscope glass cover slip (size 26 x 76mm & 0.1mm thick) on the haemocytometer.

3. Under the 'Olympus bx51' microscope on lens 'UPLSAPO 4 x 0.16', count the number of spores in the centre grid and four corner grids of the haemocytometer (Figure 5.12).

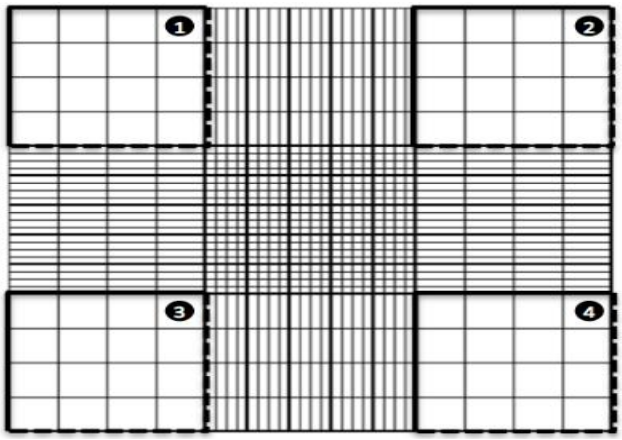


Figure 5.12 Haemocytometer grid. 1 to 4: four corner grids

4. Calculate the average spore number: Average the 5 grids and multiply by 25, as there are 25 squares in the haemocytometer.

5. Calculate the number of final spores per mL (volume of slide) by multiplying $1x10^4$. i.e. the number of spore counts (mean) x 25 grids x $10^4$ = spores $mL^{-1}$

The final results of the haemocytometer counts and spore concentrations for the two varieties were as below (Table 5.1 and 5.2);

Table 5.1 Haemocytometer counts and spore concentration ($mL^{-1}$) of the four *Trichoderma* isolates from French Breakfast seeds.

| FB | Haemocytometer counts | Spore concentration ($ml^{-1}$) |
|---|---|---|
| LU132 | 20 | $5.0 \times 10^8$ |
| LU785 | 22 | $5.5 \times 10^8$ |
| LU1347 | 36 | $9.0 \times 10^8$ |
| LU1358 | 45 | $11.3 \times 10^8$ |

Table 5.2 Haemocytometer counts and spore concentration ($mL^{-1}$) of the four *Trichoderma* isolates from Red Round seeds.

| RR | Haemocytometer counts | Spore concentration ($ml^{-1}$) |
|---|---|---|
| LU132 | 45 | $11.3 \times 10^8$ |
| LU785 | 11 | $2.8 \times 10^8$ |
| LU1347 | 45 | $11.3 \times 10^8$ |
| LU1358 | 23 | $5.6 \times 10^8$ |

**v) Thiram fungicide seed coating and control**

A product called 'Thiram Fungus Control D.F. Granules' was used for fungicide seed coating. This product contains 400g/litre thiram in the form of a suspension concentrate (Young, 2013). 0.3g dry granular Thiram was mixed with 1ml sterilized water in a falcon tube using a vortex for 20 seconds. Next, 20g seed was added into the tube and vortexed for one minute. After that, 0.28µL of polymer were added using a pipette, and mixed using a vortex for one minute.

For the control, only 0.28µL polymer using a pipette and 1ml sterilized water were added to each 20g of seed in a falcon tube. The seed was mixed by hand and vortexed for one minute. Both the fungicide seed coating and control seeds were dried in a separate tray for two hours in a laminar flow cabinet. Treated seeds were stored in sterilized plastic containers at 4°C.

- **2nd Glasshouse experiment (*Trichoderma* seed coating)**

The 2nd Glasshouse experiment was conducted using the same method as 'Experiment 3: 5.2.1: 1st glasshouse experiment'. Natural *R. solani*-infested soil was mixed with pumice (size 3 - 4mm) and used as the plant growth medium (ratio of soil to pumice = 4:1). Each pot was filled with 1.5L, and 10 coated seeds were sown per pot. A total of 112 pots were used and set out in a randomized block design in the Horticulture glasshouse, Lincoln University, in September 2017. The temperature ranged from 10.3 - 27.2ºC (average 17.1ºC). Pots were regularly watered.

There was a total of 112 pots (= two varieties x seven treatments x eight replications).

- Seven treatments: Four different *Trichoderma*-coated seed treatments, two controls (+ve controls) and one Thiram fungicide coated seed treatment.

After sowing, the plant observations and measurements, as in 'Experiment 3: 5.2.1: 1st glasshouse experiment (Data collection)' were conducted every two days for 33 days, and assessment was carried out at 20 and 30 DAS.

## 5.3 Results

### 5.3.1 Colony-forming units (1st and 2nd glasshouse experiments)

In the first glasshouse experiment, for both varieties LU132 and LU1347 had significantly higher numbers of colony-forming units than the control (Table 5.3 and Table 5.4). Neither LU1358 nor LU785 differed significantly from the control. However, LU1347 for French Breakfast had a significantly higher number of colony-forming units than the other isolates (Table 5.3).

In the second glasshouse experiment, LU132 and LU785 for French Breakfast had a significantly higher number of colony-forming units than the control (Table 5.5). LU1347, LU785 and the thiram control did not differ significantly from the control. In Red Round, none of the treatments differed significantly from the control (Table 5.6).

Table 5.3 Number of *Trichoderma* colony-forming units per gram of soil for French Breakfast, at 20 DAS.

| Isolate | Variety | Number of colony-forming units per g at 20 DAS |
|---|---|---|
| Control | FB | 900 |
| LU132 | FB | 805,000* |
| LU1347 | FB | 1,852,500* |
| LU1358 | FB | 43,500 |
| LU785 | FB | 110,375 |
| LSD 5% | - | 506,000 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$).

Table 5.4 Number of *Trichoderma* colony-forming units per gram of soil for Red Round, at 20 DAS.

| Isolate | Variety | Number of colony-forming units per g at 20 DAS |
|---|---|---|
| Control | RR | 800 |
| LU132 | RR | 1,205,000* |
| LU1347 | RR | 1,502,500* |
| LU1358 | RR | 109,188 |
| LU785 | RR | 91,125 |
| LSD 5% | - | 522,000 |

Note: '*' indicates an isolate that differs significantly from the control ($P<0.05$).

Table 5.5 Number of *Trichoderma* colony-forming units per gram of soil for French Breakfast, at 20 DAS.

| Isolate | Variety | Number of colony-forming units per g at 20 DAS |
|---|---|---|
| Control | FB | 200 |
| LU132 | FB | 66,812* |
| LU1347 | FB | 12,250 |
| LU1358 | FB | 18,438 |
| LU785 | FB | 63,625* |
| Thiram | FB | 225 |
| LSD 5% | - | 39,400 |

Note: '*' indicates an isolate that differs significantly from the control ($P<0.05$).

Table 5.6 Number of *Trichoderma* colony-forming units per gram of soil for Red Round, at 20 DAS.

| Isolate | Variety | Number of colony-forming units per g at 20 DAS |
|---|---|---|
| Control | RR | 150 |
| LU132 | RR | 58,438 |
| LU1347 | RR | 89,000 |
| LU1358 | RR | 25,250 |
| LU785 | RR | 10,688 |
| Thiram | RR | 200 |
| LSD 5% | - | 96,700 |

Note: '*' indicates an isolate that differs significantly from the control ($P<0.05$).

### 5.3.2 Glasshouse experiment 1: Assessment at 24 DAS

As only one of the eight replicates was assessed at 24 DAS, data could not be analysed.

Maximum numbers of seedlings for the two varieties emerged between 6 and 12 DAS (Table 5.7). The number of seedlings for the *R. solani* control for FB had decreased by 24 DAS, whereas the four *Trichoderma* isolates (LU132, LU785, LU1347 and LU1358) had higher numbers of seedlings than the *R. solani* control (Figure 5.13).

For Red Round, only LU1358 had a higher number of seedlings (Figure 5.13). All the other isolates had the same number of seedlings as the *R. solani* control.

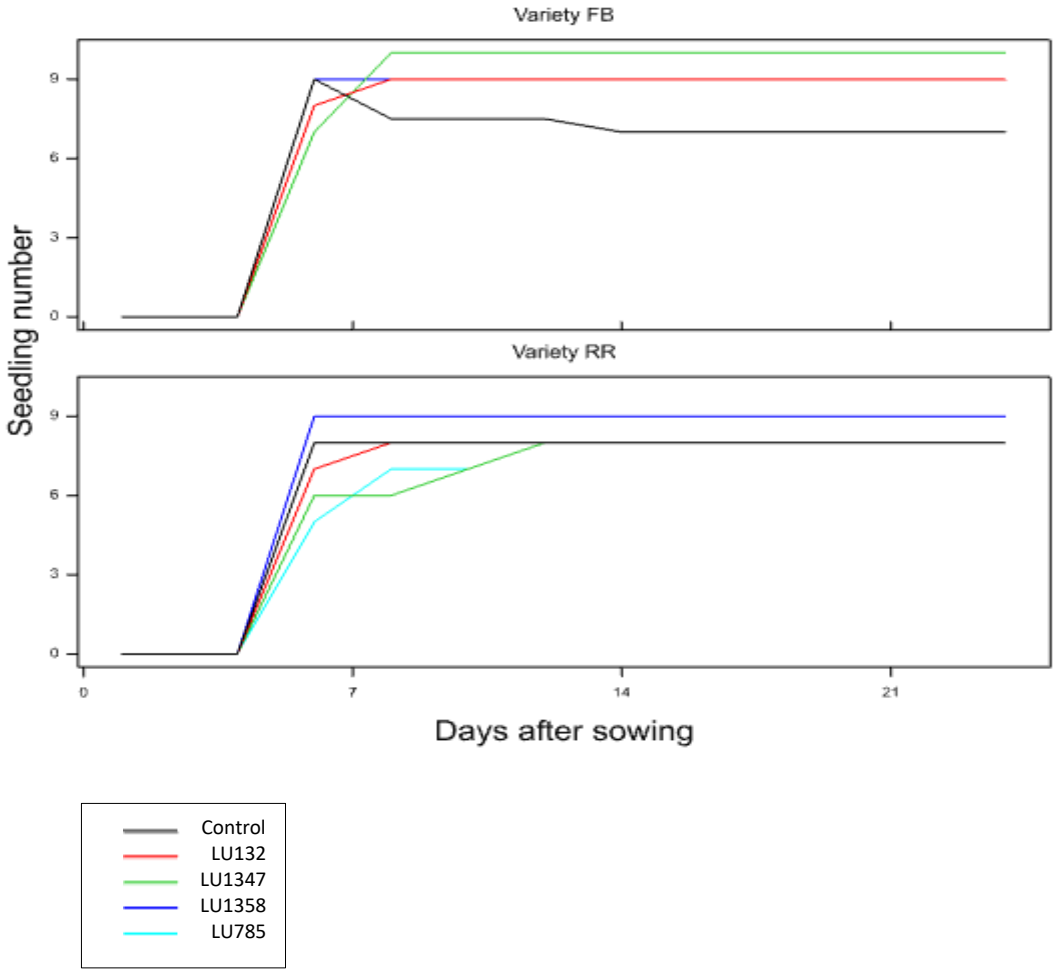


Figure 5.13 Effects of *Trichoderma* isolate and variety on the number of seedlings per pot (out of 10 seeds sown) from 1 to 24 DAS in the Horticulture Research Glasshouse, Lincoln University in September, 2017 (one replicate only).

For French Breakfast, isolate LU1347 had the highest number of seedlings among the isolates, both at maximum emergence and at 24 DAS (Table 5.7 and 5.8). No diseased or dead seedlings occurred in any of the isolates at the maximum emergence date. However, the numbers of diseased and dead seedlings for all the isolates had increased by 24 DAS. In particular, LU785 had the highest number of diseased seedlings at 24 DAS of all the isolates (Table 5.7). The four *Trichoderma* isolates had a disease score of 1, whereas the *R. solani* control had a disease score of 2. There was only one dead seedling in the *R. solani* control (Table 5.8).

The numbers of Red Round seedlings did not decrease after maximum emergence (Table 5.7 and 5.8). Isolate LU1358 had a higher number of seedlings than all other isolates, both at the maximum emergence date and at 24 DAS. LU1358 and LU785 had no diseased or dead seedlings at 24 DAS, while the diseased and dead seedlings of the other treatments (*R. solani* control, LU132 and LU1347) had increased by 24 DAS (Table 5.8). The *R. solani* control had two diseased seedlings and the highest disease score of 2, while the other isolates scored only 1. There was only one dead seedling in the *R. solani* control. The SPAD (Soil Plant Analysis Development) values of the two varieties ranged from 22.9 - 29.2 (Table 5.8). Isolates LU1347 for FB, and LU132 for RR, had the highest SPAD values among the isolates.

Table 5.7 Effects of *Trichoderma* isolate on maximum number of seedlings to emerge, and number of days after sowing to achieve maximum emergence and number of diseased and dead seedlings at maximum emergence (one replicate only).

| Isolate | Variety | Maximum number of seedlings to emerge | Days after sowing to achieve maximum emergence | Number of diseased seedlings at time of maximum emergence | Number of dead seedlings at time of maximum emergence |
|---|---|---|---|---|---|
| Control | FB | 9 | 6 | 0 | 0 |
| LU132 | FB | 9 | 8 | 0 | 0 |
| LU1347 | FB | 10 | 8 | 0 | 0 |
| LU1358 | FB | 9 | 6 | 0 | 0 |
| LU785 | FB | 9 | 8 | 0 | 0 |
| Control | RR | 8 | 10 | 0.5 | 0 |
| LU132 | RR | 8 | 8 | 0 | 0 |
| LU1347 | RR | 8 | 12 | 2 | 0 |
| LU1358 | RR | 9 | 6 | 0 | 0 |
| LU785 | RR | 8 | 12 | 0 | 0 |

Table 5.8 Effects of *Trichoderma* isolate on number of surviving seedlings, number of diseased seedlings, disease score, SPAD value, and number of dead seedlings post-emergence at 24 DAS (one replicate only).

| | | Assessment at 24 DAS | | | | |
|---|---|---|---|---|---|---|
| Isolate | Variety | Number of surviving seedlings | Number of diseased seedlings | Disease score | Number of dead seedlings post-emergence | SPAD value |
| Control | FB | 7 | 2 | 2 | 1 | 23.5 |
| LU132 | FB | 9 | 1 | 1 | 0 | 22.9 |
| LU1347 | FB | 10 | 2 | 1 | 0 | 27.8 |
| LU1358 | FB | 9 | 1 | 1 | 0 | 23.0 |
| LU785 | FB | 9 | 3 | 1 | 0 | 26.5 |
| Control | RR | 8 | 2 | 2 | 1 | 27.7 |
| LU132 | RR | 8 | 1 | 1 | 0 | 29.2 |
| LU1347 | RR | 8 | 2 | 1 | 0 | 25.2 |
| LU1358 | RR | 9 | 0 | 0 | 0 | 25.6 |
| LU785 | RR | 8 | 0 | 0 | 0 | 26.0 |

All the *Trichoderma* isolates had higher healthy seedling percentages than the *R. solani* control (50%) (Figure 5.14). For FB LU132, LU1347 and LU1358 had a healthy seedling percentage of 80%, whereas LU785 had a healthy seedling percentage of 60% (Figure 5.14).

The *Trichoderma* isolates for RR had higher healthy seedling percentages than the *R. solani* control, with the exception of LU1347, which had the same percentage of diseased seedlings as the *R. solani* control. Isolate LU1358 had the highest healthy seedling percentage (90%) among the isolates.

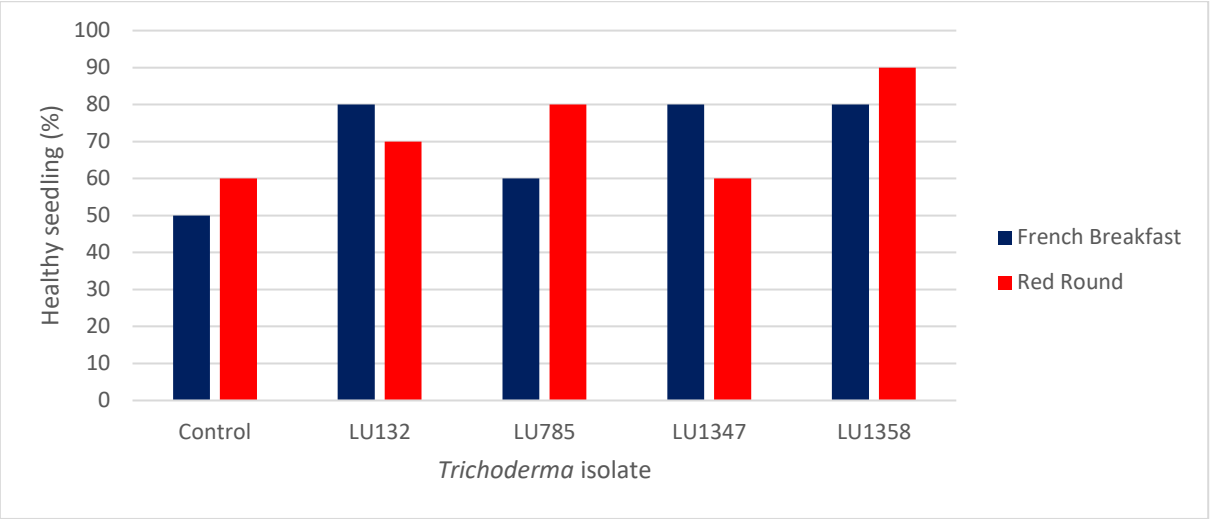


Figure 5.14 Effects of *Trichoderma* isolate on healthy seedling percentage, at 24 DAS in the Horticulture Research Glasshouse, Lincoln University in September 2017 (one replicate only).

Isolates LU132, LU1347, LU1358 and LU785 on FB had higher shoot lengths, hypocotyl radial sizes and fibrous root lengths than the *R. solani* control (Table 5.9). Isolate LU132 had the highest shoot length (129.69mm), and LU1347 had the highest hypocotyl length (34.43mm) and radial size (5.67mm), as did LU785 for fibrous root length (130.62mm).

For RR, isolates LU132 and LU1358 had higher shoot lengths than the *R. solani* control (93.63mm) (Table 5.10). In addition, LU1358 had the highest hypocotyl radial size (9.87mm) among the isolates, as did LU132 for the fibrous root length. However, the *R. solani* control had a higher hypocotyl length (20.29mm) than the *Trichoderma* isolates.

Table 5.9 Effects of *Trichoderma* isolate on plant parameters (shoot length, hypocotyl length, hypocotyl radial size and fibrous root length) of French Breakfast, at 24 DAS in the Horticulture Research Glasshouse, Lincoln University in September 2017 (one replicate only).

| **French Breakfast** | | | | |
|---|---|---|---|---|
| Isolate | Shoot length (mm) | Hypocotyl length (mm) | Hypocotyl radial size (mm) | Fibrous root length (mm) |
| Control | 94.70 | 25.16 | 2.59 | 76.09 |
| LU132 | 129.69 | 22.35 | 3.26 | 118.52 |
| LU1347 | 116.12 | 34.43 | 5.67 | 118.00 |
| LU1358 | 108.41 | 27.10 | 3.45 | 118.65 |
| LU785 | 108.84 | 20.85 | 3.34 | 130.62 |

Table 5.10 Effects of *Trichoderma* isolate on plant parameters (shoot length, hypocotyl length, hypocotyl radial size and fibrous root length) of Red Round at 24 DAS in the Horticulture Research Glasshouse, Lincoln University in September 2017 (one replicate only).

| **Red Round** | | | | |
|---|---|---|---|---|
| Isolate | Shoot length (mm) | Hypocotyl length (mm) | Hypocotyl radial size (mm) | Fibrous root length (mm) |
| Control | 93.63 | 20.29 | 8.27 | 69.10 |
| LU132 | 103.87 | 11.27 | 8.37 | 107.65 |
| LU1347 | 69.87 | 10.24 | 2.05 | 74.18 |
| LU1358 | 116.48 | 14.61 | 9.87 | 61.71 |
| LU785 | 89.31 | 17.19 | 8.25 | 100.43 |

The *R. solani* control of French Breakfast had lower fresh shoot and root weights, and dry shoot weight, per pot than the *Trichoderma* isolates (Table 5.11). Isolate LU132 produced higher fresh shoot and root weights per pot (18.99g and 2.86g respectively), as well as a higher dry shoot weight per pot (1.22g), than the other isolates. Isolate LU785 had a higher dry root weight than the other isolates (Table 5.11). However, the *R. solani* control had lower fresh shoot and root weights per plant than the *Trichoderma* isolates apart from isolate LU785, which had lower weights than the *R. solani* control (Table 5.12). In addition, the *R. solani* control had lower dry shoot and root weights per plant than the *Trichoderma* isolates.

