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Allelopathic interactions between wheat, selected crop species and the weed *Lolium multiflorum* x *perenne*

by

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CONTENTS

I, Michael Ignatius Ferreira declare that the thesis/dissertation, which I hereby submit for the degree Ph D: Agronomy at the University of Pretoria, is my own work, except where acknowledged, and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: .....…………………………..
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Allelopathic interactions between wheat, selected crop species and the weed *Lolium multiflorum* x *perenne*

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ABSTRACT

No information is available on the role of allelopathy in crop rotation systems of the Western Cape Province of South Africa, where more than 100 000 ha are under threat from herbicide-resistant rye grass. A study which investigated the use of allelopathic properties for the suppression of rye grass hybrid type *(Lolium multiflorum* x *perenne)* was undertaken. These objectives were accomplished by: a) exploring the use of allelopathic properties of crop residues for rye grass suppression; b) evaluation of the role of allelopathy from seeds, seedlings, roots and above-ground plant material of rotational crops; c) assessing the distribution of genetic and morphological variability of rye grass and d) determining the interactions among micro-organisms and allelopathic root leachates from rotational crops and rye grass. In the field trials, growth inhibitory or stimulatory effects were observed on crops exposed to the residues of others. Medic suppressed the weed type rye grass. The radicle length of rye grass was inhibited by seed leachates from wheat and lupine. Growth inhibition from lupine seed and seedling leachates was evident in rye grass radicle length and cumulative germination percentage. Morphologically, 50% of the total number of specimens was classified as rigid rye grass, 48% as the hybrid, namely *L. multiflorum* x *perenne* and 2% as perennial rye grass. The wide genetic and morphological variation detected in rye grass may be due to high genotypic plasticity and hybridisation for producing the weed type *L. multiflorum x perenne*. The faster growth rate of rye grass on Langgewens soil treated with barley root leachates was revealed by Principal Component Analysis (PCA) as a probable association with growth-promoting soil micro-organisms. Crop cultivars and weeds may modify the soil micro-organism populations to their advantage and to the disadvantage of other species by the release of root exudates that apparently differ in composition between plant species. The effect on microbial communities varied with source of exudates and between soils.

Allelopatiese wisselwerking tussen koring, gekose gewasspesies en die onkruid *Lolium multiflorum* x *perenne*

deur

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UITTREKSEL

Daar bestaan geen inligting oor die rol van allelopatie in wisselboustelsels van die Wes-Kaap Provinsie in Suid-Afrika, waar meer as 100 000 ha bedreig word deur raaigras met weerstand teen onkruiddoders, nie. ’n Studie wat die gebruik van allelopatiese eienskappe vir die onderdrukking van onkruidtipe hibriediese raaigras *(Lolium multiflorum* x *perenne)* ondersoek het, was onderneem. Hierdie doelwitte is bereik deur: a) ’n ondersoek na die gebruik van allelopatiese eienskappe van gewasreste vir raaigrasonderdrukking; b) evaluasie van die rol van die allelopatie van saad, saailinge, wortels en plantmateriaal van die bogrondse dele van wisselbougewasse; c) assessering van die verspreiding van genetiese en morfologiese veranderlikheid van raaigras en d) bepaling van die wisselwerking tussen mikroörganismes en allelopatiese wortelloog van wisselbougewasse en raaigras. In die veldproewe is groei-inhiberende of stimulerende invloede op gewasse wat blootgestel was aan ander gewasreste, waargeneem. Medic het die onkruidtipe-raaigras onderdruk. Saadloog van koring en lupiene het die lengte van raaigras se kiemwortel geïnhibeer. Raaigras se groei is ook geïnhibeer deur lupiensaad en lupiensaailingloog, soos waargeneem kon word in kiemwortellengte en kumulatiewe ontkiemingspersentasie. Morfologies was 50% van die totale aantal plantmonsters geklassifiseer as raaigras *(Lolium rigidum)*, 48% as ‘n hibried, naamlik *L. multiflorum* x *perenne* en 2% as meerjarige raaigras. Die wye genetiese en morfologiese variasie wat vir raaigras waargeneem is, mag weens hoë genotipiese plastiesiteit en verbastering wees om die onkruidtipe *L. multiflorum x perenne* te vorm. Die vinniger groeitempo van raaigras op grond van Langgewens en wat met garsloog behandel was, is deur Prinsipiële Komponent Analise (PKA) onthul as ’n moontlike assosiasie met grond-mikroörganismes wat groei bevorder. Gewaskultivars en onkruide kan die grondmikroörganisme-populasies tot hul voordeel en tot die nadeel van ander spesies modifiseer, deur die vrystelling van wortelafskeidings waarvan die samestelling blykbaar tussen spesies verskil. Hierdie invloed op mikroörganisme-gemeenskappe het varieër met bron van afskeiding en tussen grondsoorte.

