# Studies on the Efficacy of Three *Trichoderma atroviride* Isolates for Controlling Take-all (*Gaeumannomyces graminis* var. *tritici*) in Grasses

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A thesis
submitted in partial fulfilment
of the requirements for the Degree of
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by
Abdullah Umar

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# Studies on the Efficacy of Three *Trichoderma atroviride* Isolates for Controlling Take-all (*Gaeumannomyces graminis* var. *tritici*) in Grasses

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Take-all, caused by the fungal pathogen *Gaeumannomyces graminis* var. *tritici* (*Ggt*), is an internationally important widespread root disease of cereals and grasses. The disease is suspected as the main cause of the increasing occurrence of light seeds in ryegrass seed crops. This study was carried out to investigate its impact on grass seed yield using prairie grass (*Bromus wildenowii*) as a model system, and examined the interaction responses of *Ggt*, biocontrol by *Trichoderma* and moisture stress. Another study was used to assess if *Trichoderma* isolates could deliver effective biocontrol activity and growth promotion of perennial ryegrass grown in a soil naturally infected with *Ggt*.

Take-all delayed the time to first seedhead emergence (TFSE) of prairie grass by 1.5 days, reduced the number of seeds, total seed weight, and the weight of machine dressed seeds (MDS) by 14%, 14%, and 13%, respectively. Seed yield loss occurred mainly through a reduced number of seeds, instead of weight of the individual seeds. However, seed yield reduction did not occur in plants with a restricted access to soil nutrients. The disease also caused a substantial root infection and reduced root dry weight. Soil inoculation with *Trichoderma* isolates increased all seed yield components. *Trichoderma* isolates also accelerated plant growth and continuously increased shoot dry weight for up to 7 months after their introduction. *Trichoderma* also enhanced drought resistance of the plants by increasing leaf relative water content under moisture stress conditions. This experiment confirmed that moisture stress decreased all seed yield components, including the number of fertile tillers, the number of seeds, the total seed weight, the weight of MDS, and thousand seed weight by 13%, 32%, 41%, 46%, and 11%, respectively. The stress also severely reduced shoot dry weight throughout the experimental period. Take-all did not reduce seed yield

in the plants protected by *Trichoderma*. On the other hand, growth promotion effects by *Trichoderma* isolates occurred only in the presence of *Ggt*. Moisture stress reduced the root disease severity, but did not affect the biocontrol efficacy of *Trichoderma* against *Ggt*. Instead, a greater increase in the weight of MDS occurred in moisture stressed (42%) than non-stressed (24%) plants. Without protection by *Trichoderma* isolates, take-all combined with moisture stress exacerbated the seed yield loss, reducing the weight of MDS by 51%. Moisture stress was the primary determinant of *Trichoderma*-plant-pathogen interactions, reducing the shoot and seed yields in the absence/presence of *Trichoderma* or *Ggt*.

The second study confirmed that in the presence of *Ggt* all *Trichoderma* isolates significantly increased perennial ryegrass shoot dry weight by 46-73%, which was strongly correlated with an increase in root dry weight by 42-62%. *Trichoderma* isolates also significantly decreased root disease severity. This study did not support the hypothesis that a combination of the three *Trichoderma* isolates could improve the biocontrol efficacy over that of any individual isolate. However, the mixtures of isolates and isolate LU140 produced a greater root dry weight than the control, which could possibly give the plant a greater ability to cope with adverse environmental conditions than the two other isolates.

**Keywords:** *Gaeumannomyces graminis* var. *tritici*, *Ggt*, take-all, root disease, *Trichoderma atroviride*, LU132, LU140, LU584, mixture of isolates, rhizosphere-competent, biological control, plant growth promotion, moisture stress, drought, prairie grass, perennial ryegrass, grass seed crops, pasture, seed yield, soil nutrients.

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Sekali lagi, Terima kasih.

"*For indeed, with hardship [will be] ease. Indeed, with hardship [will be] ease.*" (Ash-Sharh 94: 5-6).

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# Glossary

- Ascospore : a spore contained in an ascus or that was produced inside an ascus.
- Ascus : spore sack for ascospores.
- Perithecia : sexual fruiting bodies.
- Hyphopodia : attachment lobes on mycelia.
- Prill : dry sphere formed from a melted liquid.
- Culm : The aerial (above-ground) stem of a grass. Originally referred to the stem of any type of plant.
- Seminal roots : lateral roots, root that develop from the radicle.
- Radicle : the first part of a seedling (a growing plant embryo) to emerge from the seed during the process of germination.

# Chapter 1

# Introduction

## 1.1 General issues of take-all

Take-all, caused by the root-infecting fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*), is an important disease in cereal production leading to severely reduced productivity and yields. The pathogen is best known and is important to cause root rot on wheat (Cook, 2003). It is arguably the most-studied root disease of any crop and remains the most critical root disease of wheat worldwide (Cook, 2003). Along with wheat, barley and triticale are the cereals most susceptible to take-all, but numerous forage grass species can also be infected, with various degrees of susceptibility (Chng et al., 2005; Wherrett & MacLeod, 2018). According to Nilsson and Smith (1981), as many as 402 grass species have been reported as hosts of *Ggt*, indicating the importance of take-all in pastures or meadows. For instance, a severe case of take-all was reported in a *Lolium perenne* (perennial ryegrass) crop grown in a high pH soil in Ireland (O'Rourke, 1976). Also, grass seed crops may be severely infected by the disease (Nilsson & Smith, 1981). In New Zealand, take-all is of major concern as many susceptible grass species are cultivated as annual forage crops or as part of a rotation in sequence with cereal crops, allowing carry-over of *Ggt* inoculum and allowing the infection to persist successfully from one host to another (Bithell et al., 2011a). Furthermore, the disease can affect crops grown under both high and low precipitation (Cook, 2003). Hence, it is possible that attacks and yield losses caused by *Ggt* are common in pastures or meadows.

## 1.2 Pathogen biology

*Gaeumannomyces graminis* var. *tritici* belongs to the phylum *Ascomycota,* sub-division *Ascomycotina,* class *Ascomycetes,* order *Diaporthales,* and family *Magnapothaceae* (Hornby et al., 1998). It tends to produce a *Phialophora*-like anamorph, allowing its identification in agar medium (Hornby et al., 1998; Walker, 1981). Other varieties of *G. graminis* are var. *avenae* (*Gga*), var. *graminis* (*Ggg*), and var. *maydisal* (*Ggm*). The morphological characteristic of these four varieties are similar but they can be distinguished through the size of their ascospores, pathogenicity and extent of infection on the host roots. Wheat, barley and many grass species are susceptible to *Ggt*, while oats are resistant to the pathogen mainly because of the presence of four antifungal avenacins, i.e. A-1, A-2, B-1 and B-2, in their root system (Crombie & Crombie, 1986; Hornby et al.,

1998; Wiese, 1998). *Ggg* is pathogenic to turf grasses but is weakly pathogenic to wheat. Meanwhile, *Ggm* is the most recently proposed *Gaeumannomyces* variety as a pathogen for maize in China (Hornby et al., 1998; Wiese, 1998). Simple hyphopodia are produced by *Ggt, Gga* and *Ggm* on the surfaces of host tissues, while *Ggg* produces both simple and lobed hyphopodia. Both *Ggt* and *Ggg* produce ascospores with a size of 27 µm to 124 µm, shorter than the 65 µm to 176 µm for those produced by *Gga*. Meanwhile, *Ggm* has ascospore sizes of 55.5 µm to 85.5 µm with one end being pointed (Hornby et al., 1998).

The fungus produces dark, flask-shaped fruiting bodies called perithecia (Figure 1.1a) (Bockus & Tisserat, 2005). Each perithecium (Figure 1.1b) contains many asci (sacs). Meanwhile, eight long, thin ascospores (Figure 1.1c) are located inside the ascus.

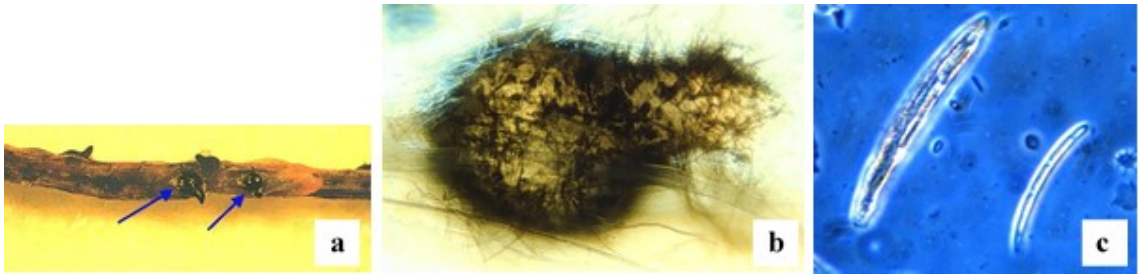


**Figure 1.1 (a) Perithecia of *Gaeumannomyces graminis* var. *tritici* indicated by the blue arrows. (b) Perithecium of *Gaeumannomyces graminis* var. *tritici*. (c) Ascus (left) and ascospore (right) of *Gaeumannomyces graminis* var. *tritici*. Source: (Bockus & Tisserat, 2005).**

## 1.3 Isolation techniques for *Ggt*

The fungus can be easily isolated from infected plant parts, such as stem bases, sub-coronal internodes, crown roots or seminal roots (Cunningham, 1981). While direct isolation from soil is difficult, it can be done by using susceptible hosts grown as baits and afterwards isolating the fungus from the infected roots. Ingredients which can be used to assist the isolation and identification processes are potato-dextrose agar (PDA) amended with antibiotics, semi-selective media SM-*GGT*3, R-PDA and SM-*GGT*4 (Chng, 2009). Depending on the preferences, carrot agar can also be used as more aerial mycelial will grow in this medium than in PDA (Cunningham, 1981). Both SM-*GGT*3 and SM-*GGT*4 contain PDA amended with L-DOPA, an amino acid, which results in a black colour around the growing hyphae of *Ggt*, plus antibiotics and antifungal agents to inhibit the growth of other competing microorganisms. R-PDA consists of diluted PDA amended with rifampicin, such that its colour will be altered by *Ggt* from orange to purple within 24 h (Chng, 2009).

Young *Ggt* colonies grown on PDA display an apparent whorled pattern, as a result of the curling back of hyphal tips, after 2-4 h at 20°C (Cunningham, 1981). The hyphae colour are generally pale and later on become darker with age. While the intensity of pigmentation is a heritable characteristic, the substrate can become the determinant factor on its appearance. Another feature of the colony is the wavy fascicles of dark macrohyphae on the agar surface, which may not be produced by some isolates (Cunningham, 1981).

## 1.4 The disease and its symptoms

Symptoms of take-all include root rot, stunted growth, yellowing of above-ground plant parts, nutrient deficiency, and prematurely ripened heads (whiteheads) (Kwak & Weller, 2013). Below the ground, disease infection can be identified by characteristic black to chocolate brown lesions on the roots. At first, small black lesions occur in the infected roots. These lesions expand and eventually merge. Heavily infected roots are very brittle, and much of them remains in the soil when the plant is pulled from the ground. Under a microscope, dark brown `runner hyphae` (Figure 1.2a) and mycelial mats (Figure 1.2b) on roots or stolon surfaces are easily seen and are also helpful in diagnosing this root disease (Bockus & Tisserat, 2005). In wheat, patches of diseased plants, if any, commonly occur after ear emergence. The patches will eventually be invaded by weeds (Clarkson & Polley, 1981). In pastures, take-all creates patches that contain yellowed or browned, weakened plants, which often have weakened root systems and can be easily uprooted. Meanwhile, dead plants may appear as dark brown or with black lesions at the shoot base (Harvey & Harvey, 2009). On closely mowed bentgrass turf, take-all has been observed to appear as roughly circular dead patches ranging from several centimetres to more than a metre in diameter (Figure 1.2c).

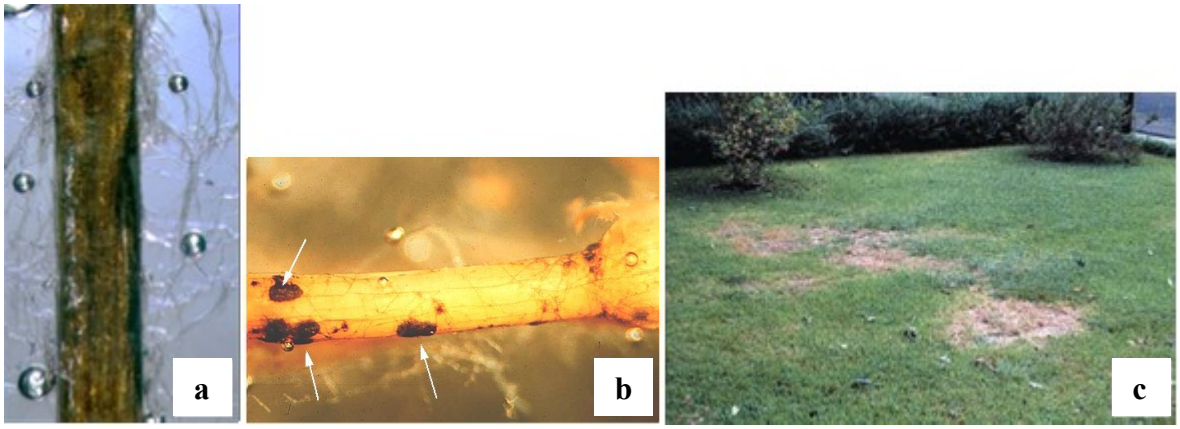


**Figure 1.2 (a) Ectotrophic runner hyphae of *Gaeumannomyces graminis* var. *graminis* on prairie grass roots. (b) Mycelial mats of *Gaeumannomyces graminis* var. *graminis* on bentgrass roots indicated by the white arrows. (c) Dead patches of take-all root rot. Source: (Bockus & Tisserat, 2005; Vann & Spurlock, Undated)**

## 1.5 Disease cycle

*Ggt* survives in the residue of the previous season's grass host. Cooler temperatures and rainfall in the autumn encourage primary root infection. The further root-to-root contact and secondary infection may increase disease pressure under higher rainfall throughout the growing season, while lower moisture will decrease the chance of a severe disease outbreak (Wherrett & MacLeod, 2018). However, the disease can still occur under conditions of low precipitation in dryland cropping systems, known as 'dryland take-all' (Cook, 2003). The term 'dryland take-all' was used to differentiate symptoms of take-all from those under wet soil conditions. Severe disease infection is commonly found in light-textured soils with low fertility, and at alkaline pH. Therefore, disease severity decreases in acidic conditions when soil pH declines to below 7.0 (Kwak & Weller, 2013). Take-all severity is also enhanced by high soil nitrogen content, especially during primary infection on seminal roots. Conversely, high nitrogen content decreases take-all severity on crown roots and during secondary infection (Kwak & Weller, 2013).

*Ggt* persists from season to season in crop debris. The mycelium colonises and infects living roots, and as the roots die, the fungus survives in the dying tissue (Bockus & Tisserat, 2005). The infected fragments can be transported by wind, water, animals, and machinery. In wheat, disease infection can take place at any growth stage of the plant (Wiese, 1998), at a soil pH of 5.5 to 8.5 under humid conditions (Kwak & Weller, 2013). The fungal hyphae penetrate the plant tissue through the cortex, endodermis and stele, and use the plant as a food base. The hyphae grow on the root surface, both upward and downward, and infect different sites along the root. Pathogen spread between plants occurs via runner hyphae growing through root bridges (Kwak & Weller, 2013). Temperature between 10 and 20°C is optimum for the penetration of hyphae, indicating that primary infection commonly occurs in autumn with very little further infection and symptom development during winter (Wiese, 1998). Lesion and disease spread via root-to-root contact begins to develop as temperatures increase in spring. After that, plants may start showing symptoms such as uneven growth, stunting, and stem blackening from late spring to summer (Hornby et al., 1998).

Dissemination of *Ggt* via ascospores is somewhat unimportant and insignificant in the epidemiology of the disease (Hornby et al., 1998; Wiese, 1998). Ascospores in the stem bases or stubble may be released into the air by active ejection from the ascus and spread by splashing rain or wind (Hornby, 1981). In the field at least 0.25 mm of rain is required to release spores and 2.03 mm to generate the maximum spore density of 3700 $m^{-3}$ within two hours. The proximal parts of the seminal roots of seed sown on the soil surface (not deep down into the soil) can be

means for initial infection, as well as root hairs by the tropical growth of the hyphae towards the roots (Hornby, 1981; Skou, 1981). Furthermore, ascospores released during summer may not be able to survive the dry and hot conditions, therefore rarely cause infection. However, susceptible grasses infected by the ascospores during the early season can serve as primary or secondary sources of disease inoculum throughout the growing season (Hornby et al., 1998; Skou, 1981; Wiese, 1998).

## 1.6 The competitive saprophytic ability of *Ggt*

*Ggt* is a root-infecting fungus with minimal ability to survive in infested plant residue in soil without the presence of living host-plant roots (Skou, 1981). However, it does survive in dead plant tissue with little competitive saprophytic ability (CSA), acting as inoculum sources for the subsequent crops or susceptible grass species. However, the level of *Ggt* inoculum available in the soil to infect the following susceptible crops is significantly affected by the ability of the fungus to compete for substrates in dead plant tissue with other soil microorganisms (Chng, 2009). The poor or decreased CSA of *Ggt* was confirmed in pot experiments in which *Ggt* inoculum and non-sterilised soils were added to sterilised soils. The experiments showed that the rate of enzymatic degradation of the walls around the apices of penetrating hyphae determined the pace of fungus penetration into mature cell walls of the straw. Other factors contributing to the poor CSA of *Ggt* include slow germination and growth, reduced production of cellulolytic and lignolytic enzymes, which is essential for cell wall degradation of the roots for hyphal penetration, and many other interacting attributes, which eventually dictate its pathogenic activity (Shipton, 1981).

All those reports indicate that, without the presence of susceptible hosts, the level of *Ggt* inoculum in infected plant debris will decline since it is a weak competitor in the soil. Thus, without adequate CSA further saprophytic colonisation is minimal.

## 1.7 Roles of temperature and moisture

Temperature and moisture are essential determinants for the survival of *Ggt* inoculum and hence take-all development (Cook, 1981). It has been demonstrated that when wheat plants were grown in sterilised soil inoculated with *Ggt*, the intensity of root infection increased from >50 up to 90% as the soil temperature increased from 13 to 23°C, or even 27°C, which is considered as its optimum growth temperature on agar medium. However, in non-sterilised soil or a natural environment, the disease severity declined as the temperature was raised up to 18°C (Deacon, 1997). The study concluded that the increased temperature gave more benefit to the growth of other soil microorganisms rather than *Ggt*. These other soil microbes may play an important role

in inhibiting the *Ggt* through antagonism mechanisms, or by competing for saprophytic degradation of the plant residues, causing the death of *Ggt*. Therefore, other interacting factors must be taken into account in natural conditions. Also, these show that temperatures of 13-18°C are sufficient for infection to occur (Chng, 2009).

Studies have indicated that take-all is commonly found in moist soil, mainly because the high water potential required for the pathogen to grow and cause infection is available (Cook, 1981). The required water potential is available in the top 25 cm of soil during abundant water supply, but, this soil layer is also the first to dry when rain or irrigation ceases. The growth of *Ggt* ceases at a soil matrix potential drier than -1.5 to -2.0 MPa, which could occur quickly in the top layer of soil during a drought without additional irrigation. Thus, water must be supplied regularly for severe take-all to develop.

A search of the literature revealed varied results on the effects of temperature and moisture on the survival of *Ggt* during the absence of a living host. A report by Shipton (1981) showed that the fungus was able to tolerate high temperatures during soil storage. When inoculated into the sterilised soil, its pathogenicity was not affected by repeated temperature alteration between 21 to 29°C and even tolerated 45°C for 30 min. A bioassay study using cores stored in various environmental conditions found that viability of *Ggt* was not affected by either a dry and cold environment (-25 MPa to -98 MPa at 15°C) or a moist and cold environment (-0.4 MPa to -0.7 MPa at 15°C) for at least nine weeks (Mac Nish, 1973). Reduction of up to 50% in inoculum viability occurred under very dry and hot soil regimes (-98 MPa at 35°C) for nine weeks and was eliminated entirely under a wet and hot soil environment (-0.01 MPa to -0.2 MPa at 35°C) for four weeks.

An experiment assessing the viability of *Ggt* in soil inoculated with infected wheat straw was carried out by Wong (1984). In this experiment, the *Ggt*-infected soil was incubated under four temperature-moisture treatments for three months and later the lesions on the roots of wheat seedlings grown as baits were assessed. The results showed that cold dry soil (15°C at < -10 MPa) was best for *Ggt* with 100% inoculum survival, followed by warm dry soil (30°C at < -10 MPa) with 63 to 97% survival. Meanwhile, warm moist soil (30°C at -0.3 MPa) eliminated the fungus, but cold moist soil (15°C at -0.3 MPa) maintained 18 to 40% inoculum survival. The author did not mention the exact method used to determine these survival rates. However, the various temperature-moisture treatments used in the experiment suggested that *Ggt* was able to survive under the arid environment (-98 MPa), which is beyond the permanent wilting point of -1.5 MPa, and that cold temperature were preferred over warm temperatures.

## 1.8 Take-all development under different soil moisture conditions

Under high precipitation or irrigation, take-all will spread to the crown and eventually to the culm base, and patches of stunted plants will be observed. Furthermore, as transport of water to above-ground plant parts will be disrupted due to the stem infection, premature death of the infected plants can occur (Moore & Cook, 1984). Conversely, under soil moisture stress, symptoms are limited to blackened roots and patches of infected plants are not typically observed (Kwak & Weller, 2013).

Under a dryland cropping system with annual precipitation of 250 to 300 mm, the growth of *Ggt* is reduced by half when the soil matrix potential reaches -1,5 to -2,0 MPa. However, the water potential inside the roots can still provide a suitable environment for pathogen development. In this case, the root disease can continue to develop internally heading to the stem base without the distinguished black lesions on the surface of the stem and culm base (Kwak & Weller, 2013). In a dryland environment, which is less favourable for fungal spread from plant to plant, disease patches may be absent. Pathogen spread to adjacent plants under these conditions may require separate infections from the primary inoculum in the soil. However, above-ground symptoms can still occur since the disease can progress into the base of the stem, but might only be observed on individual and scattered plants (Cook, 2003).

## 1.9 Effects of soil fertilisation

Root damages caused by take-all lead the plant to be less efficient in the uptake of essential nutrients, such as nitrogen, phosphorus, potassium, and other primary and trace elements (Cook, 2003). The addition of these nutrients, especially nitrogen (N) during tillering, stem elongation, or other stages in plant development, will minimise the harmful effects of nutrient deficiency due to root damage, supporting plant development to outgrow the disease (Cook, 2003). In general, increasing N decreases the disease, but a high level of soluble N in the soil favours for the survival of *Ggt* in the absence of hosts. The pathogen will continue to grow until the excess level of N accelerates decomposition of residues by a succession of other microorganisms that will compete with *Ggt* for available N. There was also a case where nitrogenous fertilization of turf increased take-all severity, but it then decreased in the subsequent years (Nilsson & Smith, 1981).

*Ggt* infection on plants that are already deficient in the relatively immobile nutrients, such as phosphorus and other trace elements (e.g. iron, zinc and manganese) worsens the damage on the plants (Huber, 1981). Their damaged roots will experience reduced absorptive capacity for

nutrients and water. The immobile nutrient deficiency becomes more severe, thus increasing the susceptibility of the host and triggering the rapid development of the disease (Cook, 2003).

In addition to the root damage due to *Ggt* infection, the ability of plants to absorb trace elements is also affected by soil pH. Many of these trace elements are more available for plants under acidic soils than alkaline soils (Huber, 1981). Conversely, the availability of many trace minerals including iron, zinc, manganese, copper and boron but not molybdenum is reduced under alkaline soils, which are also more conducive for take-all development. The level of nutrient deficiency due to the loss of absorptive capacity, however, can be very variable within the field, from one plant to another or one area to another area. Therefore, it is unnecessary to cope with these localised and sporadic deficiencies with an increased nutrient application over the entire field, especially when the crop in the region is healthy (Cook, 2003).

## 1.10 The importance of take-all in grasses

Take-all can infect many grass species and may provide the means for the disease to survive between cereal crops. Several important grass species, such as kikuyu grass (*Pennisetum clandestinum*) and prairie grass (*Bromus wildenowii*) are highly susceptible to take-all and are likely to maintain or increase pathogen inoculum concentration and disease severity (Foundation for Arable Research (FAR), 2007). Under some circumstances, the fungus spreading in the grass roots may increase its infectivity to the following cereal crop (Kidd et al., 2002). The pasture grasses, perennial ryegrass (*Lolium perenne*) and timothy (*Phleum pratense*), are known to be susceptible, which particularly for the former, potentially aids the survival of the disease inoculum between cereal crops. According to Harvey and Harvey (2009), the occurrence of the disease in ryegrass pastures is most prevalent during spring and summer but may not lead to severe damage. However, due to its importance in cereal crops, care should be taken to avoid cross-infection. Chng et al. (2005) suggest to avoid the use of highly susceptible grass species as break crops due to their ability to carry-over, or increase, disease inoculum which may increase take-all incidence in subsequent cereal crops.

It is suspected, but not confirmed, that take-all may reduce grass seed yield (Rolston, pers, comm, 2017). In some perennial ryegrass seed crops from the 2016/17 season, seed yields were reduced by the occurrence of a high number of very small or undeveloped seeds, and take-all symptoms were observed on the roots of plants in these fields (Chynoweth, pers, comm, 2017). In a study investigating fungi invading roots of perennial ryegrass, Skipp and Christensen (1989) reported that *Ggt* created more extensive damage in the grass root system than other fungal species.

Damage to the root system due to pathogen infection is likely to affect dry matter accumulation in the above-ground plant parts since less water and nutrients can be absorbed. Therefore, the yield and quality of seed produced may also be affected by the disease.

## 1.11 Existing take-all control methods

The most effective cultural practice used to control take-all is crop rotation. As reported by FAR (2007), crop rotation between wheat and commonly grown pasture grasses such as perennial ryegrass, annual ryegrass, and tall fescue effectively reduced *Ggt* inoculum levels. However, crop rotation may result in loss of a soil's natural ability to suppress take-all, which is an important matter to consider. The loss in suppressiveness may result in the rapid increase of take-all severity in following wheat crops despite the low disease inoculum in the crop (Bithell et al., 2011b). On the other hand, some grass species serve as a strong host of take-all to cause further build-up of *Ggt* inoculum in soils. For example, prairie grass grown as a break crop for wheat increased the *Ggt* inoculum levels by 18-fold compared to a 2-fold increase after a second wheat crop (FAR, 2007). Regardless of the differences in grass species susceptibility, resistant grass cultivars are not available, which makes disease control more difficult. Tillage is another effective method to control take-all, but reduced tillage, which has been adopted by many growers to control erosion, can exacerbate the disease (Kwak & Weller, 2013).

Take-all control can be achieved by using soil fumigants such as methyl bromide and chloropicrin (Kwak & Weller, 2013). However, the high fumigation cost and their adverse effect on the entire soil biota make this control method impractical. Subsequent take-all infection after soil fumigation was reported to be severe on young *Agrostis* turf (Nilsson & Smith, 1981). It was suggested that soil microflora antagonistic to *Ggt* may be killed by the fumigation. Also, methyl bromide has been banned due to its adverse effect on the ozone layer. Fumigation can be implemented only in an experimental scale, e.g. to produce a healthy check within a larger factorial experiment. Cook et al. (2002) showed soil fumigation using methyl bromide was the only method that produced a considerable reduction in the take-all incidence in wheat grown in a small plot experiment. The yield increase due to soil fumigation was approximately 1,000 kg $ha^{-1}$ and 900 kg $ha^{-1}$ for spring and winter wheat, respectively. However, it was not possible to determine which root diseases were controlled by the fumigation as another root disease was also detected.

Several fungicides have been reported to reduce take-all incidence significantly in naturally infested fields, but the results have been inconsistent and uneconomical (Kwak & Weller, 2013).

For example, Cook et al. (2002) reported that difenoconazole, metalaxyl, mefenoxam, tebuconazole and thiram applied as a seed treatment, either singly or in combination, resulted in either lower or insignificantly higher yield of wheat compared to the nontreated control. The variation of yield response due to the chemical treatment occurred across different wheat cultivars and fields. Comprehensive examination using silthiofam to control take-all by Schoeny and Lucas (1999) showed a result that was somewhat similar. Under naturally infected fields in France, this fungicide effectively reduced take-all under the occurrence of an early epidemic but was less effective in controlling the disease when the epidemic started late. In China, a formulation of N-allyl-4,5-dimethyl-2-trimethylsilylthiophene-3-carboxamide applied at various rates was reported effective in reducing root rot severity and increasing grain yields of wheat (Xiulan et al., 2001). However, the increased grain yield required increased rates of fungicide application, which may indicate the treatment was uneconomical. Freeman and Ward (2004) reported that fluquinconazole, a broad spectrum fungicide, can be used to control the disease, although there is no further report on its efficacy. Hence, although some fungicides have shown promise in controlling take-all, they seem not economical due to either lack of significant yield improvement, a high rate of application, or inconsistent results across different sites.

A study was conducted in New Zealand in the 2002/03 season, comparing the effectiveness of various fungicides in controlling take-all in wheat (Chng, 2009). Autumn-sown seeds were treated with 0.1 and 0.2 g a.i. triadimenol (Baytan Universal®), 0.07 and 0.14 g a.i. silthiofam (Latitude®), 1.5 and 3.0 g a.i. fluquinconazole and 0.3 and 0.6 g a.i. prochloraz (Jockey®), per kg seeds. The results showed that at both application rates, triadimenol and silthiofam were effective in reducing the root disease severity early in the season by 32 and 25% respectively, but did not affect disease severity or whitehead development later in the season. Furthermore, the two fungicides did not significantly increase grain yield. Meanwhile, the combined fluquinconazole and prochloraz (Jockey®) treatment effectively decreased the whitehead symptom by an average of 84% at both rates, as well as reducing the disease severity by 57 and 75%, respectively. However, seedling emergence was reduced by 47.6% and there was no yield increase due to the Jockey application (Chng, 2009).

## 1.12 The potential for biological control of take-all

### 1.12.1 Biological control of *Ggt* in TAD soil

One of the best examples of biological control of take-all is the phenomenon known as take-all decline (TAD), which occurs in wheat monocultures around the world. By definition, TAD is the spontaneous decrease of the take-all severity that occurs in monoculture wheat or other susceptible host crops following one or more severe disease outbreaks (Weller et al., 2002). In TAD soils, root-colonising bacteria, which are antagonistic to *Ggt*, build up in the soils making it suppressive to the disease, which leads to a decline in disease severity to an insignificant level. The production of an antibiotic by the bacteria is thought to be an essential aspect of the disease suppression, inhibiting the growth of the fungus in the plant roots (Bockus & Tisserat, 2005).

It is now well established from a variety of studies that take-all decline (TAD) is associated with the build-up of bacteria of the species *Pseudomonas fluorescens* (Kwak & Weller, 2013). Studies in the United States and the Netherlands confirmed that *P. fluorescens* produced the broad-spectrum antibiotic 2,4-Diacetylphlorogucinol (2,4-DAPG) during monoculture of wheat and barley, which is involved in the mechanism of TAD (Kwak & Weller, 2013). To date, many 2,4-DAPG producing strains have been isolated from both suppressive and non-suppressive soils and used to control take-all disease, primarily in wheat fields. For example, *P. fluorescens* 2-79 alone or in combination with 13-79 is reported to suppress take-all when applied as a seed treatment (Weller, 1988). Some of the 2,4-DAPG producing genotypes, especially those isolated from TAD fields, are “premier” colonists of wheat roots that can maintain their threshold population sizes needed for pathogen suppression during the growing season (Kwak & Weller, 2013). This is in accordance with the general assumption that biological control agents of root pathogens should be good root colonisers (Weller, 1988).

Although *Pseudomonas fluorescens* has been found to be effective against take-all, the bacteria alone may not be sufficient to control the disease in field soils. This is because the heterogeneity of soil flora and fauna, along with the physical properties of natural soil environments, can affect the behaviour of antagonists and pathogens. In fact, other antibiotic-producing bacteria were also found from the rhizosphere of wheat grown in take-all decline soil. Kim et al. (1997) discovered *Bacillus* sp. strain L324-92 from among approximately 2,000 rhizosphere isolates of *Bacillus* species collected from a wheat field. In the subsequent experiment, the authors reported the effectivity of the bacteria in increasing yields of spring wheat, as well as significantly suppressing take-all. The discovery suggests that other biocontrol agents can still be found to complement biological control, not only for use against take-all, but possibly for use against other root diseases.

### 1.12.2 Biological control potential by *Trichoderma* spp.

Earlier studies have indicated the involvement of *Trichoderma* spp. in the occurrence of take-all decline. Simon and Sivasithamparam (1988a) reported a soil in Western Australia in which the development of both take-all of wheat and the saprophytic growth of *Ggt* was suppressed. In this suppressive soil, as much as 71% of the total number of fungi isolated from the soil belongs to *Trichoderma* spp. A further study showed that *Trichoderma* spp. played a role in suppression of the saprophytic growth of the pathogen. When *T. koningi* isolated from a suppressive soil was added to the same soil, the saprophytic growth of *Ggt* was reduced (Simon & Sivasithamparam, 1988b). Also, the introduction of *T. koningii* in field trials of wheat in Australia, China and United States showed the protection of the plant against take-all, as well as increased grain yield (Duffy et al., 1997).

Among *Trichoderma* species, several root-colonising strains have been observed to confer optimal benefits by providing long-term disease protection while promoting growth of host plants (Vitti et al., 2015). A rhizosphere-competent strain will grow continuously with the development of the plant and induce the plant's defense system, as the induced systemic resistance (ISR) mechanism is closely related to the root colonisation (Harman et al., 2004; Vos et al., 2014). Of the *Trichoderma* strains, *T. harzianum* T-22 is reported as an effective root coloniser in many plant species. Seed treatment of maize with this strain resulted in a significant increase in plant growth, and reduced disease symptoms following inoculation of leaves by the pathogen *Colletotrichum graminicola* (Harman et al., 2004). *Trichoderma atroviride* LU132 and LU140 are also rhizosphere-competent isolates (Cripps-Guazzone, 2014). This study showed that the population density of these isolates in the rhizosphere was significantly higher than that of T-22, indicating a higher capacity for root colonisation by these two isolates compared to T-22. The better root-colonising capacity of the isolates may indicate better efficacy of biological control performance as suggested by various studies (Weller, 1988).