For Red Round, isolate LU1358 produced higher fresh and dry weights per pot (both shoot and root) than the other isolates (Table 5.11), as it did for the fresh shoot weight per plant (Table 5.12). However, isolates LU132, LU1347 and LU785 produced lower fresh and dry weights per pot (both shoot and root) than the *R. solani* control (Table 5.11), as well as fresh root and dry shoot weight per plant, apart from the LU132 dry root weight per plant (Table 5.12). LU132 had higher dry root weight per plant than the other isolates (Table 5.12).

Table 5.11 Effects of *Trichoderma* isolate (of both varieties) on plant fresh and dry weights per pot (g) containing *R. solani*, at 24 DAS in the Horticulture Research Glasshouse, Lincoln University, in September 2017 (one replicate only).

| | | Fresh weight per pot (g) | | Dry weight per pot (g) | |
|---|---|---|---|---|---|
| Isolate | Variety | Shoot (g) | Root (g) | Shoot (g) | Root (g) |
| Control | FB | 10.10 | 1.27 | 0.64 | 0.14 |
| LU132 | FB | 18.99 | 2.86 | 1.22 | 0.23 |
| LU1347 | FB | 13.98 | 2.80 | 0.99 | 0.22 |
| LU1358 | FB | 15.10 | 1.73 | 0.93 | 0.13 |
| LU785 | FB | 11.77 | 1.61 | 0.86 | 0.24 |
| Control | RR | 16.98 | 3.44 | 0.98 | 0.23 |
| LU132 | RR | 12.64 | 2.92 | 0.84 | 0.26 |
| LU1347 | RR | 9.32 | 2.26 | 0.66 | 0.20 |
| LU1358 | RR | 21.19 | 4.16 | 1.11 | 0.27 |
| LU785 | RR | 15.19 | 3.41 | 0.85 | 0.20 |

Table 5.12 Effects of *Trichoderma* isolate (of both varieties) on plant fresh and dry weights per plant (g) containing *R. solani*, at 24 DAS in the Horticulture Research Glasshouse, Lincoln University, in September 2017 (one replicate only).

| | | Fresh weight per plant (g) | | Dry weight per plant (g) | |
|---|---|---|---|---|---|
| Isolate | Variety | Shoot (g) | Root (g) | Shoot (g) | Root (g) |
| Control | FB | 1.43 | 0.181 | 0.091 | 0.020 |
| LU132 | FB | 2.11 | 0.318 | 0.136 | 0.026 |
| LU1347 | FB | 1.40 | 0.280 | 0.099 | 0.022 |
| LU1358 | FB | 1.68 | 0.192 | 0.103 | 0.014 |
| LU785 | FB | 1.31 | 0.179 | 0.096 | 0.027 |
| Control | RR | 2.28 | 0.463 | 0.131 | 0.030 |
| LU132 | RR | 1.58 | 0.365 | 0.105 | 0.033 |
| LU1347 | RR | 1.17 | 0.283 | 0.083 | 0.025 |
| LU1358 | RR | 2.35 | 0.462 | 0.123 | 0.030 |
| LU785 | RR | 1.90 | 0.426 | 0.106 | 0.025 |

For French Breakfast, LU785 produced the highest total leaf area per pot of all the isolates (Table 5.13). Isolates LU132 and LU1358 had lower leaf areas per pot than the *R. solani* control, but they had higher leaf numbers per pot. All of the *Trichoderma* isolates had higher leaf area per plant than the *R. solani* control, as did LU132 and LU785 for the leaf number per plant (Table 5.14).

For Red Round, LU1358 produced the highest total leaf area and leaf number per pot of all the isolates (Table 5.13). However, LU132, LU1347 and LU785 produced lower leaf areas per pot than the *R. solani* control (Table 5.14). None of the *Trichoderma* isolates produced higher

leaf area per plant than the *R. solani* control (Table 5.14). Only LU132 had a higher leaf number per plant than the other isolates.

Table 5.13 Effects of *Trichoderma* isolate on leaf area ($cm^2$) and leaf number per pot of both varieties, at 24 DAS in the Horticulture Research Glasshouse, Lincoln University, in September 2017 (one replicate only).

| Isolate | Variety | Total Leaf area (cm2) per pot | Total Leaf number per pot |
|---|---|---|---|
| Control | FB | 194.33 | 31 |
| LU132 | FB | 175.30 | 36 |
| LU1347 | FB | 210.59 | 31 |
| LU1358 | FB | 159.65 | 35 |
| LU785 | FB | 256.15 | 33 |
| Control | RR | 256.15 | 33 |
| LU132 | RR | 205.22 | 37 |
| LU1347 | RR | 123.84 | 34 |
| LU1358 | RR | 279.89 | 37 |
| LU785 | RR | 209.91 | 31 |

Table 5.14 Effects of *Trichoderma* isolate on leaf area ($cm^2$) and leaf number per plant of both varieties, at 24 DAS in the Horticulture Research Glasshouse, Lincoln University, in September 2017 (one replicate only).

| Isolate | Variety | Total Leaf area (cm2) per plant | Total Leaf number per plant |
|---|---|---|---|
| Control | FB | 16.05 | 3.79 |
| LU132 | FB | 30.70 | 4.00 |
| LU1347 | FB | 17.53 | 3.60 |
| LU1358 | FB | 23.40 | 3.44 |
| LU785 | FB | 17.74 | 3.89 |
| Control | RR | 34.35 | 4.38 |
| LU132 | RR | 25.65 | 4.63 |
| LU1347 | RR | 15.48 | 4.25 |
| LU1358 | RR | 31.10 | 4.11 |
| LU785 | RR | 26.24 | 3.88 |

### 5.3.3 Glasshouse experiment 1: Assessment at 40 DAS

Plant numbers per pot of both varieties from 1 to 40 DAS differed significantly among the isolates (P<0.001). Isolates LU785, LU1347 and LU1358 for French Breakfast (Figure 5.15), and all four *Trichoderma* isolates for Red Round (Figure 5.16) had significantly higher numbers of seedlings than the *R. solani* control at 20, 30 and 40 DAS (Table 5.15). There were no significant interactions (variety x isolate). Maximum seedlings for the two varieties emerged by 16 DAS (Figure 5.15 and 5.16). After that, the seedling numbers of the two varieties decreased apart from isolates LU132 and LU785 in Red Round which maintained seedling numbers, while the numbers of diseased and dead plants increased.

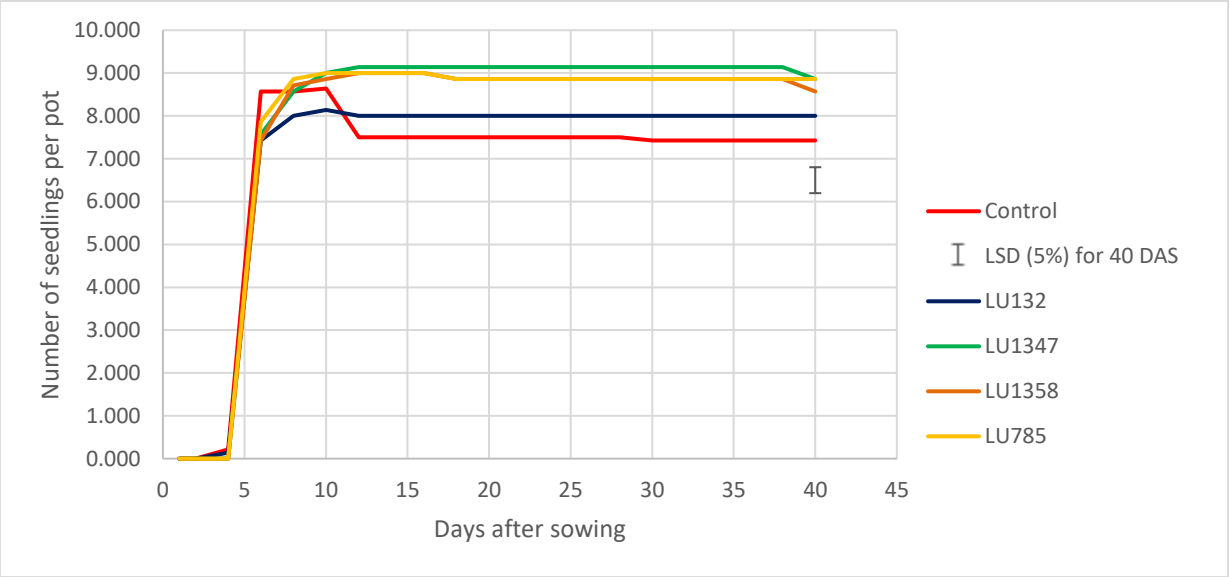


Figure 5.15 Effects of *Trichoderma* isolates (out of 10 seeds sown per pot) on seedling numbers per pot of French Breakfast, for up to 40 DAS in the Horticulture Research Glasshouse, Lincoln University, from September to October 2017.

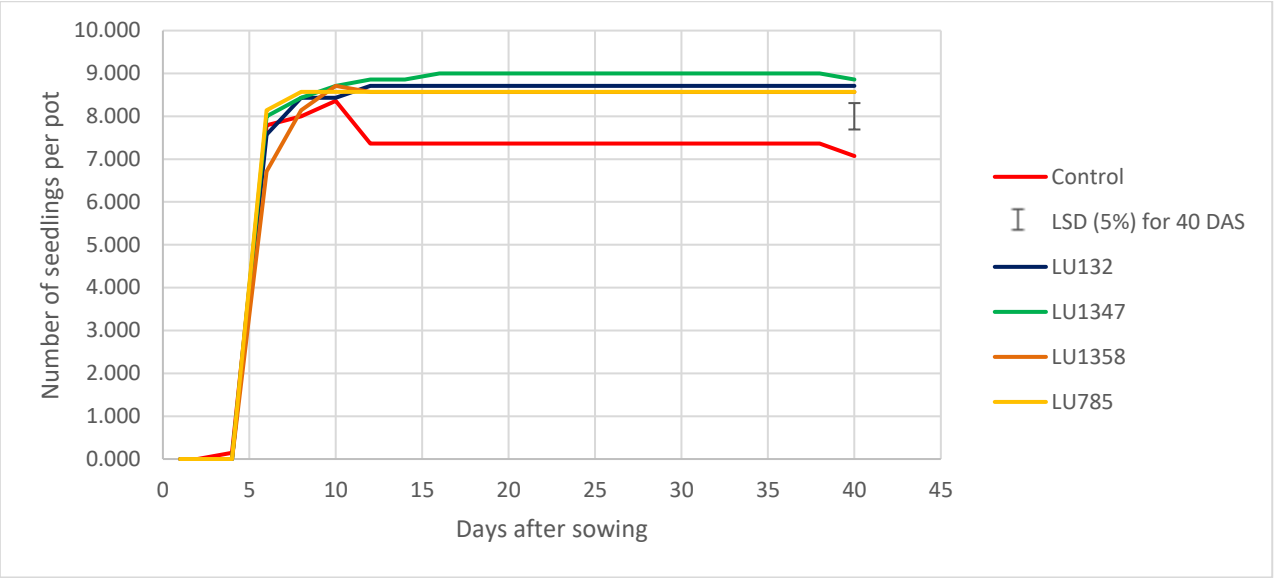


Figure 5.16 Effects of *Trichoderma* isolates (out of 10 seeds sown per pot) on seedling numbers per pot of Red Round, for up to 40 DAS in the Horticulture Research Glasshouse, Lincoln University, from September to October 2017.

Table 5.15 Least significant difference and significance of difference for the main effects of variety and isolate, and interaction for seedling numbers at 10, 20, 30 and 40 DAS.

| FB vs RR | Day 10 | Day 20 | Day 30 | Day 40 |
|---|---|---|---|---|
| Main effect of variety | 0.551 | 0.479 | 0.481 | 0.484 |
| Main effect of isolate | 0.827 | 0.719 | 0.721 | 0.726 |
| Variety x isolate interaction | 1.169 | 1.016 | 1.02 | 1.027 |
| Significance of main effect of variety | ns | ns | ns | ns |
| Significance of main effect of isolate | ns | *** | *** | *** |
| Significance of interaction | ns | ns | ns | ns |
| Ns = Not significantly different | | | | |

French Breakfast's maximum seedling emergence occurred between 10 and 12 DAS (Table 5.16). At the date of maximum emergence, both the numbers of seedlings and diseased seedlings did not differ significantly among the isolates (Table 5.16), and there were no dead seedlings.

At 20 DAS, LU1347, LU1358 and LU785 had significantly higher numbers of survived seedlings than the *R. solani* control ($P<0.05$) (Table 5.17). Moreover, all the *Trichoderma* isolates had significantly lower numbers of dead seedlings than the *R. solani* control. However, the numbers of diseased seedlings and the SPAD value for the *Trichoderma* isolates did not significantly differ from the *R. solani* control (Table 5.17).

By 40 DAS, the numbers of surviving seedlings had decreased, while the numbers of diseased and dead seedlings post-emergence increased (Table 5.18). Isolates LU1347, LU1358 and LU785 had significantly higher numbers of surviving seedlings than the *R. solani* control ($P<0.05$) (Table 5.18). The numbers of diseased seedlings for the *Trichoderma* isolates did not differ significantly from the *R. solani* control. However, the *R. solani* control had the highest disease score of 2, whereas the *Trichoderma* isolates scored only 1. LU132, LU785 and LU1347 had significantly lower numbers of dead seedlings post-emergence than the *R. solani* control ($P<0.05$). In addition, all the *Trichoderma* isolates recorded significantly higher SPAD values than the *R. solani* control ($P<0.001$).

Table 5.16 Effects of the *Trichoderma* isolates on maximum seedling emergence, days after sowing for maximum emergence, and numbers of diseased seedlings at maximum emergence for French Breakfast.

| French Breakfast | | | |
|---|---|---|---|
| Isolate | Maximum seedlings emergence | Days after sowing to achieve maximum emergence | Number of diseased seedlings at time of maximum emergence |
| Control | 8.64 | 10 | 0.86 |
| LU132 | 8.14 | 10 | 0.71 |
| LU1347 | 9.14 | 12 | 1.57 |
| LU1358 | 9.00 | 12 | 1.14 |
| LU785 | 9.00 | 10 | 0.86 |
| LSD 5% | 1.09 | - | 0.76 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$).

Table 5.17 Effects of *Trichoderma* isolates on number of surviving seedlings, number of diseased seedlings, SPAD value and number of dead seedlings post-emergence for French Breakfast at 20 DAS.

| French Breakfast | Assessment at 20 DAS | | | |
|---|---|---|---|---|
| Isolate | Number of surviving seedlings | Number of diseased seedlings | Number of dead seedlings post-emergence | SPAD value |
| Control | 7.50 | 1.57 | 0.93 | 24.66 |
| LU132 | 8.00 | 1.43 | 0.00* | 24.34 |
| LU1347 | 9.14* | 1.71 | 0.00* | 23.79 |
| LU1358 | 8.86* | 1.71 | 0.14* | 25.44 |
| LU785 | 8.86* | 1.57 | 0.16* | 26.29 |
| LSD 5% | 0.90 | 0.84 | 0.50 | 2.20 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Table 5.18 Effects of *Trichoderma* isolates on number of surviving seedlings, number of diseased seedlings, disease score, SPAD value and number of dead seedlings post-emergence for French Breakfast at 40 DAS.

| French Breakfast | Assessment at 40 DAS | | | | |
|---|---|---|---|---|---|
| Isolate | Number of survived seedlings | Number of diseased seedlings | Disease score | Number of dead seedlings post-emergence | SPAD value |
| Control | 7.50 | 2.50 | 2 | 1.00 | 22.95 |
| LU132 | 8.00 | 2.14 | 1 | 0.00* | 31.37* |
| LU1347 | 8.86* | 2.43 | 1 | 0.29* | 29.40* |
| LU1358 | 8.57* | 2.14 | 1 | 0.43 | 31.40* |
| LU785 | 8.86* | 2.29 | 1 | 0.14* | 31.20* |
| LSD 5% | 0.92 | 0.76 | | 0.67 | 2.33 |

Note: '*' indicates isolates that differ significantly from *R. solani* control (P<0.05).

Maximum seedling emergence for Red Round occurred between 8 and 16 DAS (Table 5.19). At the date of maximum emergence, numbers of seedlings did not differ significantly among the isolates. Isolates LU1358 and LU785 had significantly lower numbers of diseased seedlings ($P<0.05$) than the *R. solani* control, and there were no dead seedlings.

At 20 DAS, all of the *Trichoderma* isolates had significantly higher numbers of surviving seedlings than the *R. solani* control (Table 5.20). Numbers of diseased seedlings of the *Trichoderma* isolates did not differ significantly from the *R. solani* control. However, the *R. solani* control had one dead seedling post-emergence. The SPAD value varied from 26.01 - 27.37, with no significant difference among the treatments.

At 40 DAS, all of the *Trichoderma* isolates had significantly higher numbers of live seedlings and SPAD values, and significantly lower numbers of diseased seedlings than the *R. solani* control ($P<0.001$) (Table 5.21). All of the isolates had a disease score of 1.

The four of the *Trichoderma* isolates had significantly lower numbers of dead seedlings than the *R. solani* control ($P<0.05$) (Table 5.21). In particular, no seedlings died post-emergence for isolates LU132 and LU1358. At this time, the SPAD value of the *Trichoderma* isolates was significantly higher than the *R. solani* control ($P<0.001$).

Table 5.19 Effects of *Trichoderma* isolates on maximum seedling emergence, days after sowing for maximum emergence, and numbers of diseased seedlings at maximum emergence for Red Round.

| Red Round | | | |
|---|---|---|---|
| Isolate | Maximum seedlings emergence | Days after sowing to achieve maximum emergence | Number of diseased seedlings at maximum emergence |
| Control | 8.36 | 10 | 1.07 |
| LU132 | 8.71 | 12 | 0.71 |
| LU1347 | 9.00 | 16 | 0.86 |
| LU1358 | 8.71 | 10 | 0.14* |
| LU785 | 8.57 | 8 | 0.29* |
| LSD 5% | 1.31 | - | 0.66 |

Note: '*' indicates isolates that differs significantly from *R. solani* control ($P<0.05$).