INTRODUCTION

A weed is a plant growing where it is not desired, or a plant out of place (Ashton & Monaco, 1991). Weeds are diverse in their habit and habitats throughout the world. Although they account for not more than 1% of the total plant species on earth, they cause great problems nevertheless to humankind by interfering in food production, health, economic stability, and welfare (Qasem & Foy, 2001). In agriculture, weeds are of concern because they compete with cultivated crop plants for growth factors (Vyvyan, 2002).

Economically, there is no doubt that herbicides and herbicide-resistant crops have drastically improved agricultural efficiency and yields. However, the broad application and/or sometimes the abuse of herbicides also created problems. The major problem is the evolution of weeds with resistance to herbicides which refers to the capacity of a plant to grow and reproduce under the dose of herbicide that is normally lethal to the species (Yuan *et al.*, 2007). Weed resistance to herbicides presents one of the greatest current economic challenges to agriculture (Baucom, 2009) with more than 346 biotypes of weed known to be resistant to herbicides (Heap, 2010).

Allelopathy was considered an alternative to herbicides and an aid for weed control by Fay and Duke (1977) who found that some *Avena* spp accessions contained an allelopathic agent that reduced annual weed growth and caused chlorosis, stunting and twisting when planted in close association. According to Hoffman *et al.* (1996) competitive hierarchies often form during early stages of plant growth, and therefore interference should be measured between germinating seeds and between seedlings. Typical field studies cannot separate the effects of competition from allelopathy since they happen simultaneously between roots and shoots. In view of this, artificial environments must be devised that remove any possibility of competition while allowing chemical exchange to take place (Smith *et al.,* 2001).

Knowledge about the genetic constitution of rye grass and its populations is increasingly becoming crucial, particularly given the extent of herbicide-resistance within the Western Cape. Data on this topic will further enhance our understanding of the genetics and evolution of herbicide-resistant weeds. Descriptive studies of patterns of genetic diversity in weedy populations can be an extremely important tool for helping to minimise the evolvement of resistance to herbicides (Madhou *et al.*, 2005).

McCalla and Norstadt (1974) showed that the water soluble substances in wheat residues reduced germination and growth of wheat seedlings. Wheat residues reduced yield of the subsequent wheat crop. This was attributed to the fact that wheat contains a number of phenolic acids. Kong (2008) confirmed that variation of the soil microbial populations and community structures could be distinguished by the allelopathic and non-allelopathic rice varieties tested. Furthermore, Sozeri and Ayhan (1998) found in pot experiments, that mixing straw, which was gathered after harvesting, with soil, decreased germination of wheat seeds and increase seedling mortality. The release of phytotoxins by plants, has been proposed as an alternative theory for the success of some invasive plants and they have long been suspected of using allelopathic mechanisms to rapidly displace native species (Bais *et al.,* 2003).

Herbicide resistant rye grass is a serious problem in Western Cape grain producing areas as it is threatening more than 100 000 ha of productive grain fields. Wheat fields have become so heavily infested that economic grain production will be impossible in certain areas in the foreseeable future, leading to huge production losses and less sustainable grain production. Therefore, system-oriented approaches to weed management that make better use of alternative weed management tactics need to be developed (Liebman & Davis, 2000; Barberi, 2002). Although residue management appears to be a key factor in residue-mediated weed suppression, very few studies have systematically compared the influence of different residue management methods on germination and establishment of crop and weed species (Kruidhof, 2008). Therefore, a hypothesis was formulated: the management of crop residues, which is normally regarded as a production constraint, could be used for suppression of herbicide-resistant weeds, thereby reducing input costs and promoting the sustainability of cropping systems. Locally, the concomitant responses of the crop species in such systems have to be considered as well.