Very few studies have examined the effect of *Trichoderma atroviride* in grass crops. A study by Kandula et al. (2014) showed that application of either LU132, LU140 or LU584 significantly increased shoot weight of perennial ryegrass sown in *Ggt*-infested soils. Also, LU132-treated plants showed lower disease severity in the host root system compared to untreated plants. Cripps-Guazzone (2014) also confirmed that LU132 was among the most effective isolates to colonise the rhizosphere of perennial ryegrass.

## 1.13 Aims and objectives

The pathogenicity of *Ggt* to various pasture grasses is well recorded, yet the level of yield loss in grass seed crops is not fully understood. Similarly, there a very few studies of biocontrol as a method to manage the disease, especially in grass crops. Meanwhile, *T. atroviride* isolates LU132, LU140 and LU584 have previously been shown to have some activity against *Ggt* in grasses (Kandula et al., 2014). The aim of this project, therefore, was to study the efficacy of *T. atroviride* isolates for controlling take-all in grasses. There were four objectives as follows:

*Objective 1:* To assess the effect of take-all disease on grass seed yield by using prairie grass (*Bromus wildenowii*) as a model system.

*Objective 2:* To evaluate the efficacy of three *Trichoderma* isolates in controlling take-all disease in prairie grass, and investigate whether a mixture of isolates will provide a better result for the disease control than any of these individual isolates.

*Objective 3:* To assess the effect of moisture stress on take-all severity and seed yield of prairie grass, and investigate whether the three *Trichoderma* isolates can reduce the impact of moisture stress on grass seed yield and/or quality.

*Objective 4:* To test in perennial ryegrass the hypothesis that: (i) a combination of the three *Trichoderma* isolates will provide more effective control of *Ggt* than any individual isolate, and (ii) ryegrass grown in soil inoculated with the mixture of isolates will produce more DM than plants treated with the individual isolates.

# Chapter 2

# Effects of take-all disease on seed yield of prairie grass

## 2.1 Introduction

The seed industry plays an essential role in New Zealand's national economy as it underpins New Zealand's primary production. In 2015, the sector generated an export revenue of $198m, of which herbage seed (ryegrass and white clover seeds) contributed about 34.6% ($68m) (Chynoweth et al., 2015; Ministry for Primary Industries, 2015). This herbage seed export revenue was 37% higher than the $50m generated in 1998 (Rowarth et al., 1998), indicating successful growth in the international seed market. On an international scale, New Zealand's contribution to global herbage seed production was only 4% in 2014, yet it is a significant producer and exporter globally of both perennial ryegrass and white clover seeds (Pyke et al., 2004). Therefore, to increase herbage seed yields has always been a national priority in order to boost domestic revenue. In the case of ryegrass, part of the goal has been successfully achieved through better crop management techniques, which have allowed a substantial seed yield improvement (Chynoweth et al., 2015). However, threats to herbage seed yields continue to exist, and need to be anticipated, such as those caused by disease.

Take-all disease caused by the pathogen *Gaeumannomyces graminis* var. *tritici* (*Ggt*) has been long-standing as a severe threat for many important crop species. In wheat, for example, severe yield losses due to this pathogen occur worldwide as a result of plant stunting and premature ripening of the grain (Freeman & Ward, 2004). While its destructive effects on the cereal crop are widely known, its pathogenicity to various grass species has also been recorded, including some of the important New Zealand forage species, such as ryegrass, tall fescue and brome grasses (Chng et al., 2005). On susceptible grass species, the pathogen may cause patches in pasture several centimetres long, although they generally do not lead to severe pasture losses (Harvey & Harvey, 2009). Despite the relatively low impact of take-all on grasses compared to those of cereal crops, root damage is always found in various grass species due to *Ggt* infection (Nilsson & Smith, 1981). Damage to the root system may lower seed yield, by disrupting water and nutrient supplies to the plant. Recently, ryegrass seed growers have reported an increasing occurrence of very light seed in their crops, and there is a suspicion *Ggt* infection may be responsible (Rolston, pers, comm, 2017).

What remains unclear, however, how much influence is *Ggt* have on seed yield loss. Two early reports noted take-all in grass seed crops, with whitehead symptoms in both annual ryegrass and perennial ryegrass and take-all symptoms in a cocksfoot crop (Nilsson & Smith, 1981; O'Rourke, 1976). However, neither of these reports provided adequate information on seed yield losses or confirmed the presence of *Ggt* as the primary cause. Meanwhile, more recent studies which have noted the occurrence of *Ggt* on herbage grass crops mainly ignored seed yield assessment and focused on the build-up level of pathogen inoculum (Bithell et al., 2011a; FAR, 2007), host susceptibility of grass species (Chng et al., 2005) or the shoot and root dry weight of infected grasses (Gutteridge et al., 2005; Kandula et al., 2014).

The primary purpose of this study was to assess the effect of take-all disease on grass seed yield using prairie grass (*Bromus wildenowii*) as a model system. One advantage of using prairie grass is that it does not have a vernalization (period of temperature <10°C) requirement to trigger the switch from vegetative to reproductive growth. Therefore, it was able to produce seeds within the time frame of the experiment which started in early summer. Another advantage of this model plant is that it a highly susceptible host of *Ggt* (Chng et al., 2005). This grass, when planted as a break crop, played a more significant role in increasing *Ggt* inoculum concentration in the soil than any other grass species. The inoculum increased from 16 to 1197 picogram DNA $g^{-1}$ soil in the following a wheat crop, much higher than the 52 to 96 picogram DNA $g^{-1}$ soil from a continuous wheat planting system (Bithell et al., 2011a).

## 2.2 Materials and methods

### 2.2.1 Experimental site

The study of the effect of take-all on seed yield of prairie grass was conducted at the Aluminex glasshouse at Lincoln University, Canterbury. The glasshouse temperatures were automatically recorded every two hours using a temperature data logger. The record showed an average monthly temperature of 21°C during December – March, and 17°C during April – August (Figure 2.1). The glasshouse was equipped with an automatic fan and heating system to maintain the temperatures at this level.

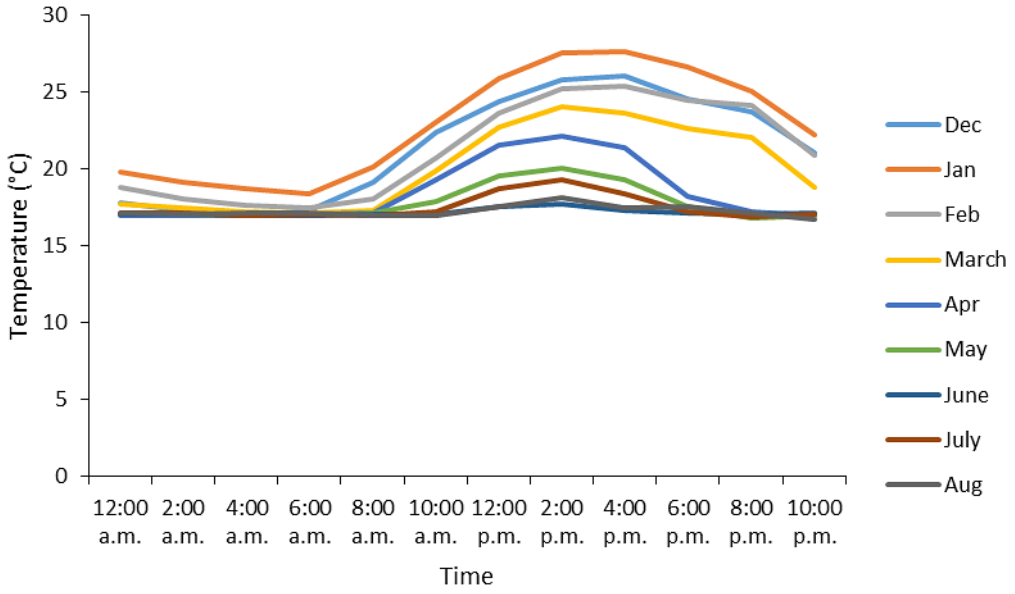


**Figure 2.1 Mean temperatures in the Aluminex glasshouse at different times of the day throughout the experimental period.**

### 2.2.2 Seed lots

Prairie grass seeds of Cv. Grasslands Matua were supplied by AgResearch Ltd. The size of the seeds was 20 mm long on average. The germination percentage of the seeds was tested before they were used in the experiment.

### 2.2.3 Initial germination test

To confirm the seed viability before being used for the main experiment, an initial germination test was carried out using the between paper method (BP) (International Seed Testing Association (ISTA), 2017). In this test, ten replicates of 50 randomly selected seeds were placed in the top half of the germination paper in two rows of 25 seeds. Then, the germination paper was sprayed with distilled water, to provide adequate moisture for germination, before the seeds were covered with the bottom half of the paper sheet. Excessive moisture was avoided to prevent too much humidity which may cause seeds to decay. The paper was rolled to about 5 cm width and placed into a zip-lock plastic bag. Five germination rolls were placed in each bag and incubated at 25°C with 8 hours of light daily for 14 days. Two weeks after incubation, the germination test was assessed by counting the number of normal and abnormal seedlings and dead seeds (ISTA, 2017).

### 2.2.4 Initial *Ggt* inoculation test on prairie grass roots

This experiment was established using germinated prairie grass seeds from the germination test. Five *Ggt*-colonised agar plugs (2.2.6) were taken from the agar medium and put in an inverted position on top of prairie grass roots growing in the germination paper. In this test, five germination papers were inoculated, and five germination papers were left uninoculated as controls. Twenty-one days later, the inoculated roots were visually compared with uninoculated ones. A small portion of grass roots from both inoculated and uninoculated treatments was placed onto a glass microscope slide for observation. Microscope images were taken to confirm the presence of the runner hyphae of *Ggt* on the roots (Figure 2.2).

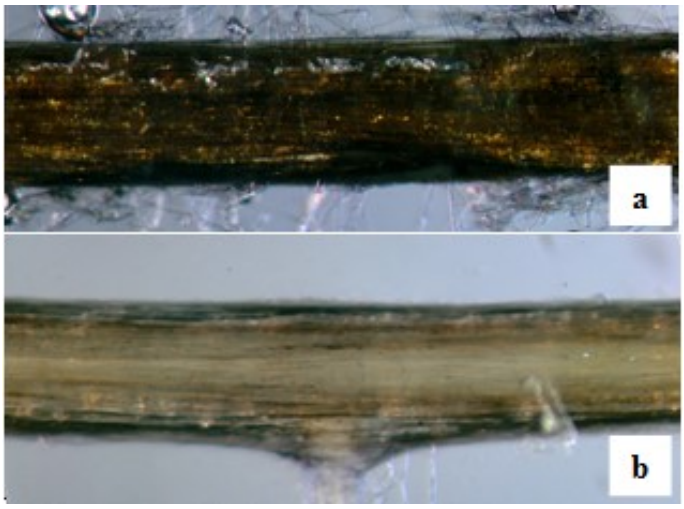


**Figure 2.2 Runner hyphae of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) and blackened root observed in the infected root of prairie grass seedling (a), and root without infection (b).**

### 2.2.5 Preparation of prairie grass seedlings

Prairie grass seeds were sown in a plastic seedling raising tray, then transplanted into main pots, to allow the selection of healthy seedlings to be used in the experiment (Figure 2.3a). The growing medium used in the trays was a potting mix, which was comprised of bark and pumice in 4:1 (v/v) ratio enriched with osmocote fertiliser 16-3-9-10 (3-4 months), horticultural lime and hydraflo in a ratio of 3:1:1 (v/v/v). The trays were filled with the medium about three-quarters full in each cell. Two seeds were sown in each of the cells followed by the addition of more potting mix to cover up the seeds. The trays were placed inside the glasshouse with a controlled temperature of 20°C ± 2°C. The moisture level of the medium during germination and seedling emergence periods was maintained by spraying water as necessary. Two seedlings were transplanted into each of the main pots at 15 days after sowing (DAS) (Figure 2.3b).

Apart from the transplanted seedlings, seeds were also sown directly into the main experiment pots as an additional experiment which represented a different method of establishment. In this direct sowing, ten seeds were sown in each pot at 5-6 mm depth, surrounding the transplanted seedlings in the centre of the pots (Figure 2.3c).

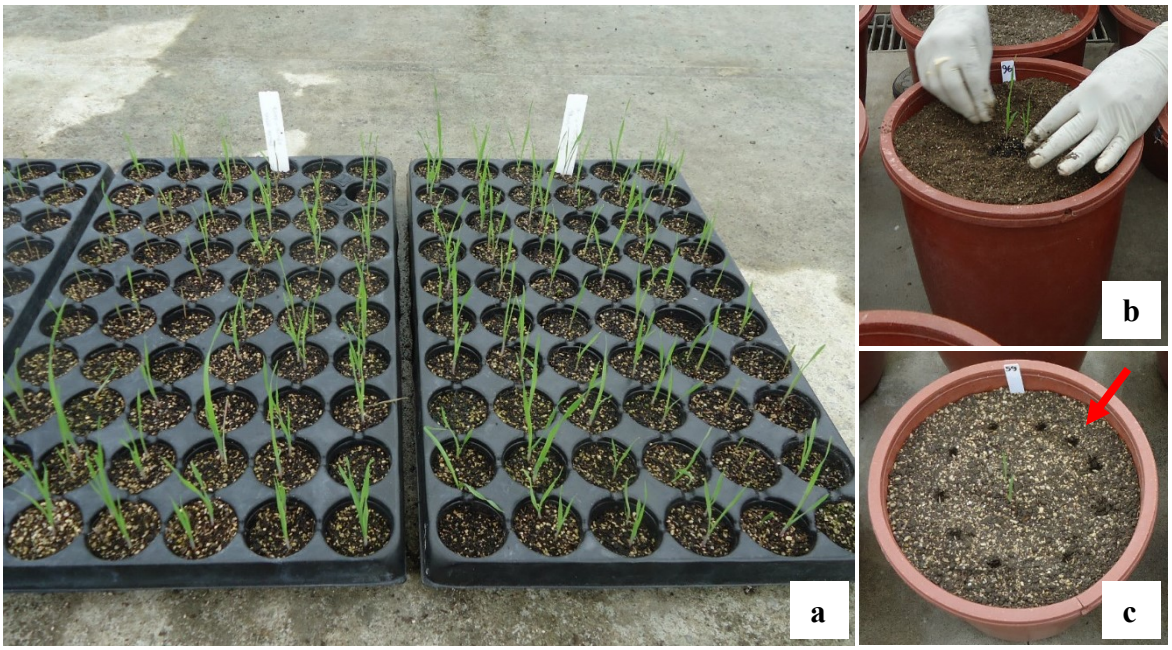


**Figure 2.3** **(a) Prairie grass seedlings grown in the trays. (b) 14 day old seedlings transplanted into the main pot. (c) Ten seeds were sown surrounding the transplanted seedlings as indicated by the red arrow.**

### 2.2.6 Preparation of growth medium for prairie grass

The growth medium was a mixture of silt loam soil and pumice with a ratio of 3:1 (v/v), intended to prevent soil compaction and allow optimum root development. The soil, a Templeton silt loam, was collected from a field that had grown grass species for many years. Basic properties of the soil were determined by R. J. Hill Laboratories Ltd., Hamilton, New Zealand. These properties included pH, resin P, Olsen phosphorus, anion storage capacity, potassium, calcium, magnesium and sodium (Appendix 1). These tests are important to understand the soil nutrient status which is often unique to a particular field (R. J. Hill Laboratories Ltd, 2018). The test results indicated that the Mg, Na and total N concentrations were low. The soil had a cation exchange capacity (CEC) within the medium range, indicating the capability for storing nutrients (Morton et al., 2000).

The non-fumigated soil was passed through a 4 mm sieve and air-dried at 20-25°C for three days before being mixed with pumice and put into a pot of 9-litre capacity. This was done to get an equivalent amount of soil volume, as well as provide an optimum growth medium for each prairie

grass plant grown in the experimental pots. Before the soil was put into the pots, a paper sheet was laid on the pot base to avoid soil leaking through the bottom hole of the pots. Initially, all pots were filled with eight kilograms of soil, and another one kilogram was added later during seedling transplanting.

### 2.2.7 Preparation of pathogen inoculum

Pathogen inoculum of *G. graminis* var. *tritici* isolate A3SL4 was acquired from the Lincoln University Microbial Culture Collection. The pathogen was grown on three Petri dishes of dilute potato-dextrose agar (PDA) (20 g dextrose, 15 g agar, and 4 g potato starch in 1 L of distilled water) and plates were kept in an incubator at a controlled temperature of 20°C for three weeks.

The growing *Ggt* cultures on plates were inoculated onto sterilised grass roots for further propagation using a method described by Kandula et al. (2015). The grass roots were collected from the same field soil used for the growth medium. Only fine roots were used and sterilised after being chopped into small sections of 2-4 mm. One hundred grams of these roots were placed in each of five 250 ml flasks and autoclaved at 121°C and 15 psi for 120 min. Once cooled, the sterile roots in each flask were inoculated with ten colonised agar plugs, 6 mm in diameter, taken from the actively growing *Ggt* culture, and gently shaken before incubation. Then, the inoculated-grass roots were put in a controlled-temperature incubator at 20°C for 14 days to allow the proliferation of the fungus before being used as the inoculum source.

### 2.2.8 Establishment of prairie grass in the main experimental pots

The experimental pots contained prairie grass plants established from two different sowing methods, i.e. transplanted and direct-seeded plants. Prairie grass seedlings at 15 DAS were transplanted into the centre of the pots along with their growth medium (i.e. potting mix). One kilogram of the pumice-mixed soils was added to each pot after being mixed with one gram of the *Ggt*-colonised grass roots and/or three grams of a formulated *Trichoderma* isolate unless they were absent from treatments (the formulation of *Trichoderma* isolates described in section 3.2.2). The next day, ten prairie grass seeds were sown directly at the edge of the pot, surrounding the transplanted seedlings. Soon after plant transplanting and seed sowing finished, a half-litre of water was given to each pot.

Watering was regularly given for all pots during the vegetative growth stage to provide equal establishment conditions. In the early growth stages, light watering was done every day until plants were three weeks old, and after that every three to five days depending on weather

conditions. To nourish the plants, urea fertiliser was applied at a dosage of 11 g/m2 (0.54 g/pot) by dissolving into water. The fertiliser was applied three times at 34, 50 and 113 DAS. Ahead of the flowering period at 42 DAS, supplemental lighting was provided using high-pressure sodium lamps to extend the daylength up to 16 hours (Figure 2.4). The daylength extension was needed to allow prairie grass plants to enter the flowering stage due to the shortening days during the experimental period. As soon as the plants entered the heading stage (ear emergence), four bamboo sticks with coiling strings were installed surrounding the plants to help the plants keep standing throughout the experimental periods. At this time, artificial wind using a desk fan was also introduced to ensure equal opportunities for pollination. Additionally, manual weeding was done regularly, especially during the vegetative growth period.

**Figure 2.4 Experimental pots arranged in a split-split-plot design, with added supplemental lighting to extend the daylength. Picture taken at 42 DAS.**

### 2.2.9 Collection of seed

Seed harvesting was done selectively for mature seeds, characterised by a hard seed coat and brown colour (Figure 2.5a and Figure 2.5b), over a span of three weeks, since the seeds matured at different times between 80 and 100 DAS. Some seeds were harvested before they entered the

fully mature stage to minimise seed yield loss through shedding (Figure 2.5c). Seeds from the centre and surrounding plants were placed in separate, labelled, paper bags and stored at ambient temperature before further assessments (Figure 2.5d).


**Figure 2.5** **(a) Fully matured seeds ready to harvest. (b) Selective harvesting done on matured seeds and to avoid yield loss. (c) Some of the seeds were not fully mature, characterised by a greenish colour. (d) Harvested seeds placed in labelled paper bags.**

### 2.2.10 Treatments and trial design

Treatments used in the experiment consist of three factors described as follows:

| **Factors** | **Treatments** |
|---|---|
| (1) Moisture | Not stressed (-) |
| | Moisture stressed (+) |
| (2) Pathogen inoculation | Not inoculated (P-) |
| | Pathogen inoculated (P+) |
| (3) *Trichoderma* isolates | Control-1 |
| | Control-2 |
| | LU132 |
| | LU140 |
| | LU584 |
| | Mixture of three isolates. |

Treatments containing the pathogen (P+) had eight replicates, and those without the pathogen (P-) had four replicates. The experiment which consisted of 144 pots, was arranged in a split-split-plot design to separate pathogen inoculated pots from non-inoculated pots, and separate moisture

stressed pots from non-stressed. This was done to minimise the contamination or treatment errors between treated and untreated plants (Figure 2.6).

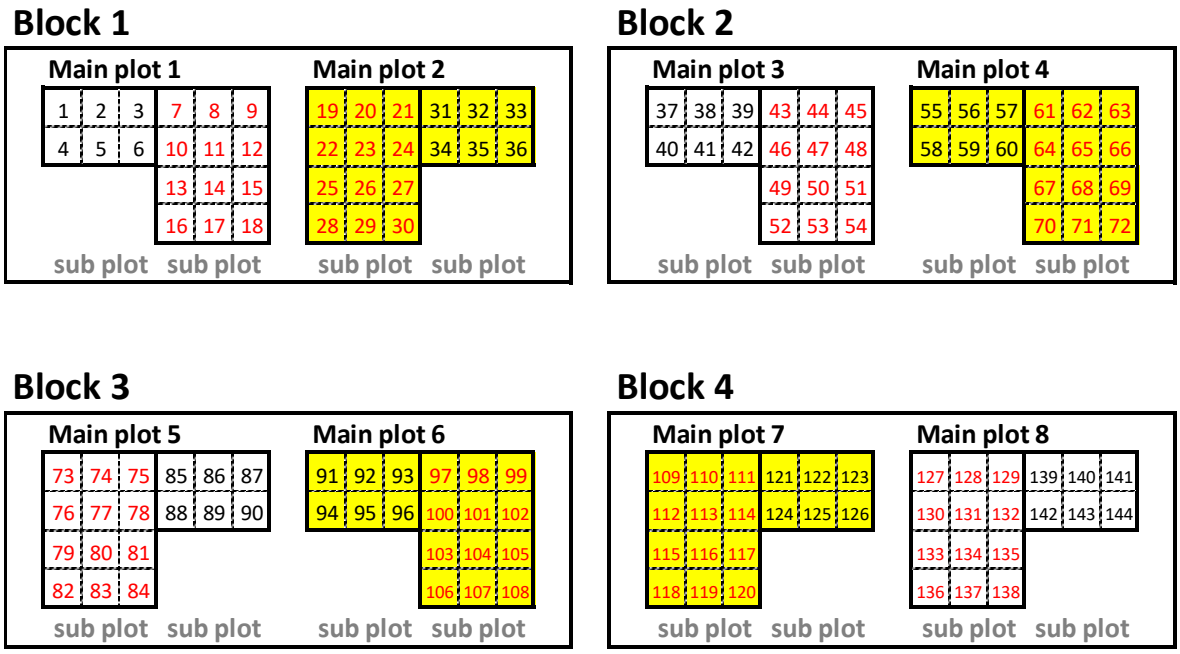


**Figure 2.6 Trial design of the prairie grass experiment. Numbers in red colour are pathogen inoculated-pots, and numbers in black are non-*Ggt* inoculated. Cells with yellow background are moisture stressed-pots, cells in white are non-stressed pots. Numbers in each sub plot represent pots treated with four *Trichoderma* isolates and two untreated controls arranged in the randomised orders. These numbers/treatments were doubled in pathogen inoculated-sub plot (red numbers) since P+ had eight replicates, while P- had only four replicates.**

## 2.3 Assessments

### 2.3.1 Seedling emergence

The seedling emergence was recorded for the direct-sown seeds at 36 DAS. The results are presented as a percentage of the seeds sown.

### 2.3.2 Time to seedhead emergence

The time of seedhead emergence was first recorded from the transplanted plants at 42 DAS. After that, newly emerged seedheads were counted every day until no more emerged in all treatments.

### 2.3.3 Number of tillers

Total number of tillers per plant were counted from the transplanted plants. All emerged tillers were included. Tillers which produced seedheads were considered as fertile, and the ones without seedheads were termed as non-fertile tillers.

### 2.3.4 Number of seeds

Seed counting was done using a seed counter Contador 2 (Pfeuffer GmbH) (Figure 2.7a), provided through the courtesy of PGG Wrightson Seeds Ltd, Kimihia-Lincoln.

### 2.3.5 Seed weight determination

Several seed weight data were obtained during the seed assessments, including the total weight of seeds, the weight of light seeds, the percentage of light seeds, the weight of machine dressed seeds (MDS), and thousand seed weight (TSW). Total seed weight was obtained after manually separating the seeds from their spikelet and removing the empty ones. The MDS was collected after separating light seeds from the seed lots using a Hoffman seed blower (Hoffman Mfg. Inc.) (Figure 2.7b), provided by PGG Wrightson Seeds Ltd Kimihia-Lincoln. The volume of the air intake gate of the blower was set to 25 (the actual gate opening from two dials was 25.6), which produced an air velocity of 7.0 ± 0.2 m/s. The blowing time was set for three minutes for each of the seed lots. The light seeds, which accumulated in a separate container during the blowing process, were also collected and weighed. Furthermore, the TSW was calculated from the average weight of eight replicates of 100-seeds (from the MDS) multiplied by ten (ISTA, 2017).

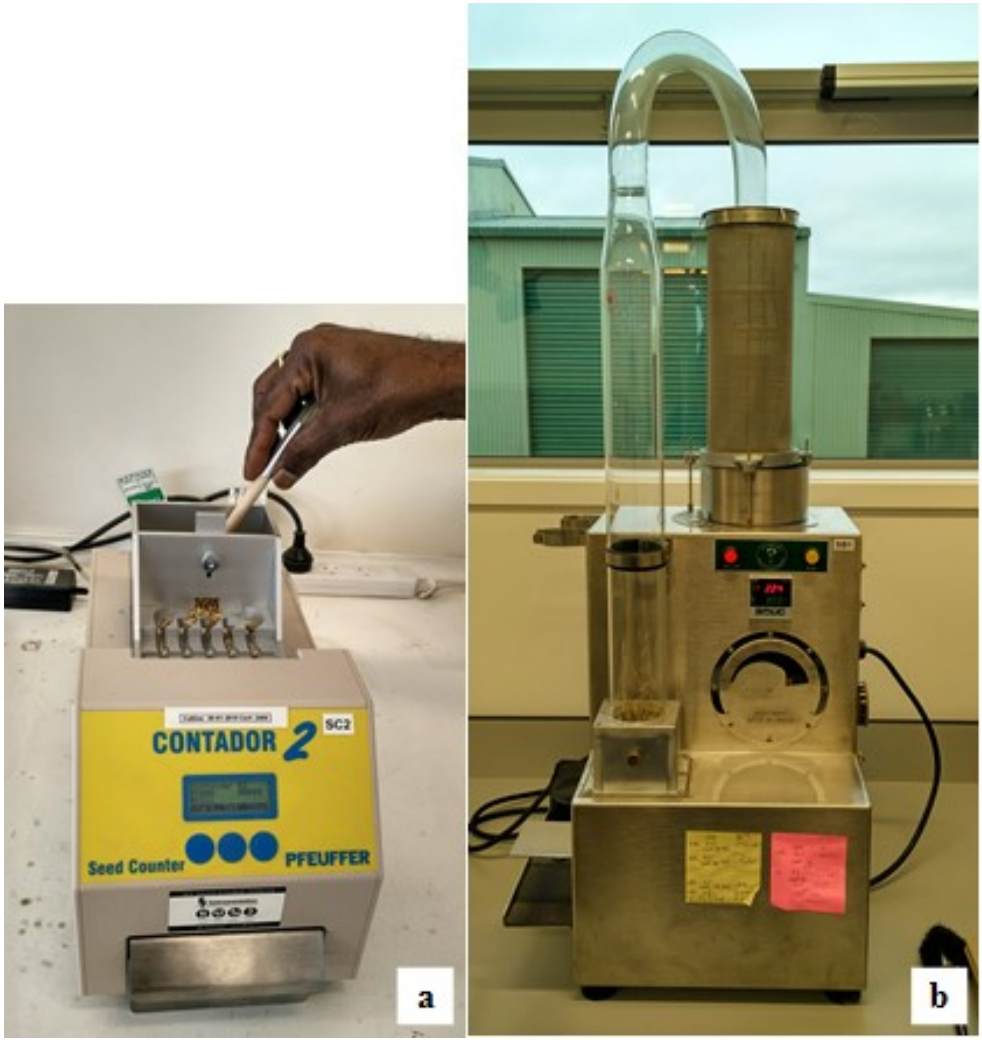


**Figure 2.7 Seed counter Contador 2 (a), and Hoffman seed blower (b) used in the assessment, provided by PGG Wrightson Seeds Ltd Kimihia-Lincoln.**

### 2.3.6 Germination of harvested seeds

The germination of MDS seeds was assessed using the method described in section 2.2.3. However, seedlings which were clearly normal were removed at the first count on the seventh day, while those requiring more time for development were counted at the fourteenth day. Abnormal seedlings and ungerminated seeds were counted at day 14. The latter included fresh seeds and dead seeds (ISTA, 2017). In the second week of the germination test, after the second count, there were still a number of fresh ungerminated seeds. These were pricked using a needle to allow water imbibition and incubated for another week before the final germination assessment.

### 2.3.7 Seed moisture content

Seed moisture was measured using the constant-temperature oven method (ISTA, 2017). Initially, an empty aluminium container with its lid used for the test was weighed (M1). Then, 4.5 g of randomly selected seeds were placed in the container and weighed jointly (M2). The oven was set at 130°C, and the containers with seeds were left to dry for one h with the lid removed (high-temperature method). The containers were then removed from the oven after covering with their lid, and then allowed to cool for 15 min in a desiccator before being weighed along with its contents (M3). The percentage SMC was calculated using the following equation:

$$SMC\ (\%) = \frac{Loss\ of\ weight}{Initial\ weight}\ x\ 100 = \frac{M_2 - M_3}{M_2 - M_1}\ x\ 100$$

Only two seed lots were measured with the assumption that all lots would have a similar SMC (Appendix 2). Therefore, these data were excluded from the statistical analysis.

### 2.3.8 Statistical analysis

The analyses were carried out using Genstat 18th edition (VSN International, Hemel Hempstead, UK). All data were analysed using an analysis of variance (ANOVA) for a split-split-plot design with eight main plots and sixteen subplots. The design was used to gather moisture-stressed and pathogen-inoculated pots in the separated main plots and subplots, while also separated from their untreated controls. Three main factors, i.e. pathogen, *Trichoderma* and moisture were included in the treatment structure of each analysis, except for seedling emergence and time of seedhead emergence. For these two parameters, the moisture factor was excluded from the analysis since they were obtained before the moisture stress treatment was applied.

Least significant difference (LSD) at $P<0.05$ was used to further investigate the difference of mean value between treatments, either as a single or their interaction with other treatments. The interaction tables are given for parameters that had significant differences in the treatment interactions.

## 2.4 Results

In this experiment, the pathogen and the *Trichoderma* treatments were applied at the beginning of the experimental period. The moisture treatment was applied two months later. The data presented for selected parameters in the following sections include all three treatments (moisture, pathogen, and *Trichoderma*). However, to match with the objectives of each chapter,

only pathogen and *Trichoderma* treatments are presented here. The description of the results and discussion for the moisture treatment are presented in Chapter 4.

### 2.4.1 Initial seed lot germination

An initial germination test to confirm the seed lot viability was done before the glasshouse experiment. The germination was 83%, with 5% abnormal seedlings (2.2% with a fungal infection) and 12% dead seeds (6.8% with a fungal infection) (Figure 2.8). An assessment of the sporulating fungi showed that seeds were infected by *Alternaria* spp., *Fusarium* spp., and *Aspergillus* spp. (Appendix 3).

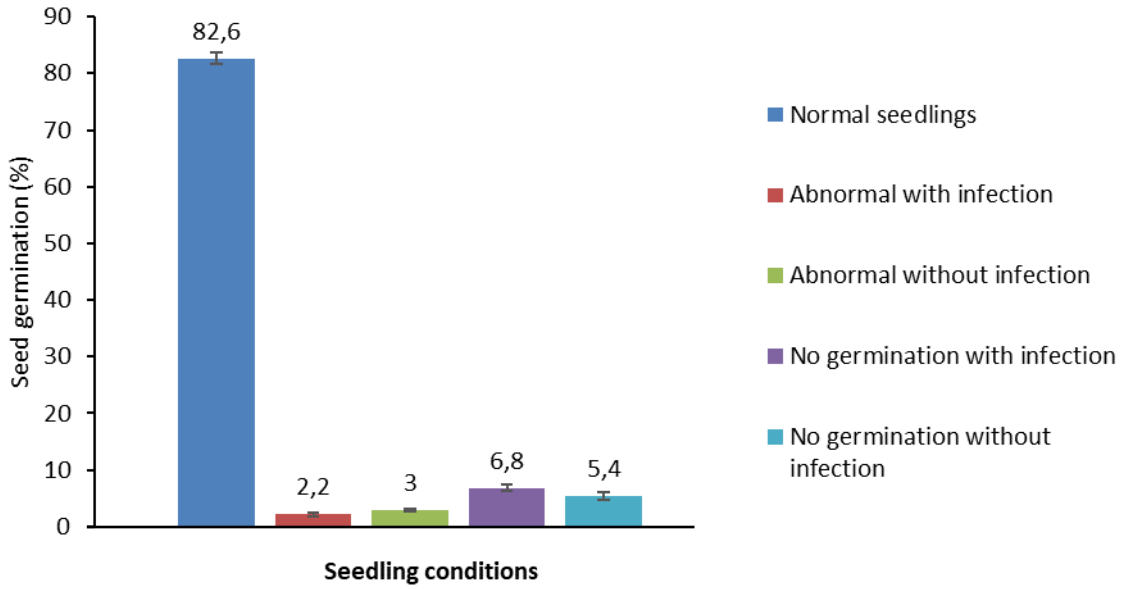


**Figure 2.8 Seed germination of prairie grass seed lot used for the glasshouse experiment. Error bars are calculated from standard error.**

### 2.4.2 Effects of take-all on seedling emergence and time to first seedhead emergence

The percentage seedling emergence did not differ between the non-inoculated soil (95%) and the *Ggt*-inoculated one (93%) (Table 2.1). Similarly, the introduction of the three individual isolates of *T. atroviride* had no significant effect on seedling emergence, but the mixture of isolates resulted in a small but significant reduction in emergence (Table 2.1).