Table 5.20 Effects of *Trichoderma* isolates on number of surviving seedlings, number of diseased seedlings, SPAD value, and number of dead seedlings post-emergence for Red Round at 20 DAS.

| Red Round | Assessment at 20 DAS | | | |
|---|---|---|---|---|
| Isolate | Number of surviving seedlings | Number of diseased seedlings | Number of dead seedlings post-emergence | SPAD value |
| Control | 7.36 | 1.36 | 1.07 | 26.01 |
| LU132 | 8.71* | 0.71 | 0.00* | 27.37 |
| LU1347 | 9.00* | 0.86 | 0.00* | 26.14 |
| LU1358 | 8.57* | 0.71 | 0.00* | 26.73 |
| LU785 | 8.57* | 0.57 | 0.00* | 26.07 |
| LSD 5% | 1.20 | 0.83 | 0.46 | 1.50 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

Table 5.21 Effects of *Trichoderma* isolates on number of surviving seedlings, number of diseased seedlings, disease score, SPAD value, and number of dead seedlings post-emergence for Red Round at 40 DAS.

| Red Round | Assessment at 40 DAS | | | | |
|---|---|---|---|---|---|
| *Trichoderma* isolate | Number of surviving seedlings | Number of diseased seedlings | Disease score | Number of dead seedlings post emergence | SPAD value |
| Control | 7.07 | 1.93 | 1 | 1.29 | 24.79 |
| LU132 | 8.71* | 1.71 | 1 | 0.00* | 34.91* |
| LU1347 | 8.86* | 2.29 | 1 | 0.14* | 34.83* |
| LU1358 | 8.57* | 2.29 | 1 | 0.00* | 34.01* |
| LU785 | 8.57* | 1.71 | 1 | 0.14* | 34.09* |
| LSD 5% | 1.19 | 0.90 | - | 0.50 | 3.36 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

Healthy plant percentages for both varieties differed significantly among the isolates (P<0.05) (Table 5.22). For French Breakfast isolates LU1347, LU785 and LU1358 had significantly higher healthy plant percentage than the *R. solani* control (50%). For Red Round isolates LU132, LU1347 and LU785 had higher healthy plant percentages than the *R. solani* control (51.4%).

Table 5.22 Effects of *Trichoderma* isolates on the percentages of healthy seedlings of both varieties at 40 DAS.

| Isolate | French Breakfast healthy plant (%) at 40 DAS | Red Round healthy plant (%) at 40 DAS |
|---|---|---|
| Control | 50.0 | 51.4 |
| LU132 | 58.6 | 70.0* |
| LU1347 | 64.3* | 65.7* |
| LU1358 | 64.3* | 62.9 |
| LU785 | 65.7* | 68.6* |
| LSD 5% | 11.7 | 13.3 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

The plant parameters for French Breakfast differed significantly among the isolates (P<0.05) (Table 5.23). Isolates LU132, LU1358 and LU785 had significantly higher shoot lengths than the *R. solani* control (109.5mm), whereas LU1347 had the lowest (103.9mm). However, LU1347 had a significantly higher hypocotyl radial size than the *R. solani* control (16.1mm), as did LU132 for the fibrous root length. Hypocotyl length did not differ significantly among the isolates.

The shoot and hypocotyl lengths for Red Round did not differ significantly from the *R. solani* control (Table 5.24). Only LU132 had a significantly higher hypocotyl radial size than the *R. solani* control, as did LU1358 for the fibrous root length.

Table 5.23 Effects of *Trichoderma* isolates on plant parameters (shoot, hypocotyl and fibrous root length, and hypocotyl radial size) of French Breakfast at 40 DAS, in the Horticulture Research Glasshouse, Lincoln University, in September 2017.

| French Breakfast | | | | |
|---|---|---|---|---|
| Isolate | Shoot length (mm) | Hypocotyl length (mm) | Hypocotyl radial size (mm) | Fibrous root length (mm) |
| Control | 109.5 | 31.0 | 12.1 | 71.7 |
| LU132 | 130.5* | 33.0 | 11.7 | 83.4* |
| LU1347 | 103.9 | 34.4 | 16.1* | 75.6 |
| LU1358 | 127.8* | 33.7 | 13.2 | 72.4 |
| LU785 | 127.0* | 28.8 | 11.6 | 79.3 |
| LSD 5% | 13.9 | 5.2 | 3.4 | 10.8 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

Table 5.24 Effects of *Trichoderma* isolates on plant parameters (shoot, hypocotyl and fibrous root length, and hypocotyl radial size) of Red Round, at 40 DAS in the Horticulture Research Glasshouse, Lincoln University, in September 2017.

| Red Round | | | | |
|---|---|---|---|---|
| Isolate | Shoot length (mm) | Hypocotyl length (mm) | Hypocotyl radial size (mm) | Fibrous root length (mm) |
| Control | 101.1 | 26.8 | 18.7 | 58.7 |
| LU132 | 114.3 | 23.0 | 21.3* | 59.2 |
| LU1347 | 104.0 | 23.5 | 18.2 | 63.7 |
| LU1358 | 105.0 | 25.7 | 18.2 | 77.0* |
| LU785 | 111.8 | 21.2 | 20.8 | 62.2 |
| LSD 5% | 15.2 | 10.0 | 3.2 | 12.9 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

Fresh and dry weights per pot for both shoot and fibrous root of the two varieties differed significantly among the isolates (P<0.05) (Table 5.25 and 5.27). For each variety, the four *Trichoderma* isolates produced significantly higher fresh and dry shoot weights per pot than the *R. solani* control. In addition, for FB, LU1347 and LU1358 had significantly heavier fresh and dry fibrous weights than the *R. solani* control (Table 5.25). However only LU132 and LU1358 had significantly higher shoot fresh weights per plant than the *R. solani* control, as did LU132 for fibrous dry weight per plant. Fresh bulb and fibrous root weight per plant, and dry shoot and bulb per plant did not differ significantly from the *R. solani* control (Table 5.26).

Only LU785 for RR had a significantly higher fibrous weight per pot than the *R. solani* control (Table 5.27) as it did for fresh fibrous weight per plant (Table 5.28). Dry fibrous weights per plant of *Trichoderma* isolates did not differ significantly from the *R. solani* control (Table 5.28). The fresh and dry bulb weights per pot and per plant did not differ significantly among the isolates for either variety (Table 5.25 and 5.27).

Table 5.25 Effects of *Trichoderma* isolates on fresh and dry weights per pot (g) for French Breakfast, at 40 DAS.

| French Breakfast | Fresh weight per pot (g) | | | Dry weight per pot (g) | | |
|---|---|---|---|---|---|---|
| Isolate | Shoot (g) | Bulb (g) | Fibrous (g) | Shoot (g) | Bulb (g) | Fibrous (g) |
| Control | 19.2 | 23.1 | 1.90 | 2.06 | 1.86 | 1.90 |
| LU132 | 32.3* | 22.1 | 2.72 | 3.04* | 1.60 | 2.72 |
| LU1347 | 28.9* | 26.6 | 2.85* | 2.87* | 1.85 | 2.85* |
| LU1358 | 30.8* | 32.1 | 2.96* | 2.82* | 2.06 | 2.96* |
| LU785 | 34.8* | 21.7 | 2.64 | 3.23* | 1.61 | 2.64 |
| LSD 5% | 7.6 | 9.5 | 0.86 | 0.60 | 0.51 | 0.86 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

Table 5.26 Effects of *Trichoderma* isolates on fresh and dry weights per plant (g) for French Breakfast, at 40 DAS.

| French Breakfast | Fresh weight per plant (g) | | | Dry weight per plant (g) | | |
|---|---|---|---|---|---|---|
| Isolate | Shoot (g) | Bulb (g) | Fibrous (g) | Shoot (g) | Bulb (g) | Fibrous (g) |
| Control | 2.52 | 3.17 | 0.257 | 0.272 | 0.253 | 0.0027 |
| LU132 | 4.06* | 2.79 | 0.344 | 0.381 | 0.201 | 0.0377* |
| LU1347 | 3.21 | 3.03 | 0.315 | 0.320 | 0.214 | 0.0264 |
| LU1358 | 3.61* | 3.83 | 0.348 | 0.331 | 0.246 | 0.0331 |
| LU785 | 3.99 | 2.50 | 0.296 | 0.366 | 0.184 | 0.0284 |
| LSD 5% | 0.81 | 1.29 | 0.101 | 0.058 | 0.073 | 0.0091 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

Table 5.27 Effects of *Trichoderma* isolate on fresh and dry weights per pot (g) for Red Round, at 40 DAS.

| Red Round | Fresh weight per pot (g) | | | Dry weight per pot (g) | | |
|---|---|---|---|---|---|---|
| Isolate | Shoot (g) | Bulb (g) | Fibrous (g) | Shoot (g) | Bulb (g) | Fibrous (g) |
| Control | 18.50 | 44.02 | 1.45 | 1.56 | 2.55 | 0.158 |
| LU132 | 27.89* | 48.85 | 1.97 | 2.26* | 2.47 | 0.186 |
| LU1347 | 27.68* | 46.01 | 2.11 | 2.33* | 2.37 | 0.176 |
| LU1358 | 27.70* | 45.74 | 2.03 | 2.24* | 2.29 | 0.189 |
| LU785 | 31.31* | 52.04 | 2.96* | 2.51* | 2.66 | 0.258* |
| LSD 5% | 5.97 | 11.24 | 0.72 | 0.54 | 0.58 | 0.071 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

Table 5.28 Effects of *Trichoderma* isolate on fresh and dry weights per plant (g) for Red Round, at 40 DAS.

| Red Round | Fresh weight per plant (g) | | | Dry weight per plant (g) | | |
|---|---|---|---|---|---|---|
| Isolate | Shoot (g) | Bulb (g) | Fibrous (g) | Shoot (g) | Bulb (g) | Fibrous (g) |
| Control | 2.63 | 6.38 | 0.205 | 0.218 | 0.365 | 0.0219 |
| LU132 | 3.23 | 5.88 | 0.222 | 0.263 | 0.292 | 0.0213 |
| LU1347 | 3.15 | 5.39 | 0.241 | 0.265 | 0.276 | 0.0201 |
| LU1358 | 3.29 | 5.59 | 0.231 | 0.263 | 0.276 | 0.0222 |
| LU785 | 3.72* | 6.23 | 0.34* | 0.295* | 0.315 | 0.0296 |
| LSD 5% | 0.76 | 1.98 | 0.074 | 0.066 | 0.090 | 0.0078 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

All of the *Trichoderma* isolates for both varieties produced significantly higher leaf areas per pot than the *R. solani* control (Table 5.29 and 5.31). Isolates LU132, LU1358 and LU785 for French Breakfast had significantly higher leaf area per plant than the *R. solani* control (Table 5.30) as did only LU785 for RR (Table 5.32).

The leaf numbers per pot for both varieties differed significantly from the *R. solani* control (P<0.05). For French Breakfast isolate LU1358 produced a significantly higher number of leaves per pot than the *R. solani* control (Table 5.29), whereas all the *Trichoderma* isolates for Red Round had significantly higher numbers of leaves per plant than the *R. solani* control (Table 5.31). However, none of the *Trichoderma* isolates for FB's leaf number per plant differed significantly from the *R. solani* control (Table 5.30), and only LU785 for Red Round had a significantly higher leaf number per plant (Table 5.32).

Table 5.29 Effects of *Trichoderma* isolates on French Breakfast leaf area ($cm^2$) and leaf number per pot, at 40 DAS.

| French Breakfast | | |
|---|---|---|
| Isolate | Total Leaf area (cm2) per pot | Leaf number per pot |
| Control | 310 | 29.9 |
| LU132 | 558* | 35.9 |
| LU1347 | 474* | 35.4 |
| LU1358 | 520* | 37.6* |
| LU785 | 587* | 35.9 |
| LSD 5% | 132 | 6.0 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

Table 5.30 Effects of *Trichoderma* isolates on French Breakfast leaf area ($cm^2$) and leaf number per plant, at 40 DAS.

| French Breakfast | | |
|---|---|---|
| Isolate | Total Leaf area (cm2) per plant | Leaf number per plant |
| Control | 40.53 | 4.05 |
| LU132 | 71.34* | 4.53 |
| LU1347 | 52.46 | 3.98 |
| LU1358 | 61.21* | 4.39 |
| LU785 | 67.42* | 4.07 |
| LSD 5% | 15.22 | 0.74 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Table 5.31 Effects of *Trichoderma* isolates on Red Round leaf area ($cm^2$) and leaf number per pot, at 40 DAS.

| Red Round | | |
|---|---|---|
| Isolate | Total Leaf area (cm2) per pot | Leaf number per pot |
| Control | 333 | 26.6 |
| LU132 | 482* | 32.6* |
| LU1347 | 474* | 36.7* |
| LU1358 | 498* | 33.9* |
| LU785 | 545* | 40.1* |
| LSD 5% | 106 | 5.5 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

Table 5.32 Effects of *Trichoderma* isolates on Red Round leaf area ($cm^2$) and leaf number per plant, at 40 DAS.

| Red Round | | |
|---|---|---|
| Isolate | Total Leaf area (cm2) per plant | Leaf number per plant |
| Control | 46.83 | 3.81 |
| LU132 | 55.76 | 3.75 |
| LU1347 | 54.12 | 4.16 |
| LU1358 | 59.37 | 3.99 |
| LU785 | 64.68* | 4.75* |
| LSD 5% | 13.16 | 0.66 |

Note: '*' indicates isolates that differs significantly from *R. solani* control (P<0.05).

### 5.3.4 Glasshouse experiment 2: Assessment at 20 DAS

Maximum emergence of the two varieties was between 4 and 6 DAS (Figure 5.17 and Table 5.33). After the maximum emergence date, the numbers of seedlings did not decrease for either the *Trichoderma* isolates, or the thiram control of either variety (Figure 5.17). Only the *R. solani* control (of both varieties) had a decrease in the number of seedlings by 20 DAS (Figure 5.17), and there was one dead seedling for each variety (Table 5.34). The numbers of diseased seedlings for both varieties increased by 20 DAS (Table 5.34). All the isolates had a disease score of 1, with the exception of isolate LU785 in Red Round which had a disease score of 2 (Table 5.34).

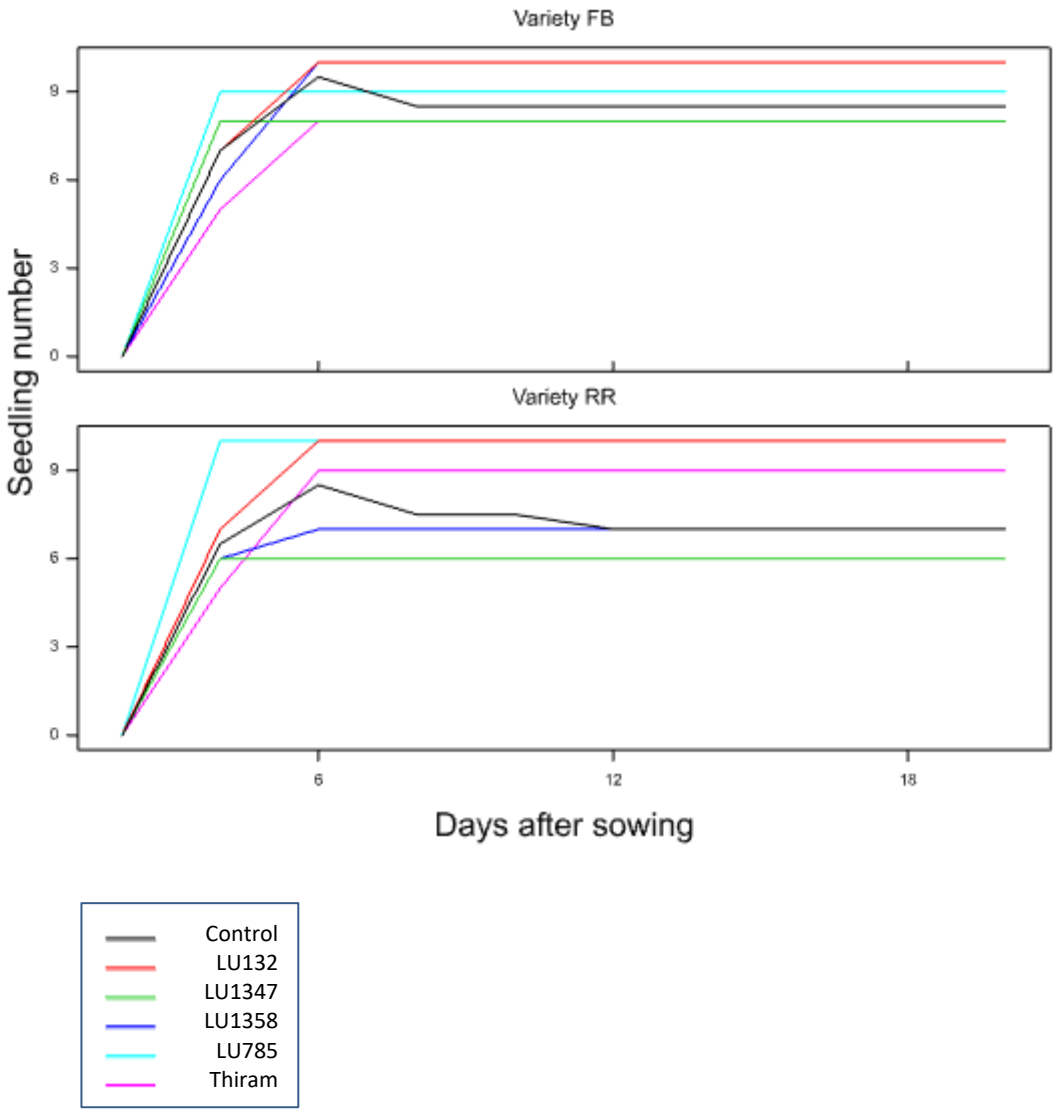


Figure 5.17 Effects of *Trichoderma* isolate and variety (out of 10 seeds per pot) on the number of seedlings per pot, from 1 to 20 DAS (one replicate only).

Table 5.33 Effects of *Trichoderma* isolates on maximum seedling emergence, days after sowing for maximum emergence, and number of diseased seedlings at maximum emergence (one replicate only).

| Isolate | Variety | Maximum seedling emergence | Days after sowing to achieve maximum emergence | Number of diseased seedlings at maximum emergence |
|---|---|---|---|---|
| Control | FB | 10 | 6 | 1.5 |
| LU132 | FB | 10 | 6 | 1.0 |
| LU1347 | FB | 8 | 4 | 0.0 |
| LU1358 | FB | 10 | 6 | 0.0 |
| LU785 | FB | 9 | 4 | 0.0 |
| Thiram | FB | 8 | 6 | 0.0 |
| Control | RR | 9 | 6 | 1.0 |
| LU132 | RR | 10 | 6 | 1.0 |
| LU1347 | RR | 6 | 4 | 0.0 |
| LU1358 | RR | 7 | 6 | 1.0 |
| LU785 | RR | 10 | 4 | 0.0 |
| Thiram | RR | 9 | 6 | 0.0 |

Table 5.34 Effects of *Trichoderma* isolates on number of surviving seedlings, number of diseased seedlings, and number of dead seedlings post-emergence at 20 DAS (one replicate only).

| Isolate | Variety | Number of surviving seedlings at 20 DAS | Number of diseased seedlings at 20 DAS | Disease score at 20 DAS | Number of dead seedlings post-emergence |
|---|---|---|---|---|---|
| Control | FB | 8.5 | 2.5 | 1 | 1.0 |
| LU132 | FB | 10.0 | 2.0 | 1 | 0.0 |
| LU1347 | FB | 8.0 | 2.0 | 1 | 0.0 |
| LU1358 | FB | 10.0 | 2.0 | 1 | 0.0 |
| LU785 | FB | 9.0 | 1.0 | 1 | 0.0 |
| Thiram | FB | 8.0 | 1.0 | 1 | 0.0 |
| Control | RR | 7.0 | 2.5 | 1 | 1.5 |
| LU132 | RR | 10.0 | 2.0 | 1 | 0.0 |
| LU1347 | RR | 6.0 | 2.0 | 1 | 0.0 |
| LU1358 | RR | 7.0 | 2.0 | 1 | 0.0 |
| LU785 | RR | 10.0 | 7.0 | 2 | 0.0 |
| Thiram | RR | 9.0 | 1.0 | 1 | 0.0 |

For French Breakfast the thiram control and isolates LU132, LU1358 and LU785 had higher healthy seedlings percentages than the *R. solani* control (60%) (Figure 5.18). However, for Red Round, only the thiram control and isolates LU132 and LU1358 had higher healthy

seedlings percentages than the *R. solani* control (45%), whereas LU1347 and LU785 had lower percentages (40% and 30% respectively) (Figure 5.18).