The presence of *Ggt* delayed the time to first seedhead emergence (TFSE) in prairie grass plants by 1.5 days ($P<0.05$) (Table 2.1). The application of *Trichoderma* LU132 and LU140, on the other hand, significantly reduced the TFSE by 1.71 and 1.54 days compared to the non-inoculated control ($P<0.05$), while LU584 and the mixture of isolates did not affect the TFSE of the plants.

There was a moderate negative correlation between the TFSE and the seed yield of prairie grass as indicated in Figure 2.9 ($R^2 = 0.50$). Early emerged seedheads in plants treated with different pathogen and *Trichoderma* treatments can be seen in Figure 2.10.

**Table 2.1 Main effect means of percentage seedling emergence and days to first seedhead emergence of prairie grass for the different pathogen and *Trichoderma* treatments.**

| Main effect means | Seedling emergence (%) | Days to first seedhead emergence |
|---|---|---|
| Moisture | | |
| Non-stressed | 93.87 | 46.04 |
| Stressed | n/a | n/a |
| LSD (5%) | n/a | n/a |
| Sig. of diff. | | |
| Pathogen | | |
| Not inoculated | 94.79 | 45.04 |
| Inoculated | 93.40 | 46.54 |
| LSD (5%) | 2.51 | 0.71 |
| Sig. of diff. | ns | * |
| *Trichoderma* | | |
| Control | 94.47 | 46.71 |
| LU132 | 95.42 | 45.00 * |
| LU140 | 94.58 | 45.17 * |
| LU584 | 93.84 | 45.79 |
| Mixture | 90.42 * | 46.88 |
| LSD (5%) | | |
| (Ctrl v Trtd) | 3.40 | 0.92 |
| (Trtd v Trtd) | 3.93 | 1.06 |

ns = not significant; * = $P<0.05$; ** = $P<0.01$. n/a = data were not included in the analysis (moisture treatment was not yet applied).

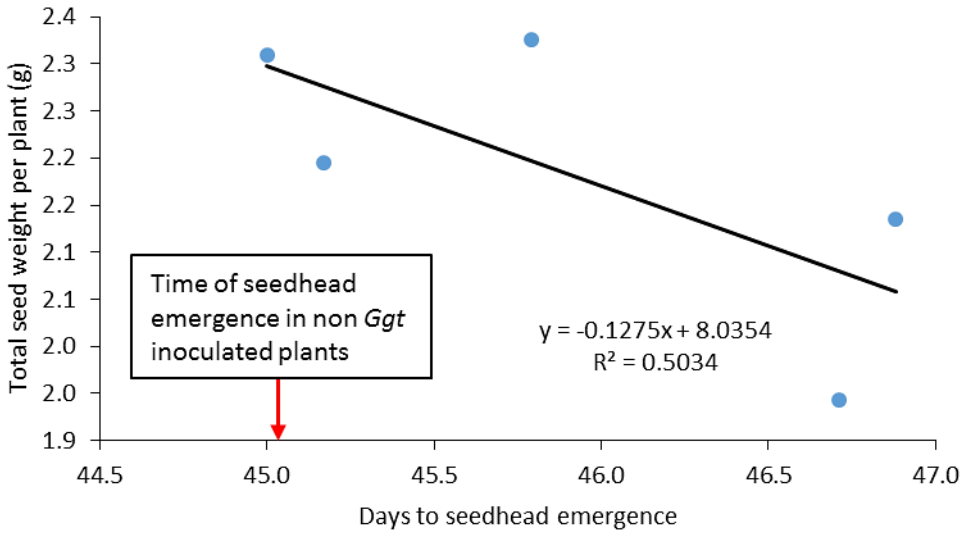


**Figure 2.9 Correlation between days to first seedhead emergence and total seed weight per plant for transplanted prairie grass plants growing in the glasshouse.**

### 2.4.3 The production of tillers, seed yield and seed quality of *Ggt*-infected prairie grass

*Ggt* infection of prairie grass did not significantly affect the total production of tillers or fertile tillers per plant in transplanted or direct-sown plants (Table 2.2). However, *Ggt* significantly reduced ($P<0.05$) the total seed weight and the number of seeds per plant of transplanted plants. There were no significant differences for both seed weight and the number of seeds per plant between *Ggt*-inoculated and uninoculated plants for direct-sown plants. There was a positive correlation ($R^2 = 0.61$) between the percentage of fertile tillers and the total seed weight per plant for transplanted plants (Figure 2.11).

The introduction of *Trichoderma* gave significant positive effects ($P<0.05$) for the production of tillers per plant of transplanted plants, but not for the direct-sown plants (Table 2.2). In transplanted plants, plants treated with *Trichoderma* isolates LU132, LU140 and LU584 produced 0.42 to 0.48 more tillers per plant than the control ($P<0.05$). *Trichoderma* application did not affect the number of fertile tillers per plant for both planting systems. However, the *Trichoderma* increased both the number of seeds and the seed weight per plant. In the transplanted plants, only LU132 and LU584 increased the number of seeds per plant, but all three isolates and the mixture of isolates increased the seed weight per plant ($P<0.05$) (Table 2.2). In the direct-sown plants, isolates LU584 and the mixture of isolates increased both the number of seeds and the seed weight per plant ($P<0.05$).

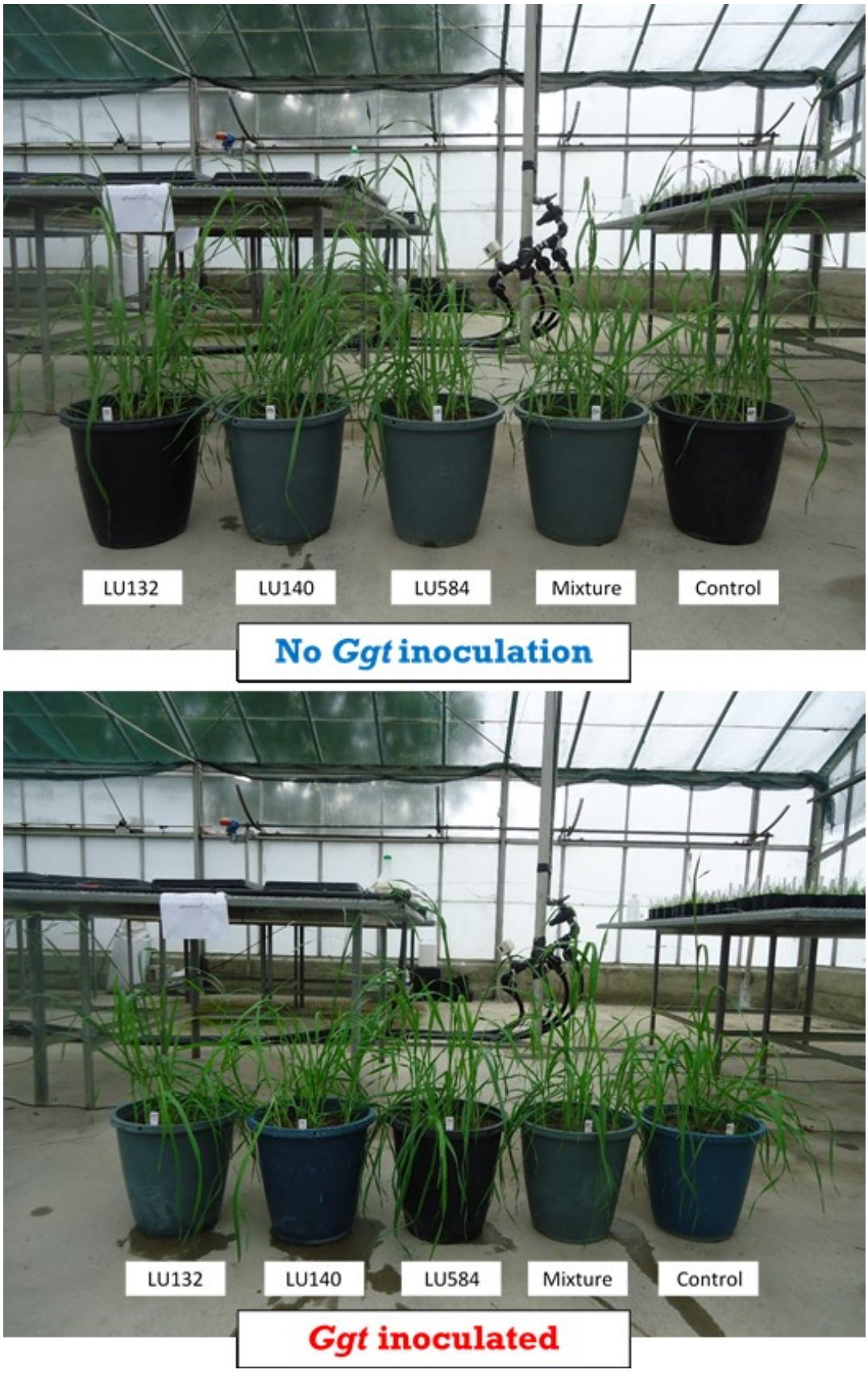


**Figure 2.10** **Early seedhead emergence in prairie grass plants in the experimental pots with and without *Gaeumannomyces graminis* var. *tritici* and *Trichoderma* inoculation at 55 days after sowing.**

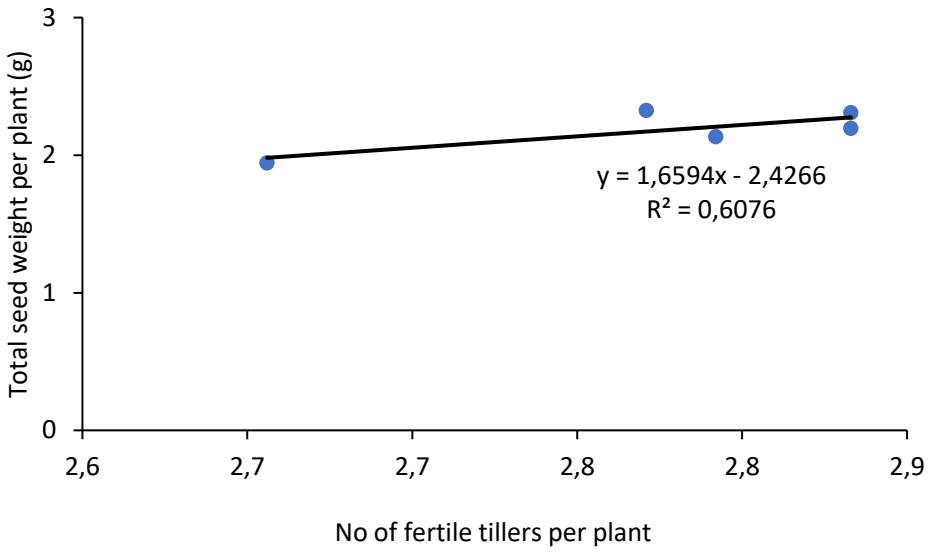


**Figure 2.11 Correlation between the number of fertile tillers and total seed weight per plant for prairie grass growing in the glasshouse.**

There were no significant differences in the number of seeds per plant and total seed weight among *Trichoderma* isolates (Table 2.2). However, isolate LU584 performed best in the transplanted plants, producing the highest number of seeds (238) as well as the highest total seed weight per plant (2.33 g). The mixture of isolates was the most effective growth promoter in the direct-sown plants, generating the biggest number of seeds (52.18) as well the biggest seed weight per plant (0.49 g). There was a strong positive correlation ($R^2$ = 0.97) between the number of seeds per plant and the total seed weight per plant (Figure 2.12).

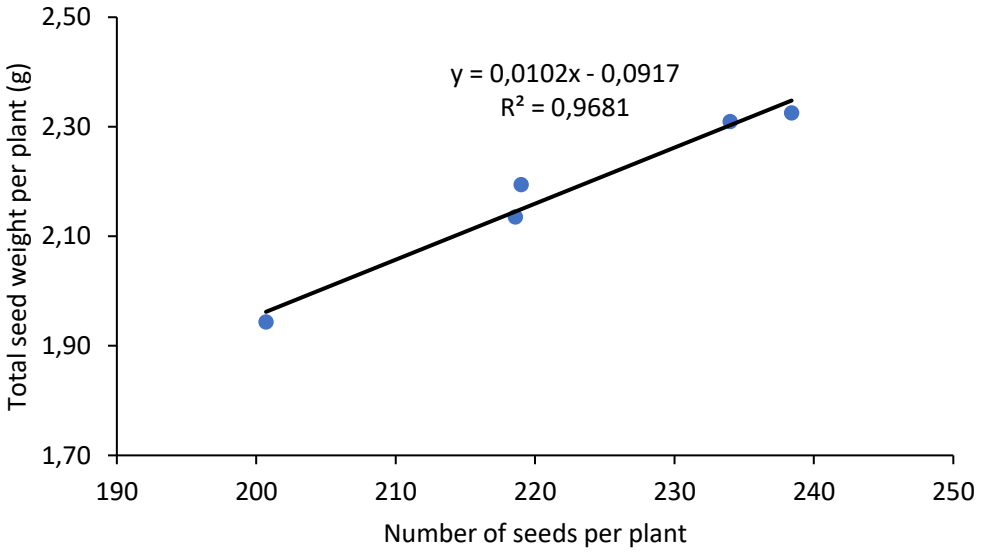


**Figure 2.12 Correlation between the number of seeds per plant and the total seed weight per plant for prairie grass growing in the glasshouse.**

*Ggt* inoculation did not reduce the weight of light seeds per plant, the percentage of light seeds per plant, or the thousand seed weight (TSW) for both planting systems (Table 2.3). However, *Ggt* significantly reduced ($P$<0.05) the weight of machine dressed seeds (MDS) of transplanted plants by 13% as well as lowering the germination percentage of the seeds produced by 6% ($P$<0.01). The weight of MDS of direct-sown plants was not affected by pathogen inoculation.

There was a variation in the effects of *Trichoderma* isolates on the weight of light seeds per plant (Table 2.3). Isolate LU132 and the mixture of isolates did not affect the weight of light seeds in either transplanted or direct-sown plants. Meanwhile, isolates LU140 and LU584 increased the weight of light seeds in the transplanted plants ($P$<0.05), but not in the direct-sown plants.

Overall, the percentage of light seed per plant (both transplanted and direct-sown plants) was not affected by the *Trichoderma* isolates, except for LU584. Isolate LU584 significantly decreased the percentage of light seeds per plant of the direct-sown plants, but significantly increased ($P$<0.05) the percentage of light seeds per plant of the transplanted plants.

All the *Trichoderma* treatments significantly increased ($P$<0.05) the weight of MDS per plant of the transplanted plants by an average of 14%, with LU132 gave the highest increase of 18% (Table 2.3). Meanwhile, only isolate LU584 and the mixture of isolates significantly increased the weight of MDS per plant in the direct-sown plants by 16% and 17%, respectively. However, both the TSW and the germination percentage were not affected by *Trichoderma* treatments.

**Table 2.2 Main effect means of total number of tillers per plant, number of fertile tillers per plant, number of seeds per plant, and total seed weight per plant for the different moisture, pathogen and *Trichoderma* treatments.**

| Main effect means | Total No of tillers per plant[1] | | No of fertile tillers per plant | | No of seeds per plant | | Total seed weight (g) per plant | |
|---|---|---|---|---|---|---|---|---|
| | Transplanted[2] | Direct sown[3] | Transplanted | Direct sown | Transplanted | Direct sown | Transplanted | Direct sown |
| Moisture | | | | | | | | |
| Non-stressed | 4.06 | 1.95 | 2.95 | 1.11 | 261 | 56.54 | 2.69 | 0.57 |
| Stressed | 3.68 | 1.76 | 2.56 | 0.99 | 176 | 39.50 | 1.60 | 0.33 |
| LSD (5%) | 0.48 | 0.11 | 0.31 | 0.07 | 33 | 8.92 | 0.46 | 0.07 |
| Sig. of diff. | ns | * | * | * | ** | ** | ** | ** |
| Pathogen | | | | | | | | |
| Not inoculated | 4.02 | 1.81 | 2.87 | 1.04 | 242 | 47.66 | 2.36 | 0.45 |
| Inoculated | 3.79 | 1.88 | 2.70 | 1.06 | 207 | 48.20 | 2.03 | 0.45 |
| LSD (5%) | 0.31 | 0.11 | 0.37 | 0.07 | 29 | 7.85 | 0.27 | 0.07 |
| Sig. of diff. | ns | ns | ns | ns | * | ns | * | ns |
| *Trichoderma* | | | | | | | | |
| Control | 3.60 | 1.87 | 2.66 | 1.04 | 201 | 44.88 | 1.94 | 0.42 |
| LU132 | 4.02 * | 1.77 | 2.83 | 1.06 | 234 * | 47.69 | 2.31 * | 0.45 |
| LU140 | 4.02 * | 1.88 | 2.83 | 1.05 | 219 | 48.63 | 2.19 * | 0.45 |
| LU584 | 4.08 * | 1.83 | 2.77 | 1.05 | 238 * | 49.85 * | 2.33 * | 0.47 * |
| Mixture | 3.88 | 1.92 | 2.79 | 1.05 | 219 | 52.18 * | 2.14 * | 0.49 * |
| LSD (5%) | | | | | | | | |
| (Ctrl v Trtd) | 0.34 | 0.11 | 0.24 | 0.06 | 18 | 4.55 | 0.18 | 0.04 |
| (Trtd v Trtd) | 0.39 | 0.13 | 0.28 | 0.06 | 21 | 5.25 | 0.20 | 0.05 |

ns = not significant; * = $P<0.05$; ** = $P<0.01$. [1] = All emerged tillers per plant, including fertile and non-fertile tillers. [2] = Results of the centre plants, which represent transplanted plants. [3] = Results of the surrounding plants, which represent the direct-sown plants. No = number.

**Table 2.3 Main effect means of the weight of light seeds, the percentage of light seeds, the weight of machine dressed seeds (MDS), the thousand seed weight (TSW), and the germination percentage of harvested seeds for the different moisture, pathogen and *Trichoderma* treatments.**

| Main effect means | Weight of light seeds (g) per plant | | % of light seeds per plant | | Weight of MDS (g) per plant | | TSW (g) | Germination percentage (%) |
|---|---|---|---|---|---|---|---|---|
| | Transplanted | Direct sown | Transplanted | Direct sown | Transplanted | Direct sown | | |
| Moisture | | | | | | | | |
| Non-stressed | 0.15 | 0.22 | 2.82 | 4.48 | 2.53 | 0.54 | 10.29 | 61.20 |
| Stressed | 0.23 | 0.19 | 7.01 | 6.37 | 1.37 | 0.31 | 9.11 | 61.10 |
| LSD (5%) | 0.09 | 0.14 | 1.75 | 4.02 | 0.49 | 0.06 | 0.33 | 7.86 |
| Sig. of diff. | ns | ns | ** | ns | ** | ** | ** | ns |
| Pathogen | | | | | | | | |
| Not inoculated | 0.22 | 0.22 | 4.93 | 5.90 | 2.14 | 0.42 | 9.74 | 65.20 |
| Inoculated | 0.17 | 0.20 | 4.91 | 5.19 | 1.86 | 0.43 | 9.68 | 59.10 |
| LSD (5%) | 0.08 | 0.09 | 1.22 | 2.09 | 0.21 | 0.07 | 0.23 | 2.89 |
| Sig. of diff. | ns | ns | ns | ns | * | ns | ns | ** |
| *Trichoderma* | | | | | | | | |
| Control | 0.16 | 0.23 | 4.54 | 6.31 | 1.79 | 0.39 | 9.61 | 59.10 |
| LU132 | 0.20 | 0.19 | 4.80 | 4.87 | 2.11 * | 0.43 | 9.85 | 63.80 |
| LU140 | 0.21 * | 0.20 | 5.17 | 4.84 | 1.99 * | 0.43 | 9.77 | 63.80 |
| LU584 | 0.26 * | 0.17 | 6.22 * | 4.24 * | 2.07 * | 0.45 * | 9.76 | 62.90 |
| Mixture | 0.16 | 0.23 | 4.23 | 5.99 | 1.98 * | 0.46 * | 9.61 | 58.20 |
| LSD (5%) | | | | | | | | |
| (Ctrl v Trtd) | 0.05 | 0.07 | 1.07 | 1.73 | 0.16 | 0.04 | 0.23 | 5.97 |
| (Trtd v Trtd) | 0.06 | 0.08 | 1.24 | 2.00 | 0.19 | 0.05 | 0.27 | 6.89 |

ns = not significant; * = $P<0.05$; ** = $P<0.01$. TSW = Thousand seed weight, assessed from transplanted plants. Germination percentage was assessed from transplanted plants.

### 2.4.4 Treatment interactions

The interaction between two or three factors was significant for some traits measured in this study. The significant interaction for seed yield components was found in the transplanted plants (Appendix 4), but not in the direct-sown plants.

Take-all disease significantly reduced the total seed weight of transplanted plants in both moisture-stressed and non-stressed plants, by 33% and 26%, respectively, but only in *Trichoderma*-untreated pots (Table 2.4). The pathogen did not reduce the seed weight in plants treated with *Trichoderma* isolates. On the other hand, *Trichoderma* isolates significantly increased the total seed weight in both moisture-stressed and non-stressed plants only in the presence of *Ggt* infection. The BCAs did not affect the total seed weight while the pathogen was absent.

Take-all significantly increased the weight of light seeds of transplanted plants treated with isolates LU132 and LU584, but only under moisture stress condition (Table 2.5). The pathogen did not affect the weight of light seeds in non-stressed plants, with or without *Trichoderma* treatments.

Take-all reduced the weight of MDS of transplanted plants in both stressed (34%) and non-stressed (25%) plants only when *Trichoderma* isolates were absent (Table 2.6). On the other hand, *Trichoderma* treatments increased the weight of MDS relative to control plants, in both stressed (42% by LU132) and non-stressed (24% by LU132) plants, only in the presence of take-all. None of the isolates affected the weight of MDS in the absence of the pathogen.

The disease did not affect the TSW in moisture-stressed plants (Table 2.7). However, in non-stressed plants, the disease caused a small but significant 5% reduction in the TSW, but only when the *Trichoderma* isolates were absent. The *Trichoderma* treatments increased the TSW only in *Ggt*-infected and well-watered plants. The *Trichoderma* application did not increase the TSW when the pathogen was absent or with the presence of the pathogen under moisture stress conditions.

**Table 2.4 Interaction effects between *Trichoderma* isolates, pathogen, and moisture treatments on total seed weight (g/plant) of transplanted plants.**

| | Control (Double reps) | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (p+)*** **(double reps)** | | | | | |
| (1) Non-stressed | 2.23 + | 2.76 * | 2.65 * | 2.81 * | 2.76 * |
| (2) Stressed | 1.20 + x | 1.74 * x | 1.72 * x | 1.69 * x | 1.40 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (1) & (2) | - | | 0.31 | | 0.35 |
| Between rows (1) & (2) | 0.44 | | 0.46 | | 0.48 |
| ***Un-inoculated (p-)*** | | | | | |
| (3) Non-stressed | 3.01 | 2.73 | 3.00 | 3.01 | 2.76 |
| (4) Stressed | 1.80 x | 2.14 | 1.44 x | 1.96 x | 1.74 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (3) & (4) | - | | 0.43 | | 0.50 |
| Between rows (3) & (4) | 0.53 | | 0.58 | | 0.63 |

| Other comparison LSD (5%) values | C v C | Cp+ v T | Cp- v T | T v T |
|---|---|---|---|---|
| Between rows (1) & (3) | 0.42 | 0.48 | 0.45 | 0.51 |
| Between rows (1) & (4) | 0.48 | 0.53 | 0.51 | 0.56 |
| Between rows (2) & (3) | 0.48 | 0.53 | 0.51 | 0.56 |
| Between rows (2) & (4) | 0.42 | 0.48 | 0.45 | 0.51 |

+ indicates 5% significant difference of pathogen inoculated from uninoculated within the same moisture treatment; * indicates 5% significant difference of *Trichoderma*-treated from control plants in the same row; x indicates 5% significant difference of stressed from non-stressed in the same column.

**Table 2.5 Interaction effects between *Trichoderma* isolates, pathogen, and moisture treatments on weight of light seeds (g/plant) of transplanted plants.**

| | Control (Double reps) | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (p+)*** **(double reps)** | | | | | |
| (1) Non-stressed | 0.11 | 0.13 | 0.16 | 0.12 | 0.13 |
| (2) Stressed | 0.16 | 0.26 x | 0.24 | 0.32 x | 0.19 |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (1) & (2) | - | | 0.08 | | 0.10 |
| Between rows (1) & (2) | 0.10 | | 0.11 | | 0.12 |
| ***Un-inoculated (p-)*** | | | | | |
| (3) Non-stressed | 0.19 | 0.14 | 0.29 | 0.29 | 0.16 |
| (4) Stressed | 0.23 | 0.28 | 0.16 | 0.38 | 0.14 |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (3) & (4) | - | | 0.12 | | 0.14 |
| Between rows (3) & (4) | 0.14 | | 0.15 | | 0.17 |

| Other comparison LSD (5%) values | C v C | Cp+ v T | Cp- v T | T v T |
|---|---|---|---|---|
| Between rows (1) & (3) | 0.13 | 0.14 | 0.13 | 0.15 |
| Between rows (1) & (4) | 0.12 | 0.13 | 0.13 | 0.14 |
| Between rows (2) & (3) | 0.12 | 0.13 | 0.13 | 0.14 |
| Between rows (2) & (4) | 0.13 | 0.14 | 0.13 | 0.15 |

x indicates 5% significant difference of stressed from non-stressed in the same column.

**Table 2.6 Interaction effects between *Trichoderma* isolates, pathogen, and moisture treatments on weight of machine dressed seeds (g/plant) of transplanted plants.**

| | Control (Double reps) | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (p+)*** **(double reps)** | | | | | |
| (1) Non-stressed | 2.13 + | 2.63 * | 2.49 * | 2.68 * | 2.63 * |
| (2) Stressed | 1.04 + x | 1.48 * x | 1.47 * x | 1.37 * x | 1.21 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (1) & (2) | - | | 0.28 | | 0.33 |
| Between rows (1) & (2) | 0.45 | | 0.46 | | 0.48 |
| ***Un-inoculated (p-)*** | | | | | |
| (3) Non-stressed | 2.82 | 2.58 | 2.71 | 2.71 | 2.60 |
| (4) Stressed | 1.57 x | 1.86 x | 1.27 x | 1.58 x | 1.61 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (3) & (4) | - | | 0.40 | | 0.46 |
| Between rows (3) & (4) | 0.51 | | 0.55 | | 0.59 |

| Other comparison LSD (5%) values | C v C | Cp+ v T | Cp- v T | T v T |
|---|---|---|---|---|
| Between rows (1) & (3) | 0.36 | 0.42 | 0.39 | 0.45 |
| Between rows (1) & (4) | 0.47 | 0.51 | 0.49 | 0.54 |
| Between rows (2) & (3) | 0.47 | 0.51 | 0.49 | 0.54 |
| Between rows (2) & (4) | 0.36 | 0.42 | 0.39 | 0.45 |

+ indicates 5% significant difference of pathogen inoculated from uninoculated within the same moisture treatment; * indicates 5% significant difference of *Trichoderma*-treated from control plants in the same row; x indicates 5% significant difference of stressed from non-stressed in the same column.

**Table 2.7 Interaction effects between *Trichoderma* isolates, pathogen, and moisture treatments on thousand seed weight (TSW) (g/plant) of transplanted plants.**

| | Control (Double reps) | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (p+)*** **(double reps)** | | | | | |
| (1) Non-stressed | 9.92 + | 10.37 * | 10.58 * | 10.59 * | 10.32 |
| (2) Stressed | 9.14 x | 9.22 x | 8.88 x | 9.19 x | 8.95 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (1) & (2) | - | | 0.41 | | 0.47 |
| Between rows (1) & (2) | 0.38 | | 0.44 | | 0.50 |
| ***Un-inoculated (p-)*** | | | | | |
| (3) Non-stressed | 10.40 | 10.44 | 10.03 | 10.34 | 10.22 |
| (4) Stressed | 9.18 x | 9.47 x | 9.65 | 8.65 x | 8.90 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (3) & (4) | - | | 0.57 | | 0.66 |
| Between rows (3) & (4) | 0.51 | | 0.61 | | 0.69 |

| Other comparison LSD (5%) values | C v C | Cp+ v T | Cp- v T | T v T |
|---|---|---|---|---|
| Between rows (1) & (3) | 0.44 | 0.54 | 0.49 | 0.59 |
| Between rows (1) & (4) | 0.45 | 0.55 | 0.50 | 0.60 |
| Between rows (2) & (3) | 0.45 | 0.55 | 0.50 | 0.60 |
| Between rows (2) & (4) | 0.44 | 0.54 | 0.49 | 0.59 |

+ indicates 5% significant difference of pathogen inoculated from uninoculated within the same moisture treatment; * indicates 5% significant difference of *Trichoderma*-treated from control plants in the same row; x indicates 5% significant difference of stressed from non-stressed in the same column.

## 2.5 Discussion

The presence of the take-all pathogen in field soil of grass seed crops has been suspected to cause yield reductions, as indicated by the occurrence of very light seeds in the crops (Rolston, pers, comm, 2017). In the present research, the primary objective was to assess the effect of take-all on grass seed yield with prairie grass used as a model system. *Trichoderma* effects are also included in the discussion.

### 2.5.1 Effects on seedling emergence and days to first seedhead emergence

The presence of *Ggt* inoculum in the soil had no effect on seedling emergence, which indicates the pathogen did not cause an infection of prairie grass seedlings. This can generally be understood since take-all is a typical root-inhabiting fungus (Skou, 1981), which starts to colonise the plant root system by its runner hyphae as soon as the plant roots develop. The initial infection by the pathogen occurs when seedling roots are contacting infected trash or by the fungus growing through the soil (Wherrett & MacLeod, 2018). In fact, in the long history of the pathogen, no report can be found regarding any effect of take-all on the emergence of either wheat or grass crops.

Overall, there were also no significant differences among *Trichoderma* treatments for seedling emergence, except for the mixture of isolates which slightly reduced emergence. While two isolates gave a marginal increase in emergence, LU584 and the mixture of isolates reduced the seedling emergence of prairie grass in the presence of *Ggt* inoculum, and LU132 and the mixture of isolates reduced the emergence in the absence of the pathogen inoculum, with the latter mentioned being a significant reduction ($P$.<05) (Appendix 5, 5.1). This result is similar to an earlier study, which showed only 3 out of 9 isolates of *Trichoderma* spp. increased seedling emergence of perennial ryegrass grown in soils infected with the pathogen *Rhizoctonia solani* (Kandula et al., 2015). In this experiment, two *Trichoderma* isolates (*Trichoderma hamatum* LU740 and *T. koningiopsis* LU713) reduced seedling emergence in the presence of the pathogen, and four isolates reduced the seedling emergence in the absence of the pathogen. Numerous secondary metabolites are produced by *Trichoderma* spp., which can be beneficial to promote plant growth (Harman et al., 2004). However, some strains can produce phytotoxins that are harmful to plant growth, such as the phytotoxin viridol released by *T. virens*. The compound can reduce emergence when placed near the seeds by causing severe necrosis of the radicle and lead to seedling death (Kandula et al., 2015).

Plants grown in *Ggt*-inoculated soil had a longer TFSE, indicating that the pathogen may have affected the function of the plant system. Take-all disease causes root rot, and infected roots are very brittle (Kwak & Weller, 2013). According to Pillinger et al. (2005), the reduction in either the size of the root system or the efficiency of uptake function can reduce the ability of the root system to supply the plant with adequate water and nutrients. The damaged or weakened root system, which likely occurred before the heading stage, interrupted the plant's normal function (Shurtleff et al., 2018), including prolonging the time until first seedhead emergence.

Interestingly, the application of most of the *Trichoderma* isolates reduced the TFSE, indicating the BCAs was able to alleviate the harmful effects of the *Ggt* infection. *Trichoderma* spp. have been reported to confer numerous benefits for the host plant, including systemic resistance to diseases through the induction of jasmonic acid and salicylic acid signalling, improved adaptation to abiotic stress including drought, salt and temperature, enhanced nutrient solubility, and regulation of root system architecture (Contreras-Cornejo et al., 2013). *Trichoderma* can promote plant growth through increased root development, increased root formation, and increased root size (Hermosa et al., 2012; Samolski et al., 2012). Thus, the growth promotion induced by *Trichoderma* spp., along with other possible antagonism mechanisms, may be able to compensate for the root damage caused by *Ggt* that caused prolonged TFSE in prairie grass plants.

The reduction of the TFSE by *Trichoderma* isolates was significant in *Ggt*-inoculated plants, but not in non-inoculated plants (Appendix 5, 5.2). This result indicates that the BCAs had better biological activities during unfavourable conditions. This accords with earlier studies, which showed the influence of *T. harzianum* T22 was more prominent under abiotic stress conditions and during plant contact with the pathogen *Pythium ultimum* (Mastouri et al., 2010). This report showed that the production of several defence-related enzymes increased further with pathogen infections. The result also suggests that *Trichoderma* alleviated the damage caused by *Ggt* infection early in the season to be able to increase the seed yield at harvest.

Another interesting finding is a strong negative correlation between TFSE and seed yield. It is possible, therefore, to predict the prairie grass seed yield by measuring the days required to seedhead emergence. The longer the number of days would mean lower seed yield (Figure 2.9), and may also indicate the presence of disease as the cause of the prolonged TFSE.