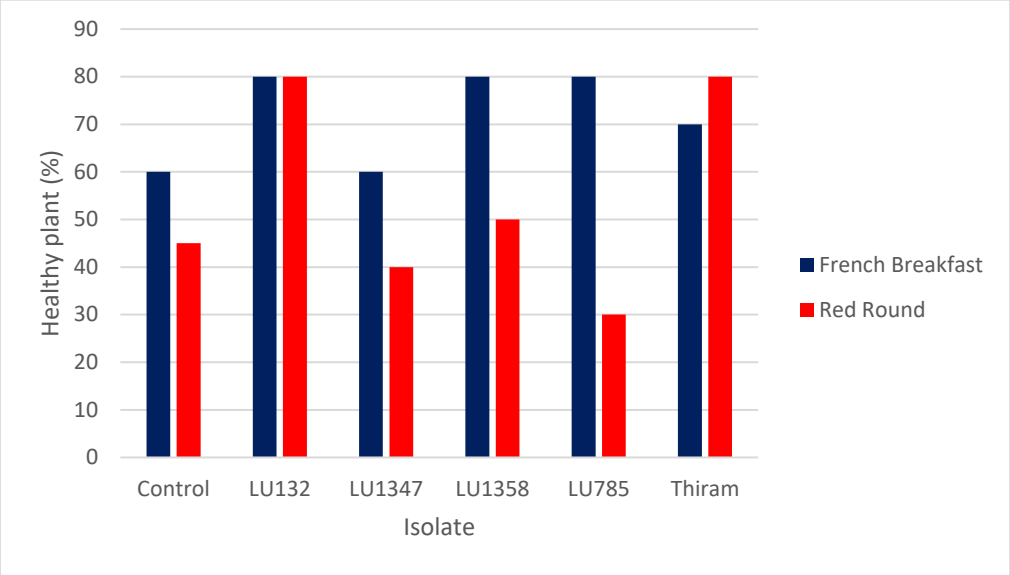


Figure 5.18 Effects of *Trichoderma* isolate on healthy seedling percentages of both varieties, at 20 DAS (one replicate only).

All the *Trichoderma* isolates, and the thiram control of the two varieties, produced higher shoot lengths than the *R. solani* control (77.16mm for FB and 77.78mm for RR) (Table 5.35).

The FB thiram control produced the longest hypocotyl length (35.11mm), greater than that of any of the isolates (Table 5.35), as did LU785 for RR (16.81mm). However, LU132, LU1347 and LU1358 and the thiram control for RR had lower hypocotyl lengths than the *R. solani* control (Table 5.35).

Isolates LU1358 for FB (3.46mm) and LU1347 for RR (5.50mm) had the highest hypocotyl radial size among the isolates. In addition, LU1347 for FB had a longer fibrous root length than the *R. solani* control, as did LU1347 for Red Round.

Table 5.35 Effects of *Trichoderma* isolate on plant parameters (shoot, hypocotyl and fibrous root length, and hypocotyl radial size) at 20 DAS (one replicate only).

| Isolate | Variety | Shoot length(mm) | Hypocotyl length (mm) | Hypocotyl radial size (mm) | Fibrous root length (mm) |
|---|---|---|---|---|---|
| Control | FB | 77.16 | 28.07 | 2.30 | 93.03 |
| LU132 | FB | 91.51 | 30.46 | 1.63 | 80.52 |
| LU1347 | FB | 91.05 | 29.43 | 1.58 | 94.59 |
| LU1358 | FB | 110.49 | 30.22 | 3.46 | 64.89 |
| LU785 | FB | 93.74 | 21.33 | 2.22 | 94.55 |
| Thriam | FB | 98.58 | 35.11 | 1.49 | 58.17 |
| Control | RR | 77.78 | 16.03 | 2.89 | 80.52 |
| LU132 | RR | 87.94 | 15.93 | 2.12 | 82.16 |
| LU1347 | RR | 92.29 | 13.03 | 5.50 | 105.42 |
| LU1358 | RR | 83.71 | 12.69 | 4.08 | 92.64 |
| LU785 | RR | 80.17 | 16.81 | 4.12 | 88.19 |
| Thriam | RR | 93.28 | 15.21 | 4.10 | 79.85 |

The four *Trichoderma* isolates, and the thiram control produced higher fresh shoot weights per pot than the *R. solani* control in both varieties (Table 5.36). They also produced higher shoot fresh weights per plant for Red Round, and LU132, LU1347 and the thiram control did so for French Breakfast (Table 5.37). In particular, for FB isolate LU785, and for RR the thiram control had the highest fresh and dry shoot weights per pot (Table 5.36). However, the thiram control for FB had higher dry shoot weight per pot than the *R. solani* control (Table 5.36), whereas LU785 and the thiram control for FB, and all the isolates for Red Round had higher shoot dry weight per plant than the *R. solani* control (Table 5.37).

For FB, isolate LU1358 had the highest fresh and dry bulb weights per pot (3.36 and 0.23g respectively) of all the isolates (Table 5.36). In addition, LU1358 had a higher fresh and dry bulb weight per plant than the *R. solani* control (Table 5.37). Interestingly, the *R. solani* control for RR had a higher fresh bulb weight (2.23g) per pot than the other treatments, as did LU1347 for the dry bulb weight (0.15g) (Table 5.36). Moreover, only LU1347 had a higher fresh and dry bulb weight per plant than the *R. solani* control, whereas LU132 had lower dry bulb weights per plant (Table 5.37).

Isolate LU785 for FB had a higher fresh fibrous weight per pot and per plant than the *R. solani* control (Table 5.36), and isolate LU1347 had the highest dry fibrous weight per pot among the isolates (Table 5.36), as did LU1347 for dry fibrous weight per plant (Table 5.37). However, LU1347 for RR had a higher fresh fibrous weight per plant than the *R. solani*

control (Table 5.37), as did LU1358 per pot for RR which had highest fresh dry fibrous weights in both per pot and per plant than the *R. solani* control.

Table 5.36 Effects of *Trichoderma* isolate (of both varieties) on plant fresh and dry weights per pot (g), at 20 DAS (one replicate only).

| | | Fresh weight per pot (g) | | | Dry weight per pot (g) | | |
|---|---|---|---|---|---|---|---|
| Isolate | Variety | Shoot (g) | Bulb (g) | Fibrous (g) | Shoot (g) | Bulb (g) | Fibrous (g) |
| Control | FB | 6.72 | 1.34 | 1.12 | 0.79 | 0.11 | 0.068 |
| LU132 | FB | 9.95 | 1.12 | 0.86 | 0.84 | 0.08 | 0.055 |
| LU1347 | FB | 8.27 | 0.89 | 0.95 | 0.77 | 0.07 | 0.091 |
| LU1358 | FB | 7.61 | 3.36 | 1.25 | 0.64 | 0.23 | 0.079 |
| LU785 | FB | 12.53 | 1.25 | 1.45 | 0.96 | 0.10 | 0.066 |
| Thiram | FB | 16.90 | 1.09 | 0.61 | 1.37 | 0.09 | 0.034 |
| Control | RR | 6.51 | 2.23 | 0.77 | 0.62 | 0.08 | 0.040 |
| LU132 | RR | 10.40 | 0.77 | 0.77 | 0.88 | 0.06 | 0.052 |
| LU1347 | RR | 8.01 | 2.10 | 1.24 | 0.70 | 0.15 | 0.053 |
| LU1358 | RR | 8.91 | 1.43 | 1.36 | 0.70 | 0.11 | 0.063 |
| LU785 | RR | 9.27 | 1.45 | 1.05 | 0.74 | 0.11 | 0.042 |
| Thiram | RR | 12.04 | 1.79 | 0.96 | 0.96 | 0.13 | 0.059 |

Table 5.37 Effects of *Trichoderma* isolate (of both varieties) on plant fresh and dry weights per plant (g), at 20 DAS (one replicate only).

| | | Fresh weight per plant (g) | | | Dry weight per plant (g) | | |
|---|---|---|---|---|---|---|---|
| Isolate | Variety | Shoot (g) | Bulb (g) | Fibrous (g) | Shoot (g) | Bulb (g) | Fibrous (g) |
| Control | FB | 0.83 | 0.167 | 0.140 | 0.099 | 0.0141 | 0.0084 |
| LU132 | FB | 1.00 | 0.112 | 0.086 | 0.084 | 0.0081 | 0.0055 |
| LU1347 | FB | 1.03 | 0.111 | 0.119 | 0.096 | 0.0084 | 0.0114 |
| LU1358 | FB | 0.76 | 0.336 | 0.125 | 0.064 | 0.0234 | 0.0079 |
| LU785 | FB | 1.39 | 0.139 | 0.162 | 0.107 | 0.0113 | 0.0074 |
| Thiram | FB | 2.11 | 0.136 | 0.076 | 0.171 | 0.0111 | 0.0042 |
| Control | RR | 0.93 | 0.318 | 0.111 | 0.089 | 0.0118 | 0.0057 |
| LU132 | RR | 1.16 | 0.086 | 0.086 | 0.098 | 0.0065 | 0.0058 |
| LU1347 | RR | 1.34 | 0.350 | 0.206 | 0.117 | 0.0245 | 0.0088 |
| LU1358 | RR | 1.27 | 0.204 | 0.195 | 0.101 | 0.0154 | 0.0090 |
| LU785 | RR | 1.16 | 0.181 | 0.131 | 0.092 | 0.0133 | 0.0053 |
| Thiram | RR | 1.34 | 0.199 | 0.106 | 0.106 | 0.0144 | 0.0065 |

The thiram control had the highest leaf area per pot for both varieties (239.42 mm for FB and 149.76 mm for RR), whereas the *R. solani* control had the lowest (Table 5.38). Moreover, LU132, LU1347, LU785 and the thiram control for FB had higher leaf area per plant than the *R. solani* control (Table 5.39), as did all the *Trichoderma* isolates and the thiram control for Red Round.

Leaf numbers per pot for the two varieties ranged from 17 – 28 (Table 5.38). LU785 for FB and the thiram control for RR had higher leaf number per pot than the other treatments (Table 5.36). In addition, LU785 for FB had higher leaf number per plant than the *R. solani* control, as did LU1347, LU1358 and the thiram control for Red Round (Table 5.39).

The SPAD value varied for both varieties. All of the *Trichoderma* isolates, and the thiram control of both varieties, had higher SPAD values than the *R. solani* control, with the exception of FB LU132, which had a lower value (21.3) (Table 5.38).

Table 5.38 Effects of *Trichoderma* isolate on SPAD value, leaf area ($cm^2$) and leaf numbers per pot of both varieties, at 20 DAS (one replicate only).

| Isolate | Variety | Leaf number per pot | Leaf area ($cm^2$) per pot | SPAD value |
|---|---|---|---|---|
| Control | FB | 17 | 91.95 | 24.5 |
| LU132 | FB | 24 | 142.59 | 21.3 |
| LU1347 | FB | 16 | 114.38 | 26.9 |
| LU1358 | FB | 20 | 110.19 | 28.3 |
| LU785 | FB | 26 | 179.57 | 24.8 |
| Thiram | FB | 25 | 239.42 | 25.4 |
| Control | RR | 22 | 93.41 | 25.5 |
| LU132 | RR | 18 | 149.08 | 27.2 |
| LU1347 | RR | 18 | 118.31 | 29.6 |
| LU1358 | RR | 21 | 110.24 | 29.4 |
| LU785 | RR | 21 | 134.04 | 28.1 |
| Thiram | RR | 28 | 149.76 | 28.1 |

Table 5.39 Effects of *Trichoderma* isolate on leaf area ($cm^2$) and leaf numbers per plant of both varieties, at 20 DAS (one replicate only).

| Isolate | Variety | Leaf number per plant | Leaf area ($cm^2$) per plant |
|---|---|---|---|
| Control | FB | 2.6 | 11.49 |
| LU132 | FB | 2.0 | 14.26 |
| LU1347 | FB | 2.0 | 14.30 |
| LU1358 | FB | 2.3 | 11.02 |
| LU785 | FB | 3.1 | 19.95 |
| Thiram | FB | 2.0 | 29.93 |
| Control | RR | 2.9 | 13.34 |
| LU132 | RR | 2.9 | 16.56 |
| LU1347 | RR | 3.0 | 19.72 |
| LU1358 | RR | 3.0 | 15.75 |
| LU785 | RR | 2.0 | 16.76 |
| Thiram | RR | 3.3 | 16.64 |

### 5.3.5 Glasshouse experiment 2: Assessment at 33 DAS

The numbers of seedlings from 1 to 33 DAS differed significantly among the treatments for both varieties (P<0.001). All the *Trichoderma* isolates and the thiram control of French Breakfast had significantly higher seedling numbers than the *R. solani* control (Figure 5.19). Also, LU132, LU1347 and LU1358, and the thiram control for Red Round had significantly higher seedling numbers than the *R. solani* control, but LU785 had a significantly lower seedling number than the *R. solani* control (Figure 5.20).

At 10, 20 and 33 DAS, there was significant interactions between the two varieties and among the isolates (Table 5.40). LU132 for FB and LU1347 for RR had significantly higher numbers of seedlings at 10, 20 and 33 DAS than the *R. solani* control.

Maximum numbers of seedlings for the two varieties had emerged by 10 DAS (Table 5.41 and 5.44) but then decreased in all treatments except for LU1358 and LU785 for FB, and LU132 and LU1347 for RR because of post-emergence seedling death (Figure 5.20).

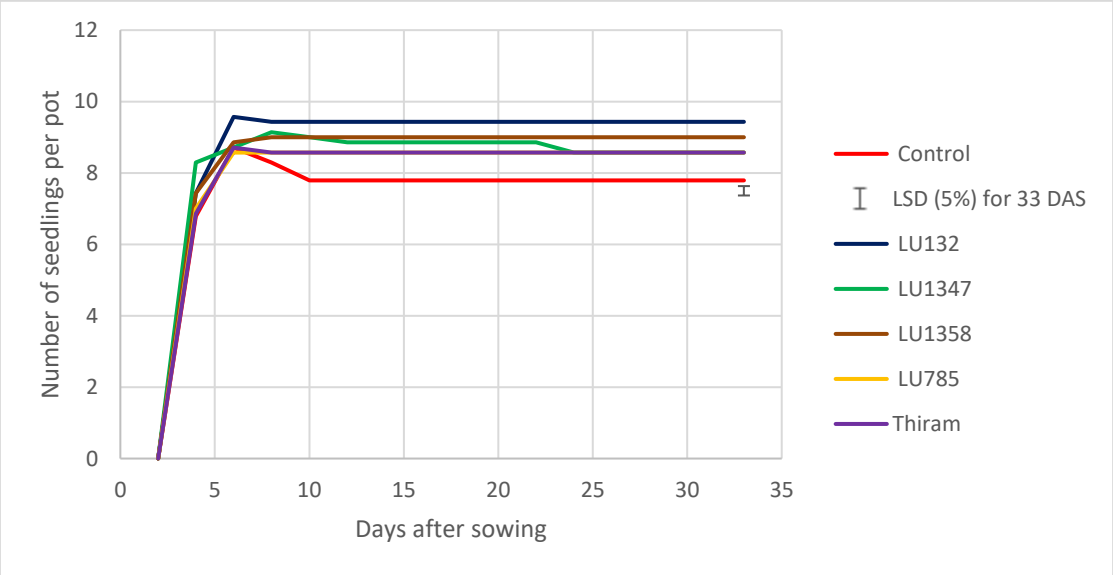


Figure 5.19 Effects of *Trichoderma* isolate on seedling numbers per pot (out of 10 seeds sown per pot) of French Breakfast, up to 33 DAS.

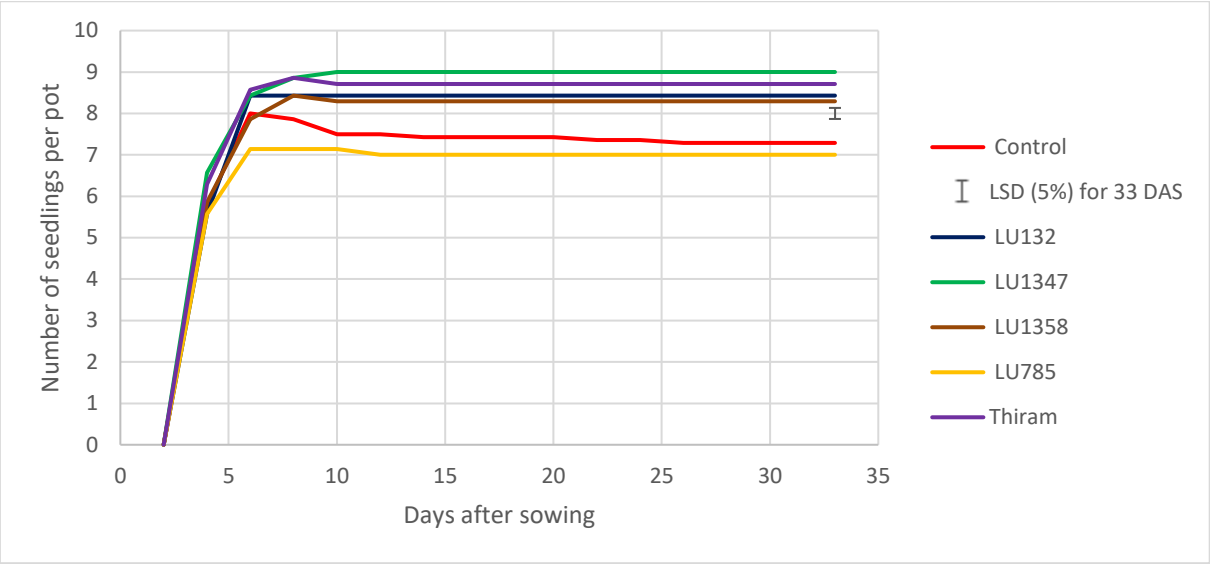


Figure 5.20 Effects of *Trichoderma* isolate on seedling numbers per pot (out of 10 seeds sown per pot) of Red Round, up to 33 DAS.

Table 5.40 Least significant difference and significance of difference for the main effects of variety and isolate, and interaction for seedling number at 10, 20 and 33 DAS.

| FB vs RR | Day 10 | Day 20 | Day 33 |
|---|---|---|---|
| Main effect of variety | 0.443 | 0.463 | 0.466 |
| Main effect of isolate | 0.717 | 0.75 | 0.756 |
| Variety x isolate interaction | 1.015 | 1.06 | 1.069 |
| Significance of main effect of variety | * | * | * |
| Significance of main effect of isolate | *** | *** | *** |
| Significance of interaction | ns | ns | ns |
| Ns = Not significantly different | | | |

Maximum number of seedlings for FB emerged from 6 to 8 DAS (Table 5.41). LU132, LU1347 and LU1358 had significantly higher numbers of seedlings than the *R. solani* control ($P<0.05$). However, the numbers of diseased seedlings did not differ significantly among the treatments (Table 5.41).

At 20 DAS, isolates LU132 and LU1358 had significantly higher numbers of seedlings than the *R. solani* control ($P<0.05$). At this time, there were no significant differences for the numbers of diseased seedlings, numbers of dead seedlings post-emergence or SPAD values (Table 5.42). However, the number of diseased seedlings had increased.

At 33 DAS, LU132 and LU1358 still had significantly higher seedling numbers than the *R. solani* control. However, none of the treatments differed significantly from the *R. solani* control in terms of the numbers of diseased and dead seedlings (Table 5.43). All the treatments had a disease score of 1.

All of the treatments had significantly higher SPAD values than the *R. solani* control, with thiram having the highest SPAD value.

Table 5.41 Effects of *Trichoderma* isolates on maximum seedling emergence, days after sowing for maximum emergence, and numbers of diseased seedlings at maximum emergence for French Breakfast.

| French Breakfast | | | |
|---|---|---|---|
| Isolate | Maximum seedlings emergence | Days after sowing to achieve maximum emergence | Number of diseased seedlings at maximum emergence |
| Control | 8.71 | 6 | 0.6 |
| LU132 | 9.57* | 6 | 0.7 |
| LU1347 | 9.14* | 8 | 0.7 |
| LU1358 | 9.00* | 8 | 0.7 |
| LU785 | 8.57 | 6 | 0.3 |
| Thiram | 8.71 | 6 | 0.3 |
| LSD 5% | 0.14 | - | 0.7 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$).