### 2.5.2 Effects on tillering and seed yield

All aspects of the vegetative and the reproductive growths of plants infected by *Ggt* are affected to some extent (Clarke & Eagling, 1994; Manners & Myers, 1981). In this trial, however, the differences in tillering between *Ggt*-infected and non-infected plants were not significant. Both treatments produced a similar number of total and fertile tillers. Tillering is a continuous process, and thus at any one time grass plants comprise a collection of tillers, differing in age, position on the plant, and size (Langer, 1980). The number of fertile tillers is an important component in determining the seed yield potential. However, its importance varies among grass species. Grass species with a high vernalisation requirement greatly depend on tiller number and tiller size in autumn to maintain high yield potential in first-year seed crops. Bahmani (1999) reported that tiller dynamics of perennial ryegrass were affected by cultivar and environmental factors such as nitrogen fertiliser and irrigation. In this experiment, most of the seedheads that contributed to seed yield emerged from early developed tillers. Only a small portion of tillers which came next produced seedheads, and the later emerged tillers were either non-fertile or had produced immature seeds at the time of seed harvesting. This has been found earlier by Langer (1980) who reported tillers produced later in the spring by prairie grass plants did not contribute to seed yield.

The number of seeds and the total seed weight per plant were significantly lower in infected than in healthy plants in transplanted plants. The yield loss was presumably influenced by the root damage due to *Ggt* infection, which would lower water and nutrient absorption through either a reduction in the size of the root system or a reduction in the efficiency of uptake (Pillinger et al., 2005). However, the reduction did not occur in direct-sown plants. It is possible that these related to different nutrients acquired, since the transplanted plants had more nutrients available in the potting mix during their early stages of development. The infection potential of *Ggt* on direct-sown plants might be reduced because the plants received less nutrients, resulting in no significant reduction of seed yield compared to control plants. However, nutrient status of the direct-sown and transplanted plants was not determined. Supporting evidence for this was reported by Manners and Myers (1981) who found that wheat plants grown in a pot experiment with low N concentration developed only half the root infection than in other treatments, suggesting that lack of N reduced the infection potential of the pathogen. Unfortunately, the present study did not assess the root disease of the transplanted and the direct-sown plants separately, and therefore, it was not possible to confirm the differences in the root disease severity between the two. Another likely explanation is that plant stress associated with transplant shock created vulnerable condition to exacerbate the *Ggt* problems in transplanted

plants. However, further research is required to confirm the actual cause of yield loss in transplanted plants, but not in direct-sown plants.

*Trichoderma* isolates can promote plant growth by improving the plant's ability to take up nutrients (Martínez-Medina et al., 2009) and through the secretion of growth-promoting metabolites (Contreras-Cornejo et al., 2009). In the present experiment, the increased number of tillers in *Trichoderma*-treated plants in transplanted plants indicates the BCAs have the potential to further increase the seed yield of prairie grass by allowing the production of more fertile tillers per plant when ample nutrients are readily available in the soil. Some of the last tillers to emerge also produced seedheads, but could not be harvested because they failed to fully develop and did not contribute to seed yield. More time most likely would have increased the number of fertile tillers and added more seed yield for *Trichoderma*-treated plants. Therefore, the differences in the effects of *Trichoderma* on tillering in transplanted and direct-seeding plants can presumably be attributed to the difference in nutritional status, rather than the different planting systems. In fact, the transplanted plants produced more tillers, and were bigger and taller than the direct-sown plants, indicating nutritional differences between the two.

Overall, there was a big gap in the yield components between the transplanted and the direct-sown plants (Table 2.2 and 2.3). In addition to the nutrition availability, the growth and yield differences between the two planting system can be attributed to plant competition for growth space. The transplanted plants grown in the centre of the pot had the advantage for soil nutrient access, as well as water, over the direct-sown plants grown at the edge of the pot. The plants grown at the edge also developed fewer roots than plants in the centre, which was observed during root assessment. Thus, the limited growth spaces and nutrients were the main factors causing low productivity of the direct-sown plants. Additionally, the differences could be an artefact of the experimental setup, as the direct sown plants were more crowded compared to the transplanted plants in every pot.

All *Trichoderma* isolates significantly increased total seed weight in transplanted plants, but only two isolates (LU584 and the mixture of isolates) gave a significant increase in direct-sown plants. This result further supports the suggestion that the greater nutrient availability in transplanted plants allowed a better performance of *Trichoderma* in promoting plant growth. For example, both LU132 and LU140 did not increase the total seed weight of the direct-sown plants that received less nutrients, but did so for transplanted plants that received more nutrients (Table 2.2).

The increase in the number of seeds per plant in transplanted plants by *Trichoderma* isolates was strongly correlated with the increase in seed yield (Figure 2.12). Although the two isolates (LU140 and the mixture of isolates) did not significantly increase the number of seeds, the end result showed the two isolates increased the total seed weight per plant as well as the other isolates, with no differences statistically among *Trichoderma* treatments. This means that a marginal increase in the number of seeds can contribute to a significant increase in the seed yield, emphasizing the importance of this component. These results are in agreement with Oliva et al. (1994) which showed that the number of seeds was the most important yield component for red clover, directly affecting seed yield per plant. Langer (1980) noted fertile tiller number per unit area as the primary determinant of the yield of grass seed crops, which also indicates the importance of seed numbers to the yield.

No differences in the TSW between *Trichoderma*-treated and untreated plants further confirms that the increased number of seeds was the primary means through which the BCAs increased the seed yield. This is similar with the work of Rolston et al. (2004), which showed that the plant growth regulator trinexapac-ethyl (TE) increased the seed yield of perennial ryegrass without affecting the TSW. These authors concluded that the increased yield was achieved through either disease reduction and/or increased green leaf area. *Trichoderma* isolates promote plant growth and increase yield through various mechanisms. These include synthesis of phytohormones, either by the microbe or the plant; production of vitamins; enhanced solubilisation of soil nutrients; increased uptake and translocation of nutrients; enhanced root development; and increases in the rate of carbohydrate metabolism, photosynthesis and plant defense mechanisms (Stewart & Hill, 2014).

Only LU584 and the mixture of isolates significantly increased seed yield in direct-sown plants, indicating the two isolates performed better than LU132 and LU140 when nutrient supply was limited. According to Stewart and Hill (2014), some *Trichoderma* species/isolates have a better chance of promoting plant growth under suboptimal conditions, such as light, water, nutrient, or temperature limitations. Under nutrient-stress conditions, *Trichoderma* can improve plant growth through nutrient solubilisation (Shoresh et al., 2010).

### 2.5.3 Effects on seed quality

In wheat crops, obvious reductions in grain yield and quality were recorded from severely infected *Ggt* plants, but less severe infection resulted in more variable yield and quality (Manners & Myers, 1981). These authors reported the thousand grain weight and/or the grain protein

content from *Ggt*-infected wheat plants was sometimes greater than in the control. In the present study, *Ggt* infection resulted in a reduction of total seed weight and weight of MDS (Table 2.2 and 2.3), but the infection did not affect the weight of light seeds or the percentage of light seeds. The result indicate that seed yield loss by *Ggt* infection occurred through a reduction in the number of seeds produced per plant rather than a reduction in individual seed weight. A reduced number of grains in *Ggt*-infected wheat plants was reported by Manners and Myers (1981), who later suggested that the reduction resulted in a proportional increase of nitrogen content of the grains of the infected plants.

*Ggt* infection also affected seed quality by reducing the germination percentage of the seed produced. Seed viability or the ability of the embryo to germinate is influenced by a variety of factors, including seed maturity (Ghive et al., 2007). The stage of maturity at harvest influences both viability and longevity of the seeds. Other factors can also influence seed viability, such as storage conditions (Hampton & Hill, 2002) and moisture content (Shaban, 2013). As mentioned earlier in the method section, most of the seeds were not fully ripe when harvested, and pathogen infection may have contributed in disturbing seed maturity through water and nutrient disturbance and/or interruption in plant's normal function. The immaturity of some of the harvested seeds also explains why the overall germinations were poor.

The growth and yield responses of host plants to *Trichoderma* spp. have been highly variable. The same plant species can respond positively or negatively to the BCAs even when inoculated with the same *Trichoderma* isolate (Harman et al., 2004). Some factors that can affect growth promotion include crop type, growing conditions, inoculum type/formulation, and inoculum rate/concentration (Stewart & Hill, 2014). One or more of these factors, or interaction with other external factors, may contribute to the variation of growth promotion efficacy. In the present study, for example, adequate nutrient availability along with the high *Trichoderma* inoculum concentration in the soil stimulated the transplanted prairie grass plants to produce greater seed yields. However, the occurrence of moisture stress in the interaction caused the plants to generate more light seeds (Table 2.5). As a consequence, transplanted plants treated with *Trichoderma* isolates had more light seeds than control plants when exposed to moisture stress. These finding may also explain the contradictory results in the percentage of light seeds, where isolate LU584 increased the percentage in the transplanted plants, but decreased the percentage in the direct-sown plants.

### 2.5.4 Interaction responses

The reduction in total seed weight, the weight of MDS, and TSW in *Ggt*-infected plants occurred only in the control. This shows that without the interruption of the pathogen by the biocontrol activity of the *Trichoderma*, take-all developed to a level that caused damage to the plant root system. The damaged roots then decreased water and nutrient uptake through either reducing root growth or decreasing the efficiency of the root system (Pillinger et al., 2005).

Among the *Ggt*-infected plants, plants with moisture stress suffered a greater reduction in the total seed weight and the weight of MDS than well-watered plants. This result implies that the occurrence of the two stresses (moisture and the pathogen) can result in further reduction in seed yield. It was suggested by Hornby et al. (1998), that if the infection occurs at the advanced stage of plant development, when natural substantial root growth has ceased, the additional stress of dry conditions may combine with a diseased root system to exacerbate yield loss. However, TSW of stressed plants was not affected by *Ggt*, and only a small reduction occurred in non-stressed plants. This further supports the earlier result which showed that reduced total seed weight by *Ggt* was primarily caused by the reduction of the number of seeds per plant and not the weight of individual seeds. Whereas, increased weight of light seeds in LU132- and LU584-treated plants under moisture stress was possibly related to the high seed yield of both treatments as explained earlier.

The beneficial effect of *Trichoderma* application on seed yield parameters occurred only in the presence of take-all infection. The result is in accordance with that of Mastouri et al. (2010) who reported that the effect of *Trichoderma*-plant associations are greater under various environmental stresses, including pathogen infection. Whereas, in the absence of stresses, plant growth promotion may (Bae et al., 2009) or may not (Yildirim et al., 2006) occur. It was reported that the production of defense-related enzymes increased further with pathogen infections (Harman et al., 2004). Furthermore, recent findings suggest that the ability of the fungi to reprogram plant gene expression, by which induced systemic resistance (ISR) is activated, is considered as the primary mechanism for controlling plant pathogens (Shoresh et al., 2010). Their report demonstrated that the changes associated with *Trichoderma*-induced resistance in red pepper were observed mainly in plants inoculated with the pathogens *R. solani* or *P. capsici*. Therefore, it is possible that *T. atroviride* isolates used in the present study activated the plant's ISR mechanism to control take-all, beside other possible mechanisms.

# Chapter 3

# Efficacies of three *Trichoderma* isolates in controlling take-all in prairie grass

## 3.1 Introduction

Current methods for controlling take-all disease have several limitations. In wheat growing areas, the most effective method to control take-all is through crop rotation with a grass crop. For instance, a crop rotation between wheat and commonly grown pasture grasses such as perennial ryegrass, annual ryegrass, and tall fescue can reduce *Ggt* inoculum levels, even though the grasses can also become infected (FAR, 2007). However, the rotation may result in the loss of a soil's natural ability to suppress take-all. Also, some grass species such as prairie grass, could not be used in the crop rotation because of their susceptibility to *Ggt* resulting in increasing disease inoculum levels. Tillage can also be used to control take-all but reduced tillage, which has been adopted by many growers to control erosion, can exacerbate the disease (Kwak & Weller, 2013). Furthermore, soil fumigation using methyl bromide or chloropicrin, while an effective control method, is impractical due to its adverse effect on the environment along with its high cost. The problems associated with these current control methods have contributed to increasing interest in developing an environmentally friendly approach using biological control agents (BCAs).

Fungi from the genus *Trichoderma* show great potential as BCAs of numerous plant pathogens, including *Ggt*. Their broad spectrum of host plants and antagonism to plant pathogens have been indicated in numerous reports (Harman et al., 2004). In a study which set out to determine the biological control potential of *Trichoderma* spp. against *Ggt*, Kandula et al. (2014) demonstrated that the introduction of *T. atroviride* isolates reduced root disease severity of perennial ryegrass grown in *Ggt*-infected soils. The *Trichoderma* isolates also increased shoot and root dry weight of perennial ryegrass. However, effectivities of the BCAs can be very variable within species, or even isolates, when tested in different host plants and growing conditions (Stewart & Hill, 2014).

One factor believed to be the major determinant in their effectivity is the ability to colonise plant roots (i.e. to be rhizosphere-competent) (Harman, 2006). The most effective strains are those which are quickly able to invade the first or second layers of root cells, or even the vascular system, and provide benefits for host plants (Harman et al., 2004; Weller, 1988). *Trichoderma atroviride* isolates LU132, LU140 and LU584 have been previously reported as being either a

reliable rhizosphere-competent strain or an effective BCA. As reported by Cripps-Guazzone (2014), the population density of LU132 and LU140 in sweet corn roots outpaced the population of *T. harzianum* Rifai T22, a well-known highly competent rhizosphere strain. The two isolates also significantly increased seedling emergence of perennial ryegrass by 60-150% in the presence of the common root pathogen *Rhizoctonia solani* (Kandula et al., 2015). Meanwhile, LU584 gave greater shoot fresh weight compared to that of the pathogen control. For these reasons, the three isolates were selected to evaluate their ability to control the root pathogen *Ggt* in a glasshouse experiment.

A mixture of *Trichoderma* isolates is believed to deliver better performance for biological control (Harman et al., 2004; Stewart, 2001). However, there is conflicting evidence on the biological control performance of isolate mixtures. Kandula et al. (2014) found that a mixture of *Trichoderma* isolates LU132, LU140 and LU584 resulted in poorer disease control compared to those of single-formulated isolates. Therefore, further study is required to confirm the efficacy of an isolate mixture in controlling the disease in a different host plant. The study presented in this chapter is one of the first investigations of the effects of *T. atroviride*, both as single and a mixture of isolates, against take-all in a grass seed crop. There are two primary aims of this study: i) to evaluate the efficacy of the three *Trichoderma* isolates in controlling take-all disease in prairie grass; ii) to investigate whether a mixture of three *Trichoderma* isolates will provide a better result for disease control than any of the individual strains.

## 3.2 Materials and methods

### 3.2.1 Plant and disease establishments

The establishment of prairie grass plants and take-all disease has been described in Chapter 2 (section 2.2.5 – 2.2.8).

### 3.2.2 *Trichoderma* isolates

Three *Trichoderma* isolates LU132, LU140 and LU584, were acquired from the Lincoln University Microbial Culture Collection. The isolates were formulated as a prill by Agrimm Technologies Ltd. (Lincoln) as a single isolate for each and a combination of the three isolates. The formulations contained initial cfu of 1 x $10^7$ cfu/g. During the preparation periods, the prills were kept in cold storage (4°C) for several weeks before being introduced into the experimental pots (3 g/pot) at the time of plant transplanting as described in section 2.2.8.

## 3.3 Assessments

### 3.3.1 Plant height

Plant height was assessed three times during the experiment, i.e. 47, 63, and 110 DAS, by measuring from the crown to the highest point using a ruler.

### 3.3.2 Enumeration of colony forming units (cfu) of *Trichoderma*

Sampling for enumeration of *Trichoderma* cfu was conducted at 70 DAS for non-stressed pots. Each of four *Trichoderma* treatments plus one control in each of four blocks, a total of 40 pots, were selected as samples. One prairie grass plant was removed along with 10 grams of rhizosphere soil from each of the assessed pots. Adhering soil was shaken off from the plant roots. Ten grams of soil was added into 90 ml of sterilised water (autoclaved at 121°C and 15 psi for 120 min) to obtain a $10^{-1}$ dilution and put into a laboratory shaker for 10 min. Then, the solution was diluted to $10^{-2}$ by adding 1 ml of the $10^{-1}$ dilution into 9 ml of sterilised water. After that, 200 µl of each dilution were pipetted onto the surface of two replicate *Trichoderma* selective medium (TSM) plates and spread with a sterilised glass rod. Plates were incubated at room temperature for two weeks. After two weeks, the number of *Trichoderma* colonies growing in the medium were counted (Figure 3.1), and the number of *Trichoderma* cfu/gram of rhizosphere soil was calculated.

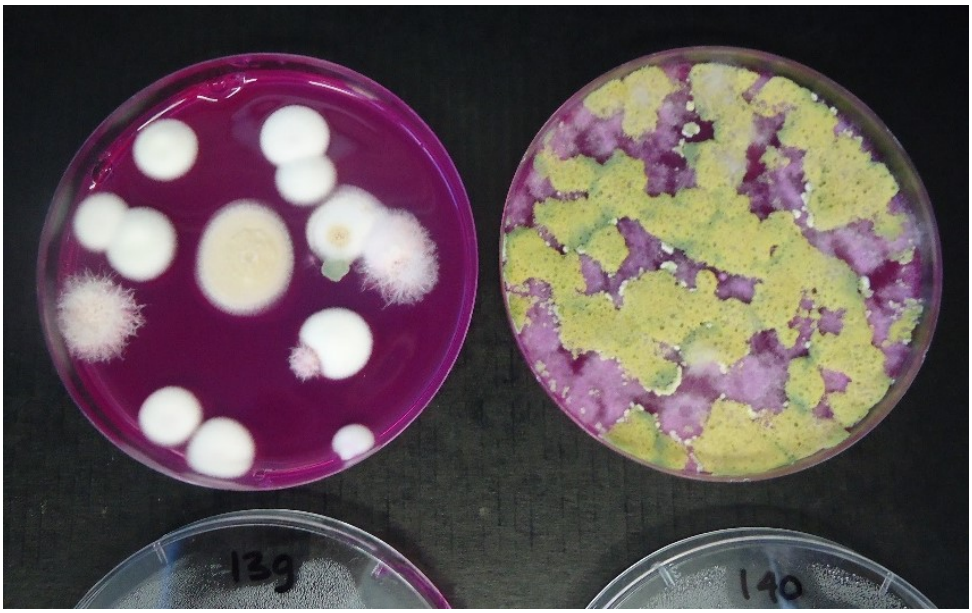


**Figure 3.1 After two weeks of incubation, soil sample isolated from an untreated control showed no *Trichoderma* colonies growing in the medium (left). Abundant colonies of *Trichoderma* grown from a soil treated with isolate LU132 (right).**

The TSM was prepared using the following components (g/1 distilled water): $K_2HPO_4$, 1; $MgSO^4$, 0.5; peptone, 5.0; glucose, 10.0; rose-bengal (tetrachlorotetradiodofluorescein, BDH Chemicals Ltd., England), 0.15; agar (Difco Laboratories, USA), 20. All ingredients were placed into two 500 ml flasks and autoclaved at 121°C and 15 psi for 120 min. Once cooled, 0.2 ml formaldehyde and 3 ml of 1% streptomycin stock solution were added to the solution (Martin, 1950).

### 3.3.3 Plant dry matter

Above-ground dry weight was obtained from all the above-ground plant parts. Plant dry matter (DM) was first measured after seed harvest (post-seed harvest) and then from the vegetative regrowth. The post-seed harvest DM was taken at 113 DAS by cutting the plant at 6 cm above the soil surface. The fresh weight of the post-seed harvest plant materials was measured before DM measurement as a comparison. Dry matter of the vegetative regrowth was measured three times at a duration of 36 days for each. Once harvested, the plant materials were placed in paper bags, separated between the centre and the surrounding plants, labelled with treatment numbers, before being dried in a forced-draft oven at 65°C. After 48 h, the dry weight was determined using a digital scale with two decimal places.

After three periods of vegetative regrowth, plant roots were removed from the pots and washed free of soil for root assessment and root dry matter measurement. Small portions of the plant roots (±0.5 g) were collected from each treatment for microscope assessment. After that, the roots were placed in paper bags labelled with treatment numbers and put in the oven at 65°C. After 96 h, the root dry weight was obtained by weighing using a digital scale with two decimal places.

### 3.3.4 Assessment of root disease severity

The severity of take-all disease on plant roots was assessed visually using the method described by Weller and Cook (1983). After washing the roots free of soil, the root disease severity was rated on a score of 0 – 5: 0 = no disease symptoms; 1 = one or two lesions on the roots of the given plant; 2 = 50 – 100% of the roots with one or more lesions each; 3 = all roots with lesions and some evidence of infection on the stem; 4 = lesions abundant and beginning to coalesce on the stem; and 5 = plant dead or nearly so (Figure 3.2).

Root assessment for disease severity was also done microscopically. The root systems were inspected under a binocular microscope (10x magnification), for the presence of *Ggt* runner hyphae and/or hyphodia. Disease score was then rated based on root deterioration and pathogen colonisation using a 0 (clean) to 5 (heavily infected) score (Figure 3.3): 0 = root intact and no

runner hyphae; 1 = root intact with runner hyphae on some roots; 2 = root intact with runner hyphae on most roots; 3 = disintegrated root with hyphae on all the roots and hyphodia on some roots; 4 = disintegrated root with hyphae and hyphodia on most roots; 5 = totally disintegrated root with hyphae and hyphodia on all the roots (Kandula et al., 2014).

### 3.3.5 Statistical analysis

Statistical analyses were done as detailed in section 2.3.8.


**Figure 3.2 Prairie grass roots infected by *Gaeumannomyces graminis* var. *tritici*, with abundant lesions on the roots, which reached up to the crowns and stems, indicated by the darker colour (left). Brighter roots indicate no *Ggt* infection (right).**

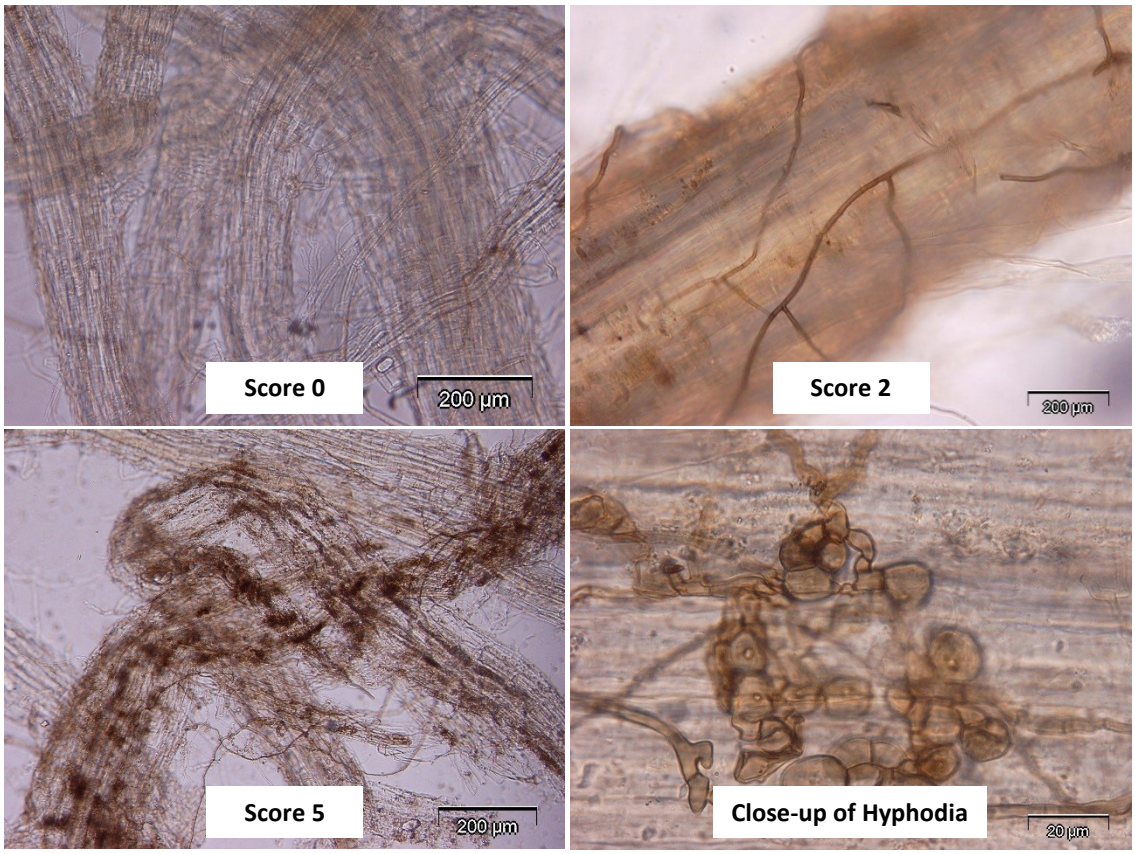


**Figure 3.3 Microscopic images of ryegrass roots infected with *Gaeumannomyces graminis* var. *tritici* at three disease scores. Close-up of hyphodia was taken from disease score 5. [Images reproduced by kind permission of Diwakar Kandula].**

## 3.4 Results

Data presented in the following sections include those for moisture, pathogen and *Trichoderma* treatments. The effects of pathogen and *Trichoderma* treatments are presented here, while the elucidation for moisture treatment is given in Chapter 4.

### 3.4.1 Effects of *Ggt* and *Trichoderma* inoculation on agronomic parameters of *Ggt*-infected prairie grass

Several agronomic parameters were assessed to measure the effects of the pathogen and *Trichoderma* treatments, including plant height, shoot fresh weight, shoot dry weight, and root dry weight. Inoculation of *Ggt* significantly ($P$<0.05) reduced plant height at 47 DAS, but did not change the plant height at 63 DAS, and unexpectedly, significantly ($P$<0.05) increased the plant height at 110 DAS (Table 3.1). Meanwhile, *Ggt* infection did not affect shoot fresh weight and shoot dry weight in transplanted plants, but significantly ($P$<0.001) increased the fresh weight and dry weight of the shoots in direct-sown plants.

*Trichoderma* application significantly ($P$<0.05) increased the height of prairie grass plants only at 47 DAS, at which LU132, LU140, and LU584-treated plants were 9%, 4%, and 6%, respectively, taller than non-treated plants (Table 3.1). The mixture of isolates, on the other hand, did not increase the plant height at all during the experimental period. All *Trichoderma* isolates also increased shoot fresh weight in transplanted plants (by 15% on average), but in direct-sown plants isolate LU140 reduced the shoot fresh weight by 9%, whereas the other isolates did not affect the shoot fresh weight. The effects of *Trichoderma* on shoot dry weight showed a similar result to those for shoot fresh weight. The three isolates significantly increased the shoot dry weight in transplanted plants (by 13% on average), but in direct-sown plants isolates LU140 and LU584 reduced the shoot dry weight (by 9% on average) (Table 3.1).

There was a variation in the effects of *Ggt* inoculation on shoot dry weight during vegetative regrowth. At the first vegetative regrowth cut, the pathogen increased shoot dry weight of direct-sown plants, and at the second regrowth cut had increased the shoot dry weight of both transplanted and direct-sown plants (Table 3.2). However, at the third vegetative regrowth cut *Ggt* did not affect the shoot dry weight of plants in both planting systems. Root assessment showed that *Ggt* infection significantly ($P$<0.05) reduced the root dry weight.

Overall, all *Trichoderma* isolates significantly ($P$<0.05) increased the shoot dry weight of prairie grass in both transplanted (Figure 3.4) and direct-sown plants at the three periods of vegetative regrowth (Table 3.2). The only exception was that the mixture of isolates did not increase the shoot dry weight of direct-sown plants at the first vegetative regrowth cut. However, the mixture of isolates was also the only treatment to significantly ($P$<0.05) increase the root dry weight of prairie grass. The differences between shoot dry weights of plants treated with *Trichoderma* isolates were not significant (Table 3.2). The percentage of increased shoot dry weight, by all isolates, had improved from one period to the next of vegetative regrowth (Figure 3.5).

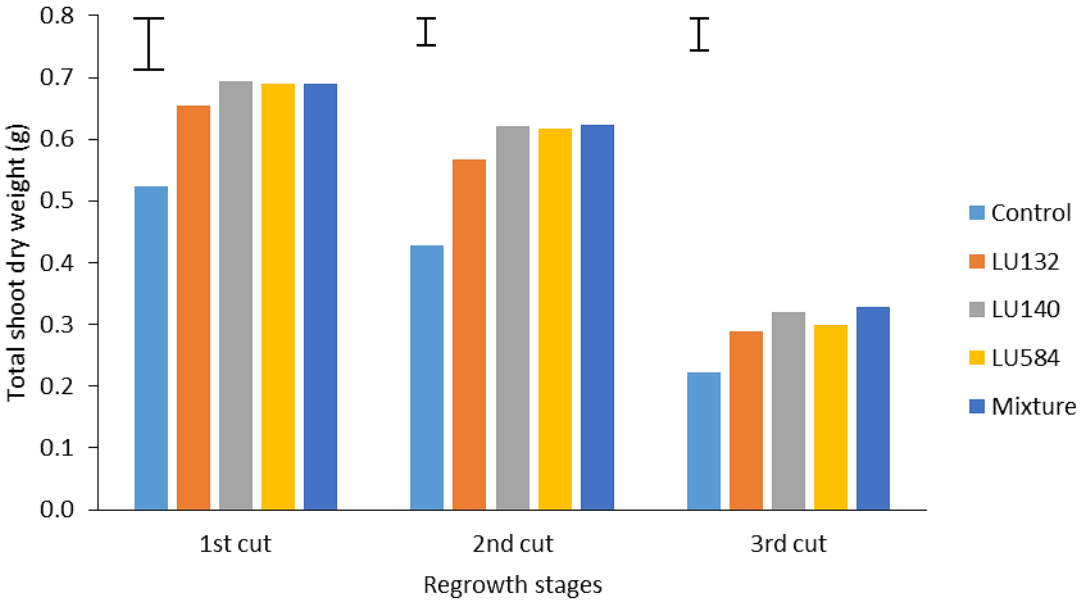


**Figure 3.4** **Mean total shoot dry weight of transplanted prairie grass treated with *Trichoderma atroviride* isolates compared to untreated control at three stages of vegetative regrowth. Error bars are the least significant difference (LSD) at the 5% level.**

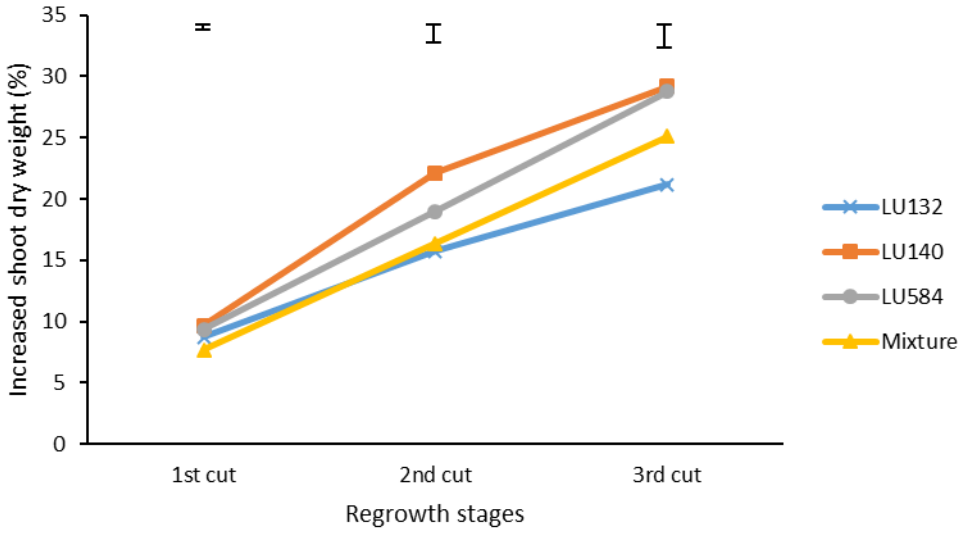


**Figure 3.5** **The percentage increased shoot dry weight relative to control plants in prairie grass treated with different *Trichoderma* isolates. Error bars are calculated from standard error.**

**Table 3.1 Main effect means of plant height, shoot fresh and dry weight of prairie grass for the different moisture, pathogen and *Trichoderma* treatments.**

| Main effect means | Plant height (cm) | | | Shoot fresh weight (g) after seed harvest | | Shoot dry weight (g) after seed harvest | |
|---|---|---|---|---|---|---|---|
| | 47 DAS | 63 DAS | 110 DAS | Transplanted | Direct sown | Transplanted | Direct sown |
| Moisture | | | | | | | |
| Non-stressed | 64.01 | 96.21 | 110.76 | 9.51 | 2.88 | 3.89 | 1.00 |
| Stressed | n/a | 94.68 | 86.00 | 4.99 | 1.45 | 2.84 | 0.69 |
| LSD (5%) | n/a | 3.11 | 9.38 | 2.03 | 0.54 | 0.33 | 0.11 |
| Sig. of diff. | | ns | ** | ** | ** | ** | ** |
| Pathogen | | | | | | | |
| Not inoculated | 66.56 | 96.23 | 94.10 | 7.29 | 1.94 | 3.46 | 0.79 |
| Inoculated | 62.73 | 95.05 | 100.52 | 7.23 | 2.27 | 3.32 | 0.88 |
| LSD (5%) | 3.12 | 2.36 | 5.40 | 0.67 | 0.22 | 0.33 | 0.05 |
| Sig. of diff. | * | ns | * | ns | ** | ns | ** |
| *Trichoderma* | | | | | | | |
| Control | 62.23 | 94.25 | 98.29 | 6.61 | 2.20 | 3.13 | 0.88 |
| LU132 | 67.88 * | 96.92 | 101.96 | 7.70 * | 2.16 | 3.52 * | 0.83 |
| LU140 | 64.71 * | 96.58 | 97.54 | 7.50 * | 2.00 * | 3.50 * | 0.81 * |
| LU584 | 66.25 * | 96.25 | 97.33 | 7.78 * | 2.12 | 3.56 * | 0.80 * |
| Mixture | 60.75 | 94.42 | 96.88 | 7.31 * | 2.28 | 3.36 | 0.88 |
| LSD (5%) | | | | | | | |
| (Ctrl v Trtd) | 2.19 | 3.67 | 5.61 | 0.67 | 0.18 | 0.28 | 0.06 |
| (Trtd v Trtd) | 2.53 | 4.24 | 6.48 | 0.77 | 0.20 | 0.32 | 0.07 |

ns = not significant; * = $P<0.05$; ** = $P<0.01$. DAS = days after sowing. n/a = data were not included in the analysis (moisture treatment was not yet applied).