Table 5.42 Effects of *Trichoderma* isolates on number of surviving seedlings, number of diseased seedlings, SPAD value, and number of dead seedlings post-emergence for French Breakfast radish at 20 DAS.

| French Breakfast | Assessment at 20 DAS | | | |
|---|---|---|---|---|
| Isolate | Number of surviving seedlings | Number of diseased seedlings | Number of dead seedlings post-emergence | SPAD value |
| Control | 7.79 | 0.8 | 0.92 | 24.8 |
| LU132 | 9.43* | 1.1 | 0.14 | 26.0 |
| LU1347 | 8.86 | 0.9 | 0.28 | 23.8 |
| LU1358 | 9.00* | 1.0 | 0.00 | 24.5 |
| LU785 | 8.57 | 1.1 | 0.00 | 24.5 |
| Thiram | 8.57 | 0.6 | 0.14 | 25.9 |
| LSD 5% | 1.12 | 0.8 | - | 2.2 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Table 5.43 Effects of *Trichoderma* isolates on number of surviving seedlings, number of diseased seedlings, disease score, SPAD value, and number of dead seedlings post-emergence for French Breakfast radish at 33 DAS.

| French Breakfast | Assessment at 33 DAS | | | | |
|---|---|---|---|---|---|
| Isolate | Number of survived seedlings | Number of diseased seedlings | Disease score | Number of dead seedlings post-emergence | SPAD value |
| Control | 7.79 | 1.1 | 1 | 0.9 | 22.9 |
| LU132 | 9.43* | 1.1 | 1 | 0.1 | 27.6* |
| LU1347 | 8.57 | 1.0 | 1 | 0.6 | 28.6* |
| LU1358 | 9.00* | 1.3 | 1 | 0.0 | 25.9* |
| LU785 | 8.57 | 1.1 | 1 | 0.0 | 28.6* |
| Thiram | 8.57 | 0.6 | 1 | 0.1 | 29.6* |
| LSD 5% | 1.12 | 1.0 | - | - | 2.9 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Maximum seedling emergence for Red Round was between 6 and 10 DAS and did not differ significantly among the treatments (Table 5.44). The number of diseased seedlings at maximum emergence did differ significantly among the isolates (P<0.05), and LU132 and the thiram control had significantly lower numbers of diseased seedlings than the *R. solani* control.

At 20 DAS, isolate LU1347 and the thiram control had significantly higher numbers of surviving seedlings than the *R. solani* control (P<0.05). There were no significant differences for the number of diseased seedlings, number of dead seedlings post-emergence, or the SPAD

value (Table 5.45). However, the numbers of diseased seedlings had increased after the date of maximum emergence.

At 33 DAS, the thiram control and isolates LU132 and LU1347 had significantly higher numbers of live seedlings than the *R. solani* control (Table 5.46). However, only the thiram control had a significantly lower number of diseased seedlings than the *R. solani* control. All of the treatments had a disease score of 1 and had lower numbers of dead seedlings post-emergence than the *R. solani* control. In particular, isolate LU132 and LU1347 had no dead seedlings at 33 DAS. All of the treatments had significantly higher SPAD values than the *R. solani* control.

Table 5.44 Effects of *Trichoderma* isolates on maximum seedling emergence, days after sowing for maximum emergence, and numbers of diseased seedlings at maximum emergence for Red Round.

| Red Round | | | |
|---|---|---|---|
| Isolate | Maximum seedlings emergence | Days after sowing to achieve maximum emergence | Number of diseased seedlings at maximum emergence |
| Control | 8.00 | 6 | 1.4 |
| LU132 | 8.43 | 6 | 0.6* |
| LU1347 | 9.00 | 10 | 1.4 |
| LU1358 | 8.43 | 8 | 1.3 |
| LU785 | 7.14 | 6 | 0.7 |
| Thiram | 8.86 | 10 | 0.6* |
| LSD 5% | 1.05 | - | 0.7 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Table 5.45 Effects of *Trichoderma* isolates on number of surviving seedlings, number of diseased seedlings, SPAD value, and number of dead seedlings post-emergence for Red Round at 20 DAS.

| Red Round | Assessment at 20 DAS | | | |
|---|---|---|---|---|
| Isolate | Number of surviving seedlings | Number of diseased seedlings | Number of dead seedlings post-emergence | SPAD value |
| Control | 7.43 | 2.1 | 0.6 | 25.71 |
| LU132 | 8.43 | 1.7 | 0.0 | 27.34 |
| LU1347 | 9.00* | 1.6 | 9.0 | 27.33 |
| LU1358 | 8.29 | 2.1 | 0.1 | 26.57 |
| LU785 | 7.00 | 1.4 | 0.1 | 26.73 |
| Thiram | 8.71* | 0.9 | 0.1 | 26.21 |
| LSD 5% | 1.09 | 0.9 | - | 2.37 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Table 5.46 Effects of *Trichoderma* isolates on number of surviving seedlings, number of diseased seedlings, disease score, SPAD value, and number of dead seedlings post emergence for Red Round at 33 DAS.

| Red Round | Assessment at 33 DAS | | | | |
|---|---|---|---|---|---|
| Isolate | Number of surviving seedlings | Number of diseased seedlings | Disease score | Number of dead seedlings post-emergence | SPAD value |
| Control | 7.29 | 2.2 | 1 | 0.7 | 23.8 |
| LU132 | 8.43* | 1.7 | 1 | 0.0 | 30.8* |
| LU1347 | 9.00* | 1.6 | 1 | 0.0 | 32.8* |
| LU1358 | 8.29 | 2.1 | 1 | 0.1 | 32.4* |
| LU785 | 7.00 | 1.4 | 1 | 0.1 | 32.9* |
| Thiram | 8.71* | 0.9* | 1 | 0.1 | 31.0* |
| LSD 5% | 1.10 | 0.9 | - | - | 4.3 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

The healthy plant percentages for French Breakfast did not differ significantly among the treatments (Table 5.47). However, for Red Round, the thiram control had a higher (P<0.05) healthy seedling percentage (81.4%) than the *R. solani* control (67.9%).

Table 5.47 Effects of *Trichoderma* isolates on healthy seedling percentages of both varieties at 33 DAS.

| Isolate | French Breakfast healthy plant (%) at 33 DAS | Red Round healthy plant (%) at 33 DAS |
|---|---|---|
| Control | 77.1 | 67.9 |
| LU132 | 78.6 | 72.9 |
| LU1347 | 80.0 | 74.3 |
| LU1358 | 77.1 | 68.6 |
| LU785 | 78.6 | 75.7 |
| Thiram | 84.3 | 81.4* |
| LSD (5%) | 9.7 | 9.1 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

The shoot lengths of both varieties differed significantly from the *R. solani* control for all treatments (P<0.05) (Table 5.48 and 5.49). All of the *Trichoderma* isolates and the thiram control produced higher shoot lengths than the *R. solani* control (91.6mm for FB and 82.7mm for RR).

For FB, isolate LU1347 had a significantly higher hypocotyl length (15.35mm) than the *R. solani* control (P<0.05) (Table 5.48) and for RR, isolates LU1347, LU1358 and LU785 produced higher hypocotyl lengths than the *R. solani* control (Table 5.49).

The French Breakfast thiram control had the greatest (P<0.05) hypocotyl radial size (42.62mm), but there were no significant differences for Red Round. Only LU132 for FB (Table 5.48) and the thiram control for RR (Table 5.49) produced significantly higher fibrous root lengths than the *R. solani* control.

Table 5.48 Effect of *Trichoderma* isolates on plant parameters (i.e. shoot, hypocotyl and fibrous root length, and hypocotyl radial size) of French Breakfast at 33 DAS.

| French Breakfast | | | | |
|---|---|---|---|---|
| Isolate | Shoot length (mm) | Hypocotyl length (mm) | Hypocotyl radial size (mm) | Fibrous root length (mm) |
| Control | 91.6 | 7.92 | 33.49 | 81.73 |
| LU132 | 128.4* | 8.44 | 37.24 | 94.90* |
| LU1347 | 124.8* | 15.35* | 34.45 | 82.24 |
| LU1358 | 122.3* | 7.09 | 39.81 | 92.23 |
| LU785 | 124.1* | 11.73 | 36.60 | 89.85 |
| Thiram | 134.7* | 8.89 | 42.62* | 89.57 |
| LSD 5% | 18.8 | 5.30 | 8.32 | 12.52 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Table 5.49 Effect of *Trichoderma* isolates on plant parameters (i.e. shoot, hypocotyl and fibrous root length, and hypocotyl radial size) of Red Round at 33 DAS.

| Red Round | | | | |
|---|---|---|---|---|
| Isolate | Shoot length (mm) | Hypocotyl length (mm) | Hypocotyl radial size (mm) | Fibrous root length (mm) |
| Control | 82.7 | 11.98 | 17.83 | 80.01 |
| LU132 | 122.5* | 15.05 | 19.98 | 89.38 |
| LU1347 | 132.2* | 17.42* | 20.50 | 88.66 |
| LU1358 | 122.6* | 16.16* | 18.76 | 91.94 |
| LU785 | 113.1* | 17.59* | 18.81 | 83.70 |
| Thiram | 131.1* | 15.62 | 20.76 | 102.02* |
| LSD 5% | 15.1 | 4.07 | 3.66 | 15.96 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

All treatments increased the fresh and dry shoot weights per pot for French Breakfast (Table 5.50) and fresh shoot weight per plant (Table 5.51). However, only LU1347, LU785 and the thiram control had significantly higher dry shoot weight per plant than the *R. solani* control (Table 5.51). Isolates LU132 and LU785 had significantly higher fresh bulb and dry fibrous root weights per pot than the *R. solani* control (Table 5.50). In addition, LU132, LU785, and the thiram control had significantly higher fibrous fresh weights per pot than the *R. solani* control. The dry bulb weight per pot was significantly increased by LU785 and the thiram. Fresh bulb and fibrous weight per plant for all the isolates did not differ significantly from the *R. solani* control as did dry bulb weights per plant (Table 5.51). Only LU785 had a significantly higher dry fibrous weight per plant than the *R. solani* control (Table 5.51).

For Red Round, all of the treatments had significantly higher fresh and dry shoot weights per pot and per plant than the *R. solani* control (Table 5.52 and Table 5.53). In addition, LU1347, LU1358, LU785 and the thiram control produced significantly higher fresh and dry bulb weights per pot than the *R. solani* control (Table 5.52). Also, LU1347, LU1358 and LU785 had significantly higher fresh bulb weights per plant than the *R. solani* control, as did LU785 for Red Round per plant (Table 5.53). The fresh fibrous root weight per pot (Table 5.52) and per plant (Table 5.53) did not differ significantly from the *R. solani* control, while only LU132 had a significantly higher dry fibrous root weight per pot and per plant than the *R. solani* control.

Table 5.50 Effect of *Trichoderma* isolates on fresh and dry plant weights per pot (g) for French Breakfast, at 33 DAS.

| French Breakfast | Fresh weight per pot (g) | | | Dry weight per pot (g) | | |
|---|---|---|---|---|---|---|
| Isolate | Shoot (g) | Bulb (g) | Fibrous root (g) | Shoot (g) | Bulb (g) | Fibrous root (g) |
| Control | 12.08 | 10.35 | 2.54 | 1.41 | 0.87 | 0.15 |
| LU132 | 26.28* | 17.64* | 3.50* | 2.40* | 1.28 | 0.22* |
| LU1347 | 31.65* | 16.42 | 3.08 | 2.56* | 1.12 | 0.15 |
| LU1358 | 29.80* | 11.38 | 2.99 | 2.32* | 0.98 | 0.20 |
| LU785 | 32.20* | 17.83* | 3.60* | 2.81* | 1.43* | 0.23* |
| Thiram | 35.61* | 16.38 | 3.46* | 3.05* | 1.48* | 0.20 |
| LSD 5% | 6.79 | 6.99 | 0.83 | 0.49 | 0.47 | 0.06 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control ($P<0.05$).

Table 5.51 Effect of *Trichoderma* isolates on fresh and dry plant weights per plant (g) for French Breakfast, at 33 DAS.

| French Breakfast | Fresh weight per plant (g) | | | Dry weight per plant (g) | | |
|---|---|---|---|---|---|---|
| Isolate | Shoot (g) | Bulb (g) | Fibrous root (g) | Shoot (g) | Bulb (g) | Fibrous root (g) |
| Control | 1.67 | 1.38 | 0.327 | 0.189 | 0.1139 | 0.0191 |
| LU132 | 2.89* | 2.04 | 0.383 | 0.263 | 0.1475 | 0.0241 |
| LU1347 | 3.70* | 2.08 | 0.372 | 0.305* | 0.1460 | 0.0186 |
| LU1358 | 3.36* | 1.29 | 0.335 | 0.258 | 0.1108 | 0.0224 |
| LU785 | 3.79* | 2.08 | 0.417 | 0.330* | 0.1680 | 0.0266* |
| Thiram | 4.17* | 2.16 | 0.416 | 0.364* | 0.1886 | 0.2503 |
| LSD 5% | 1.07 | 1.13 | 0.099 | 0.079 | 0.0766 | 0.0073 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Table 5.52 Effect of *Trichoderma* isolates on fresh and dry plant weights per pot (g) for Red Round, at 33 DAS.

| Red Round | Fresh weight per pot (g) | | | Dry weight per pot (g) | | |
|---|---|---|---|---|---|---|
| Isolate | Shoot (g) | Bulb (g) | Fibrous root (g) | Shoot (g) | Bulb (g) | Fibrous (g) |
| Control | 12.40 | 16.12 | 3.29 | 1.34 | 1.20 | 0.18 |
| LU132 | 29.45* | 28.43 | 4.08 | 2.22* | 1.69 | 0.47* |
| LU1347 | 32.82* | 35.76* | 4.01 | 2.43* | 2.13* | 0.20 |
| LU1358 | 30.79* | 37.27* | 4.15 | 2.23* | 2.00* | 0.16 |
| LU785 | 25.14* | 35.20* | 4.14 | 2.01* | 1.95* | 0.18 |
| Thiram | 30.54* | 31.64* | 4.39 | 2.30* | 1.87* | 0.18 |
| LSD 5% | 8.18 | 12.33 | 1.36 | 0.58 | 0.62 | 0.24 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Table 5.53 Effect of *Trichoderma* isolates on fresh and dry plant weights per plant (g) for Red Round, at 33 DAS.

| Red Round | Fresh weight per plant (g) | | | Dry weight per plant (g) | | |
|---|---|---|---|---|---|---|
| Isolate | Shoot (g) | Bulb (g) | Fibrous root (g) | Shoot (g) | Bulb (g) | Fibrous root (g) |
| Control | 1.71 | 2.23 | 0.454 | 0.181 | 0.1664 | 0.0025 |
| LU132 | 3.43* | 3.28 | 0.474 | 0.259* | 0.1943 | 0.0556* |
| LU1347 | 3.65* | 4.01* | 0.446 | 0.270* | 0.2383 | 0.0225 |
| LU1358 | 3.77* | 4.62* | 0.499 | 0.274* | 0.2482 | 0.0195 |
| LU785 | 3.64* | 5.25* | 0.573 | 0.287* | 0.2857* | 0.0248 |
| Thiram | 3.51* | 3.77 | 0.499 | 0.264* | 0.2189 | 0.0210 |
| LSD 5% | 0.99 | 1.75 | 0.134 | 0.065 | 0.0826 | 0.0262 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Total leaf area and leaf numbers per pot for both varieties differed significantly from the *R. solani* control (P<0.001) (Table 5.54 and 5.56). All treatments for both varieties had significantly higher leaf areas and leaf numbers per pot than the *R. solani* control, with the exception of isolate LU132 in FB for total leaf area per pot. LU1347, LU785 and the thiram control for French Breakfast had significantly higher leaf area per plant than the *R. solani* control (Table 5.55), as did all the isolates for Red Round (Table 5.57). In addition, all the isolates of the two varieties had significantly higher leaf number per plant than the *R. solani* control (Table 5.55 and 5.57) apart from LU132 for FB which did not differ significantly.

Table 5.54 Effect of *Trichoderma* isolates on leaf area ($cm^2$) and leaf numbers per pot for French Breakfast, at 33 DAS.

| French Breakfast | | |
|---|---|---|
| Isolate | Leaf area (cm2) per pot | Leaf number per pot |
| Control | 259.7 | 23.4 |
| LU132 | 423.8 | 33.4* |
| LU1347 | 565.2* | 34.4* |
| LU1358 | 503.4* | 35.1* |
| LU785 | 558.4* | 34.0* |
| Thiram | 611.1* | 36.0* |
| LSD 5% | 166.0 | 4.2 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Table 5.55 Effect of *Trichoderma* isolates on leaf area ($cm^2$) and leaf numbers per plant for French Breakfast, at 33 DAS.

| French Breakfast | | |
|---|---|---|
| Isolate | Leaf area (cm2) per plant | Leaf number per plant |
| Control | 36.28 | 3.06 |
| LU132 | 45.93 | 3.63 |
| LU1347 | 67.04* | 4.02* |
| LU1358 | 56.56 | 3.94* |
| LU785 | 65.48* | 4.01* |
| Thiram | 73.61* | 4.30* |
| LSD 5% | 24.62 | 0.69 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Table 5.56 Effect of *Trichoderma* isolates on leaf area ($cm^2$) and leaf numbers per pot for Red Round, at 33 DAS.

| Red Round | | |
|---|---|---|
| Isolate | Leaf area (cm2) per pot | Leaf number per pot |
| Control | 209.4 | 25.8 |
| LU132 | 509.7* | 39.0* |
| LU1347 | 556.0* | 40.6* |
| LU1358 | 545.5* | 40.9* |
| LU785 | 462.2* | 34.0* |
| Thiram | 520.3* | 38.0* |
| LSD 5% | 141.0 | 6.5 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

Table 5.57 Effect of *Trichoderma* isolates on leaf area ($cm^2$) and leaf numbers per plant for Red Round, at 33 DAS.

| Red Round | | |
|---|---|---|
| Isolate | Leaf area (cm2) per plant | Leaf number per plant |
| Control | 29.25 | 3.59 |
| LU132 | 59.17* | 4.60* |
| LU1347 | 61.80* | 4.50* |
| LU1358 | 67.31* | 4.98* |
| LU785 | 66.08* | 4.88* |
| Thiram | 61.24* | 4.36* |
| LSD 5% | 18.28 | 0.66 |

Note: '*' indicates an isolate that differs significantly from *R. solani* control (P<0.05).

## 5.4 Discussion

*Rhizoctonia solani* is an economically important soil-borne pathogen with a wide range of hosts (e.g. root crops); it causes damage to plant growth with a resulting reduction in yield (Miller & Baysal-Gurel, 2010). *Trichoderma* spp. are known as effective biocontrol agents worldwide, with the ability to promote plant growth and induce plant resistance against pathogens (Agrimm Technologies, 2014; Samules & Hebbar, 2015) in the agriculture and horticulture industry. *Trichoderma* can be applied as a seed treatment (Sharma, 2016).

Two glasshouse experiments were conducted to test the four selected *Trichoderma* isolates for their biocontrol ability in naturally *R. solani*-infested soil. The first glasshouse pot experiment used *Trichoderma* in wheat-bran. The second glasshouse pot experiment used *Trichoderma* seed coatings and a current fungicide seed treatment (thiram) in naturally-*R. solani* infested soil. This was carried out to determine if the *Trichoderma* seed coating could be used instead of prills in the field experiment (Kandula et al., 2015).