**Table 3.2 Main effect means of shoot and root dry weight of prairie grass for the different moisture, pathogen and *Trichoderma* treatments.**

| Main effect means | Shoot dry weight (g) of 1st regrowth | | Shoot dry weight (g) of 2nd regrowth | | Shoot dry weight (g) of 3rd regrowth | | Root dry weight (g) |
|---|---|---|---|---|---|---|---|
| | Transplanted | Direct sown | Transplanted | Direct sown | Transplanted | Direct sown | |
| Moisture | | | | | | | |
| Non-stressed | 0.92 | 0.45 | 0.73 | 0.38 | 0.34 | 0.18 | 5.72 |
| Stressed | 0.33 | 0.18 | 0.36 | 0.18 | 0.22 | 0.12 | 6.37 |
| LSD (5%) | 0.06 | 0.10 | 0.14 | 0.16 | 0.16 | 0.03 | 2.19 |
| Sig. of diff. | *** | ** | * | * | ns | * | ns |
| Pathogen | | | | | | | |
| Not inoculated | 0.61 | 0.29 | 0.52 | 0.25 | 0.28 | 0.13 | 7.12 |
| Inoculated | 0.64 | 0.33 | 0.56 | 0.30 | 0.28 | 0.15 | 5.51 |
| LSD (5%) | 0.07 | 0.03 | 0.04 | 0.04 | 0.08 | 0.03 | 1.11 |
| Sig. of diff. | ns | * | * | * | ns | ns | * |
| *Trichoderma* | | | | | | | |
| Control | 0.52 | 0.30 | 0.43 | 0.25 | 0.22 | 0.12 | 5.75 |
| LU132 | 0.65 * | 0.33 * | 0.57 * | 0.29 * | 0.29 * | 0.15 * | 6.18 |
| LU140 | 0.69 * | 0.33 * | 0.62 * | 0.31 * | 0.32 * | 0.16 * | 6.14 |
| LU584 | 0.69 * | 0.33 * | 0.62 * | 0.30 * | 0.30 * | 0.16 * | 6.08 |
| Mixture | 0.69 * | 0.32 | 0.62 * | 0.29 * | 0.33 * | 0.16 * | 6.37 * |
| LSD (5%) | | | | | | | |
| (Ctrl v Trtd) | 0.08 | 0.02 | 0.05 | 0.04 | 0.05 | 0.02 | 0.58 |
| (Trtd v Trtd) | 0.10 | 0.03 | 0.05 | 0.04 | 0.06 | 0.03 | 0.67 |

ns = not significant; * = $P<0.05$; ** = $P<0.01$. *** = $P<0.001$.

### 3.4.2 The number of *Trichoderma* colony forming units and its effects on Take-all root disease severity

The number of *Trichoderma* cfu in the rhizosphere of prairie grass plants was assessed from 40 pots of non-moisture stressed plants, comprised of *Ggt*-inoculated and non-inoculated treatments. There was no significant difference in the *Trichoderma* populations between *Ggt*-inoculated and non-inoculated soils (Table 3.3). However, the cfu between *Trichoderma*-treated and non-treated pots and within pots treated with *Trichoderma* isolates differed significantly ($P<0.05$). The cfu of isolates LU140 and LU583 was significantly greater than that of isolates LU132 and the mixture of isolates.

**Table 3.3 Main effect means of the *Trichoderma* colony forming units (cfu), and root disease severity of prairie grass for the different moisture, pathogen and *Trichoderma* treatments.**

| Main effect means | CFUs/g of soil | Root disease score (direct observation) | Root disease score (microscope) |
|---|---|---|---|
| Moisture | | | |
| Non-stressed | 57,883 | 2.60 | 1.31 |
| Stressed | n/a | 2.42 | 1.27 |
| LSD (5%) | n/a | 0.11 | 0.22 |
| Sig. of diff. | | * | ns |
| Pathogen | | | |
| Not inoculated | 59,797 | 0.00 | 0.15 |
| Inoculated | 55,969 | 3.76 | 1.86 |
| LSD (5%) | 8,339 | 0.09 | 0.19 |
| Sig. of diff. | ns | *** | *** |
| *Trichoderma* | | | |
| Control | (188) | 2.54 | 2.21 |
| LU132 | 54,250 * | 2.46 | 0.71 * |
| LU140 | 60,656 * | 2.46 | 0.79 * |
| LU584 | 63,812 * | 2.50 | 1.06 * |
| Mixture | 52,812 * | 2.54 | 0.75 * |
| LSD (5%) | | | |
| (Ctrl v Trtd) | 4,820 | 0.18 | 0.27 |
| (Trtd v Trtd) | - | 0.21 | 0.308 |

ns = not significant; * = $P<0.05$; ** = $P<0.01$; n/a = data were not included in the analysis (moisture treatment was not yet applied). Disease score was rated using a 0 (clean) to 5 (heavily infected) score. The brackets () indicate that the Control has been omitted from the analysis of variance (this was necessary to achieve homogeneity of variance).

The effects of *Trichoderma* application on root disease severity on the *Ggt*-infected plants were assessed through direct observation and microscopically using a 0 to 5 disease score. For both methods, the root disease severity was significantly ($P$<0.001) different between plants grown in the *Ggt*-inoculated and non-inoculated soils (Table 3.3).

Plants treated with all *Trichoderma* isolates had significantly lower root disease score when assessed microscopically with disease scores of 0.71, 0.79, 1.06, and 0.75 for LU132, LU140, LU584, and the mixture of isolates, respectively, compared to a score of 2.21 for the untreated control plants (Table 3.3). Among the *Trichoderma* isolates, isolate LU132 and the mixture of isolates had significantly lower disease scores than LU584, but did not differ significantly with isolate LU140. However, no significant differences for root disease score were recorded between *Trichoderma*-treated and untreated plants in the direct observation (Figure 3.6).

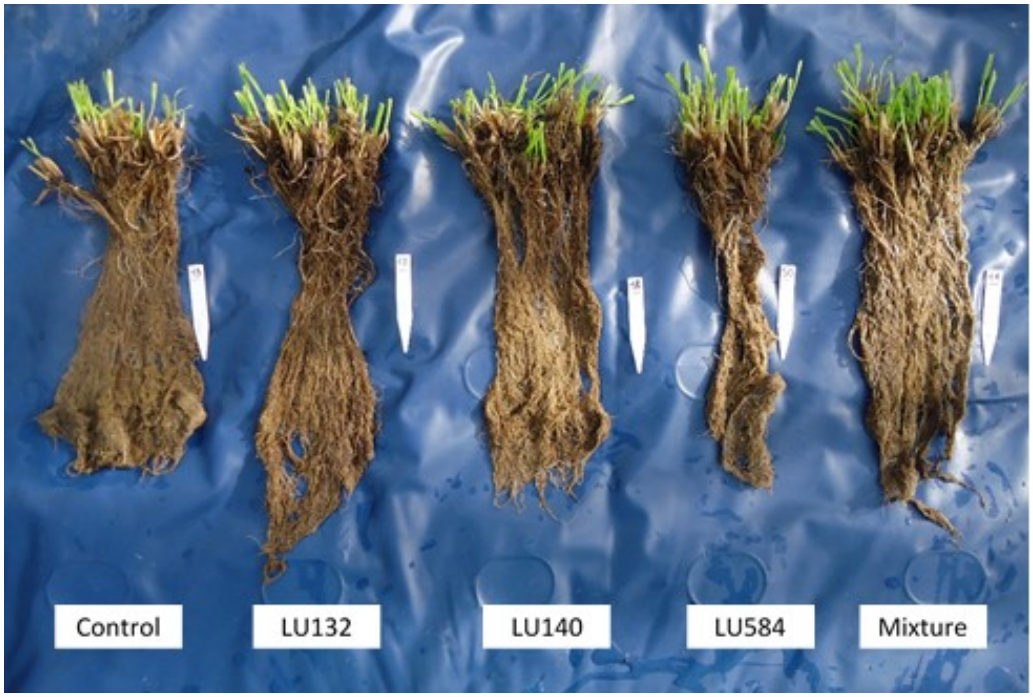


**Figure 3.6 Appearance of prairie grass roots grown in *Ggt*-inoculated soils treated with different *Trichoderma* isolates.**

### 3.4.3 Treatment interactions

The presence of *Ggt* infection and the occurrence of moisture stress significantly affected the ability of *Trichoderma* to promote plant growth. In transplanted plants, *Trichoderma* isolates increased shoot dry weight after seed harvest only in *Ggt*-infected plants in both moisture stress and non-stress conditions (Table 3.4). In direct-sown plants, *Trichoderma* increased shoot dry weight only in well-watered plants infected by the pathogen (Table 3.5). *Trichoderma* isolates did not increase the shoot dry weight in both transplanted and direct-sown plants when *Ggt* was absent. Also, in plants protected by *Trichoderma* isolates, take-all did not reduce the production of shoot dry weight relative to uninoculated plants, under moisture stress or non-stress conditions, either in transplanted (Table 3.4) or direct-sown (Table 3.5) plants.

At vegetative regrowth, *Trichoderma* isolates also increased the shoot dry weight in the presence of the pathogen, but only in well-watered conditions. The isolates had no effect on the shoot dry weight when plants were exposed to moisture stress, either in transplanted (Table 3.6) or direct-sown (Table 3.7) plants.

All *Trichoderma* isolates reduced root disease score in plants infected with *Ggt*, in both moisture stress and non-stress conditions (Table 3.8). No *Ggt* infection was found in non-inoculated plants.

**Table 3.4 Interaction effects between *Trichoderma* isolates, pathogen, and moisture treatments on shoot dry weight (g/plant) of transplanted plants after seed harvest.**

| | Control (Double reps) | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (p+)*** **(double reps)** | | | | | |
| (1) Non-stressed | 3.25 + | 3.83 * | 4.33 * | 4.05 * | 4.19 * |
| (2) Stressed | 2.51 x | 3.16 * x | 3.08 * x | 3.01 * x | 2.61 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (1) & (2) | - | | 0.48 | | 0.55 |
| Between rows (1) & (2) | 0.43 | | 0.51 | | 0.58 |
| ***Un-inoculated (p-)*** | | | | | |
| (3) Non-stressed | 4.30 | 3.99 | 3.81 | 4.00 | 3.77 |
| (4) Stressed | 2.92 x | 3.12 x | 2.39 x | 3.24 | 2.77 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (3) & (4) | - | | 0.68 | | 0.78 |
| Between rows (3) & (4) | 0.63 | | 0.73 | | 0.83 |

| Other comparison LSD (5%) values | C v C | Cp+ v T | Cp- v T | T v T |
|---|---|---|---|---|
| Between rows (1) & (3) | 0.57 | 0.68 | 0.63 | 0.73 |
| Between rows (1) & (4) | 0.54 | 0.66 | 0.60 | 0.71 |
| Between rows (2) & (3) | 0.54 | 0.66 | 0.60 | 0.71 |
| Between rows (2) & (4) | 0.57 | 0.68 | 0.63 | 0.73 |

+ indicates 5% significant difference of pathogen inoculated from uninoculated within the same moisture treatment; * indicates 5% significant difference of *Trichoderma*-treated from control plants in the same row; x indicates 5% significant difference of stressed from non-stressed in the same column.

**Table 3.5 Interaction effects between *Trichoderma* isolates, pathogen, and moisture treatments on shoot dry weight (g/plant) of direct-sown plants after seed harvest.**

| | Control (Double reps) | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (p+)*** **(double reps)** | | | | | |
| (1) Non-stressed | 1.14 + | 1.00 * | 0.97 * | 0.94 * | 1.00 * |
| (2) Stressed | 0.75 + x | 0.69 x | 0.70 x | 0.72 x | 0.75 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (1) & (2) | - | | 0.11 | | 0.12 |
| Between rows (1) & (2) | 0.11 | | 0.12 | | 0.14 |
| ***Un-inoculated (p-)*** | | | | | |
| (3) Non-stressed | 0.93 | 0.97 | 0.91 | 0.87 | 1.10 * |
| (4) Stressed | 0.58 x | 0.66 x | 0.62 x | 0.62 x | 0.70 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (3) & (4) | - | | 0.15 | | 0.18 |
| Between rows (3) & (4) | 0.14 | | 0.16 | | 0.18 |

| Other comparison LSD (5%) values | C v C | Cp+ v T | Cp- v T | T v T |
|---|---|---|---|---|
| Between rows (1) & (3) | 0.10 | 0.14 | 0.12 | 0.15 |
| Between rows (1) & (4) | 0.12 | 0.15 | 0.14 | 0.16 |
| Between rows (2) & (3) | 0.12 | 0.15 | 0.14 | 0.16 |
| Between rows (2) & (4) | 0.10 | 0.14 | 0.12 | 0.15 |

+ indicates 5% significant difference of pathogen inoculated from uninoculated within the same moisture treatment; * indicates 5% significant difference of *Trichoderma*-treated from control plants in the same row; x indicates 5% significant difference of stressed from non-stressed in the same column.

**Table 3.6 Interaction effects between *Trichoderma* isolates, pathogen, and moisture treatments on shoot dry weight (g/plant) of transplanted plants at the 3rd regrowth.**

| | Control (Double reps) | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (p+)*** **(double reps)** | | | | | |
| (1) Non-stressed | 0.20 | 0.34 * | 0.44 * | 0.42 * | 0.50 * |
| (2) Stressed | 0.18 | 0.25 | 0.24 x | 0.19 x | 0.21 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (1) & (2) | - | | 0.09 | | 0.11 |
| Between rows (1) & (2) | 0.15 | | 0.16 | | 0.16 |
| ***Un-inoculated (p-)*** | | | | | |
| (3) Non-stressed | 0.32 | 0.31 | 0.35 | 0.33 | 0.29 |
| (4) Stressed | 0.25 | 0.23 | 0.22 | 0.24 | 0.26 |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (3) & (4) | - | | 0.13 | | 0.15 |
| Between rows (3) & (4) | 0.17 | | 0.18 | | 0.20 |

| Other comparison LSD (5%) values | C v C | Cp+ v T | Cp- v T | T v T |
|---|---|---|---|---|
| Between rows (1) & (3) | 0.13 | 0.14 | 0.13 | 0.15 |
| Between rows (1) & (4) | 0.16 | 0.17 | 0.17 | 0.18 |
| Between rows (2) & (3) | 0.16 | 0.17 | 0.17 | 0.18 |
| Between rows (2) & (4) | 0.13 | 0.14 | 0.13 | 0.15 |

* indicates 5% significant difference of *Trichoderma*-treated from control plants in the same row; x indicates 5% significant difference of stressed from non-stressed in the same column.

**Table 3.7 Interaction effects between *Trichoderma* isolates, pathogen, and moisture treatments on shoot dry weight (g/plant) of direct-sown plants at the 3rd regrowth.**

| | Control (Double reps) | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (p+)*** **(double reps)** | | | | | |
| (1) Non-stressed | 0.13 | 0.21 * | 0.24 * | 0.23 * | 0.21 * |
| (2) Stressed | 0.12 | 0.12 x | 0.11 x | 0.12 x | 0.13 x |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (1) & (2) | - | | 0.04 | | 0.04 |
| Between rows (1) & (2) | 0.04 | | 0.04 | | 0.05 |
| ***Un-inoculated (p-)*** | | | | | |
| (3) Non-stressed | 0.16 | 0.14 | 0.15 | 0.14 | 0.14 |
| (4) Stressed | 0.10 | 0.12 | 0.11 | 0.11 | 0.12 |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (3) & (4) | - | | 0.05 | | 0.06 |
| Between rows (3) & (4) | 0.06 | | 0.06 | | 0.07 |

| Other comparison LSD (5%) values | C v C | Cp+ v T | Cp- v T | T v T |
|---|---|---|---|---|
| Between rows (1) & (3) | 0.05 | 0.06 | 0.05 | 0.06 |
| Between rows (1) & (4) | 0.05 | 0.06 | 0.05 | 0.06 |
| Between rows (2) & (3) | 0.05 | 0.06 | 0.05 | 0.06 |
| Between rows (2) & (4) | 0.05 | 0.06 | 0.05 | 0.06 |

* indicates 5% significant difference of *Trichoderma*-treated from control plants in the same row;
x indicates 5% significant difference of stressed from non-stressed in the same column.

**Table 3.8 Interaction effects between *Trichoderma* isolates, pathogen, and moisture treatments on root disease severity observed microscopically.**

| | Control (Double reps) | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (p+)*** **(double reps)** | | | | | |
| (1) Non-stressed | 3.27 | 1.13 * | 0.88 * | 1.50 * | 1.13 * |
| (2) Stressed | 3.13 | 1.00 * | 1.38 * | 1.44 * | 1.13 * |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (1) & (2) | - | | 0.46 | | 0.53 |
| Between rows (1) & (2) | 0.37 | | 0.46 | | 0.54 |
| ***Un-inoculated (p-)*** | | | | | |
| (3) Non-stressed | 0.50 + | 0.00 + | 0.00 + | 0.25 + | 0.00 + |
| (4) Stressed | 0.00 + | 0.00 + | 0.25 + | 0.25 + | 0.00 + |
| LSD (5%) values: | Control(C) vs C | | C vs Treated (T) | | T vs T |
| Within rows (3) & (4) | - | | 0.65 | | 0.75 |
| Between rows (3) & (4) | 0.52 | | 0.65 | | 0.76 |

| Other comparison LSD (5%) values | C v C | Cp+ v T | Cp- v T | T v T |
|---|---|---|---|---|
| Between rows (1) & (3) | 0.45 | 0.60 | 0.53 | 0.66 |
| Between rows (1) & (4) | 0.45 | 0.59 | 0.53 | 0.66 |
| Between rows (2) & (3) | 0.45 | 0.59 | 0.53 | 0.66 |
| Between rows (2) & (4) | 0.45 | 0.60 | 0.53 | 0.66 |

+ indicates 5% significant difference of pathogen inoculated from uninoculated within the same moisture treatment; * indicates 5% significant difference of *Trichoderma*-treated from control plants in the same row.

## 3.5 Discussion

### 3.5.1 Effects of *Ggt* on the agronomic parameters of prairie grass

In the field, older plants infected by *Ggt* are stunted, tillering is reduced, and they produce 'whiteheads' (Manners & Myers, 1981). In the present study, stunting of the infected plants occurred at the early stage of growth (47 DAS), but the infected plants had recovered from the stunting by 63 DAS (no difference in plant height), and eventually became taller than the non-infected plants. There was also no reduction of either fresh weight or dry weight of shoots after seed harvest in transplanted plants. Several factors could explain this observation. **Firstly**, the recovery of infected plants may be related the recovery of the roots. Manners and Myers (1981) reported that *Ggt* infection on cereal crops triggers the production of adventitious roots if the infection is not too severe. For example, artificially infected wheat plants grown in a pot experiment recovered from *Ggt* infection by producing sufficient adventitious roots to replace those ineffective roots (Davis, 1925). This new root growth may result in enhancement of the above ground growth and development, resulting in similar agronomic growth between pathogen-infected and control plants. **Secondly**, limited growth spaces may also limit the growth and development of roots, especially for non-infected plants, because prairie grass is a deep-rooted grass which growth best on fertile and well-drained soils (Farm Information Services, 2002). The growth medium limitation may have contributed to the insignificant differences between the *Ggt*-infected and control plants. **Thirdly**, during reproductive stages, most of the sugars produced from photosynthesis are utilised for seed filling (George & Rice, 2016). Therefore, at this stage, diseased plants suffered losses more in seed yields rather than in shoot production.

In direct-sown plants, *Ggt* infection resulted in greater fresh weight and dry weight of the shoots after seed harvest. This increased shoot dry weight continued for the vegetative regrowth stages. Deacon (1981) observed that infection by the pathogen *Phialophora graminicola* significantly increased shoot dry weight of wheat seedlings grown under a low phosphorus regime. This author proposed that the pathogen and similar fungi, might have increased mineral nutrient uptake by plants in the same way as do mycorrhizal fungi. The severity of infection also affects the growth and development of the plants, and as discussed in Chapter 2, *Ggt* infection is decreased in plants receiving less nutrients. Manners and Myers (1981) reported that a non-severe attack of *Ggt* on wheat plants resulted in an increase in tillering, grain weight, or number of grain per ear, which presumably was a result of decreased competition. Alternatively, Simon (1989) reported an increase in dry weight of shoots and roots of 4 week old wheat seedlings infected by take-all

disease. He suggested that pathogen infection may trigger the release of nutrients, e.g. nitrate, by the infected plants, allowing the plants to better cope with the disease.

In the present study, however, the damage caused by *Ggt* infection was clearly demonstrated through the decreased root dry weight. The infection is initiated by the runner hyphae growing on the root surface, both upward and downward, and infecting different sites along the roots (Kwak & Weller, 2013). Initially, this infection created small black lesions in the infected roots, which expanded and eventually merged. This infection causes cessation of the root growth, reduction in seminal root dry weights, root death below the point of infection, and a reduction in total root length (Pillinger et al., 2005). Additionally, infected roots become very brittle, so that some of them could have remained in the soil when plants were pulled from the pots, and some may have been lost during root washing.

### 3.5.2 Effects of *Trichoderma* application

A single application of a biocontrol agent that could simultaneously confer protection from disease infection and promote plant growth would be of importance to agricultural plant production. In this chapter, the ability of *Trichoderma* to increase plant agronomic parameters as well as reduce take-all disease severity in prairie grass was demonstrated.

The application of *Trichoderma* isolates initially accelerated plant growth by increasing the height of prairie grass plant at 47 DAS, but plant heights between *Trichoderma* treatments and the control did not differ significantly afterwards. This can be explained by the fact that the growth of *Trichoderma*-treated plants was faster during the first 6 – 7 weeks. The differences between plant heights of treated and untreated plants became less apparent after nine weeks after sowing because the plant heights reached their maximum, or else, there was a shift of energy allocation from shoot growth during the vegetative phase to seed production during the reproductive phase (George & Rice, 2016). This result is consistent with research that found no significant difference in the plant height of *Miscanthus* x *giganteus* treated with *T. atroviride* compared to the control at seventeen weeks after establishment (Chirino-Valle et al., 2016).

In addition to the increased seed yield (Chapter 2), *Trichoderma* isolates also increased the shoot fresh weight and dry weight in transplanted plants. Therefore, the beneficial effects of greater nutrient availability (such as in transplanted plants) in *Trichoderma*-treated plants also positively influenced the shoot fresh weight and dry weight of prairie grass. Conversely, limited nutrient availability (such as in direct-sown plants) can limit the colonisation of plant tissues by

*Trichoderma* spp (Bailey & Melnick, 2013). Other influences of nutrient availability on *Trichoderma* effects have been explained in the discussion of Chapter 2.

*Trichoderma* isolates increased shoot dry weight at three periods of vegetative regrowth, indicating the consistency of growth promotion activity by the BCAs. This also suggests that the isolates had colonised plant roots and were still active at least 7 months after their introduction, which demonstrated their ability as rhizosphere-competent strains to give long-term benefits, both as plant growth promoter and biocontrol agents. The percentage of increased shoot dry weight by *Trichoderma* isolates relative to control plants continuously improved along the periods of vegetative regrowth. Plant growth promotion may occur as a result of increased root development, increased secondary root formation (Samolski et al., 2012), increased root size (Hermosa et al., 2012), or increased root absorptive capacity (Contreras-Cornejo et al., 2013).

Growth promotion activity by *Trichoderma* isolates was prominent in the presence of *Ggt* (Appendix 5, 5.3 to 5.8), suggesting a greater impact under unfavourable conditions. There are some reports of enhanced plant growth in non-stress environments as a result of the association of *Trichoderma* strains with plants, but the responses are usually greater during the presence of biotic, abiotic, or physiological stresses (Mastouri et al., 2010). It is likely that the presence of the pathogen induced the activity of the BCA to reprogramme plant gene expression. The genetic reprogramming supports ISR, as well as inducing mechanisms in the plant that further improve plant nitrogen use efficiency (NUE) (Shoresh et al., 2010).

While all *Trichoderma* isolates demonstrated their ability to promote plant growth through increased above-ground agronomic parameters, i.e. seed yield and shoot weight, only the mixture of isolates significantly increased root dry weight. The results indicate that three *Trichoderma* isolates promoted plant growth of prairie grass through increased root absorptive capacity more than increased root development or size to acquire water and nutrients from the soil. In maize plants, root colonisation by *Trichoderma* was observed to enhance root absorptive capacity to compete for sugar, which benefitted both plant and the BCA (Contreras-Cornejo et al., 2013). These authors also reported that cucumber plants treated with *T. harzianum qid74* gene had longer root hairs compared to those inoculated with the wild-type strain. The root hairs play an important role in absorbing soluble nutrients that have low diffusion in the soil, such as phosphate (Contreras-Cornejo et al., 2013).

The most important factor determining the efficacy of *Trichoderma* to control root pathogens is its ability to be rhizosphere-competent, which is related to a high population density of the BCAs

in the rhizosphere (Ahmad & Baker, 1988). While the number of *Trichoderma* cfu can be an important indicator for biocontrol efficacy, Hohmann et al. (2011) showed that increased *Trichoderma* population may not always be beneficial. Paulitz (2000) reported a threshold of $10^5$ cfu/g of root for effective biocontrol for most BCA, and that increased population density above that level did not give an improvement in disease control. In the present study, the greater cfu of isolates LU140 and LU584 did not necessarily mean greater reduction in root disease score, nor greater increase in shoot dry weight. Instead, isolates LU132 and the mixture of isolates, which had lower cfu $g^{-1}$ of soil, were more effective than LU584 in reducing root disease severity. However, the mechanism of how the *Trichoderma* population density affects the extent of disease control or plant growth promotion is still unknown (Hohmann et al., 2011).

Disease severity between *Trichoderma*-treated and untreated plants was clearly recognized in the microscope observation, but not by direct observation. The reason was related to the characteristics of the plant roots. The severity of the take-all symptoms observed depends on the ability of the host to produce new roots when the infection occurred. When new root development is quicker than the pathogen can destroy roots, then disease severity will be less (Manners & Myers, 1981). Prairie grass has a large root system and higher root growth than either smooth brome grass or tall fescue, developing a root length of up to 80 cm deep in soil (Shaffer et al., 1994). These characteristics created similar root appearance of *Ggt*-infected prairie grass plants grown in pots between *Trichoderma*-treated and untreated plants, and as a result accurate root assessment by eye was somewhat difficult. Additionally, assessing root disease by looking at the extent of root discoloration may not reflect the true effect of the disease because it portrays only the amount of root area affected not the intensity of discoloration (Clarkson & Polley, 1981).

### 3.5.3 Interaction responses

The beneficial effect of *Trichoderma* to the host plant was greater under the occurrence of pathogen infection, resulting in a higher seed yield (Chapter 2). Similarly, the increased shoot dry weight, after seed harvest and at vegetative regrowth, also occurred when *Ggt* was present. On the other hand, there was no significant effect on seed yield or shoot dry weight following *Trichoderma* application when the pathogen was absent. These results reflect those of Kandula et al. (2015) who also found that LU132 and LU140 did not increase shoot weight of perennial ryegrass grown in pathogen-free medium. Therefore, it seems that plant growth promotion by *T. atroviride* isolates LU132, LU140, LU584, and the mixture of isolates on prairie grass occurred as a result of biocontrol activity against the pathogen. Much of the previously published literature also reported that enhanced plant growth by *Trichoderma* spp. has been attributed to direct control of

one or more plant pathogens, because it is said that all nonsterile soils are to some extent diseased (Stewart & Hill, 2014). For example, a strain of *T. harzianum* has been shown to enhance the plant biomass of tomato seedlings grown in soil, but did not increase or even reduced the plant biomass when introduced in disease-free hydroponic experiments (Li et al., 2015).

It has been reported that *Trichoderma atroviride* LU132 promoted plant growth of perennial ryegrass in the absence of pathogen infection (Daryaei et al., 2016). However, the growth promotion efficacy is not only dependent on the *Trichoderma* isolate, but is also plant species specific (Singh et al., 2011). Take-all caused yield loss by damaging the plant root system, therefore, reducing nutrient uptake. Meanwhile, growth enhancement by *Trichoderma* spp. occurs partly through increased plant nutrient uptake (Yedidia et al., 2001). Therefore, it is possible that shoot dry weight and seed yield of *Ggt*-infected prairie grass increased because the *Trichoderma* isolates helped to recover the plant's ability to uptake soil nutrients.

Generally, the growth promotion effects by *Trichoderma* isolates occurred under both moisture stress and non-stress conditions. However, better shoot dry weight and seed yield were achieved in well-watered plants, emphasizing the importance of soil moisture for optimum bioactivity. This is because *Trichoderma* also requires soil moisture for its survival (Srivastava et al., 2015). The importance of soil moisture became more profound in the direct-sown plants as the BCAs did not increase the shoot dry weight in moisture-stressed plants. In the same way, *Trichoderma* isolates did not affect the shoot dry weight of vegetative regrowth under moisture stress in both planting system. The results may be explained by the fact that the direct-sown plants had received less nutrients, while nutrient availability for the transplanted plants was already depleted during vegetative regrowth. Therefore, besides the presence of the pathogen, moisture stress and lack of soil nutrients also affected the growth promotion effect provided by *Trichoderma* isolates.

# Chapter 4

# Effects of moisture stress and *Trichoderma* on take-all severity and seed yield of prairie grass

## 4.1 Introduction

Soil moisture is a critical factor in the spread of take-all. Under high precipitation conditions in pasture the disease can progress up to the culm base, and eventually, patches of stunted plants appear (Kwak & Weller, 2013). Meanwhile, limited soil moisture, while limiting pathogen development in the roots, does allow the development of blackened root symptoms without any patches that can be noticed above ground. Moore and Cook (1984) reported that take-all incidence was more common in irrigated wheat crops than in rainfed areas. The disease is prevalent in moist soil mainly due to the high water potential in the top 25 cm of soil, required by the pathogen to grow and infect the host. This layer is the first to dry when rain or irrigation ceases (Cook, 1981). Dry soil conditions do not necessarily eliminate *Ggt* inoculum from the soil. Wong (1984) found that *Ggt* survived for at least three months under cool dry soil and warm dry soil with 100% and 63-97% inoculum survival respectively. Hence, take-all infection under dry soil conditions can still cause yield losses, even without any visual symptoms above the ground surface.

While moisture stress does not support pathogen development, seed yield of the grass crop, on the other hand, is threatened by these conditions. The general response of plants to a water shortage are the shortening of the reproductive growth stage and decreasing seed yield (Rowarth, 1997). Moisture stress affects seed yield even when other essential elements are not limiting. For instance, a ryegrass seed crop grown under dryland conditions produced lower seed yields regardless of the amount of nitrogen given to the crop, compared to those grown in irrigated soils, even though the stressed crop generated more reproductive heads (Rolston et al., 1994). Also, the detrimental effects could be more apparent if water stress conditions occur during critical periods, such as the seed development and maturation phases (Hebblethwaite, 1977).

Root colonisation by *Trichoderma* spp. can reduce diseases as well as alleviate the effect of environmental factors, such as moisture stress. The BCAs may enhance plant tolerance to moisture stress by improving root development (Contreras-Cornejo et al., 2009). In maize, the presence of root-colonising strain T22 was reported to stimulate root growth up to 25-75 cm in

depth, resulting in increased drought tolerance (Harman et al., 2004). Additionally, plant roots colonised by *Trichoderma* spp. produce various chemical compounds that facilitate tolerance against abiotic stresses as well as enhance the branching capacity of the host root system (Kashyap et al., 2017). More interestingly, several previous reports showed that biological control performance of *Trichoderma* spp. was not severely affected by soil moisture level, allowing the BCAs to effectively reduce disease severity caused by several plant pathogens even under soil moisture stress (Hung-Chang & Scott, 2008; Innocenti et al., 2015). Considering that the *Trichoderma* isolates used in this experiment are identified as good root colonisers, it was of interest to determine their impacts on both take-all disease and grass seed yield in the presence of moisture stress.

The effect of moisture stress on the ability of *Trichoderma atroviride* isolates LU132, LU140, and LU584 to control take-all of prairie grass has not been investigated. This study, therefore, set out to assess: i) the effect of moisture stress on take-all severity and seed yield of prairie grass, and ii) the effect of the three *Trichoderma* isolates on disease severity and seed yield of prairie grass under moisture stress conditions.

## 4.2 Materials and methods

### 4.2.1 Plant, disease and *Trichoderma* establishment

The establishment of prairie grass plants, take-all and *Trichoderma* has been detailed in Chapters 2 and 3 (section 2.2, 3.2).

### 4.2.2 Moisture stress

The moisture stress treatment was applied according to a method described by Hofmann (pers, comm, 2018). At 54 DAS, all pots were watered until they reached soil field capacity. At first, water was poured into each pot until it reached the pot edge. After half an hour, pots were watered in the same way for the second time, and this was repeated for the third time after one hour, before pots were left overnight. The next day, pots were weighed as the soils had reached their field capacity level. After that, watering was stopped for moisture-stressed pots until drought symptoms occurred, as evidenced by plants starting to wilt. At this point, the moisture stressed pots were weighed, and water was added at a rate of 4% of soil weight which was 350 ml for each pot. The same amount of water was added each time the stressed plants returned to the wilting point. Meanwhile, the moisture level of non-stressed pots was maintained by adding 500

ml water per pot every 3-4 days, depending on climatic conditions, to avoid any water limiting problems. These procedures were continued until the end of the experiments. The performance of prairie grass plants under drought conditions is displayed in Figure 4.1.


**Figure 4.1** **Moisture-stressed prairie grass plants grown in the experimental pots with and without *Gaeumannomyces graminis* var. *tritici* and *Trichoderma* treatments at 64 days after sowing.**

## 4.3 Assessments

### 4.3.1 Determination of relative water content (RWC)

Relative water content (RWC) was measured to see if there were any differences in plant water status in response to *Trichoderma* inoculation and *Ggt* infection. The assessment on moisture-stressed and non-stressed pots was done separately since the comparison between moisture treatments was unnecessary. The measurement was done before seed harvesting at 78 DAS.

Three sections of leaf (4 cm long) were cut from each measured plant and immediately weighed using a four-digit-decimal balance to obtain the fresh weight (FW). After that, the leaf samples were immediately hydrated to full turgidity by placing in closed Petri dishes flanked by two wet paper sheets and kept in cold storage overnight. After hydration, the leaf samples were taken out of the water, surface moisture blotted using tissue paper, and weighed to determine the turgid weight (TW). Samples were then oven dried at 60°C for 2-3 days and weighed to obtain the dry weight (DW). The RWC is calculated using the following formula described by González and González-Vilar (2001):

$$\text{RWC}\ (\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}}\ \text{x}\ 100$$

### 4.3.2 Chlorophyll content measurement

Leaf chlorophyll content (ChlC) was measured using a Minolta Soil Plant Analysis Development (SPAD)-502 chlorophyll meter (SPAD units) (Figure 4.2). Chlorophyll content is essential for the absorption of light energy during photosynthesis which is indicated by the number of chlorophyll pigments. The probe measures the ChlC of leaves via light transmittance, i.e. absorption of red light at 650 nm and infrared light at 940 nm. However, data collected by the SPAD meter are not absolute chlorophyll values, they are a 'chlorophyll concentration index' instead (CCI; ranging from 0 to 99.9) (Pask et al., 2012). The measurement was done pre-harvest (110 DAS) and for the subsequent vegetative regrowth (142 DAS).

The sensor was placed around the centre of the leaf for an accurate reading. The reading was done three times on three different leaves, which were randomly selected from the transplanted plants in each pot. The measurements using the SPAD meter were conducted during a sunny day between 11:00 to 14:00 although it can be done under any environmental conditions and at any time of the day (Pask et al., 2012).

**Figure 4.2 Chlorophyll content measurement pre-harvest, and the measurement at vegetative regrowth (insert) using a Minolta SPAD-502 chlorophyll meter.**

### 4.3.3 Seed collection and plant dry matter

Seed collection and plant dry matter measurement have been detailed in Chapters 2 and 3 (section 2.2.8, 3.3.3).

### 4.3.4 Statistical analysis

Statistical analyses were done as detailed in section 2.3.8.

## 4.4 Results

### 4.4.1 Effects of moisture stress, take-all and *Trichoderma* treatments on relative water content (RWC) and chlorophyll content (ChlC)

Relative water content (RWC) was measured once before seed harvesting, while leaf ChlC was measured twice, i.e. at pre-harvest and during vegetative regrowth. Pathogen infection decreased RWC of well-watered plants, but not in moisture-stressed plants (Table 4.1). Conversely, *Trichoderma* isolates improved the leaf RWC in stressed plants, with no significant differences among the isolates. In well-watered plants, *Trichoderma* isolates did not affect the RWC. Moisture stress treatment did not affect the leaf ChlC at pre-harvest, but significantly ($P$<0.01) increased the ChlC of the vegetative regrowth. Likewise, *Ggt* infection caused a decline in leaf ChlC only for the vegetative regrowth ($P$<0.05). Most of the *Trichoderma* isolates did not affect the plant ChlC. However, LU584 increased the ChlC at pre-harvest, while the mixture of isolates caused a significant ($P$<0.05) reduction during vegetative regrowth.

**Table 4.1 Main effect means of chlorophyll content (ChlC) and relative water content (RWC) of prairie grass for the different moisture, pathogen and *Trichoderma* treatments.**

| Main effect means | RWC (%) | | ChlC (SPAD unit) | |
|---|---|---|---|---|
| | Moisture stressed | Non-stressed | Pre-harvest | Vegetative regrowth |
| Moisture | | | | |
| Non-stressed | n/a | 88.85 | 37.02 | 35.83 |
| Stressed | 51.00 | n/a | 41.00 | 45.11 |
| LSD (5%) | n/a | n/a | 4.34 | 4.35 |
| Sig. of diff. | | | ns | ** |
| Pathogen | | | | |
| Not inoculated | 46.64 | 89.90 | 39.26 | 41.21 |
| Inoculated | 55.33 | 87.80 | 38.88 | 40.10 |
| LSD (5%) | 11.98 | 1.50 | 1.02 | 0.87 |
| Sig. of diff. | ns | * | ns | * |
| *Trichoderma* | | | | |
| Control | 43.50 | 89.09 | 38.16 | 41.72 |
| LU132 | 52.56 * | 88.32 | 39.07 | 40.07 |
| LU140 | 54.80 * | 88.56 | 39.51 | 40.66 |
| LU584 | 53.76 * | 88.43 | 39.94 * | 40.40 |
| Mixture | 50.32 * | 89.83 | 39.20 | 38.23 * |
| LSD (5%) | | | | |
| (Ctrl v Trtd) | 6.41 | 2.43 | 1.38 | 1.82 |
| (Trtd v Trtd) | - | - | 1.60 | 2.10 |

ns = not significant; * = $P$<0.05; ** = $P$<0.01. n/a = data were not included in the analysis. Data collected from transplanted plants. Relative water content was assessed pre-harvest.

There was a strong positive correlation between the RWC of drought-stressed plants with the seed yield of prairie grass ($R^2$ = 0.78), where total the seed yield increased with the increased RWC (Figure 4.3). Similarly, a strong positive correlation ($R^2$ = 0.70) was also found between the ChlC at pre-harvest and the seed yield of prairie grass (Figure 4.4).

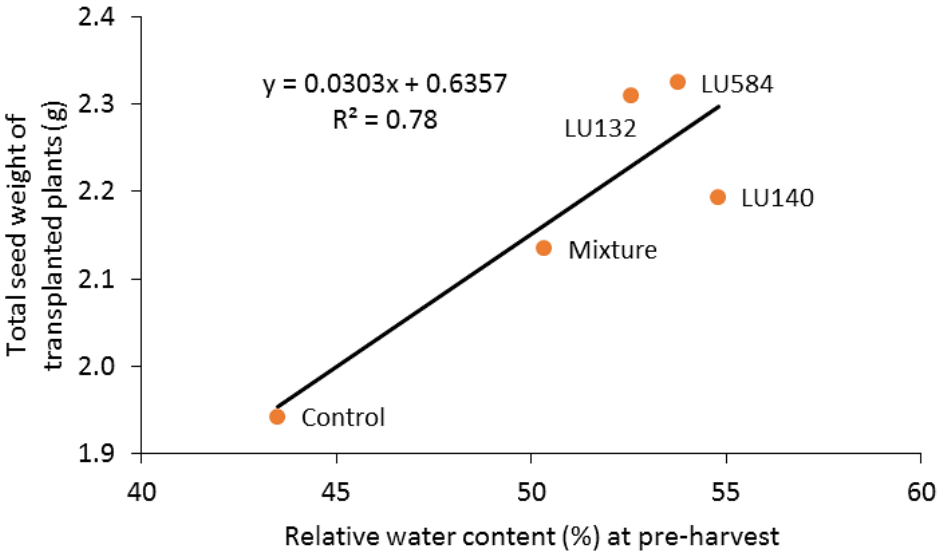


**Figure 4.3 Correlation between relative water content (RWC) and seed yield of prairie grass growing in the experimental pots.**

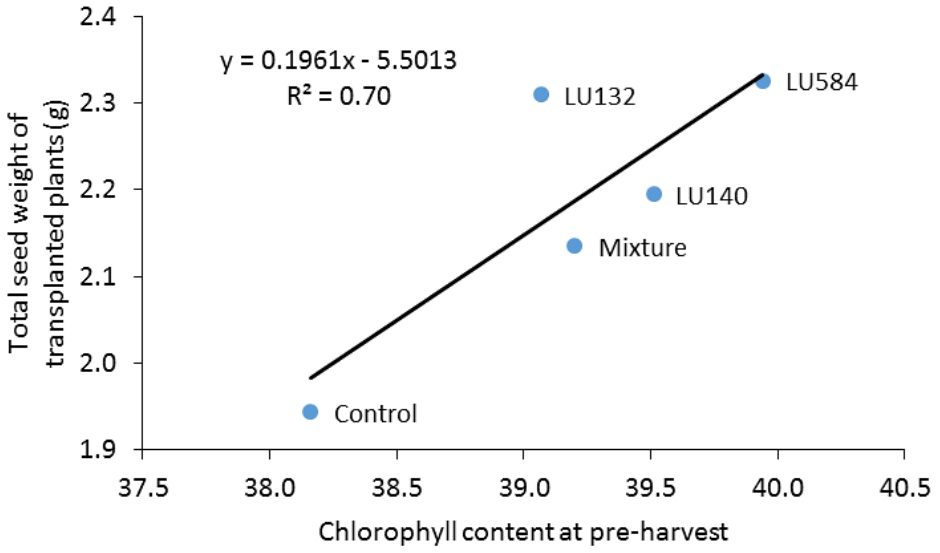


**Figure 4.4 Correlation between leaf chlorophyll content at pre-harvest and seed yield of prairie grass growing in the experimental pots.**

### 4.4.2 Effects of moisture stress and *Trichoderma* application on vegetative and reproductive growth

Other effects of moisture and *Trichoderma* treatments on agronomic parameters of prairie grass have been presented in the previous chapters. The overall observation was that the drought treatment affected nearly all agronomic parameters measured, with small variations. Moisture stress significantly ($P$<0.05) reduced the number of tillers in the direct-sown plants, but not in the transplanted plants (Chapter 2, Table 2.2). The stressed plants had a significantly ($P$<0.05) lower number of fertile tillers in both transplanted and direct-sown plants. The drought stress greatly reduced the number of seeds per plant in both planting systems, and also significantly ($P$<0.01) reduced the total seed weight per plant in both transplanted and direct-sown plants by 41% and 42%, respectively.

There was no significant difference in the weight of light seeds between the moisture treatments (Chapter 2, Table 2.3). However, the stress significantly ($P$<0.01) increased the percentage of light seeds per plant in the transplanted plants, but not in the direct-sown plants. The stressed plants produced lower yields ($P$<0.01) of MDS in both the transplanted and direct-sown plants. The drought stress also reduced the TSW, but did not affect the germination percentage of the seeds (Chapter 2, Table 2.3).

Moisture stress gave a significant ($P$<0.01) reduction in plant height at 110 DAS (Chapter 3, Table 3.1). Also, the shoot fresh weight and shoot dry weight after seed harvest were greatly affected by water stress. Shoot fresh weights in the transplanted and direct-sown plants were significantly ($P$<0.01) reduced by 48% and 50%, respectively. Meanwhile, shoot dry weights in the transplanted and direct-sown plants were significantly ($P$<0.01) reduced by 27% and 31%, respectively.

Moisture stress also decreased the dry matter production for the first, second, and third periods of vegetative regrowth with a small variation in significance level (Chapter 3, Table 3.2). At first regrowth, moisture stress highly ($P$<0.001) reduced the shoot dry weight in transplanted plants and significantly ($P$<0.01) reduced the shoot dry weight in direct-sown plants. At second regrowth, the treatment reduced ($P$<0.05) the shoot dry weight in both the transplanted and direct-sown plants. At third regrowth, a significant ($P$<0.05) reduction in shoot dry matter occurred only in the direct-sown plants. Meanwhile, root dry weight were not affected by the moisture stress treatment (Chapter 3, Table 3.2).

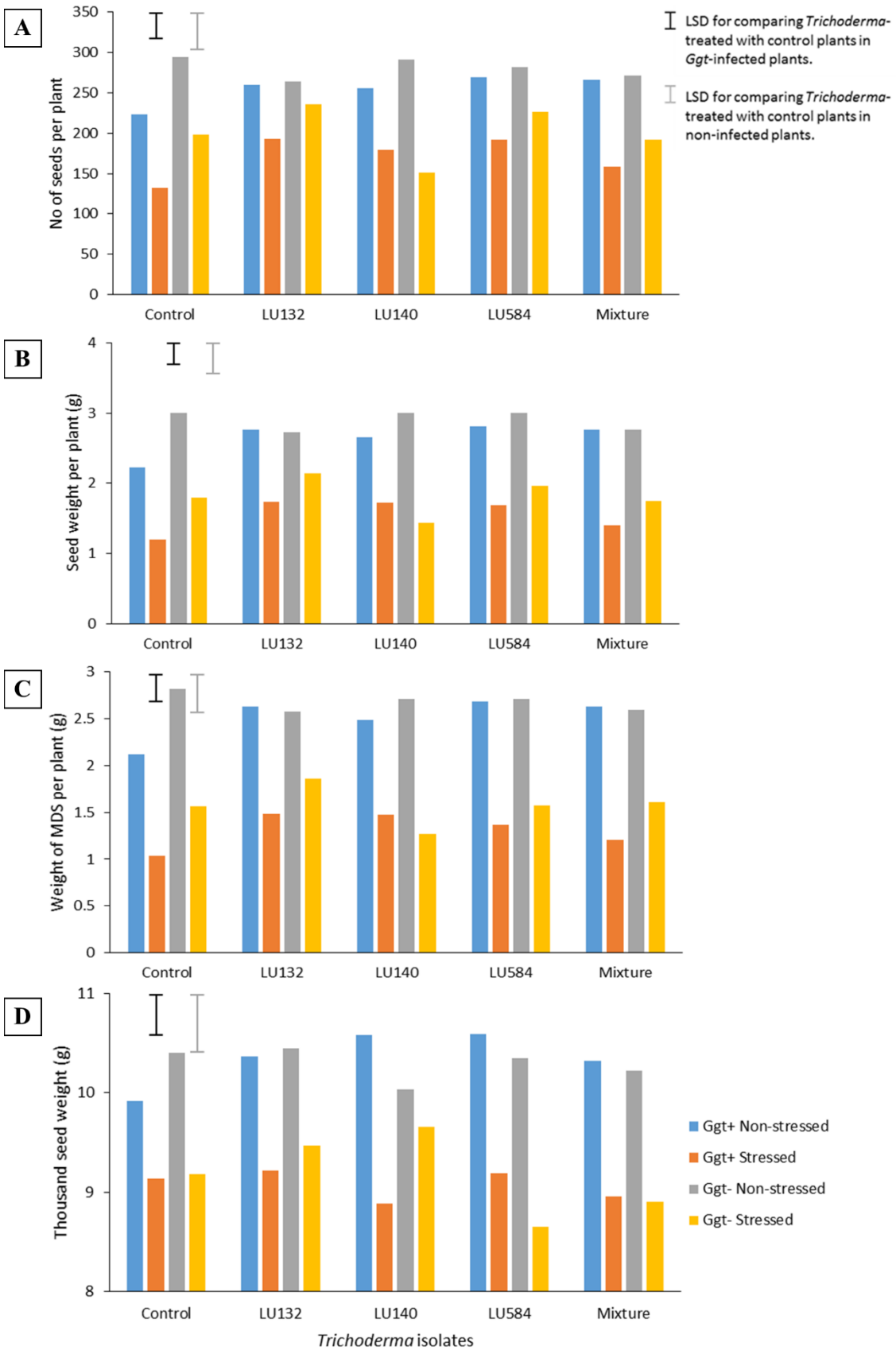


**Figure 4.5** **Effects of moisture stress and *Trichoderma* on seed yield components of *Ggt*-infected prairie grass plants. Error bars are the least significant difference (LSD) at the 5% level. MDS = machine dressed seeds.**

Generally, moisture stress decreased seed yield components, while *Trichoderma* isolates increased the yield components of prairie grass in both *Ggt*-infected and non-infected plants (Figure 4.5). In *Ggt*-infected plants, moisture stressed-plants produced 26-41% fewer seeds, 35-49% less total seed weight, 41-54% lower weight of MDS, and 8-16% lower TSW relative to control plants. In non-infected plants, moisture stress resulted in 11-33% fewer seeds, 22-52% less total seed weight, 28-53% lower weight of MDS, and 4-16% lower TSW.

In the presence of *Ggt*, plants protected with *Trichoderma* isolates generated 15-21% more seeds in well-watered plants, and 20-46% more in moisture-stressed plants, compared to control plants (Figure 4.5). The protected plants also produced a total seed weight 19-26% higher in well-watered plants and 16-45% higher in moisture-stressed plants. The *Trichoderma* isolates also gave a MDS yield 17-26% higher in non-stressed plants and 16-42% higher in stressed plants. For the TSW, *Trichoderma* treatments gave 4-7% increase only in non-stressed plants and not in non-stressed plants. In the absence of *Ggt*, *Trichoderma* isolates did not increase the seed yield components.

### 4.4.3 Effects of moisture stress on disease severity

Moisture stress significantly ($P$<0.05) reduced the root disease severity as observed by direct observation, but the reduction was not observed microscopically (Chapter 3, Table 3.3). However, reduced root disease severity of prairie grass in the presence of *Trichoderma* was detected through microscopic observation (Figure 4.6). Root disease scores of *Trichoderma*-inoculated plants were significantly ($P$<0.05) reduced in both non-stressed plants and moisture-stressed plants (Chapter 3, Table 3.8). There was no significant difference in root disease score between non-stressed and stressed plants.

### 4.4.4 Treatment interactions

Interactions between treatments showed that moisture stress reduced several seed yield components of the transplanted plants in both *Ggt*-inoculated and non-inoculated plants, and in both *Trichoderma*-treated and untreated plants. Those yield components include the total seed weight (Table 2.4), the weight of MDS (Table 2.6), and the TSW (Table 2.7).

Moisture stress also reduced several agronomic traits in both *Ggt*-inoculated and non-inoculated plants, and in both *Trichoderma*-treated and untreated plants. Those agronomic traits are the shoot dry weight of the transplanted plants (Table 3.4), shoot dry weight of the direct-sown plants (Table 3.5), shoot dry weight of the transplanted plants at the third vegetative regrowth (Table 3.6), and shoot dry weight of the direct-sown plants at the third vegetative regrowth (Table 3.7).

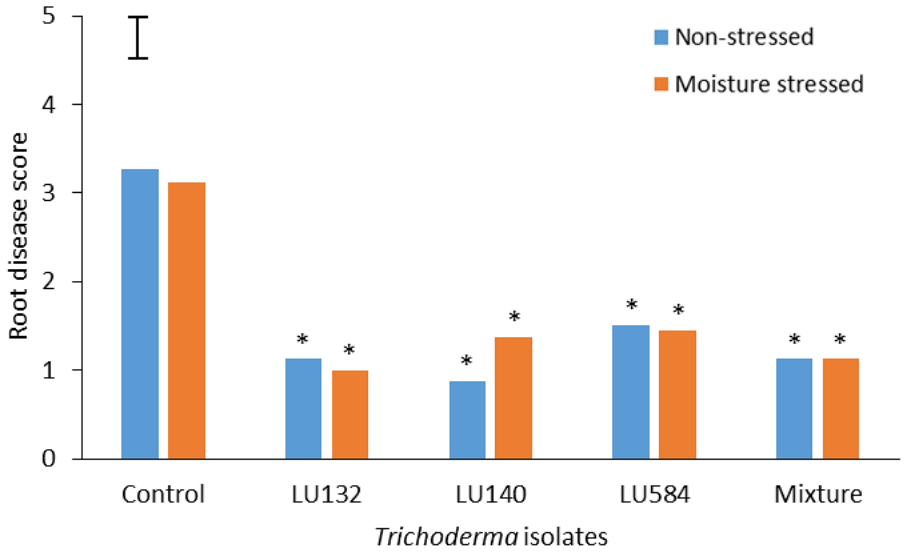


**Figure 4.6 Effects of *Trichoderma* and moisture stress treatments on root disease severity (0 – 5 scores) of prairie grass observed microscopically. Bars with stars indicate 5% significant difference from control.**

## 4.5 Discussion

### 4.5.1 Effects on relative water content (RWC) and chlorophyll content (ChlC)

Moisture stress is one of the most important factors affecting almost every aspect of plant growth (Khayatnezhad & Gholamin, 2012), and is also important in determining microbial activity in the soil (Shipton, 1981). One of the principal effects of *Ggt* infection is the detrimental effect on plant water uptake, which eventually lowers the percentage leaf water content (Manners & Myers, 1981). In the present study, take-all reduced the RWC of non-moisture stressed plants, but not the stressed plants, both at pre-harvest and during vegetative regrowth. This result may be explained by the fact that adequate soil moisture level is required for take-all to develop (Cook, 1981), presumably, up to a level that impairs water absorption. Drought conditions, on the other hand, are not conducive for the pathogen to spread and cause further damage to the plant water uptake system. This finding supports previous studies, which showed that *Ggt* growth ceases under dry soil conditions (Cook, 1981; Cook, 2003). If soil moisture is limited, take-all symptoms are limited to blackened roots (Kwak & Weller, 2013).

Moisture stress is reported to inhibit chlorophyll a/b synthesis of cereal crops, but another report showed no significant reduction in ChlC of chickpea due to drought stress (Rahbarian et al., 2011). This suggests that the effect of moisture stress on ChlC may differ from plant to plant. The present study found an increase of ChlC in moisture-stressed plants during vegetative regrowth, which

may be related to the plant's resistance to drought, an important trait of prairie grass (Charlton & Stewart, 1999). An increased ChlC of drought-stressed plants was also reported by Khayatnezhad and Gholamin (2012). These authors stated that the green tissues (chlorophyll) of a drought-resistant cultivar increase under drought stress, as a mechanism to adapt to the stress and produce the most yield.

As a result of damaged roots following pathogen colonisation, plant physiological processes – those connected with water and nutrient uptake – become disrupted (Warzecha et al., 2015). This disruption may cause stress to the extent of damaging to the photosynthesis apparatus due to lowered ChlC level (Warzecha et al., 2015). The significant reduction of ChlC by *Ggt* occurred only during vegetative regrowth, not pre-harvest, indicating the degree of chlorophyll reduction may vary over time. For example, Saric-Krsmanovic et al. (2018) reported that relative ChlC of sugar beet was significantly reduced by the field dodder parasite only at 7 days after pathogen infestation, and that no significant reduction was observed up to fourteen days later.

Among the beneficial outcomes of the *Trichoderma*-plant interaction is increased plant resistance to abiotic stress (Bae et al., 2009; Mastouri et al., 2010). This study present evidence that *Trichoderma* application enhanced the drought resistance of prairie grass by increasing the RWC during the presence of moisture stress. Under water limited conditions, the *Trichoderma*-colonised plants have a higher activity of drought resistance-related enzymes, enhanced redox buffer capacity, and lower lipid peroxide content than untreated plants (Mastouri et al., 2012). Lipid peroxidation is commonly associated with oxidative damage as a result of increased levels of reactive oxygen species (ROS), a common condition in stressed plants. Furthermore, no improvement in RWC of non-stressed plants by *Trichoderma* application shows that such a mechanism did not occur in well-watered conditions. This may be because the RWC of well-watered plants was at the optimum level, and could not be increased further by *Trichoderma* treatment.

The increased chlorophyll content induced by isolate LU584 at pre-harvest indicates that the BCA can increase the photosynthetic capacity of the host plant. This finding was also reported previously (Azarmi et al., 2011; Stewart & Hill, 2014; Zaidi & Singh, 2013). For example, ChlC of chickpea leaves treated with *T. harzianum* 25-92 was increased to 33 spad units, compared to 27 in control plants, which was correlated with the increased number of root tips (Jyotsna et al., 2008). The authors postulated that increased uptake of soil minerals was the primary mechanism responsible in increasing the ChlC. However, the result of the interactions may be influenced by other factors, such as growth stage, *Trichoderma* strain/isolate, or plant species.

Azarmi et al. (2011) reported no significant increase in ChlC of tomato seedlings grown in soil amended with either *Trichoderma* spp. or *T. harzianum* T969, but a reduction of ChlC did occur as a result of *T. harzianum* T447 inoculation. It was suggested that the *Trichoderma* inoculum survival or the depletion of nutrients available may play a role in diminishing the growth promotion effects (Azarmi et al., 2011; Macías-Rodríguez et al., 2018). This explains the reduction of ChlC by the mixture of isolates (Table 4.1), which was measured during the second period of vegetative regrowth, five months after sowing. Presumably, either *Trichoderma* inoculum reduction or nutrient depletion or a combination of both played a role in decreased leaf ChlC.

Increased chlorophyll content was associated with increased seed yield (Figure 4.4). This finding supports the work of other studies in this area linking ChlC and seed yield. Guendouz and Maamari (2012) suggested that changes in leaf ChlC are often correlated with changes in leaf N status, photosynthetic capacity, and RuBP carboxylase activity. Also, Ramesh et al. (2002) reported ChlC of rice plants before and after flowering was highly correlated with the grain yield ($R^2$ = 0.91 at 79 DAS). These authors concluded that ChlC is the best indicator of photosynthetic activity, and hence, is able to predict grain yield. Leaf RWC can also be a good indicator for seed yield potential of prairie grass, but only during the presence of drought stress (Figure 4.3).

### 4.5.2 Effects on vegetative and reproductive growth

The present study demonstrated that moisture stress affected almost every aspect of growth and yield of prairie grass, regardless of the planting system or the plant access to soil nutrients. Of the vegetative growth parameters, tillering of direct-sown plants was reduced by the moisture stress. The plant responds to water stress by reducing leaf and tiller numbers to reduce transpiration losses (Koech et al., 2014; Volaire et al., 2009). However, no significant effect of water stress on tillering of transplanted plants may indicate the plant was able to escape the drought effect, in terms of tiller production, where soil nutrients are sufficient. According to Knapp et al. (2001) nutrient and canopy light limitation may reduce the importance of water limitation to productivity. Drought-tolerant plants, such as prairie grass, have complex mechanisms to limit the adverse effect of water stress (Xu et al., 2010). One of them is drought abandoning by removing part of the individual, e.g. shedding elder leaves, which was commonly observed in the experiment of the present study. Such a mechanism may be utilised, so that the plant can maintain the normal rate for other aspects of growth, such as tiller production.

The main seed yield components affected by the moisture stress were number of fertile tillers, number of seeds per plant, seed weight per plant, weight of MDS, and TSW. The water stress

limited plant physiological processes which subsequently caused low productivity of biomass and eventually translated into low seed yield (Koech et al., 2014). The reduction in the number of fertile tillers by moisture stress was the main cause for the reduction in the overall seed yield. This was also reported by Langer (1980) who found that fertile tiller number was the primary determinant of grass seed yield. The proportion of light seeds did not contribute to the yield reduction of moisture-stressed plants since there was no significant difference in the weight of light seeds between stressed and non-stressed plants (Table 2.3). This result is similar with the previous result (Chapter 2) which showed that the light seeds was not the cause of yield reduction by take-all, it was mainly caused by the reduced number of seeds per plant instead.

Moisture stress during seed development had no effect on the germination viability of the seeds. Previous reports showed drought during seed development reduced seed dormancy and hence increased germination viability of *Avena fatua* (Peters, 1982; Sawhney & Naylor, 1982). It was suggested that the drought reduced dormancy by interfering with the synthesis of a germination inhibitor. However, another report showed no consistent effect of drought on germination viability of *Stylosanthes hamata* (Argel & Humphreys, 1983). Fenner (1991) showed that drought tended to increase the thickness of the seed coat, decreasing its permeability and hence reducing germination viability at least in short-term tests. One of those findings may provide an explanation for the result of the present study. However, since some portion of seeds were harvested early (immature) and mechanical injury was used to allow imbibition during the germination test (Chapter 2), the actual percentage of germination viability may have changed.

Moisture stress severely affected the prairie grass growth. The plant height at 63 DAS was not affected because it was only at the beginning of the drought treatment. The percentage reduction in plant dry matter both after seed harvest and during vegetative regrowth between both transplanted and direct-sown plants was comparable. For example, the fresh weight of the stressed plants in the transplanted and the direct-sown plants was 48% and 50% less, respectively (Table 3.1). This implies that the drought stress had an equal effect on the growth of plants regardless of the amount of nutrients received by the plants.

The non-significance different in root dry weight due to moisture treatment was most likely related to the drought-tolerance traits of prairie grass. Harman (2000) suggested that a drought-tolerant plant increases its root growth under water limited conditions. This finding is consistent with that of Izanloo et al. (2008) who found that bread wheat cv. Excalibur (drought-tolerant) produced more root mass (6.5 g) when imposed with severe cyclic water-limiting conditions than when it was well-watered (5.6 g). Under the water stress conditions, cv. Excalibur had a high level of osmotic

adjustment, high stomatal conductance, and rapid recovery from stress, which may partly contribute to the increased root growth. In the present study, stressed plants had more root dry weight (6.4 g) compared to non-stressed plants (5.7 g), but it was statistically not significant.

*Trichoderma* isolates alleviated the effect of drought stress as indicated by the increased seed yield components of the stressed plants. This result further emphasized the ability of the isolates to promote plant growth and increase yield under unfavourable conditions. *Trichoderma* can alter plant responses to drought through morphological adaptations, increase plant tolerance through physiological and biochemical adaptations, and enhance drought recovery (Zaidi & Singh, 2013). The drought tolerance is induced by the BCA partly through root colonisation and subsequent increase in the root growth and of the entire plant, thereby increasing plant productivity (Singh et al., 2011). Clearly, *Trichoderma* isolates effectively induced the prairie grass to be more efficient and to better resist the moisture stress.

### 4.5.3 Effects on disease severity

Soil moisture is an important determinant for take-all development (Cook, 1981), and moisture stress reducing disease severity was confirmed in the present study. The reduced root disease severity under moisture stress, observed through the direct assessment, aided in assessing the spread of *Ggt* in the overall root system. The intensity of 'the black to chocolate brown' lesions of infected roots of moisture-stressed plants was lower compared to the non-stressed plants. This suggests that the stress reduced the spread of the pathogen infection and, therefore, reduced root disease severity. Meanwhile, microscopic observation was useful in sighting the presence of runner hyphae and/or hyphodia of *Ggt*. The runner hyphae and/or hyphodia persisted in the roots of moisture-stressed plants at the same intensity as in the non-stressed plants. This suggests that the water inside the roots remained available to support the development of *Ggt* during the water stress periods.

Limited soil moisture causes the growth of *Trichoderma* to cease, as it does for most microbes (Bailey & Melnick, 2013). It was reported that population density of *T. harzianum* was positively correlated with soil moisture level (Eastburn & Butler, 1991). Moreover, a report by Innocenti et al. (2015) demonstrated that *T. harzianum* T22 reduced lettuce wilt disease severity caused by the soil-borne pathogen *Fusarium*, and the reduction was greater under wet than under dry conditions. However, there was also a report by Hung-Chang and Scott (2008), demonstrating that the efficacy of *T. virens* against *Sclerotinia sclerotiorum* was not affected by moisture level. In accordance with this report, the microscope assessment showed that root disease scores were

significantly reduced by *Trichoderma* isolates in both stressed and non-stressed conditions (Figure 4.6). The *Trichoderma* efficacy in both soil moisture conditions did not differ. A likely explanation for this might be that the activity of both *Trichoderma* isolates and *Ggt* was suppressed already by moisture stress. Therefore, lower disease reduction in non-stressed conditions, where take-all development was not limited by soil moisture, than in stressed conditions, was not possible. It is possible, therefore, soil moisture played a more important role than the *Trichoderma* isolates in the take-all development.

### 4.5.4 Interaction responses

Soil moisture influences host exudation and exudate diffusion and therefore the nutrition of the entire community of soil-borne microorganism, including pathogenic and antagonistic fungi, their interaction, and thus, plant development (Innocenti et al., 2015). Fungal spore germination, the rate of the germination and hyphal extension are also determined by soil moisture (Santamarina & Roselló, 2006). Soil moisture also affects the availability of soil nutrients, and thus, the competition between soil microflora and their ability to infect plants (Magan & Lacey, 1984).

The interactions recorded further emphasized the importance of soil moisture on plant growth and yield. Moisture stress greatly reduced seed yield and plant dry matter of prairie grass, whether the pathogen or *Trichoderma* was present or absent. This result is consistent with that of Innocenti et al. (2015) who found that dry soil conditions reduced the dry weight of lettuce plants, either when *T. harzianum* T22 and/or the pathogen *F. oxysporum* were absent or present. Moreover, moisture stress has been shown to exacerbate seed yield loss when combined with *Ggt* infection (Chapter 2, Section 2.5.4). The stress also reduced the growth promotion effect of the *Trichoderma* isolates (Chapter 3, Section 3.5.3).

The effect of moisture on *Trichoderma*-plant-pathogen interactions depends on the adaptability of the biocontrol agents to different soil moisture levels (Innocenti et al., 2015). The present study provides more evidence that *T. atroviride* isolates LU132, LU140, LU584 and the mixture of these isolates could be effective against take-all, an important root disease of grass crops, under moisture stress conditions already present in New Zealand, which may become more frequent in the near future (Intergovernmental Panel on Climate Change (IPCC), 2007).

# Chapter 5

# Effects of *Trichoderma* on dry matter production of perennial ryegrass grown in a *Ggt*-infected soil

## 5.1 Introduction

Perennial ryegrass (*Lolium perenne*) is the most widely sown pasture grass in New Zealand as it grows well under many conditions (Charlton & Stewart, 1999). In an arable cropping rotation, it is often grown after wheat, which is highly susceptible to *Gaeumannomyces graminis* var. *tritici* (*Ggt*). Perennial ryegrass is also included among susceptible hosts of *Ggt*, allowing the carry-over of *Ggt* inoculum from wheat to the grass (Bithell et al., 2011a; Chng et al., 2005). The rotation, therefore, can lead to the disease persisting in soils, maintaining *Ggt* inoculum, and providing inoculum for subsequent crops (Bithell et al., 2011b). The symptoms of *Ggt* infection of perennial ryegrass are frequently observed above-ground as "bare-patch", although other fungal pathogens present may also contribute to these symptoms (Kandula et al., 2014). The infection damages the plant root system and reduces the production of plant dry matter (DM), which may lead to economically important yield losses.

*Ggt* is considered a pathogen that is vulnerable to antagonism by other soil microflora (Cook, 2003). This vulnerability may account for the natural decline of the disease in fields where other soil microflora build up and develop natural antagonism against the pathogen. Therefore, there is a good potential to employ biological control agents (BCAs) as an effective tool to control take-all disease. An earlier study showed that *Phialophora* sp. isolate I-52, isolated from a take-all decline (TAD) soil, provided promising take-all control in five years of field experiments (Mathre et al., 1999). Another study reported that the presence of Diacetylphlorogucinol (DAPG)-producing *P. fluorescens*, at $10^5$ to $10^6$ $g^{-1}$ of roots, in TAD soils was able to suppress take-all (Chng, 2009). However, one type of soil fungus or bacterium may not be sufficient to control diseases in field soils, because the diversity of soil microorganisms and environmental factors can significantly affect biocontrol efficacy. Hornby et al. (1998) reported that the overall performance of *Pseudomonas* and *Bacillus* species, when used for biocontrol of take-all in wheat crops, had been inconsistent, as only 60% of biocontrol treatments resulted in significant yield improvement. Also, the authors pointed out that one of the major impediments for a successful bacterial biocontrol product is that these BCAs cannot promote plant growth directly.