The hypothesis was that the *R. solani* would reduce numbers of radish seedlings and their yields in both experiments. The negative effects of *R. solani* on radish seedling emergence and their growth was evident in Chapter 3 (Experiment 1), a result also previously reported by Kandula et al. (2015) for forage species and by Hua et al. (2014) for Brassica crops. *Trichoderma* spp. were expected to promote radish seedling growth, and effectively control the pathogen. Both the wheat-bran and seed coat application methods were expected to promote plant growth and enhance plant health.

Strains of the selected *Trichoderma* spp. (*T. atroviride*, *T. hamatum*, *T. harzianum* and *T. polysporum*) were identified as efficient strains for disease control and promotion of plant growth from Chapter 4 (Experiment 2). Isolates of *T. atroviride* and *T. harzianum* are known worldwide as parasitic fungi capable of controlling *R. solani*, by releasing cell wall-degrading enzymes and antibiotics (Henis et al., 1978; Lorito, Hayes, di Petro, Woo, & Harman., 1994).

Two experiments showed that *Trichoderma* isolates had significantly increased the numbers of seedlings in both radish varieties over that of the *R. solani* control (first glasshouse experiment: FB LU1347, LU1358 and LU785; RR LU132, LU1347, LU1358 and LU785; second glasshouse experiment: FB LU132 and LU1358; RR LU132 and LU1347). In the first glasshouse experiment, for French Breakfast *Trichoderma* isolates LU1347, LU1358 and LU785 had 10.7 - 13.6% more seedlings than the *R. solani* control at 40 DAS; for Red Round, these isolates had 15.0 - 17.7% more seedlings. *T. atroviride* increased cauliflower emergence

by 14% (Rehman, Lawrence, Kumar, & Badri, 2012), and Kandula et al. (2015) reported that perennial ryegrass seedling emergence was increased by 60 - 150%. Application of *Trichoderma* spp. in wheat bran is likely to act through myco-parasitism of pathogen hyphae and sclerotia in the soil (Grinyer, Hunt, McKay, Herbert, & Nevalainen., 2005).

The second glasshouse experiment showed that the *Trichoderma* and thiram seed coatings effectively increased seedling emergence and enhanced plant growth in the presence of the pathogen. LU132 and LU1358 for French Breakfast had significantly higher percentages of survived seedlings (16.4% and 12.1% respectively) than the *R. solani* control, as did LU132, LU1347 and the thiram control for Red Round, ranging from 11.4 - 17.1%. *Trichoderma* seed treatments play a vital role in improving establishment, and promoting plant growth and plant resistance against pathogen (International Seed Federation, 2015; Rakholiya & Jadeja, 2010; Roberts, 2012; Sharma et al., 2015). Treatment of seeds with *Trichoderma* can ameliorate physiological stresses to the seeds and seedlings (Sharma et al., 2015).

The numbers of diseased and dead seedlings post-emergence increased after maximum emergence in both experiments. The mature *R. solani* mycelium (approx. 20 days old) can penetrate into the plant cell tissue and damage various seedling parts (damping off and rot in seeds; wilting and root cracking in seedlings) (Brown & Ogle, 1997; Rimmer et al., 2007). Although the numbers of diseased seedlings had increased at 40 DAS, they did not differ significantly among the isolates for either variety. Only the Red Round thiram control for the second glasshouse experiment had a significantly lower number of diseased seedlings than the *R. solani* control. Fungicide seed treatments (e.g. thiram) can have a positive influence on the numbers of emerging seedlings and protecting seedlings against fungal pathogens (Falloon, 1980).

LU1347, LU1358 and LU785 for French Breakfast, and LU132, LU1347 and LU785 for Red Round, had significantly higher healthy seedling percentages than the *R. solani* control. The mechanisms used by *Trichoderma* (i.e. antibiotic, mycoparasitism and induced systemic resistance) were not determined in this experiment. However, Harman et al. (2004), Mendoza et al. (2015) and Shi et al. (2012) reported *Trichoderma* isolates induced resistance by producing an antibiosis barrier (e.g. azaphilones, petailbols and viridian) against the development of *R. solani* hyphae growth.

No New Zealand research has reported that *Trichoderma* wheat-bran inoculation or seed coating improves radish seedling chlorophyll (i.e. SPAD value), its leaf area, or its leaf number. Chlorophyll content is a significant factor in determining the quantity of dry matter

produced (Kibe, Kirui, Wagara, & Thagana., 2017). *Trichoderma* isolates in both experiments and the thiram control, had significantly higher SPAD values than the *R. solani* control. Volatile organic *Trichoderma* compounds (e.g. 6 pentyl-2H-pyran-2-one from *T. atroviride*) promote chlorophyll content (Garnica-Vergara et al., 2016).

*Trichoderma* isolates of both varieties produced significantly higher total leaf areas and leaf numbers per pot than the *R. solani* control. *Trichoderma* can improve plant growth (Samules & Hebbar., 2015), and induce resistance in the host against pathogen attacks (Harman et al., 2004). However, the leaf numbers of FB for isolates LU132, LU1347 and LU785 did not differ significantly from the *R. solani* control in the first experiment; nor did the leaf areas per pot for the FB isolate LU132 in the second experiments. A dense plant canopy resulted in inter-specific competition for light between the seedlings.

In the first glasshouse experiment, for French Breakfast the *Trichoderma* isolates LU132, LU1358 and LU785 produced significantly higher shoot lengths than the *R. solani* control. However, the Red Round *Trichoderma* isolates did not differ significantly. This may be because the two radish varieties have different physical characteristics. French Breakfast grows erect branches with simple alternating leaves (Kings Seeds, 2014), and Red Round produces compact compound leaves with small stems (Egmont Seeds, 2017). However, the shoot length of all isolates of both varieties in the second experiment was increased significantly compared to the *R. solani* control. Seed treatment of *Trichoderma* isolates and the thiram control effectively promoted plant growth (Falloon, 1980; Harman et al., 2004; Vargas et al., 2009).

Hypocotyl length for French Breakfast did not differ significantly among the isolates. Nor was hypocotyl radial size increased significantly compared to the *R. solani* control, apart from LU1347 for French Breakfast and LU132 for Red Round in the first experiment, and the thiram control for French Breakfast in the second experiment. However, in Red Round the isolates LU1347, LU1358 and LU785 in the second experiment had significantly increased hypocotyl lengths compared with the *R. solani* control. Fibrous root length did not differ significantly among the isolates with the exception of LU132 for FB and LU1358 for RR in the first experiment, and LU132 for FB and the thiram control for RR in the second experiment. The effects of growth promotion on root length were not the same in all the tested pots. Small pot (size 13 x 14cm) and higher sowing density between seedlings (2 – 3cm) restricted the root growth. In particular, parts of the fibrous root may have been removed

during extraction of samples from the pots, and the shallow pot caused several curvatures of the fibrous root and hypocotyl in several cases.

In both experiments, all *Trichoderma* isolates of both varieties had significantly higher fresh and dry shoot weights per pot in the presence of the pathogen. Similar results of plant biomass increase resulting from *Trichoderma* spp. inoculations were also reported by Inbar, Abramsky, Cohen, and Chet (1994) for cucumber and pepper, and by Kandula et al. (2015) for perennial ryegrass. *Trichoderma* enhances plant growth by increasing nutrient uptake and the production of auxin-like compounds (Harman et al., 2004).

Maximising radish bulb weight is an important goal, as this is the edible part. However, the fresh and dry bulb weights per pot and per plant in the first glasshouse experiment did not differ significantly from the *R. solani* control for either variety, whereas the second glasshouse experiments showed that some *Trichoderma* isolates (in both varieties) improved bulb weights, as did the thiram control for Red Round. Wheat-bran inoculations of *Trichoderma* isolates had more *Trichoderma* colony forming units than the *Trichoderma* seed coating. However, seed treatment of *Trichoderma* spp. allows establishment in the plant rhizosphere more quickly than wheat-bran inoculation, and provide nutrients to the host plant as a symbiont. Therefore, *Trichoderma* seed treatment improves root development and plant health in seedlings, confirming that of Henis et al., (1978). In addition, the harvest date in the first glasshouse experiment was delayed by seven days, compared to the 33 DAS harvest date in the second experiment. This later harvest date caused an increase in bulb production, but it resulted in no significant differences among the treatments because the small spacing of the pots restricted bulb growth.

*Trichoderma* is able to control a wide range of pathogens and interrupt pathogen growth by improving plant health (Dubey et al., 2011; Harman et al., 2004; Samules & Hebbar, 2015). The application of *Trichoderma* spp. contributed positively to radish development (e.g. seedling emergence and yield). Harman (2000) reported that the most useful *Trichoderma* strains for disease control and growth promotion are rhizosphere-competent strains, which are able to colonise plant roots and grow in them.

In the second glasshouse experiment, the thiram control aided seedling establishment and performed beneficial effects as a seed coating when compared to the *R. solani* control (Aamil, Zaidi, & Khan., 2012). The results show that *Trichoderma* increased seedling emergence and effectively improved plant growth, as did the thiram control. This study did not reveal any differences between the chemical compounds and *Trichoderma* spp. in terms of pathogen

attack mechanisms. However, *Trichoderma* species offered effective biocontrol activity against *R. solani* by increasing plant emergence and inducing resistance against the pathogen (Henis et al., 1978; Kandula et al., 2015).

# Chapter 6: Experiment 4

# Field experiment

## 6.1 Introduction

Edible radish is grown year-round in New Zealand and radish seed production is taking an increasingly major role in the vegetable seed export market. However, soil-borne plant pathogens including *R. solani*, are of increasing concern for production annually because current control measures, including seed treatment fungicides, are not always effective.

Seed treatment using *Trichoderma* spp. can promote seed germination, reduce the impact of plant pathogens and enhance plant growth (Rakholiya & Jadeja, 2010; Sharma et al., 2015). Harman, Chet, and Baker (1980) reported that seed treatment with *T. harzianum* in radish and peas protected seeds and seedlings from *R. solani* nearly as effectively as fungicide seed treatments.

A few commercial *Trichoderma* products are available in New Zealand in different forms (e.g. foliar spray and prill) (Agrimm Technologies, 2014). However, the effect of *Trichoderma* spp. applied via a seed coating on radish production has not been specifically researched in New Zealand. Having established that *Trichoderma* seed coating offered protection from *R. solani* in glasshouse experiments, a field trial was conducted to determine if similar responses could be obtained when radish was sown in a field known to have its soil infected by *R. solani* (Kandula, et al., 2015).

## 6.2 Materials and methods

### i) Site

A site at the Lincoln University research farm was used. The soil type was a Temuka silt loam. The soil was cultivated at a tillage depth of 10cm and was sprayed with glyphosate (ratio 40ml glyphosate per 10L) to kill weeds five weeks prior to sowing.

### ii) Seed coating

Seeds of both varieties were coated with the *Trichoderma* isolates and thiram fungicide, using the method described in Experiment 3. 5.2.3: Preparation of 2nd glasshouse experiment (seed coating). The inoculum rates of *Trichoderma*, the thiram and the control were identical to Experiment 3: 5.2.3, as follows;

- Four sets of *Trichoderma*-coated seeds (LU132, LU785, LU1347 and LU1358): 0.3g spores per 20g seed with 0.28µL seed coating polymer.
- Thiram Fungus Control D.F. Granules - coated seeds: 0.3g dry granular Thiram per 20g seed with 0.28µL of seed coating polymer.
- The control: 0.28µL of seed coating polymer and 1ml sterilized water per 20g seeds.

A total of 60g of seeds per treatment were coated and dried in a laminar flow cabinet for two hours. Next, the coated seeds were weighed to 0.675g for seed sowing each row, packed in separate zip-lock bags (size 50 x 75mm), and stored at a temperature of 4ºC before sowing.

### iii) Colony forming Units

At 20 DAS, colony forming units were counted to determine the *Trichoderma* colony in naturally infested *R. solani* soil. Preparation of *Trichoderma* selective media, soil sampling and colony forming units analysing were identical to Experiment 3 (see 5.22 Colony forming units).

### iv) Experimental design

The design was a completely randomized block (Figure 6.1), with eight blocks and 14 treatments per block. The plot size was 1m x 3m, plots rows were spaced 15 cm apart, and each row was one metre long. An irrigation pathway (size 2 x 3m, reel gun-spray) ran through each block (Figure 6.1).

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| T1 | T2 | T3 | T4 | Irrigation | T5 | T6 | T7 |
| T8 | T9 | T10 | T11 | Irrigation | T12 | T13 | T14 |

**Note: T1 to T 7** = T1: FB control, T2: FB control, T3: FB Thiram, T4: FB LU132, T5: FB LU785, T6: FB LU1347, T7: FB LU1358.

**T8 to T14: T8** = RR control, T9: RR control, T10: RR Thiram, T11: RR LU132, T12: RR LU785, T13: RR LU1347, T14: RR LU1358.

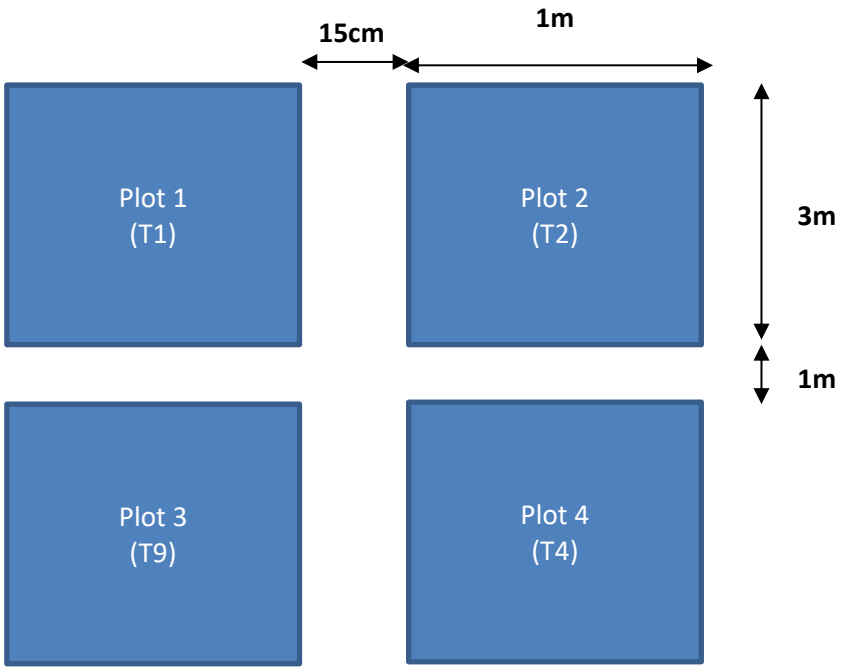


Figure 6.1 An example of experimental design (Block one).

### v) Seed sowing

Approximately 4.05g seed per plot (size 1 x 3m) was sown at 15 kg seed/ha using a seed cone planter (Hans-Ulrich Hege 901) at a depth of 2cm in rows spaced 15 cm apart, and with approximately 3cm between seeds within a row on 4th October 2017. 0.675g seed was sown in each row (0.675g was approx. 87 seeds for FB, and approx. 79 seeds for RR).

After sowing, each plot was marked with wood lane marker sticks and marker posts (Figure 6.2). Irrigation was carried out using a reel gun-spray when required (approx. 10mm per application). 3kg Westminster slug bait (15g/kg metaldehyde) was applied to all the plots after rain on 17th October 2017. The insecticide 'Attack (Chemical group: pyrethroid and organophosphate)' was applied at a rate of l L as pirmiphos-methyl and permethrin in 700L of water per ha at 34 DAS to control leaf miner (*Bedellia somnulentella*) (Young, 2013). The field experiment air temperature range was 8 – 26°C (average 18°C), from 4nd October to 27th November 2018 (Accu Weather, 2017).

Figure 6.2 Field trial in the H1 block, Lincoln University in October 2017.

### vi) Data collection at 33 and 43 DAS

At 33 and 43 DAS, four of the eight replicates were assessed to record the plant growth development and leaf miner damage. The damage caused to leaves by leaf miner was severe (Figure 6.3).

Figure 6.3 Leaf miner damage on radish leaves in the H1 block, Lincoln University in November 2017.

All seedlings were extracted from a 30 x 60cm quadrat in each plot using a spade, placed in plastic mini-grip bags (size 180 x 255mm), and stored at a temperature of -8ºC for one day. The plants were then washed using a water-spray gun to remove soil from the root and leaf surfaces.

After washing, the healthy seedlings and leaf miner damaged seedlings were counted. In addition, the numbers of healthy and diseased leaves were counted. Next, the healthy seedlings and damaged leaf areas were measured using a leaf area meter (Li- 3,100C area meter). Each different plant part (shoot and root) was cut separately using scissors, and the fresh weights of roots and shoots were measured using an electronic scale.

All the roots of a size greater than 20mm were sliced using a knife so that they could be rapidly dried in an oven. Shoots and roots were enclosed in separate paper bags (size 90 x 50 x 205 mm) and dried in an oven at 65ºC for three days. After drying, the dry weights were determined using an electronic scale that weighed up to two decimal points.

After the assessment, data were analysed by GenStat version 18 (ANOVA and Fisher's unprotected LSD test). The area sampled was 30 x 60cm quadrat, and data were converted to per $m^2$ as follows;

- Data value in $0.18m^2$

  $= \chi$ in $1m^2$

- Data value x $1 \div 0.18$

  = Data value/$m^2$

### vii) Data collection at 54 DAS

At 54 DAS, the same measurements and observation were done using the method described in Experiment 3 (5.2.4. Preparation of 2nd glasshouse experiment). The numbers of seedlings and diseased seedlings were counted in a metre of each of two seedling rows up to 54 DAS.

At 54 DAS, all the seedlings from the rows (one metre each of the two seedling rows) were extracted from the soil using a spade and washed with a spray-gun, then stored at -8ºC for one day. Each plant part was cut separately using scissors. Disease score was assessed using the categories given in Experiment 1 (see 3.2.4 Data collection). After that, the plant root parameters (hypocotyl length and hypocotyl radial size) were measured using a digital caliper; and the total leaf area was measured using a Li- 3,100C leaf area metre. Finally, the total number of leaves was counted. After measuring, plant parts were enclosed in paper bags and dried at 65ºC for three days. After drying, the dry weights were measured using an electronic scale that weighted to two decimal points.

Data per metre of the two seedling rows were converted to square metre ($m^2$) as follows:

- Area sampled is 1m x 30cm

  = 1m x 0.3m

  = $0.3m^2$

- Data value in $0.3m^2$

  = $\chi$ in $1m^2$

  = Data value x 1 ÷ 0.3

  = Data value/$m^2$

## 6.3 Results

### 6.3.1 Colony-forming units

The French Breakfast isolates LU132 and LU1347 had significantly more colony-forming units than the control and thiram (Table 6.1). For Red Round, only LU1347 had significantly more colony-forming units than the control and thiram (Table 6.2). LU132, LU1358 and LU785 did not differ significantly from the control or thiram (Table 6.2).

Table 6.1 Number of *Trichoderma* colony-forming units per gram of soil for French Breakfast, at 20 DAS.

| Isolate | Variety | Number of colony forming units per g at 20 DAS |
|---|---|---|
| Control | FB | 219 |
| LU132 | FB | 49,469* |
| LU1347 | FB | 70,969* |
| LU1358 | FB | 13,719 |
| LU785 | FB | 19,781 |
| Thiram | FB | 219 |
| LSD 5% | - | 37,600 |

Note: '*' indicates an isolate that differs significantly from the control ($P<0.05$).

Table 6.2 Number of *Trichoderma* colony-forming units per gram of soil for Red Round, at 20 DAS.

| Isolate | Variety | Number of colony forming units per g at 20 DAS |
|---|---|---|
| Control | RR | 1,125 |
| LU132 | RR | 65,094 |
| LU1347 | RR | 130,938* |
| LU1358 | RR | 17,250 |
| LU785 | RR | 24,312 |
| Thiram | RR | 375 |
| LSD 5% | - | 65,100 |

Note: '*' indicates an isolate that differs significantly from the control ($P<0.05$).