*Trichoderma* spp. are widely used BCAs for controlling a wide range of pathogens and promoting plant growth in various host plants (Harman et al., 2004). The introduction of a BCA to the plant ecosystem can confer benefits to the host, especially in the presence of plant pathogens. In perennial ryegrass plants, these beneficial effects include, but are not limited to, improvement in seedling emergence and plant dry matter (DM) production (Chohan et al., 2011). The same report demonstrated that the inoculation of four *Trichoderma* isolates gave a significant improvement of root and shoot DM of perennial ryegrass in the presence of the soil-borne pathogen *Rhizoctonia solani*. More recently, a pot study by Kandula et al. (2014) specifically demonstrated the potential for suppression of *Ggt* by *Trichoderma* spp. In that study, ryegrass grown in naturally-infected-*Ggt* soils produced more shoot and root dry matter when inoculated with *Trichoderma* spp. compared to the uninoculated control. However, it is generally understood that the performance of the BCAs may be highly variable among seasons and sites, emphasising the need for further studies for their application.

This research complements an earlier study which showed that *T. atroviride* isolates LU132, LU140 and LU584 along with other *Trichoderma* species positively influenced the DM production of ryegrass grown in *Ggt*-infected soils (Kandula et al., 2014). Therefore, the present study set out to test the hypothesis that: (i) a combination of the three *Trichoderma* isolates will provide more effective control of *Ggt*, if present, than any individual isolate, and (ii) ryegrass plants grown in *Ggt*-affected soil inoculated with the mixture of isolates will produce more DM than those inoculated with any of the individual isolates.

## 5.2 Materials and methods

### 5.2.1 Experimental site

This study of the effect of *Trichoderma* on the dry matter production of perennial ryegrass grown in a soil believed to be naturally infested *by Ggt* was conducted at the Challenger glasshouse at Lincoln University, Canterbury. Automatic fan and heating system are integrated with the glasshouse to control relative humidity and temperature and provide the stable environmental conditions needed for the experiment. A temperature data logger recorded an average temperature of 19°C throughout the experimental period from April to September (Figure 5.1).

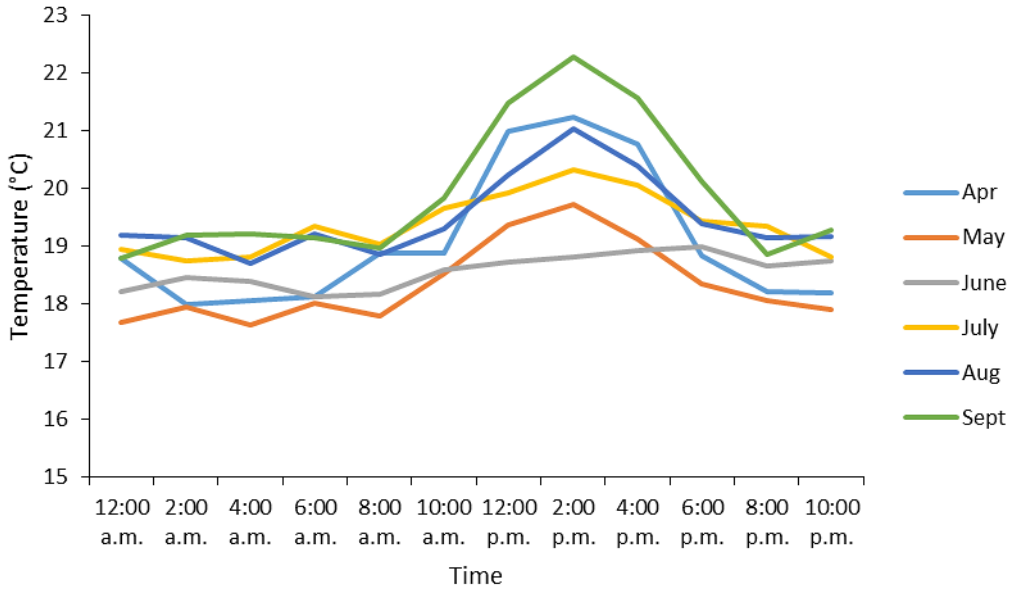


**Figure 5.1 The mean temperature in the Challenger glasshouse at different times of the day throughout the six months of the experiment.**

### 5.2.2 Seed lot

Perennial ryegrass (*Lolium perenne* L.) seeds of Cv. Grasslands Nui grown in 2016 were supplied by the Foundation for Arable Research (FAR). The germination percentage was 96%. No further seed treatment was given before the seeds were sown in the present experiment.

### 5.2.3 Inverted sward technique

The soil used in the experiment was collected from a farm in the Greendale / Darfield area, Selwyn District, Canterbury, in April 2018. Take-all disease was allegedly present in this field as the grower reported some problems with light seed, but the actual cause was unknown (Rolston, pers, comm, 2018). The soil was prepared as a growth medium for perennial ryegrass using an inverted sward technique described by Kandula et al. (2014). Square-shaped swards (size 20 x 20 x 5 cm) were taken from various spots (randomly selected) in a field which was cropped with perennial ryegrass (Figure 5.2a). Soil blocks were cut using a spade and then placed into each of 40 two-litre ice cream containers in an inverted position (Figure 5.2b). Additional soil from other spots was also collected using a 20-litre bucket. Later during the preparation, this additional soil was added to each of the ice cream containers to give an equal volume of 2-litres of soil for all containers.

On the top of the inverted sward, forty-eight ryegrass seeds were sown in each container, arranged in three rows. Then, formulated *Trichoderma* isolates were spread in the sowing paths, while blank prill was used as a control treatment (1 g/pot). After that, about 30 g/pot of potting mix was lightly distributed on the surface of the growth medium to cover the sown seeds as well as provide additional nutrients for the plants. The potting mix is comprised of bark and pumice in 4:1 (v/v) ratio and enriched with osmocote exact 16-3-9-10 (3-4 months), horticultural lime and hydraflo in a ratio of 3:1:1 (v/v/v). The containers were placed in the glasshouse under a controlled temperature of 20°C ± 2°C, and watering was done regularly to provide favourable conditions for plants and soil microflora (Figure 5.2c).


**Figure 5.2 (a) Collection of soil in the Greendale / Darfield area. (b) a square-shaped sward before being put in inverted position into the container. (c) twelve-day-old ryegrass seedlings growing in the containers.**

### 5.2.4 Experimental design

Four formulated *Trichoderma* isolates, i.e. LU132, LU140, LU584, and the mixture of these isolates, were the BCA-inoculated treatments, and there was an uninoculated control. Eight replicates were provided for each treatment to give 40 containers of the ryegrass plants. Containers were arranged in a randomised complete block design (Figure 5.3).

**Block 1**

| | | |
|---|---|---|
| 1 | 2 | 3 |
| 4 | 5 | |

**Block 2**

| | | |
|---|---|---|
| 6 | 7 | 8 |
| 9 | 10 | |

**Block 3**

| | | |
|---|---|---|
| 11 | 12 | 13 |
| 14 | 15 | |

**Block 4**

| | | |
|---|---|---|
| 16 | 17 | 18 |
| 19 | 20 | |

**Block 5**

| | | |
|---|---|---|
| 21 | 22 | 23 |
| 24 | 25 | |

**Block 6**

| | | |
|---|---|---|
| 26 | 27 | 28 |
| 29 | 30 | |

**Block 7**

| | | |
|---|---|---|
| 31 | 32 | 33 |
| 34 | 35 | |

**Block 8**

| | | |
|---|---|---|
| 36 | 37 | 38 |
| 38 | 40 | |

**Figure 5.3 Experimental design of the pasture experiment. Numbers in each block represent pots treated with four *Trichoderma* isolates and one untreated control arranged in the randomised orders. Blocks served as replicates.**

## 5.3 Assessments

### 5.3.1 Seedling emergence

Seedling emergence was recorded at 7, 9, and 39 days after sowing (DAS). The results are presented as a percentage of the sown seeds.

### 5.3.2 Plant dry matter

Above-ground dry weight was measured three times at 52-day intervals by cutting the plants at around 3 cm above the soil surface. Harvested plant material was put into labelled paper bags before being dried in a forced-draft oven for 48 h at 65°C, after which they were weighed using a digital scale to two decimal places.

After the third harvest, plant roots were collected, washed thoroughly free of soil and placed in separate paper bags before being oven dried for 96 h at 65°C to obtain the root dry weight.

### 5.3.3 Root disease

Root assessment was done microscopically as described in section 3.3.4.

### 5.3.4 Statistical analysis

Data were analysed using an analysis of variance (ANOVA) with *Trichoderma* as the only factor in the treatment structure. Seedling emergence was analysed using data collected at 7 and 9 DAS. The seedling numbers until 39 DAS were accumulated to observe the maximum number in each container, then analysed and presented as the final seedling emergence for each treatment.

The least significant difference (LSD) test at $P<0.05$ was used to further investigate the difference of mean value between treatments. All analyses were carried out using Genstat 18th edition (VSN International, Hemel Hempstead, UK).

## 5.4 Results

### 5.4.1 Effects of *Trichoderma* on seedling emergence, plant dry matter and root disease severity of ryegrass grown in *Ggt*-infected soils

No significant differences were found in the seedling emergence of ryegrass between plants treated with the three *Trichoderma* isolates and control plants at the first and second count, or for final plant numbers (Table 5.1). The mixture of isolates, however, delayed emergence at the first count, but by the final count, the mixture of isolates had significantly ($P<0.05$) increased the number of plants compared with those in the untreated control.

Overall, perennial ryegrass treated with *Trichoderma* isolates produced higher shoot dry weight compared to control plants. At 52 DAS, only LU132 had significantly ($P<0.05$) increased the shoot dry weight (by 61%). At 104 DAS, cumulative shoot dry weight among treatments did not differ significantly. However, at 156 DAS, plants protected by the *Trichoderma* isolates LU132, LU140, LU584, and the mixture of isolates had produced a significantly ($P<0.05$) greater cumulative shoot dry weight than the control, with increases of 73%, 66%, 46%, and 68%, respectively (Figure 5.4).

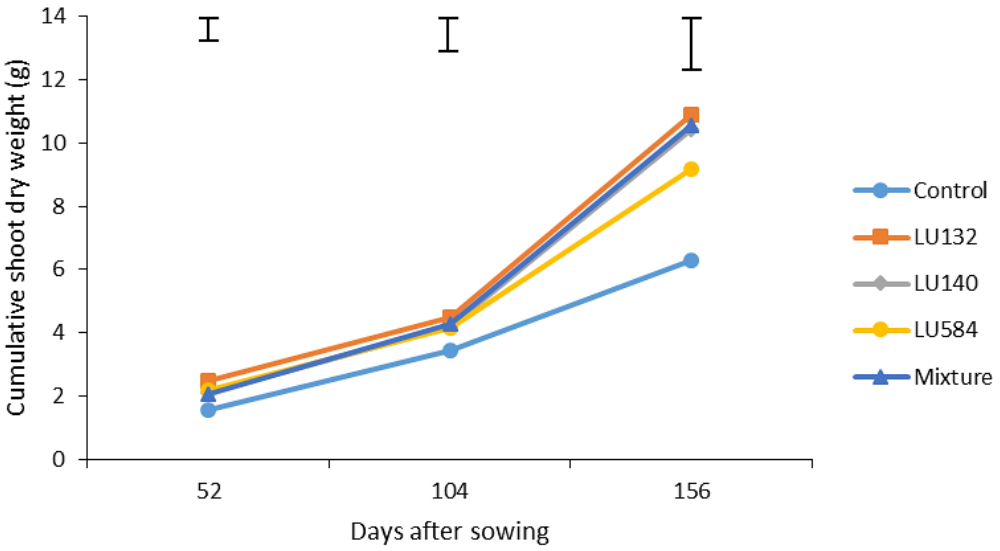


**Figure 5.4** **Cumulative shoot dry weight of perennial ryegrass grown in a *Ggt*-affected soil treated with different *Trichoderma* isolates. Error bars are the least significant difference (LSD) at the 5% level.**

*Trichoderma* application protected the plant root system from severe damage caused by *Ggt* infection as indicated by the increased root dry weight of treated plants (Table 5.1). However, differences were significant only in plants treated with LU140 and the mixture of isolates, which increased the root dry weight by 60% and 62%, respectively, over that of untreated plants ($P<0.05$). Nevertheless, isolates LU132 and LU584 had increased the root dry weight by 44% and 42%, respectively. There was a strong correlation ($R^2 = 0.87$) between the increased root dry weight and cumulative shoot dry weight (Figure 5.5).

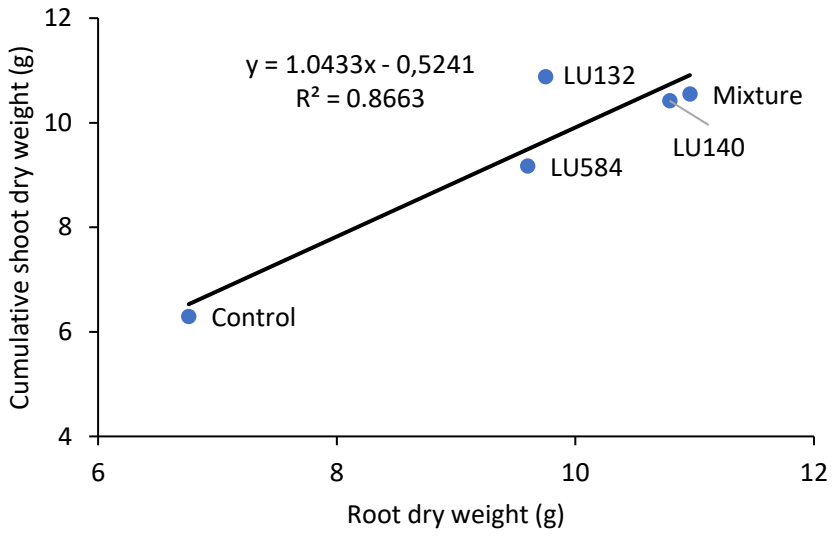


**Figure 5.5** **Correlation between cumulative shoot dry weight and root dry weight of ryegrass grown in a *Ggt*-affected soil.**

Root infection by *Ggt* was confirmed by the presence of the 'runner hyphae' and hyphodia of the pathogen in the microscope assessment (Figure 5.6a and Figure 5.6b). *Trichoderma* isolates significantly ($P$<0.05) reduced the root disease severity of treated plants (Figure 5.6c). From a root disease score of 3 in untreated control plants, root protection by *Trichoderma* LU132, LU140, LU584, and the mixture of isolates decreased the disease score to 1, 1, 1.75, and 1.12, respectively (Figure 5.7). Isolate LU584 was the least effective in protecting ryegrass from *Ggt* infection as indicated by the lowest cumulative shoot dry weight (9.17) and root dry weight (9.60), and the highest disease score (1.75) (Table 5.1). Shoot dry weight, root dry weight, and root disease severity for the mixture of isolates did not differ from that of the individual isolates. The root disease score was negatively correlated ($R^2$ = 0.89) with the root dry weight (Figure 5.8).

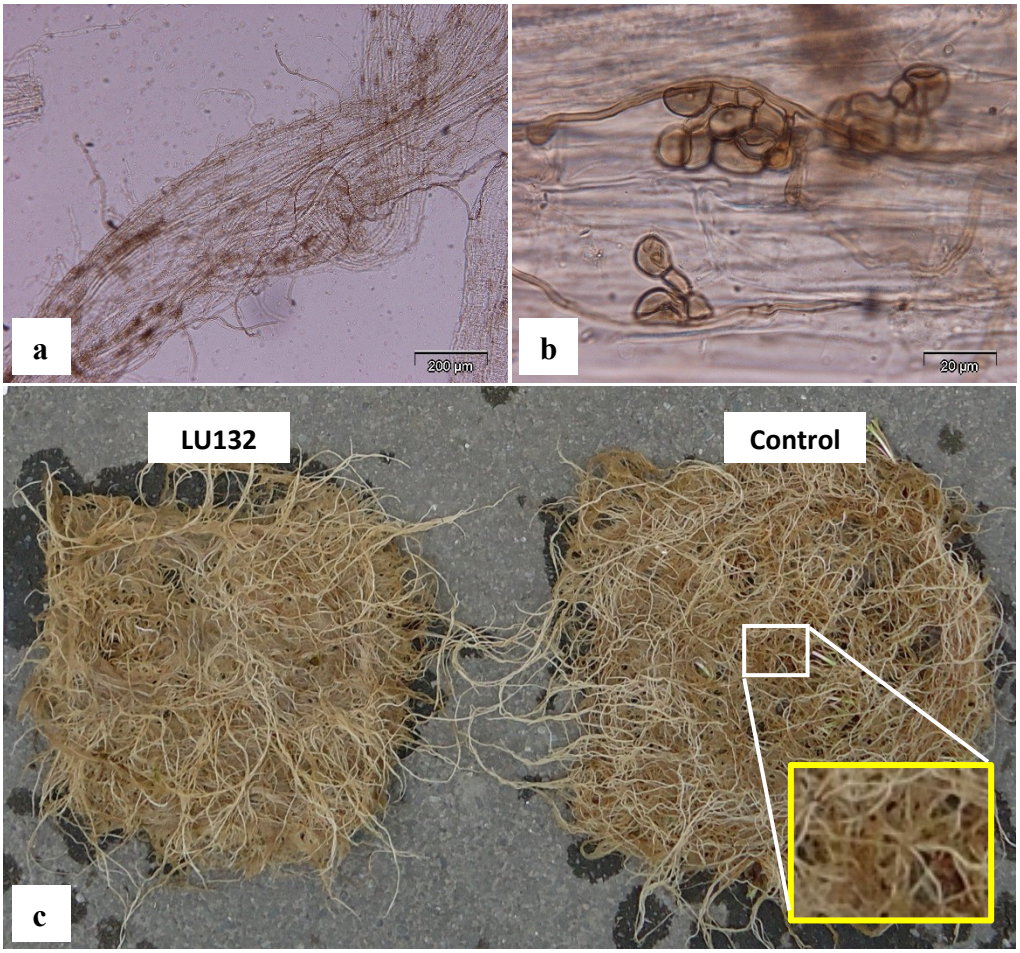


**Figure 5.6** **The runner hyphae (a) and hyphodia (b) of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) in ryegrass roots. [Images reproduced by kind permission of Diwakar Kandula]. The infected roots treated with *Trichoderma* LU132 was thicker (more root hairs) and had less blackened roots, a typical symptom of *Ggt*-infected roots, compared to the control where the roots were thinner because many of the root hairs were damaged by the pathogen (c). The blackened roots were very brittle and thinner because much of root hairs depleted (inset).**

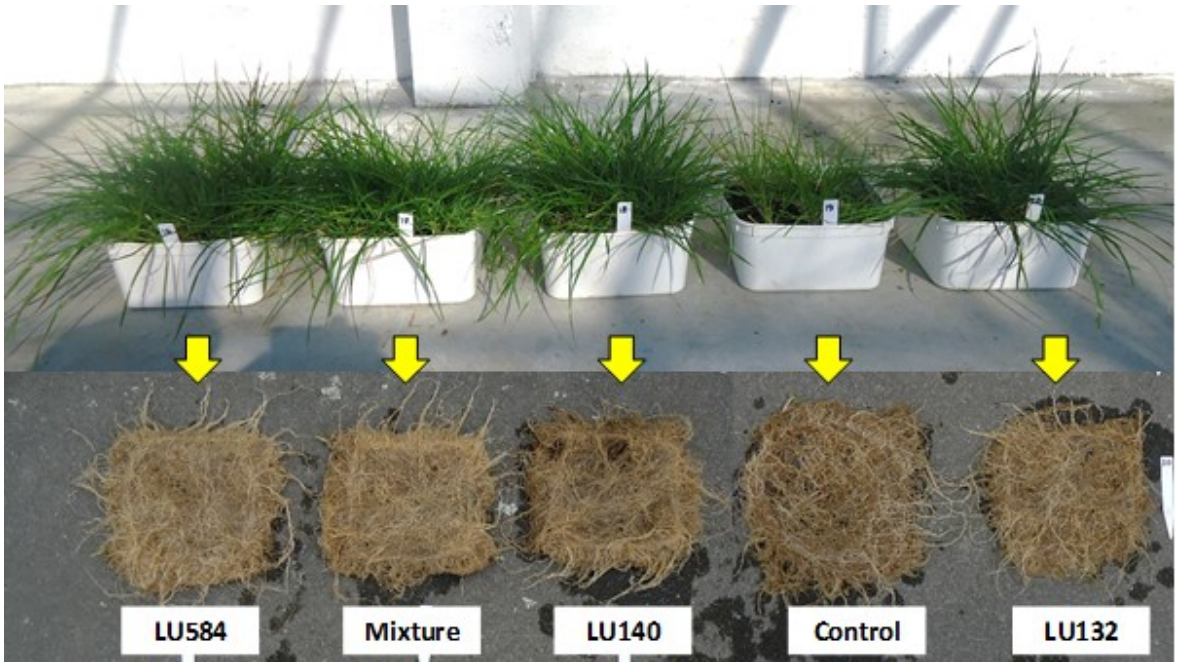


**Figure 5.7** **Effects of *Trichoderma* application on the shoot and root growth of perennial ryegrass grown in a *Ggt*-infected soil.**

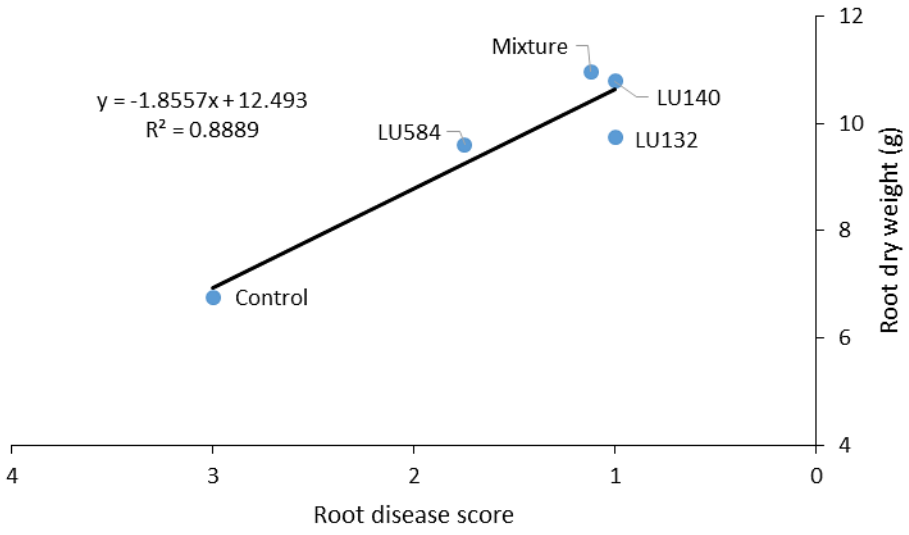


**Figure 5.8** **Correlation between root disease score and root dry weight of perennial ryegrass grown in *Ggt*-infected soil.**

**Table 5.1** **Main effect means of seedling emergence, cumulative shoot dry weight, root dry weight, and root disease severity of perennial ryegrass grown in a *Ggt*-affected soil treated with different *Trichoderma* isolates.**

| Main effect means | Seedling emergence (%) | | | Cumulative shoot dry weight (g) | | | Root dry weight (g) | Root disease Score |
|---|---|---|---|---|---|---|---|---|
| | 7 DAS | 9 DAS | 39 DAS | 52 DAS | 104 DAS | 156 DAS | | |
| *Trichoderma* treatments | | | | | | | | |
| Control | 62.2 | 80.7 | 85.7 | 1.55 | 3.45 | 6.29 | 6.76 | 3.00 |
| LU132 | 60.4 | 84.4 | 88.0 | 2.49 * | 4.49 | 10.88 * | 9.75 | 1.00 * |
| LU140 | 62.0 | 85.2 | 89.8 | 2.10 | 4.19 | 10.42 * | 10.79 * | 1.00 * |
| LU584 | 55.2 | 84.8 | 88.3 | 2.18 | 4.15 | 9.17 * | 9.60 | 1.75 * |
| Mixture | 49.0 * | 85.2 | 92.5 * | 2.08 | 4.26 | 10.55 * | 10.96 * | 1.12 * |
| LSD (5%) | 12.4 | 5.6 | 5.6 | 0.69 | 1.07 | 1.66 | 3.03 | 0.69 |

* indicates 5% significant difference from control plants in the same column.

## 5.5 Discussion

In the research reported in Chapter 2 and 3, take-all inoculated artificially into soil reduced prairie grass seed yield, and the introduction of *Trichoderma atroviride* isolates effectively reduced the root disease severity. The purpose of this study was to test the hypothesis that the mixture of isolates could deliver better biocontrol efficacy than any individual isolate in perennial ryegrass grown in a soil naturally infected with *Ggt*. The presence of *Ggt* in this soil was confirmed microscopically during the root disease assessment, showing the 'runner hyphae' and hyphodia of *Ggt* developed in the ryegrass roots (Figure 5.6a and Figure 5.6b). However, it is beyond the scope of this study to precisely verify the pathogen variety.

Overall, the results from the present experiment do not support the hypothesis that a combination of biocontrol agents can improve biocontrol efficacy and increase the consistency of growth promotion over that of individual isolates (Harman et al., 2004; Stewart, 2001; Stewart & Hill, 2014). The efficacy of the mixtures of isolates was only as good as the individual isolates in increasing shoot dry weight and reducing take-all severity. However, only the mixtures of isolates and LU140 produced a greater root dry weight than the control, which may indicate a greater ability to cope with adverse environmental conditions, such as drought (Harman et al., 2004). The mixture was also the only isolate that significantly increased the root dry weight of prairie grass (Chapter 3). A greater root development will give a significant impact on the plant's capacity to take up water and minerals (Marzec et al., 2015). Additionally, deeper roots could benefit the soil structure by increasing its steady-state carbon, water and nutrient retention, leading to more sustainable plant yields (Kell, 2011). It can thus be suggested that the mixture of isolates would have the potential to deliver a better result under particular circumstances, such as when environmental factors are limiting. Additionally, the use of multiple isolates/strains is expected to deliver a better result in specific regions, as has been suggested for biocontrol of cereal root diseases (Stewart, 2001). It was suggested that multiple strains could perform better, mainly because the BCAs can exhibit different or complementary modes of action to colonise the root microsystem (Whipps, 2001), and deliver a broader spectrum of biocontrol activity (Stewart & Hill, 2014).

*Trichoderma atroviride* isolates LU132, LU140 and L584 did not increase seedling emergence (Table 5.1). This result is consistent with that of the first experiment, where the three individual isolates did not affect the emergence of prairie grass seedlings (Chapter 2). However, at the final counting, the mixture of isolates had slightly increased plant survival. These results indicate that

these *Trichoderma* isolates did not harm seedling emergence. This is similar with that of Kandula et al. (2015) who reported *T. virens* isolate LU540 did not reduce ryegrass seedling emergence, and isolate LU132 and LU140 increased the seedling emergence in the presence of *R. solani*.

All *Trichoderma* treatments had significantly increased the production of shoot dry matter by 156 DAS. These results are in agreement with those obtained by Kandula et al. (2014) who reported a greater increase of cumulative shoot dry weight of *Ggt*-infected ryegrass treated with *T. atroviride* isolates over 215 DAS, compared to untreated plants. This also supports earlier studies which showed *Trichoderma* spp. promoted growth in numerous plant species. For example, *T. atroviride* increased both shoot and root growth in tomato (Gravel et al., 2007), oil seed rape (Maag et al., 2014), and enhanced rhizome biomass of the biofuel grass *Miscanthus* x *giganteus* (Chirino-Valle et al., 2016). The production of secondary metabolites by *Trichoderma* has been reported to play a role in plant growth promotion (Stewart & Hill, 2014). As reported by Vinale et al. (2008), *T. atroviride* P1, along with other *Trichoderma* strains, produces a series of major secondary metabolites proven to increase plant growth when the compounds were extracted and tested on wheat seedlings. Alternatively, growth promotion can be induced by phytohormones produced directly by the BCAs or released by the plant due to stimulation by the BCAs (Sofo et al., 2011). These authors reported that a change in phytohormone levels was the direct mechanism by which *T. harzianum* T22 promoted shoot and root growth of cherry rootstocks.

The increased production of the above-ground dry matter was correlated with the increased root dry weight (Figure 5.5), but a significant increase of root dry weight was found only in LU140- and the mixture of isolates-treated plants. These results indicate that an isolate of *Trichoderma* that produced the highest root mass did not necessarily become the best biocontrol agent or come with the highest above-ground dry matter. A similar finding was also observed by Boughalleb-M'Hamdi et al. (2018) who reported that watermelon seedlings, infected with *Fusarium solani*, and treated with *T. helicum*, produced the highest root dry weight, but had lower shoot dry weight compared to those inoculated with *Aspergillus flavus* that had lower root dry weight. Nonetheless, the *Trichoderma*-plant interaction in the present study did result in an increase in both shoot and root growth, which can deliver more benefits to the host plants. In the field, increased root growth means increased depth of rooting, which lead to increased drought tolerance and possibly resistance to compacted soils (Harman et al., 2004).

The *Trichoderma* isolates effectively reduced the root disease severity, most probably by colonising the plant root system and thus subsequently reducing the *Ggt* infection. *Trichoderma atroviride* isolates are known to be rhizosphere-competent strains and effective BCAs (Cripps-

Guazzone, 2014; Kandula et al., 2015). A rhizosphere-competent strain of *Trichoderma* can colonise the root surface and the outer layer of the cortex and subsequently establish a zone of interaction into which the BCA releases bioactive molecules (Harman et al., 2004). These include elicitors of resistance or enzymes that enhance plant resistance. In the presence of rhizosphere-competent strains, the infection by root pathogens is reduced using molecules and cell-wall alterations produced by the plant, after being induced by the BCA. Moreover, *Trichoderma* strains can attack the pathogen by producing sensing enzymes that release cell-wall fragments from the hyphae of the target pathogen. There is also a report that *T. harzianum* and *T. koningii* produced antibiotics to attack *Ggt* (Hornby et al., 1998). *Trichoderma* spp. may also suppress the pathogen through actual parasitism in which the BCA coils around the pathogen hyphae and produces a number of synergistic cell-wall-degrading enzymes, which eventually cause infection and death of the target fungus. As a consequence of *Trichoderma*-plant interactions, the infection of the plant by the root pathogen causes less disease when the roots are colonised by the BCA (Harman et al., 2004).

The reduction of root disease severity resulted in dramatic increases in root dry weight by as much as 42 to 62% (Figure 5.8). This increased root mass may be attributed to both direct suppression of *Ggt* by *Trichoderma* isolates, as indicated by lower disease scores, and direct growth promotion by the BCAs. *Trichoderma atroviride* isolates LU132, LU140 and LU584 have been previously reported to increase perennial ryegrass root weight (by 24 to 100%) in the absence of pathogen infection (Kandula et al., 2015), demonstrating the ability of the isolates to promote plant growth directly in a grass crop. *Trichoderma atroviride* and *T. virens* were reported to enhance root biomass of *Arabidopsis thaliana* by inducing lateral root growth (Contreras-Cornejo et al., 2009).

# Chapter 6

# Final Discussion and Conclusions

Take-all, caused by the fungal pathogen *Gaeumannomyces graminis* var. *tritici* (*Ggt*), is an important widespread root disease worldwide. The pathogen causes root damage in numerous grass species (Nilsson & Smith, 1981), and has been suspected as the main cause of the increasing occurrence of light seeds in ryegrass seed crops (Rolston, pers, comm, 2017). This study set out to investigate the effect of take-all on grass seed yield using prairie grass (*Bromus wildenowii*) as a model system. The study also examined the effects of interactions among the disease, biocontrol by *Trichoderma* and moisture stress. This chapter highlights some key points based on the results of two experiments undertaken, i.e. seed production in prairie grass and vegetative production in perennial ryegrass. Some suggestions for future work are also presented.

## 6.1 Take-all effects

This study has shown that take-all decreased the number of seeds per prairie grass plant by 14%, reduced total seed weight by 14%, and the weight of machine dressed seeds (MDS) by 13%. *Ggt* attack results in both a reduction in the root size and efficiency of water and nutrient uptake (Pillinger et al., 2005), and this is the most likely cause of the seed yield loss. Seed yield loss occurred mainly through a reduced number of seeds per plant, not a reduction in individual seed weight. Those losses mainly occurred in plants which received adequate nutrients, indicating the impact of *Ggt* on crops with high yield potential, without neglecting the fact that fertiliser application is one of the agronomic practices for alleviating the effect of take-all (Hornby et al., 1998). Conversely, seed yield reduction did not occur in plants with a reduced access to soil nutrients, suggesting the infection potential of *Ggt* might be decreased in nutrient stressed plants. The yield differences, however, could also be an artefact of the experimental setup since the nutrient stressed plants were more crowded and grown in more limited spaces than those with adequate nutrient availability. Hence, it is recommended to have the same number of plants as well as vary the position of the plants for the future experimental design. Take-all also resulted in a small (6%) reduction in germination percentage of seed produced. However as the germination of seed from all the treatments was poor (ca. 60%), this result was probably not biologically important.

The presence of *Ggt* inoculum in the soil had no effect on seedling emergence and tiller production of prairie grass. The infection by *Ggt* delayed the time to first seedhead emergence by

1.5 days, which may serve as an early indicator of the presence of the pathogen and/or potential yield loss for newly-sown grass seed crops. The infection reduced the relative water holding capacity of non-stressed, but not the moisture stressed plants, because drought is not conducive for take-all development, which would further impair plant water absorption. The infection also reduced the leaf chlorophyll content only during vegetative regrowth, not pre-seed harvest.

Take-all caused stunting during early vegetative growth (47 DAS), but this effect did not last as plants became reproductive. The disease did not reduce plant vegetative fresh weight or dry weight, which can be attributed to the plant's ability to recover, to limited growth spaces, and the shift of sugar utilisation from vegetative to reproductive growth. However, *Ggt* significantly reduced root dry weight.