### 6.3.2 Field experiment

Seedling numbers per $m^2$ of French Breakfast varied between 10 and 50 DAS, but by 54 DAS did not significantly differ among the treatments (Figure 6.3). For Red Round, LU1358 consistently had the higher seedling numbers than the other treatments (Figure 6.5).

Maximum seedling emergence for the two varieties occurred between 11 and 13 DAS (Table 6.3 and 6.4). At 54 DAS, the number of seedling per $m^2$ did not differ among the treatments for either variety, with seedling survival ranging from 78 to 80% in French Breakfast, and from 81 to 95% in Red Round (Table 6.3 and 6.4). Post-emergence seedling death at 54 DAS did not differ among the treatments in French Breakfast, ranging from 12 to 22% (Table 6.3), but in Red Round, LU132 had a significantly greater post-emergence seedling death (19%) than the other treatments (range 5 - 10%) (Table 6.4).

However, the percentages of diseased seedlings differed significantly ($P<0.05$) at 54 DAS. For French Breakfast, all *Trichoderma* isolates and the thiram control had a significantly lower percentages of diseased seedlings than the control (Table 6.3). For Red Round, only LU1358 and the thiram control had significantly lower percentages of diseased seedlings (Table 6.4). All treatments for both varieties had a disease score of 1 (Table 6.3 and 6.4).

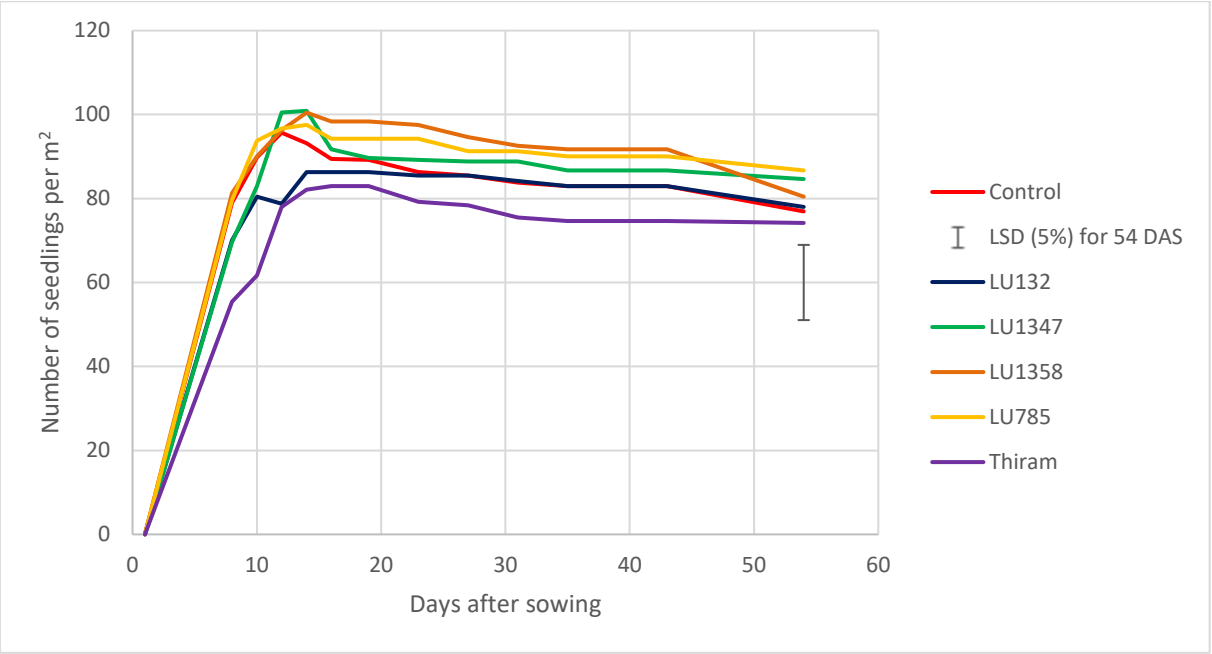


Figure 6.4 Effects of seed treatments on number of seedlings per $m^2$ for French Breakfast up to 54 DAS, at the Lincoln University research farm, from October to November 2017.

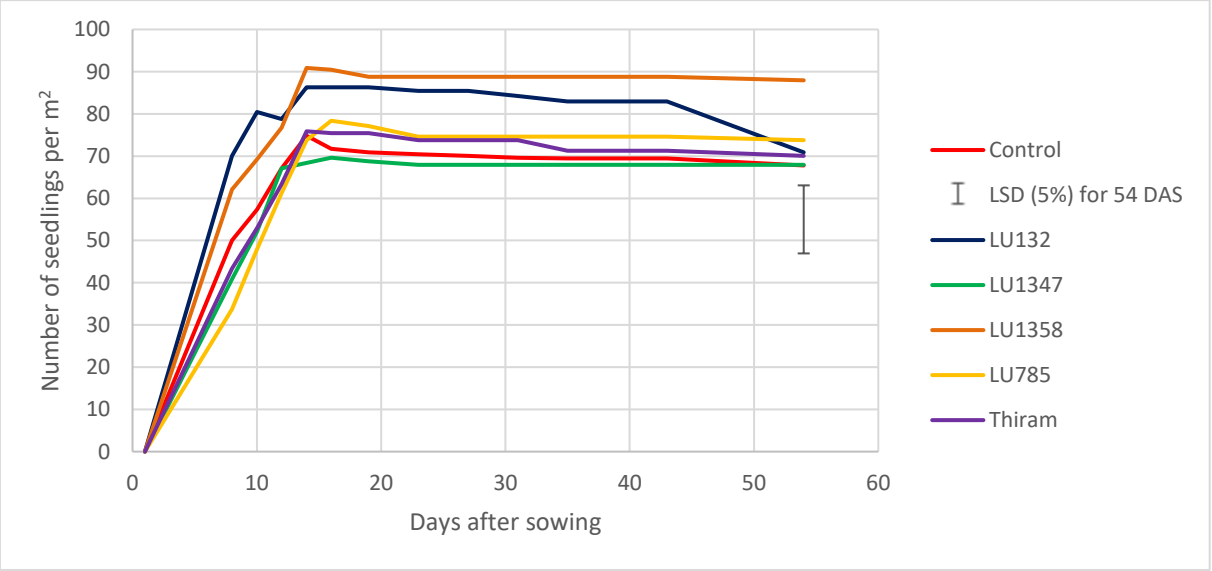


Figure 6.5 Effects of seed treatments on number of seedlings per $m^2$ for Red Round up to 54 DAS, at the Lincoln University research farm, from October to November 2017.

Table 6.3 Effects of seed treatments on maximum seedling number, days after sowing for maximum number, number and percentage of surviving seedlings at -, percentage of diseased seedlings at -, and number and percentage of dead seedlings post-emergence, all at for French Breakfast at 54 DAS.

| French Breakfast | | | | | | |
|---|---|---|---|---|---|---|
| Isolate | Maximum seedling number per $m^2$ | Days after sowing to achieve maximum number | Number and percentage of surviving seedlings per $m^2$ at 54 DAS | Percentage (%) of diseased seedlings at 54 DAS | Number and percentage of dead seedlings post-emergence per $m^2$ at 54 DAS | Disease score |
| Control | 98.00 | 11 | 76.91 (78) | 14.69 | 21.09 (22) | 1 |
| LU132 | 89.60 | 12 | 77.96 (87) | 8.13* | 11.64 (13) | 1 |
| LU1347 | 102.60 | 13 | 84.63 (83) | 8.75* | 17.97 (17) | 1 |
| LU1358 | 103.00 | 13 | 80.46 (78) | 8.13* | 22.54 (22) | 1 |
| LU785 | 98.40 | 12 | 86.71 (88) | 7.12* | 11.69 (12) | 1 |
| Thiram | 85.04 | 13 | 74.20 (87) | 4.38* | 10.84 (13) | 1 |
| LSD 5% | 23.58 | - | 23.72 | 4.92 | 15.21 | - |

Note: '*' indicates a treatment that differs significantly from the control (P<0.05).

Table 6.4 Effects of seed treatments on maximum seedling number, days after sowing for maximum number, number and percentage of surviving seedlings at -, percentage of diseased seedlings at -, and number and percentage of dead seedlings post-emergence, all at for Red Round 54 DAS.

| Red Round | | | | | | |
|---|---|---|---|---|---|---|
| Isolate | Maximum seedlings number per $m^2$ | Days after sowing to achieve maximum number | Number and percentage of surviving seedlings per $m^2$ at 54 DAS | Percentage (%) of diseased seedlings at 54 DAS | Number and percentage of dead seedlings post-emergence per $m^2$ at 54 DAS | Disease score |
| Control | 75.45 | 13 | 67.74 (90) | 11.25 | 7.71 (10) | 1 |
| LU132 | 87.13 | 13 | 70.87 (81) | 8.75 | 16.26* (19) | 1 |
| LU1347 | 72.95 | 12 | 67.95 (93) | 6.88 | 5.00 (7) | 1 |
| LU1358 | 92.55 | 13 | 87.96 (95) | 6.25* | 4.59 (5) | 1 |
| LU785 | 79.62 | 13 | 73.79 (93) | 10.00 | 5.83 (7) | 1 |
| Thiram | 73.37 | 13 | 70.03 (95) | 3.13* | 3.34 (5) | 1 |
| LSD 5% | 20.59 | - | 20.74 | 4.52 | 7.88 | - |

Note: '*' indicates a treatment that differs significantly from the control (P<0.05).

At 33 DAS, damage caused to radish by leaf miner was recorded. For French Breakfast, the number of healthy plants (= non damaged by leaf miner) per $m^2$ and leaves per plant did not differ significantly from the *R. solani* control, with the exception of LU1358, which produced a significantly higher number of healthy plants (112.59 per $m^2$) and leaves (5.23 per plant) than the *R. solani* control (67.41 per $m^2$ and 3.53 per plant respectively) (Table 6.5). However, the healthy leaf area per plant and numbers of leaf miner-damaged leaves per $m^2$ did not differ significantly; nor did the numbers of leaves or leaf areas damaged per plant.

There were no significant differences for the numbers of healthy plants per $m^2$ and their healthy leaf areas per plant for Red Round (Table 6.6). However, LU132 had a significantly lower number of leaf miner-damaged plants per $m^2$ than the *R. solani* control, whereas the number of leaf miner-damaged leaves and leaf area per plant did not differ significantly.

Table 6.5 Effects of seed treatments on number of healthy plants $m^2$, number of healthy leaves per plant, healthy leaf area ($cm^2$) per plant, number of leaf miner-damaged plants per $m^2$, number of leaf miner-damaged leaves per plant, and leaf miner-damaged leaf area ($cm^2$) per plant, for French Breakfast, at 33 DAS.

| Isolate | Number of healthy plant per $m^2$ | Number of healthy leaves per plant | Healthy leaf area ($cm^2$) per plant | Number of leaf miner-damaged plants per $m^2$ | Number of leaf miner-damaged leaves per plant | Leaf miner damaged leaf area ($cm^2$) per plant |
|---|---|---|---|---|---|---|
| Control | 67.41 | 3.53 | 11.14 | 11.12 | 4.29 | 17.48 |
| LU132 | 94.52 | 4.68 | 13.97 | 11.13 | 7.17 | 28.33 |
| LU1347 | 69.50 | 3.32 | 14.34 | 9.73 | 2.88 | 6.45 |
| LU1358 | 112.59* | 5.23* | 16.34 | 20.85 | 1.04 | 3.77 |
| LU785 | 97.30 | 4.84 | 12.41 | 20.90 | 2.18 | 7.75 |
| Thiram | 95.91 | 4.11 | 11.93 | 9.70 | 2.25 | 8.94 |
| LSD 5% | 33.17 | 1.60 | 4.89 | 11.97 | 4.23 | 13.72 |

Note: '*' indicates a treatment that differs significantly from the control ($P<0.05$).

Table 6.6 Effects of seed treatments on number of healthy plants $m^2$, number of healthy leaves per plant, healthy leaf area ($cm^2$) per plant, number of leaf miner-damaged plants per $m^2$, number of leaf miner-damaged leaves per plant, and leaf miner-damaged leaf area ($cm^2$) per plant, for Red Round, at 33 DAS.

| Isolate | Number of healthy plant per $m^2$ | Number of healthy leaves per plant | Healthy leaf area ($cm^2$) per plant | Number of leaf miner-damaged plants per $m^2$ | Number of leaf miner-damaged leaves per plant | Leaf miner damaged leaf area ($cm^2$) per plant |
|---|---|---|---|---|---|---|
| Control | 84.09 | 4.50 | 7.69 | 18.07 | 5.56 | 13.16 |
| LU132 | 91.74 | 3.42 | 12.11 | 5.56* | 5.41 | 21.71 |
| LU1347 | 70.89 | 3.55 | 6.15 | 9.73 | 4.31 | 8.13 |
| LU1358 | 94.52 | 2.61 | 5.64 | 20.85 | 3.60 | 8.08 |
| LU785 | 95.91 | 3.02 | 7.88 | 16.68 | 2.44 | 5.77 |
| Thiram | 82.01 | 2.12 | 7.14 | 11.12 | 2.42 | 6.64 |
| LSD 5% | 37.31 | 2.92 | 7.73 | 10.36 | 5.91 | 20.13 |

Note: '*' indicates a treatment that differs significantly from the control ($P<0.05$).

After spraying at 34 DAS, leaf miner thresholds decreased gradually, but all plots had severe damage.

For French Breakfast, the numbers of healthy plant $m^2$ differed significantly among the treatments ($P<0.05$) (Table 6.7). LU785 had significantly higher numbers of healthy plants than the control. However, there were no significant differences for the number of healthy leaves per plant, numbers of leaf miner damaged plants $m^2$, numbers of leaves, or damaged leaf area ($cm^2$) per plant. Only the thiram control had significantly lower healthy leaf areas ($cm^2$) per plant than the control (Table 6.7).

For Red Round, LU785 had a significantly higher number of healthy plants per $m^2$ than the control (Table 6.8). However, the numbers of healthy leaves and their leaf area per plant did not differ significantly from the control (Table 6.8). In addition, the numbers of leaf miner-damaged plants per $m^2$ and their leaves and leaf area per plant did not differ significantly from the control.

Table 6.7 Effects of seed treatments on number of healthy plants $m^2$, number of healthy leaves per plant, healthy leaf area ($cm^2$) per plant, number of leaf miner-damaged plants per $m^2$, number of leaf miner-damaged leaves per plant, and leaf miner-damaged leaf area ($cm^2$) per plant, for French Breakfast, at 43 DAS.

| French Breakfast | | | | | | |
|---|---|---|---|---|---|---|
| Isolate | Number of healthy plant per $m^2$ | Number of healthy leaves per plant | Healthy leaf area ($cm^2$) per plant | Number of leaf miner-damaged plants per $m^2$ | Number of leaf miner-damaged leaves per plant | Leaf miner damaged leaf area ($cm^2$) per plant |
| Control | 80.62 | 2.90 | 14.83 | 26.41 | 4.59 | 22.11 |
| LU132 | 104.25 | 2.50 | 12.9 | 23.63 | 4.60 | 20.34 |
| LU1347 | 86.18 | 2.39 | 12.34 | 23.60 | 4.46 | 23.73 |
| LU1358 | 98.69 | 2.67 | 14.31 | 33.36 | 2.81 | 13.32 |
| LU785 | 122.32* | 3.15 | 11.99 | 30.58 | 3.85 | 13.96 |
| Thiram | 101.47 | 2.56 | 10.86* | 19.46 | 4.32 | 17.28 |
| LSD 5% | 31.63 | 0.54 | 3.04 | 13.06 | 3.21 | 22.31 |

Note: '*' indicates a treatment that differs significantly from the control ($P<0.05$).

Table 6.8 Effects of seed treatments on number of healthy plants $m^2$, number of healthy leaves per plant, healthy leaf area ($cm^2$) per plant, number of leaf miner-damaged plants per $m^2$, number of leaf miner-damaged leaves per plant, and leaf miner-damaged leaf area ($cm^2$) per plant, for Red Round, at 43 DAS.

| Red Round | | | | | | |
|---|---|---|---|---|---|---|
| Isolate | Number of healthy plant per $m^2$ | Number of healthy leaves per plant | Healthy leaf area ($cm^2$) per plant | Number of leaf miner-damaged plants per $m^2$ | Number of leaf miner-damaged leaves per plant | Leaf miner damaged leaf area per plant |
| Control | 70.89 | 3.28 | 10.28 | 15.98 | 5.19 | 9.56 |
| LU132 | 83.40 | 3.38 | 15.04 | 19.46 | 5.25 | 18.16 |
| LU1347 | 83.00 | 2.74 | 11.82 | 13.90 | 5.08 | 20.50 |
| LU1358 | 79.23 | 4.22 | 10.52 | 18.07 | 6.17 | 13.49 |
| LU785 | 112.59* | 3.03 | 8.67 | 22.24 | 5.63 | 18.73 |
| Thiram | 73.67 | 2.97 | 7.89 | 15.29 | 5.04 | 9.95 |
| LSD 5% | 43.31 | 1.12 | 5.36 | 10.08 | 2.08 | 11.64 |

Note: '*' indicates a treatment that differs significantly from the control ($P<0.05$).

The hypocotyl length, and leaf numbers per plant for French Breakfast did not differ significantly among the treatments (Table 6.9). Isolate LU1358 and thiram produced significantly higher hypocotyl radial sizes than the control, as did LU1347 for leaf area per plant (36.85$cm^2$). SPAD value did not differ significantly among the treatments ranging from

39.64 to 42.4. For Red Round, there were no significant differences in hypocotyl length and radial size, total leaf number per plant and leaf area per plant, and SPAD value (Table 6.10).

Table 6.9 Effects of seed treatment on plant parameters (i.e. hypocotyl length and hypocotyl radial size), leaf number and leaf area per plant, and SPAD value, for French Breakfast, at 54 DAS.

| French Breakfast | | | | | |
|---|---|---|---|---|---|
| Isolate | Hypocotyl length (mm) | Hypocotyl radial size (mm) | Leaf number per plant | Leaf area per plant | SPAD value |
| Control | 39.37 | 19.29 | 4.06 | 26.17 | 39.64 |
| LU132 | 37.35 | 17.90 | 4.05 | 29.53 | 41.14 |
| LU1347 | 37.87 | 20.53 | 4.49 | 36.72* | 42.40 |
| LU1358 | 37.94 | 21.87* | 4.54 | 31.08 | 40.77 |
| LU785 | 38.09 | 20.43 | 4.26 | 28.51 | 38.33 |
| Thiram | 38.18 | 22.18* | 3.80 | 27.80 | 41.74 |
| LSD 5% | 4.28 | 2.24 | 0.67 | 8.72 | 2.07 |

Note: '*' indicates a treatment that differs significantly from the control (P<0.05).

Table 6.10 Effects of seed treatment on plant parameters (i.e. hypocotyl length and radial size), leaf number and leaf area per plant, and SPAD value, for Red Round, at 54 DAS.

| Red Round | | | | | |
|---|---|---|---|---|---|
| Isolate | Hypocotyl length (mm) | Hypocotyl radial size (mm) | Leaf number per plant | Leaf area per plant | SPAD value |
| Control | 18.42 | 22.82 | 3.80 | 21.22 | 36.08 |
| LU132 | 20.33 | 23.77 | 3.81 | 20.88 | 41.00 |
| LU1347 | 17.69 | 21.22 | 3.34 | 17.95 | 35.67 |
| LU1358 | 21.10 | 25.45 | 3.72 | 27.88 | 34.61 |
| LU785 | 19.83 | 24.44 | 3.94 | 27.09 | 40.90 |
| Thiram | 20.40 | 25.90 | 4.13 | 22.24 | 41.05 |
| LSD 5% | 3.21 | 3.96 | 0.97 | 18.7 | 6.91 |

Note: '*' indicates a treatment that differs significantly from the control (P<0.05).