## 6.2 *Trichoderma* effects

This study has identified that isolates LU132 and the mixture of isolates were more effective in reducing disease severity than LU584, which had greater cfu $g^{-1}$ of soil. Although inoculum density is an important indicator for biocontrol efficacy, increased *Trichoderma* population may not always be necessary (Hohmann et al., 2011).

Overall, *Trichoderma* isolates did not affect seedling emergence. *Trichoderma* isolates reduced the time to first seedhead emergence (TFSE), indicating the BCAs allowed the plant to recover their normal function early in the season. This recovery eventually contributed to increasing the seed yield as indicated in a strong correlation between the TFSE and seed yield. Under adequate nutrient availability, *Trichoderma* showed the potential to further increase the seed yield by allowing plants to produce more fertile tillers.

Generally, *Trichoderma* isolates increased all seed yield components, including the number of seeds, total seed weight, weight of MDS, and the TSW. However, the significances were influenced by moisture stress and *Ggt*, as well as the availability of soil nutrients. The increased seed yield mainly occurred in plants with greater nutrient availability, indicating the importance of soil nutrients for the optimum growth promotion by *Trichoderma*. Additionally, variability in the growth promotion effects can be attributed to crop type, growing conditions, inoculum type/formulation, and inoculum rate/concentration (Stewart & Hill, 2014).

*Trichoderma* isolates accelerated plant growth by increasing the plant height at 47 DAS, increased the shoot fresh weight and dry weight after seed harvest, and improved shoot dry weight during vegetative regrowth. The latter indicated that the benefits of rhizosphere-competent strains of

*Trichoderma* were still evident at least 7 months after their introduction. There was also an indication that *Trichoderma* isolates promoted the growth of prairie grass by increasing root absorptive capacity, instead of root development and size.

*Trichoderma* application enhanced the drought resistance of prairie grass by increasing the RWC under moisture stress. Isolate LU584 increased the chlorophyll content pre-harvest, indicating that the BCA can increase the plant's photosynthetic capacity. The increased uptake of soil minerals is the primary mechanism responsible for increasing chlorophyll content (Jyotsna et al., 2008). However, this ability is dependent on growth stage, *Trichoderma* strain/isolate, or plant species.

## 6.3 Moisture effects

Soil moisture is an important determinant of take-all development (Cook, 1981), and most microbes (Bailey & Melnick, 2013). Moisture stress reduced the overall root disease severity in prairie grass roots, but the water inside the plant roots remained available to support the development of *Ggt* during the periods of water stress. However, the stress did not affect the efficacy of *Trichoderma* isolates in reducing root disease severity.

These experiments confirmed that moisture stress affected almost every aspect of growth and yield of prairie grass, regardless of the plant's access to soil nutrients. Tillering of direct-sown plants was reduced by the moisture stress, but not in the transplanted plants. This may be because the larger transplanted plants, with a better competitive ability for nutrient resources in the pots, also had a larger root system, which meant water could be extracted from a greater depth in the soil. However, this was not measured. Other yield components decreased by the moisture stress were the number of fertile tillers (13%), the number of seeds per plant (32%), the seed weight per plant (41%), the weight of MDS (46%), and the TSW (11%). The loss occurred mainly through the reduction in the number of fertile tillers. The presence of light seeds was not the main cause of yield reduction by moisture stress, nor by *Ggt*. Moisture stress did not affect the germination viability of the seeds produced.

Moisture stress severely reduced all aspects of prairie grass growth, producing reductions in plant height (89%), shoot fresh weight (59%), shoot dry weight after seed harvest (88%), shoot dry weight of vegetative regrowth at first cut (64%), second cut (51%), and third cut (35%). However, the stress did not affect the root dry weight, which was likely related to the drought-tolerance traits of prairie grass.

## 6.4 Interaction responses

Take-all reduced the total seed weight (26%), the weight of MDS (25%), and TSW (5%) only in plants without *Trichoderma* treatments. Take-all combined with moisture stress caused further reduction in the weight of MDS (51%), as well as decreasing the other yield components. *Trichoderma* allowed increased seed yields only in the presence of *Ggt*, and a greater increase commonly occurred in stressed than well-watered plants. *Trichoderma*-plant interactions trigger the rapid production of defense-related enzymes when a pathogen attacks (Harman et al., 2004), and ameliorate the oxidative damage to plant cells caused by osmotic stress (Mastouri et al., 2010). These findings clearly indicate that these *Trichoderma* isolates promoted plant growth and increased yield under adverse environmental conditions, and can be reliable BCAs to control disease in dryland environments.

*Trichoderma* isolates also increased shoot dry weight, after seed harvest and during vegetative regrowth, but again only when *Ggt* was present. The shoot dry weight did not increase when the pathogen was absent, suggesting the growth promotion occurred as a result of biocontrol activity against *Ggt*. *Trichoderma* isolates promoted plant growth under both wet and dry conditions, but higher shoot dry weight and seed yield were produced by well-watered plants, emphasizing the importance of soil moisture for optimum bioactivity. Moisture stress greatly reduced the shoot and seed yields, whether the pathogen or *Trichoderma* was present or absent. This shows that soil moisture is the primary factor determining the result of *Trichoderma*-plant-pathogen interactions.

## 6.5 Pasture experiment

The main purpose of this study was to test the hypothesis that a combination of the three *Trichoderma* isolates will provide more effective disease control and generate higher production of DM, than any individual isolate, in perennial ryegrass grown in a soil naturally infected with *Ggt*.

In general, the present study does not support the hypothesis that a combination of BCAs can improve biocontrol efficacy over that of individual isolates (Harman et al., 2004; Stewart, 2001; Stewart & Hill, 2014). However, the mixtures of isolates and LU140 produced a greater root dry weight than the control, which could possibly give the plant a greater ability to cope with adverse environmental conditions than the two other isolates. In the field, greater root growth may lead to better drought tolerance and possibly resistance to compacted soils (Harman et al., 2004).

Results showed that the *Trichoderma* isolates used did not affect seedling emergence, which was consistent with that of the prairie grass experiment. All *Trichoderma* treatments had significantly

increased the production of shoot dry matter by 156 DAS. These results are in line with those obtained by Kandula et al. (2014) who reported increased cumulative shoot dry weight of ryegrass over 215 DAS, under similar conditions. The increased shoot dry weight was correlated with increased root dry weight, but a significant increase in root dry weight was found only in LU140- and the mixture of isolates-treated plants. These results were similar to the first experiment, where only the mixture of isolates significantly increased the root dry weight. All *Trichoderma* isolates also significantly reduced root disease severity.

The findings of this thesis could give valuable information for the New Zealand's grass seed industries. The results confirmed that take-all has caused a significant seed yield reduction of grass crops, and biological control using *Trichoderma* proven to be a reliable method to control the disease and increase the yield. Moreover, the results also indicate that the occurrence of yield reduction by take-all in the existing pastures could be more common than previously expected. This has been demonstrated in the ryegrass experiment, where much of the soil sample was taken from spots where plants were physically healthy. Yet, yield loss occurred in plants grown in those soils. Therefore, the findings could be used to help growers integrating *Trichoderma* in their crop management to avoid yield losses caused by take-all.

## 6.6 Recommendations for future research

This study has confirmed that the presence of *Ggt* caused substantial yield reduction in prairie grass seed yield and perennial ryegrass vegetative growth in the glasshouse experiments. Additionally, *Trichoderma* isolates was shown as reliable BCAs to control the disease and promote plant growth in these controlled environment conditions. Further research is needed before these results can be translated into practical solutions in the field.

1. *Field trials to confirm the biocontrol efficacy of Trichoderma isolates against Ggt in grass seed crops grown under natural conditions.*

   This could be done in the Greendale / Darfield area, where soil used for the pasture experiment was collected. Alternatively, trials could be done in other fields in which the presence of *Ggt* inoculum was detected (Chng, 2009). For comparison purposes, it is important to use the same *Trichoderma* formulation as used in the present study.

2. *Field trials in a grass seed crop affected by moisture stress, whether Ggt is present or absent, comparing individual Trichoderma isolates against the mixture of isolates.*

The mixture of isolates tended to produce a greater root dry weight under moisture stress conditions in both the prairie grass, as well as in the perennial ryegrass experiment, compared to at least some individual isolates. The mixture may deliver more benefits under dryland environments.

3. *Field trials in pastures when Ggt is present to confirm the ability of Trichoderma to increase pasture production.*

   *Trichoderma,* though controlling/reducing the impact of *Ggt* allowed increased root and vegetative tiller production. *Trichoderma* could be overdrilled as prills into existing pasture, and exclusion cages used to allow assessment of pasture production (yield and quality).

## 6.7 Concluding remarks

This study has enhanced our understanding of the take-all effects on various aspects of growth and yield of grass seed crops, including seed yield. It has also shown the positive influence of *Trichoderma* and significant effect of moisture stress on the plant – pathogen interactions. This study, therefore, provided a good prospect on the use of *Trichoderma* as biological control of take-all, in both pastures and grass seed crops, under both limited and adequate soil moisture conditions. Apart from that, soil nutrient availability and plant competition for growth space have been identified as potential factors to affect the interaction. Further work that more closely examines the effect of the two variables on take-all development is likely to be useful to extend the work presented here.

**Appendix 1. Mineral and nutrient status of the growth medium for the prairie grass experiment. Analysis carried out by RJ Hill Laboratories Limited, New Zealand.**

**Sample Name:** Lincoln Soil 17/12 (PNV) **Lab Number:** 1900613.1
**Sample Type:** SOIL Mixed Pasture (S1)

| Analysis | | Level Found | Medium Range | Low | Medium | High |
|---|---|---|---|---|---|---|
| pH | pH Units | 5.6 | 5.8 - 6.2 | | | |
| Resin P | mg/kg | 34 | 40 - 75 | | | |
| Olsen Phosphorus | mg/L | 24 | 20 - 30 | | | |
| Anion Storage Capacity* | % | 15 | | | | |
| Potassium | me/100g | 0.46 | 0.40 - 0.60 | | | |
| Calcium | me/100g | 6.8 | 4.0 - 10.0 | | | |
| Magnesium | me/100g | 0.72 | 1.00 - 1.60 | | | |
| Sodium | me/100g | 0.17 | 0.20 - 0.50 | | | |
| CEC | me/100g | 14 | 12 - 25 | | | |
| Total Base Saturation | % | 59 | 50 - 85 | | | |
| Volume Weight | g/mL | 1.07 | 0.60 - 1.00 | | | |
| Sulphate Sulphur | mg/kg | 5 | 10 - 12 | | | |
| Extractable Organic Sulphur* | mg/kg | 3 | 15 - 20 | | | |
| Total Carbon* | % | 2.5 | | | | |
| Total Nitrogen* | % | 0.23 | 0.30 - 0.60 | | | |
| C/N Ratio* | | 10.9 | | | | |
| 'Total' Phosphorus | mg/kg | 632 | 700 - 1600 | | | |
| 'Total' Sulphur* | mg/kg | 344 | 600 - 1000 | | | |
| Base Saturation % | | K 3.4 | Ca 50 | Mg 5.3 | Na 1.3 | |
| MAF Units | | K 10 | Ca 9 | Mg 17 | Na 8 | |

The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information. IANZ Accreditation does not apply to comments and interpretations, i.e. the 'Range Levels' and subsequent graphs.

## Appendix 2. Seed Moisture Content (SMC) Assessment

*Date: 18/7/2019*

| Sample | Container (g) | Container + Seeds (g) | Container + Dried Seeds (g) | SMC (%) |
|---|---|---|---|---|
| | *(M1)* | *(M2)* | *(M3)* | |
| 1 | 22.700 | 27.210 | 26.830 | **8.426** |
| 2 | 22.690 | 27.200 | 26.820 | **8.426** |

$$SMC\ (\%) = \frac{M_2 - M_3}{M_2 - M_1}\ x\ 100$$

## Appendix 3. Additional documentation of some activities in the experiments.

**Figure A1.** Germination test for the prairie grass seed lot. The non-germinated seeds (red arrows) were commonly infected by seed-borne fungi, including *Alternaria* spp., *Fusarium* spp., *Aspergillus* spp. (*A. niger*). [27.12.2017].

**Figure A2.** Seven-day-old prairie grass seedlings during the germination test. [27.12.2017].

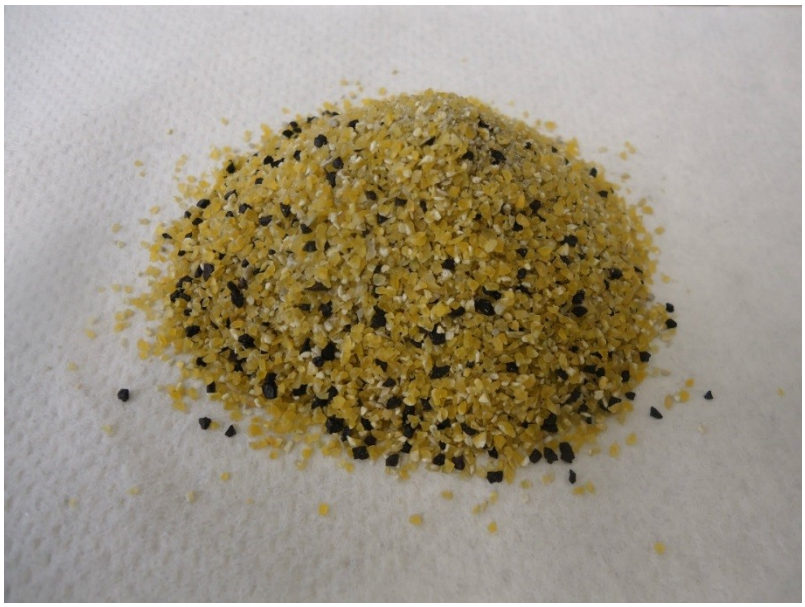

**Figure A3.** The prill formulation of *Trichoderma atroviride* isolates used in the experiments, provided by Agrimm Technologies Ltd. (Lincoln).

**Figure A4.** Supervisor visits in the prairie grass experiments at 49 DAS (left) and 71 DAS (right).

## Appendix 4. Anovas containing significant interactions for the prairie grass experiment

Variate: total seed weight of transplanted plants

| Source of variation | d.f. | s.s. | m.s. | F | p value | |
|---|---|---|---|---|---|---|
| Block.Mainplot stratum | | | | | | |
| Block | 3 | 0.5777 | 0.1926 | 0.25 | 0.857 | |
| Moisture | 1 | 42.8479 | 42.8479 | 55.95 | 0.005 | ** |
| Residual | 3 | 2.2976 | 0.7659 | 1.98 | | |
| Block.Mainplot.Subplot stratum | | | | | | |
| Pathogen | 1 | 3.5267 | 3.5267 | 9.12 | 0.023 | * |
| Moisture.Pathogen | 1 | 0.0040 | 0.0040 | 0.01 | 0.923 | ns |
| Residual | 6 | 2.3199 | 0.3867 | 3.06 | | |
| Block.Mainplot.Subplot.*Units* stratum | | | | | | |
| Trichoderma | 4 | 3.4323 | 0.8581 | 6.79 | <.001 | ** |
| Moisture.Trichoderma | 4 | 0.4370 | 0.1093 | 0.86 | 0.488 | ns |
| Pathogen.Trichoderma | 4 | 2.1248 | 0.5312 | 4.20 | 0.003 | ** |
| Moisture.Pathogen.Trichoderma | 4 | 1.0346 | 0.2587 | 2.05 | 0.093 | ns |
| Residual | 112 | 14.1593 | 0.1264 | | | |
| Total | 143 | 72.7619 | | | | |

Variate: total weight of light seeds of transplanted plants

| Source of variation | d.f. | s.s. | m.s. | F | p value | |
|---|---|---|---|---|---|---|
| Block.Mainplot stratum | | | | | | |
| Block | 3 | 0.145023 | 0.048341 | 1.77 | 0.326 | |
| Moisture | 1 | 0.186696 | 0.186696 | 6.82 | 0.080 | ns |
| Residual | 3 | 0.082105 | 0.027368 | 0.75 | | |
| Block.Mainplot.Subplot stratum | | | | | | |
| Pathogen | 1 | 0.078838 | 0.078838 | 2.17 | 0.191 | ns |
| Moisture.Pathogen | 1 | 0.039083 | 0.039083 | 1.08 | 0.340 | ns |
| Residual | 6 | 0.218074 | 0.036346 | 3.84 | | |
| Block.Mainplot.Subplot.*Units* stratum | | | | | | |
| Trichoderma | 4 | 0.203655 | 0.050914 | 5.37 | <.001 | ** |
| Moisture.Trichoderma | 4 | 0.102115 | 0.025529 | 2.69 | 0.034 | * |
| Pathogen.Trichoderma | 4 | 0.060634 | 0.015158 | 1.60 | 0.179 | ns |
| Moisture.Pathogen.Trichoderma | 4 | 0.041757 | 0.010439 | 1.10 | 0.359 | ns |
| Residual | 112 | 1.061145 | 0.009475 | | | |
| Total | 143 | 2.219125 | | | | |

Variate: Machine dressed seed weight of transplanted plants

| Source of variation | d.f. | s.s. | m.s. | F | p value | |
|---|---|---|---|---|---|---|
| Block.Mainplot stratum | | | | | | |
| Block | 3 | 0.7766 | 0.2589 | 0.30 | 0.823 | |
| Moisture | 1 | 48.6913 | 48.6913 | 57.10 | 0.005 | ** |
| Residual | 3 | 2.5583 | 0.8528 | 3.45 | | |
| Block.Mainplot.Subplot stratum | | | | | | |
| Pathogen | 1 | 2.5510 | 2.5510 | 10.33 | 0.018 | * |
| Moisture.Pathogen | 1 | 0.0181 | 0.0181 | 0.07 | 0.796 | ns |
| Residual | 6 | 1.4817 | 0.2470 | 2.25 | | |
| Block.Mainplot.Subplot.*Units* stratum | | | | | | |
| Trichoderma | 4 | 2.2832 | 0.5708 | 5.21 | <.001 | ** |
| Moisture.Trichoderma | 4 | 0.2765 | 0.0691 | 0.63 | 0.641 | ns |
| Pathogen.Trichoderma | 4 | 1.8243 | 0.4561 | 4.16 | 0.004 | ** |
| Moisture.Pathogen.Trichoderma | 4 | 0.8216 | 0.2054 | 1.88 | 0.120 | ns |
| Residual | 112 | 12.2690 | 0.1095 | | | |
| Total | 143 | 73.5516 | | | | |

Variate: Thousand seed weight (TSW) of transplanted plants

| Source of variation | d.f. | s.s. | m.s. | F | p value | |
|---|---|---|---|---|---|---|
| Block.Mainplot stratum | | | | | | |
| Block | 3 | 1.8883 | 0.6294 | 1.59 | 0.357 | |
| Moisture | 1 | 49.7417 | 49.7417 | 125.39 | 0.002 | ** |
| Residual | 3 | 1.1901 | 0.3967 | 1.36 | | |
| Block.Mainplot.Subplot stratum | | | | | | |
| Pathogen | 1 | 0.0921 | 0.0921 | 0.32 | 0.594 | ns |
| Moisture.Pathogen | 1 | 0.0330 | 0.0330 | 0.11 | 0.748 | ns |
| Residual | 6 | 1.7483 | 0.2914 | 1.30 | | |
| Block.Mainplot.Subplot.*Units* stratum | | | | | | |
| Trichoderma | 4 | 1.2582 | 0.3145 | 1.40 | 0.238 | ns |
| Moisture.Trichoderma | 4 | 1.6340 | 0.4085 | 1.82 | 0.130 | ns |
| Pathogen.Trichoderma | 4 | 1.6928 | 0.4232 | 1.89 | 0.118 | ns |
| Moisture.Pathogen.Trichoderma | 4 | 2.9501 | 0.7375 | 3.29 | 0.014 | * |
| Residual | 112 | 25.1280 | 0.2244 | | | |
| Total | 143 | 87.3566 | | | | |

Variate: Shoot dry weight of transplanted plants after seed harvest

| Source of variation | d.f. | s.s. | m.s. | F | p value | |
|---|---|---|---|---|---|---|
| Block.Mainplot stratum | | | | | | |
| Block | 3 | 10.8459 | 3.6153 | 9.33 | 0.050 | |
| Moisture | 1 | 39.5012 | 39.5012 | 101.93 | 0.002 | ** |
| Residual | 3 | 1.1625 | 0.3875 | 0.65 | | |
| Block.Mainplot.Subplot stratum | | | | | | |
| Pathogen | 1 | 0.6546 | 0.6546 | 1.09 | 0.336 | ns |
| Moisture.Pathogen | 1 | 0.1458 | 0.1458 | 0.24 | 0.639 | ns |
| Residual | 6 | 3.5905 | 0.5984 | 1.93 | | |
| Block.Mainplot.Subplot.*Units* stratum | | | | | | |
| Trichoderma | 4 | 4.6905 | 1.1726 | 3.78 | 0.006 | ** |
| Moisture.Trichoderma | 4 | 1.8388 | 0.4597 | 1.48 | 0.213 | ns |
| Pathogen.Trichoderma | 4 | 7.0327 | 1.7582 | 5.66 | <.001 | ** |
| Moisture.Pathogen.Trichoderma | 4 | 1.6229 | 0.4057 | 1.31 | 0.272 | ns |
| Residual | 112 | 34.7663 | 0.3104 | | | |
| Total | 143 | 105.8517 | | | | |

Variate: Shoot dry weight per plant of direct-sown plants after seed harvest

| Source of variation | d.f. | s.s. | m.s. | F | p value | |
|---|---|---|---|---|---|---|
| Block.Mainplot stratum | | | | | | |
| Block | 3 | 0.19740 | 0.06580 | 1.52 | 0.370 | |
| Moisture | 1 | 3.45515 | 3.45515 | 79.75 | 0.003 | ** |
| Residual | 3 | 0.12997 | 0.04332 | 3.80 | | |
| Block.Mainplot.Subplot stratum | | | | | | |
| Pathogen | 1 | 0.26906 | 0.26906 | 23.61 | 0.003 | ** |
| Moisture.Pathogen | 1 | 0.00318 | 0.00318 | 0.28 | 0.616 | ns |
| Residual | 6 | 0.06839 | 0.01140 | 0.72 | | |
| Block.Mainplot.Subplot.*Units* stratum | | | | | | |
| Trichoderma | 4 | 0.18103 | 0.04526 | 2.85 | 0.027 | * |
| Moisture.Trichoderma | 4 | 0.10291 | 0.02573 | 1.62 | 0.175 | ns |
| Pathogen.Trichoderma | 4 | 0.21132 | 0.05283 | 3.32 | 0.013 | * |
| Moisture.Pathogen.Trichoderma | 4 | 0.03250 | 0.00813 | 0.51 | 0.728 | ns |
| Residual | 112 | 1.78072 | 0.01590 | | | |
| Total | 143 | 6.43163 | | | | |

Variate: Shoot dry weight (g) of transplanted plants (3rd regrowth)

| Source of variation | d.f. | s.s. | m.s. | F | p value | |
|---|---|---|---|---|---|---|
| Block.Mainplot stratum | | | | | | |
| Block | 3 | 0.16207 | 0.05402 | 0.57 | 0.674 | |
| Moisture | 1 | 0.51600 | 0.51600 | 5.41 | 0.103 | ns |
| Residual | 3 | 0.28620 | 0.09540 | 2.86 | | |
| Block.Mainplot.Subplot stratum | | | | | | |
| Pathogen | 1 | 0.00001 | 0.00001 | 0.00 | 0.984 | ns |
| Moisture.Pathogen | 1 | 0.03115 | 0.03115 | 0.93 | 0.371 | ns |
| Residual | 6 | 0.20028 | 0.03338 | 2.92 | | |
| Block.Mainplot.Subplot.*Units* stratum | | | | | | |
| Trichoderma | 4 | 0.26061 | 0.06515 | 5.71 | <.001 | ** |
| Moisture.Trichoderma | 4 | 0.17076 | 0.04269 | 3.74 | 0.007 | ** |
| Pathogen.Trichoderma | 4 | 0.13968 | 0.03492 | 3.06 | 0.020 | * |
| Moisture.Pathogen.Trichoderma | 4 | 0.09181 | 0.02295 | 2.01 | 0.098 | ns |
| Residual | 112 | 1.27846 | 0.01141 | | | |
| Total | 143 | 3.13703 | | | | |

Variate: Shoot dry weight of direct-sown plants (3rd regrowth)

| Source of variation | d.f. | s.s. | m.s. | F | p value | |
|---|---|---|---|---|---|---|
| Block.Mainplot stratum | | | | | | |
| Block | 3 | 0.012880 | 0.004293 | 1.05 | 0.484 | |
| Moisture | 1 | 0.135119 | 0.135119 | 33.09 | 0.010 | * |
| Residual | 3 | 0.012249 | 0.004083 | 0.76 | | |
| Block.Mainplot.Subplot stratum | | | | | | |
| Pathogen | 1 | 0.020574 | 0.020574 | 3.85 | 0.097 | ns |
| Moisture.Pathogen | 1 | 0.008764 | 0.008764 | 1.64 | 0.248 | ns |
| Residual | 6 | 0.032058 | 0.005343 | 2.73 | | |
| Block.Mainplot.Subplot.*Units* stratum | | | | | | |
| Trichoderma | 4 | 0.035399 | 0.008850 | 4.52 | 0.002 | ** |
| Moisture.Trichoderma | 4 | 0.025293 | 0.006323 | 3.23 | 0.015 | * |
| Pathogen.Trichoderma | 4 | 0.021932 | 0.005483 | 2.80 | 0.029 | * |
| Moisture.Pathogen.Trichoderma | 4 | 0.026837 | 0.006709 | 3.43 | 0.011 | * |
| Residual | 112 | 0.219237 | 0.001957 | | | |
| Total | 143 | 0.550342 | | | | |

Variate: Root disease score under microscope

| Source of variation | d.f. | (m.v.) | s.s. | m.s. | F | p value | |
|---|---|---|---|---|---|---|---|
| Block.Mainplot stratum | | | | | | | |
| Block | 3 | | 0.4648 | 0.1549 | 0.87 | 0.546 | |
| Moisture | 1 | | 0.0515 | 0.0515 | 0.29 | 0.629 | ns |
| Residual | 3 | | 0.5367 | 0.1789 | 0.92 | | |
| Block.Mainplot.Subplot stratum | | | | | | | |
| Pathogen | 1 | | 94.3021 | 94.3021 | 487.49 | <.001 | *** |
| Moisture.Pathogen | 1 | | 0.1368 | 0.1368 | 0.71 | 0.433 | ns |
| Residual | 6 | | 1.1607 | 0.1934 | 0.67 | | |
| Block.Mainplot.Subplot.*Units* stratum | | | | | | | |
| Trichoderma | 4 | | 63.2649 | 15.8162 | 54.59 | <.001 | *** |
| Moisture.Trichoderma | 4 | | 1.8575 | 0.4644 | 1.60 | 0.179 | ns |
| Pathogen.Trichoderma | 4 | | 24.3096 | 6.0774 | 20.98 | <.001 | *** |
| Moisture.Pathogen.Trichoderma | 4 | | 0.3148 | 0.0787 | 0.27 | 0.896 | ns |
| Residual | 110 | (2) | 31.8711 | 0.2897 | | | |
| Total | 141 | (2) | 214.2887 | | | | |

## Appendix 5. Treatment means of some parameters of the prairie grass experiment, referenced in the discussion sections.

### 5.1 Seedling emergence (%).

| | Control | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (P+)*** | | | | | |
| Non-stressed | 93.59 | 96.88 | 93.75 | 92.01 | 90.62 |
| Stressed | - | - | - | - | - |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 4.17 | | | | |
| Comparison within rows (Treated vs Treated) | 4.81 | | | | |
| ***Un-inoculated (P-)*** | | | | | |
| Non-stressed | 96.25 | 92.50 | 96.25 | 97.50 | 90.00 * |
| Stressed | - | - | - | - | - |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 5.89 | | | | |
| Comparison within rows (Treated vs Treated) | 6.80 | | | | |

* indicates 5% significant difference from control. - signs indicate data were not included in the analysis (moisture treatment was not yet applied).

### 5.2 Time to first seedhead emergence (TFSE) (days).

| | Control | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (P+)*** | | | | | |
| Non-stressed | 47.66 | 45.25 * | 45.50 * | 45.88 * | 47.31 |
| Stressed | - | - | - | - | - |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 1.13 | | | | |
| Comparison within rows (Treated vs Treated) | 1.30 | | | | |
| ***Un-inoculated (P-)*** | | | | | |
| Non-stressed | 44.81 | 44.50 | 44.50 | 45.62 | 46.00 |
| Stressed | - | - | - | - | - |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 1.60 | | | | |
| Comparison within rows (Treated vs Treated) | 1.84 | | | | |

### 5.3 Shoot dry weight (g/plant) of transplanted plants after seed harvest.

| | Control | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (P+)*** | | | | | |
| Non-stressed | 3.25 | 3.83 * | 4.33 * | 4.05 * | 4.19 * |
| Stressed | 2.51 | 3.16 * | 3.08 * | 3.01 * | 2.61 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.48 | | | | |
| Comparison within rows (Treated vs Treated) | 0.55 | | | | |
| ***Un-inoculated (P-)*** | | | | | |
| Non-stressed | 4.30 | 3.99 | 3.81 | 4.00 | 3.77 |
| Stressed | 2.92 | 3.12 | 2.39 | 3.24 | 2.77 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.68 | | | | |
| Comparison within rows (Treated vs Treated) | 0.78 | | | | |

### 5.4 Shoot dry weight (g/plant) of direct-sown plants after seed harvest.

| | Control | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (P+)*** | | | | | |
| Non-stressed | 3.25 | 3.83 * | 4.33 * | 4.05 * | 4.19 * |
| Stressed | 2.51 | 3.16 * | 3.08 * | 3.01 * | 2.61 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.48 | | | | |
| Comparison within rows (Treated vs Treated) | 0.55 | | | | |
| ***Un-inoculated (P-)*** | | | | | |
| Non-stressed | 4.30 | 3.99 | 3.81 | 4.00 | 3.77 |
| Stressed | 2.92 | 3.12 | 2.39 | 3.24 | 2.77 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.68 | | | | |
| Comparison within rows (Treated vs Treated) | 0.78 | | | | |

### 5.5 Shoot dry weight (g/plant) of transplanted plants (1st regrowth).

| | Control | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (P+)*** | | | | | |
| Non-stressed | 0.73 | 0.93 * | 1.01 * | 1.01 * | 1.08 * |
| Stressed | 0.25 | 0.42 * | 0.46 * | 0.43 * | 0.38 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.15 | | | | |
| Comparison within rows (Treated vs Treated) | 0.17 | | | | |
| ***Un-inoculated (P-)*** | | | | | |
| Non-stressed | 0.90 | 0.96 | 0.98 | 0.98 | 0.95 |
| Stressed | 0.28 | 0.26 | 0.25 | 0.29 | 0.27 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.21 | | | | |
| Comparison within rows (Treated vs Treated) | 0.24 | | | | |

### 5.6 Shoot dry weight (g/plant) of transplanted plants (2nd regrowth).

| | Control | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (P+)*** | | | | | |
| Non-stressed | 0.47 | 0.81 * | 0.95 * | 0.90 * | 1.01 * |
| Stressed | 0.29 | 0.39 | 0.40 | 0.39 | 0.37 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.16 | | | | |
| Comparison within rows (Treated vs Treated) | 0.18 | | | | |
| ***Un-inoculated (P-)*** | | | | | |
| Non-stressed | 0.66 | 0.63 | 0.72 | 0.69 | 0.60 |
| Stressed | 0.37 | 0.37 | 0.32 | 0.43 | 0.38 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.22 | | | | |
| Comparison within rows (Treated vs Treated) | 0.25 | | | | |

## 5.7 Shoot dry weight (g/plant) of transplanted plants (3rd regrowth).

| | Control | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (P+)*** | | | | | |
| Non-stressed | 0.20 | 0.34 * | 0.44 * | 0.42 * | 0.50 * |
| Stressed | 0.18 | 0.25 | 0.24 | 0.19 | 0.21 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.09 | | | | |
| Comparison within rows (Treated vs Treated) | 0.11 | | | | |
| ***Un-inoculated (P-)*** | | | | | |
| Non-stressed | 0.32 | 0.31 | 0.35 | 0.33 | 0.29 |
| Stressed | 0.25 | 0.23 | 0.22 | 0.24 | 0.26 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.13 | | | | |
| Comparison within rows (Treated vs Treated) | 0.15 | | | | |

## 5.8 Total seed weight (g/plant) of transplanted plants.

| | Control | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (P+)*** | | | | | |
| Non-stressed | 2.23 | 2.76 * | 2.65 * | 2.81 * | 2.76 * |
| Stressed | 1.20 | 1.74 * | 1.72 * | 1.69 * | 1.40 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.31 | | | | |
| Comparison within rows (Treated vs Treated) | 0.35 | | | | |
| ***Un-inoculated (P-)*** | | | | | |
| Non-stressed | 3.01 | 2.73 | 3.00 | 3.01 | 2.76 |
| Stressed | 1.80 | 2.14 | 1.44 | 1.96 | 1.74 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 0.43 | | | | |
| Comparison within rows (Treated vs Treated) | 0.50 | | | | |

## 5.9 Root dry weight (g) of prairie grass.

| | Control | LU132 | LU140 | LU584 | Mixture |
|---|---|---|---|---|---|
| ***Take-all inoculated (P+)*** | | | | | |
| Non-stressed | 4.54 | 5.11 | 4.26 | 5.47 | 5.42 |
| Stressed | 5.73 | 6.42 | 6.27 | 6.04 | 6.58 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 1.00 | | | | |
| Comparison within rows (Treated vs Treated) | 1.16 | | | | |
| ***Un-inoculated (P-)*** | | | | | |
| Non-stressed | 7.01 | 7.95 | 8.68 * | 6.85 | 6.80 |
| Stressed | 6.96 | 6.05 | 7.11 | 6.64 | 7.41 |
| LSD (5%) | | | | | |
| Comparison within rows (Control vs Treated) | 1.42 | | | | |
| Comparison within rows (Treated vs Treated) | 1.64 | | | | |

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