At 33 DAS, the fresh shoot weights per $m^2$ for French Breakfast did not differ significantly, except for isolate LU1358, which had a significantly higher fresh shoot and root weights (201.0g and 685.3g respectively) than the control (78.90g and 210.00g) (Table 6.11 and 6.13). LU1358 and LU785 also had a significantly higher fresh root weight than the control (Table 6.13). The fresh root weight of LU785 were 95% greater than that of the control (Table 6.15).

For Red Round, the shoot fresh weights did not differ significantly from the *R. solani* control (Table 6.12), and only isolate LU132 produced a significantly higher fresh root weight (598.4 g) (Table 6.14). The fresh shoot weight of LU132 was 95% greater than that of the *R. solani* control (Table 6.16).

At 43 DAS, the fresh shoot weights did not differ significantly among the French Breakfast treatments (Table 6.11). However, LU132, LU1358 and LU785 had higher fresh root weights than the other treatments ($P<0.05$) (Table 6.13). The root weight of LU132 (713.6g) was 83% greater than that of the *R. solani* control (Table 6.15). In addition, the root weights of LU1358 and LU785 were significantly higher than that of the *R. solani* control (by 60 and 96% respectively) (Table 6.15).

For Red Round, LU785 produced significantly higher fresh shoot (Table 6.12) and root weights (Table 6.14) than those of the *R. solani* control (by 89% and 97% respectively) (Table 6.14).

At 54 DAS, the fresh shoot and root weights for French Breakfast did not differ significantly, apart from isolate LU785, which had a significantly higher fresh root weight (744.7g) than the *R. solani* control (552.1g) (Table 6.13). The root weight of LU785 was higher than that of the *R. solani* control by 35% (Table 6.15).

However fresh shoot and root weight for Red Round did not differ significantly from the *R. solani* control. *Trichoderma* isolates LU132 and LU1358 and the thiram control had higher shoot weights than the *R. solani* control (by 5%, 21% and 18% respectively), which were not significant differences (Table 6.16). Finally, all the isolates had higher root weight than the *R. solani* control, except for LU1347, which had a root weight 8% less than that of the *R. solani* control.

Table 6.11 Effects of seed treatments on fresh shoot weight (g per $m^2$) for French Breakfast at 33, 43 and 54 DAS.

| French Breakfast | Fresh shoot weight (g) per $m^2$ | | |
|---|---|---|---|
| Isolate | 33 DAS | 43 DAS | 54 DAS |
| Control | 78.9 | 173.6 | 191.0 |
| LU132 | 123.5 | 223.5 | 190.6 |
| LU1347 | 128.1 | 165.1 | 225.8 |
| LU1358 | 201.0* | 188.0 | 190.6 |
| LU785 | 127.5 | 248.8 | 225.7 |
| Thiram | 89.3 | 149.1 | 210.3 |
| LSD 5% | 74.9 | 97.3 | 58.2 |

Note: '*' indicates a treatment that differs significantly from the control (P<0.05).

Table 6.12 Effects of seed treatments on fresh shoot weight (g per $m^2$) for Red Round at 33, 43 and 54 DAS.

| Red Round | Fresh shoot weight (g) per $m^2$ | | |
|---|---|---|---|
| Isolate | 33 DAS | 43 DAS | 54 DAS |
| Control | 96.10 | 86.0 | 125.7 |
| LU132 | 113.13 | 127.7 | 132.6 |
| LU1347 | 80.54 | 122.2 | 121.5 |
| LU1358 | 72.97 | 133.3 | 152.3 |
| LU785 | 91.50 | 164.3* | 124.3 |
| Thiram | 84.92 | 86.6 | 147.8 |
| LSD 5% | 76.00 | 53.6 | 44.1 |

Note: '*' indicates a treatment that differs significantly from the control (P<0.05).

Table 6.13 Effects of seed treatments on fresh root weight (g per $m^2$) for French Breakfast at 33, 43 and 54 DAS.

| French Breakfast | Fresh root weight (g) per $m^2$ | | |
|---|---|---|---|
| Isolate | 33 DAS | 43 DAS | 54 DAS |
| Control | 210.0 | 389.0 | 552.0 |
| LU132 | 285.0 | 713.6* | 585.0 |
| LU1347 | 293.0 | 503.0 | 712.0 |
| LU1358 | 685.3* | 621.2* | 589.0 |
| LU785 | 409.5* | 761.7* | 744.7* |
| Thiram | 226.0 | 445.0 | 646.7 |
| LSD 5% | 178.0 | 162.0 | 191.0 |

Note: '*' indicates a treatment that differs significantly from the control (P<0.05).

Table 6.14 Effects of seed treatments on fresh root weight (g per $m^2$) for Red Round at 33, 43 and 54 DAS.

| Red Round | Fresh root weight (g) per $m^2$ | | |
|---|---|---|---|
| Isolate | 33 DAS | 43 DAS | 54 DAS |
| Control | 306.5 | 457.2 | 573.7 |
| LU132 | 598.4* | 744.7 | 706.1 |
| LU1347 | 365.6 | 695.8 | 528.6 |
| LU1358 | 326.5 | 724.0 | 727.4 |
| LU785 | 422.5 | 899.9* | 632.1 |
| Thiram | 354.5 | 470.9 | 780.5 |
| LSD 5% | 199.6 | 326.3 | 225.5 |

Note: '*' indicates a treatment that differs significantly from the control (P<0.05).

Table 6.15 Effects of seed treatments on fresh shoot and root weights per $m^2$ for French Breakfast at 33, 43 and 54 DAS. Data expressed as percentage of the control.

| French Breakfast | 33 DAS | | 43 DAS | | 54 DAS | |
|---|---|---|---|---|---|---|
| Isolate | Shoot weight | Root weight | Shoot weight | Root weight | Shoot weight | Root weight |
| LU132 | 57 | 36 | 29 | 83* | 0 | 6 |
| LU1347 | 62 | 40 | -5 | 29 | 18 | 29 |
| LU1358 | 155* | 227* | 8 | 60* | 0 | 7 |
| LU785 | 62 | 95* | 43 | 96* | 18 | 35* |
| Thiram | 13 | 8 | -14 | 14 | 10 | 17 |

Note: '*' indicates a treatment that differs significantly from the control (P<0.05).

Table 6.16 Effects of seed treatments on fresh shoot and root weights per $m^2$ for Red Round at 33, 43 and 54 DAS. Data expressed as percentage of the control.

| Red Round | 33 DAS | | 43 DAS | | 54 DAS | |
|---|---|---|---|---|---|---|
| Isolate | Shoot weight | Root weight | Shoot weight | Root weight | Shoot weight | Root weight |
| LU132 | 18 | 95* | 47 | 63 | 5 | 23 |
| LU1347 | -16 | 19 | 40 | 52 | -3 | -8 |
| LU1358 | -24 | 7 | 53 | 58 | 21 | 27 |
| LU785 | -5 | 38 | 89* | 97* | -1 | 10 |
| Thiram | -12 | 16 | 0 | 3 | 18 | 36 |

Note: '*' indicates a treatment that differs significantly from the control (P<0.05).

## 6.4 Discussion

The soil-borne pathogen *Rhizoctonia solani* is commonly associated with disease in grass (Kandula et al., 2015) and *Brassicae* both in New Zealand (Agriseeds, 2017a; Rimmer et al., 2007) and worldwide (Du Toit & Pelter, 2003; Kareem & Hassan, 2015; Yang et al., 2004).

The use of fungicide seed treatments has previously been considered effective in controlling seedling pathogens such as *R. solani* (Hillocks, Chinodya, & Gunner, 1988; MSPB, 2016). They are also known to increase seedling emergence (Falloon, 1980; MSPB, 2016), and to have the potential to shorten growth rate and stand development (Smiley, Patterson, & Rhinhart, 1996). However, biological control research is essential to ensure minimal disruptive influences on the environment (USDA, n.d.), including in sustainable and environmentally-compatible disease management.

*Trichoderma* spp. have been identified worldwide as having the ability to increase seedling numbers, and induce plant resistance against pathogens (Harman, 2005; Samules & Hebbar, 2015). The effects of *Trichoderma* seed coating on radish production had not been investigated in New Zealand. In this study, the beneficial micro-organisms *Trichoderma* spp. (LU132: *T. atroviride*, LU785: *T. hamatum*, LU1358: *T. polysporum* and LU1347: *T. harzianum*) were formulated into a radish seed-coating product and tested in a field (Temuka silt loam soil) with naturally *R. solani*-infested soil, in order to examine their biocontrol ability. The hypothesis of the experiment was that (i) *Trichoderma* spp. seed coating would increase seedling emergence and ameliorate plant growth (e.g. plant growth parameters and weight); (ii) *Trichoderma* would provide control of *R. solani* as effectively as thiram; and (iii) *R. solani* would reduce seedling emergence and plant growth by causing root rot and damping-off.

Henis et al. (1983) and Kandula et al. (2015) have both reported that *Trichoderma* seed treatment improves seedling establishment. In addition, several studies have stated that fungicide seed treatments contribute positive effects towards germination and seedling emergence (Falloon, 1980; Hillocks et al., 1988; Lewis, 1988). However, in this field experiment, the seedling numbers for the *Trichoderma* isolates and the thiram treatment, at maximum emergence and at 54 DAS, did not differ significantly from the control, for either variety. Seedling growth can be affected by environment factors, including sowing season, sowing depth, soil type, water availability and the presence of pests (Kandula et al., 2015). In particular, the field experiment temperature range was from 8 – 26ºC in October to November. A warm temperature range (>20ºC) can promote seedling growth (Falloon, 1980),

but low humidity causes drought stress, causing plants to close their stomata in order to prevent moisture loss, thus having a negative impact on plant growth (Indiana University, 2016).

All *Trichoderma* isolates and the thiram treatment for French Breakfast had a significantly lower percentage of diseased seedlings than the control, as did LU1358 and the thiram control for Red Round. However, as yet the mechanism for this protection is not known. *Trichoderma* spp. may protect the host plant by inducing resistance against *R. solani* infections (Kandula et al., 2015). *Trichoderma* are able to produce antibiotic compounds to inhibit pathogen activity and produce higher concentrations of fungi-toxic metabolites at higher temperatures. In addition, chemical seed treatments containing thiram inhibit the growth of the fungus (Hillocks et al., 1988). However, this protection did not last, as seedling survival was reduced by *R. solani* at 54 DAS, resulting in a disease score of '1' (i.e. symptoms of *R. solani* infections, see Experiment 1: 3.2.4) for both varieties. *R. solani* caused damage both underground (roots) and above ground (hypocotyl, leaves and stems) (Brown & Ogle, 1997).

At 33 DAS, plant damage caused by leaf miner was observed in all plots. Leaf miner damages the leaves, leading to a reduction in photosynthesises, resulting in leaf senescence (Agriseeds, 2017b). In Red Round, isolate LU132 had significantly fewer leaf miner-damaged plants per $m^2$ than the control. However, *Trichoderma* spp. are not known to be entomopathogenic against pests such as leaf miner (Lester, 2010).

Despite the damage caused by leaf miner and *R. solani* infections, *Trichoderma* spp. significantly increased radish growth parameters (i.e. hypocotyl length, leaf number and SPAD value). The hypocotyl radial size (LU1358 and the thiram control for French Breakfast), SPAD values (LU1347 for French Breakfast), and the leaf areas per plant of the two varieties (LU1347 for French Breakfast and the Red Round thiram control) were significantly higher than the control. In the presence of *R. solani*, LU1358 for French Breakfast at 33 DAS, and LU785 for Red Round at 43 DAS, produced significantly higher fresh shoot weights per $m^2$ than the control. *Trichoderma* isolates LU132, LU1358 and LU785 had heavier fresh root weights per $m^2$ than the control at both 33 and 43 DAS for both varieties. As reported by Kumar et al. (2014), a seed treatment made by mixing strains of *T. viride* with *T. harzianum* was significantly effective in increasing chickpea growth and yield.

Radish is normally harvested between 28 - 42 days after emergence (40 – 54 DAS) (Egmont Seeds, 2017; Kings Seeds, 2014). Expected yields are between 7 – 10 tonnes/ha (Pervez, Ayyub, & Saleem, 2003). The growth analysis at 43 DAS (approximately 31 days after

emergence) occurred within the commercial harvest time frame. Radish yield for the French Breakfast control was 389 g/m$^2$ (3.9 t/ha), and for the Red Round control, it was 457 g/m$^2$ (4.6 t/ha), less than half the expected yield. Yield for the thiram seed treatment did not differ from the control (4.5 t/ha for French Breakfast and 4.7 t/ha for Red Round). However, the LU785 seed treatment increased radish yield by 96% for both varieties (7.6 t/ha for French Breakfast and 9.0 t/ha for Red Round). For French Breakfast LU132 and LU1358 also significantly increased radish yield (by 83% and 60% respectively), and although in Red Round the yields from these treatments did not differ significantly from the control, they were still 63% and 58% higher.

The reason why these large yield increases were recorded is likely to be associated with suppression of *R. solani* and growth promotion, although this cannot be determined from this study. LU785 is a strain of *T. hamatum*, and Harman et al. (1980) reported that the strain of this species they used provided good control of *R. solani* in a glasshouse study. At 43 DAS, LU785 was the only treatment to have more healthy plants per m$^2$ than the control (around 50% more), which strongly implicates activity against *R. solani*. Alternatively, as thiram (a fungicide with known activity against *R. solani*) did not increase yield, perhaps the yield response to *Trichoderma* treatments was through growth promotion. This remains to be determined.

# Chapter 7 : General discussion and conclusions

*Rhizoctonia solani* is a fungal-pathogen that negatively affects a wide range of root crops by causing rotting and cracking of roots (Miller & Baysal-Gurel, 2010; USDA, 2013), and wilting of stems following the production of brownish lesions (Cabi, 2016; Henis et al., 1978). Radish is an important vegetable crop in New Zealand, both for domestic use and the export market. Radish seed production was valued at $9,058 per tonne in 2013, which was the second-highest price for non-brassica vegetable seeds (Sanderson & Twaddle, 2014).

This research was conducted to determine (i) the effects of the pathogen on red radish production, from seedling emergence to final crop yield, and (ii) biological control of the pathogen using *Trichoderma* spp. delivered via wheat-bran and as a seed coating.

Fungicides available for foliar spraying to control *R. solani* include Amistar, Inspire, Pristine, Addstem and Cannon (Young, 2013). In addition, proprietary seed treatments are used for general treatment purposes, such as SF Force Field Plus, Superstrike Brassica, Ultrastrike Brassica, Agricote Brassica and Cropcoat Plus (Spec Seed, 2017). Chemical application can be efficient in reducing pathogen damage in the short term; however, phytotoxicity may be a problem (Paulsrud et al., 2001). There is also the risk of fungicide resistance build-up from overuse (MAF, n.d.), with consequent environmental damage. On the other hand, the demand for safe food production and sustainable farming is increasing. Therefore, introducing biocontrol agents for pathogen control in order to minimize disease thresholds is a potential solution; biocontrol agents can also be beneficial to plant growth.

*Trichoderma* spp. have been proved as beneficial biocontrol agents worldwide in agriculture and horticulture (Samules & Hebbar, 2015; Schuster & Schmoll, 2010). They are commonly found in both soil and inorganic matter such as plant wood or sapwood. *Trichoderma* produce numerous secondary metabolites that are able to enhance plant growth and induce plant resistance (Elad et al., 1980; Harman et al., 2004).

In this research, 21 *Trichoderma* strains of eight species were selected based on 'APA *Trichoderma* biocontrol capability data'. *Trichoderma* spp. were tested both in *vitro* and *in vivo* to determine their capability for biocontrol against *R. solani*. Two interactions of *Trichoderma* spp. occurred in dual culture assays. *B* type (e.g. LU132) interactions inhibited and grew over the *R. solani* colony, whereas *D* type interactions (e.g. LU1347, LU1358 and LU785) inhibited at a distance, leaving a zone and inferring the production of toxins from the

*D*-type fungi. Different *Trichoderma* spp. produce different degrees of inhibition of pathogens in the environment (Henis et al., 1983; Rahman et al., 2009). *Trichoderma* strains were tested in the glasshouse using an inoculated wheat-bran medium application. A few *Trichoderma* isolates (e.g. LU132) had significantly more seedlings and higher plant weights than the *R. solani* control. Most *Trichoderma* spp. supressed *R. solani* growth; however, plants in all *Trichoderma* treatments were damaged by *R. solani* infections, as *R. solani* grows quicker than *Trichoderma* spp. in moist soil, and in cool temperate environments (Brantner, Mickelson, & Crane, n.d.).

After evaluating disease control and plant growth parameters, four of the *Trichoderma* isolates (LU132, LU785, LU1347 and LU1358) had higher numbers of surviving plants than the *R. solani* control, and promoted plant growth (i.e. plant fresh and dry weights). *Trichoderma* spp., including *T. atroviride* (LU132) and *T. harzianum* (LU1347), are currently available in various *Trichoderma* products (such as foliar spray and prills) in New Zealand agriculture and horticulture (Agrimm Technologies, 2014).

These four selected *Trichoderma* strains were formulated into seed-coatings and their performance assessed using naturally infested with *R. solani*, both in *vitro* and *in vivo*. Thiram seed treatment was included as a second control. In the second glasshouse experiment with *R. solani* infected soil, and in the field trial, *Trichoderma* treatments significantly out yielded the control and thiram treatment, with LU785 almost doubling yield in both radish varieties. The first hypothesis for this research was that soil-borne *R. solani* would decrease radish production by negatively affecting radish seedling emergence and plant growth. In the field trial, *R. solani* did not significantly reduce seedling emergence or seedlings which died post-emergence, but did increase the percentage of diseased plants (by between 50 – 80%). The difference in radish yield (harvest 43 DAS) between the control and the best of the *Trichoderma* treatments (LU785) was 76% (a difference of 3.7 t/ha for French Breakfast and 4.4 t/ha for Red Round), implying that *R. solani* was responsible for a 50% yield reduction. However, the LU785 yield was also 60 – 70% greater than the yield from the thiram seed treatment, implying that reducing the negative impact from *R. solani* on yield was not the only factor involved in the response. The hypothesis was therefore partly proved, in that in the presence of *R. solani* and in the absence of chemical or *Trichoderma*, radish plant growth (bulb yield) was almost halved. *Trichoderma* seed treatment in the rhizosphere enhances root development (Harman, 2017; Sharma et al., 2015) and promotes plant growth as an avirulent symbiont (Samules & Hebbar, 2015).

Vegetable radish is consumed as food worldwide, and the trend is a move away from agrichemical-applied food. Food markets set strict thresholds for pesticide residues (Pertot, Alabouvette, Esteve, & Franca, n.d.). In Sweden and Norway, the introduction of such schemes as 'reduction of the use of seed treatments' and the demand for 'restrictions on chemical use' are regulated (Sharma et al., 2015). Consequently, the demand for alternatives to chemicals, such as biological control (e.g. *Trichoderma* spp.), is increasing. This study proved that *Trichoderma* spp. are beneficial micro-organisms for radish growth because they gave protection against the plant pathogen *Rhizoctonia solani* and promoted plant growth (Mendoza et al., 2015). They act against plant pathogens such as *R. solani* by releasing cell wall-degrading enzymes and antibiotics (Grinyer et al., 2005; Samules & Hebbar, 2015). Furthermore, *Trichoderma* have no adverse effects on human health or the environment (Samules & Hebbar, 2015). Therefore, biological control of *R. solani* by *Trichoderma* seed treatments is a strong potential alternative to the use of the chemicals.

In conclusion, this study demonstrated the following:

1. *Rhizoctonia solani* can significantly reduce radish seedling emergence and crop yield.

2. From the Bio-Protection Research Centre's *Tricho*-bank, several *Trichoderma* strains were able to provide biocontrol of *Rhizoctonia solani*.

3. When formulated as a seed coating, some *Trichoderma* strains out-performed the thiram seed treatment, particularly when it came to radish yield in the field trial.

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