No information is available on the role of allelopathy in crop rotation systems of the Western Cape Province, where 750 000 ha are subjected to crop rotation. Of this area, more than 100 000 ha are under threat from invasive herbicide-resistant rye grass. Because of the importance this could have on sustainability of small grain crop rotation systems in the Western Cape Province, a study with the following objectives, was undertaken:

1. Explore the possibility of using allelopathic properties of rotational crop residues for the suppression of weed establishment and then specifically that of herbicide-resistant rye grass.
2. Evaluate the possible role of allelopathy from seeds, seedlings, roots and above-ground plant material of rotational crops, under controlled conditions.
3. a) Assess the distribution of genetic variability of rye grass; b) determine its botanical classification by morphological analyses; c) determine the presence of the crown rot pathogen of barley and wheat on rye grass; and d) analyse soil samples from each collection point where rye grass were sampled to determine its preference for soil chemical properties.
4. Determine the interactions among allelopathic root leachates, from different crop cultivars and the weed type rye grass, their growth rate, and soil micro-organisms. Also assessed were the allelopathic effects of the afore-mentioned plant species on wheat and barley.

CHAPTER 1

Literature Review

Introduction

Modern agriculture relies on synthetic chemicals to control weeds as unwanted plants, because they compete with cultivated crops for growth factors (water, light, nutrients and spaces), and harbour pests and plant pathogens (Qasem & Foy, 2001). There is also clear evidence that they can affect crops through the production of toxic chemicals which have a harmful effect on crop growth and development (Qasem & Hill, 1989).

Due to increased awareness about the risks involved in the use of synthetic chemicals, much attention is being focused on the alternative methods of weed control. Overuse of synthetic herbicides for weed control over the last five decades has resulted in growing public concern over their impacts upon human health, the environment, and the evolution of herbicide resistant weeds (Vyvyan, 2002). Herbicide resistance in weeds is a rapidly expanding phenomenon resulting in higher costs of production due to the greater weed impact. With current pressures to reduce herbicide usage but maintain cost-effective weed control, the innate ability of crops or cultivars to suppress weed growth has become increasingly important (Efthimiadou *et al.,* 2009).

Allelopathic crops when used in rotational sequences, are helpful in reducing noxious weeds, improve soil quality and crop yield (Khanh *et al.,* 2005). These crop plants, particularly the legumes, can reduce weed infestation and increase rice yield by between 20 and 70 %, and are suggested for use as natural herbicides (Khanh *et al.,* 2005). Cultivating a system with allelopathic crops plays an important role in the establishment of sustainable agriculture (Khanh *et al.,* 2005). Wheat (*Triticum aestivum* L.) is known to be allelopathic against crops and weeds (Alsaadawi *et al.,* 1998). Wheat straw reduced weed densities and biomass by an average of 90 % compared with those plots without residues (Putnam and DeFrank, 1983). Narwal *et al.* (1998) reported that wheat straw caused a 16.8 % reduction of broad-leaf weeds but showed no effect on grassy weeds. In fields previously cultivated with wheat, populations of barnyard grass (*Echinochloa crus-galli*) were decreased (Alsaadawi, 2001). Lopes *et al.* (1987) reported that extracts from barnyard grass reduced rice (*Oryza sativa*) radicle and coleoptile growth. Foliage vapours and foliage washings of *Salvia reflexa* adversely affected germination and seedling growth of wheat (Lovett, 1983). Root exudates from wild oat (*Avena fatua*) reduced wheat leaf and root dry mass (Schumacher *et al.,* 1983). Results from Uppar *et al.* (1993) indicated that aqueous extracts of *Commelina benghalensis* inhibited wheat cv Kiran germination by 36.1 %.

In the Western Cape two cropping areas can be defined namely, the Swartland winter rainfall area and the Overberg year-round rainfall area. Wheat is the main crop in both areas and crop rotation systems followed have the aim of sustaining wheat production. The major crop rotation system followed over a four year cycle in the Swartland is wheat-wheat-medic-medic or the less popular system of wheat-canola-wheat-lupine. Because of year-round rainfall in the Overberg region, lucerne which grows actively in summer, is grown for a five to seven year period, followed by wheat-barley-canola-wheat-barley-lupine over a six year period.

In the sections that follow, literature references are clustered according to the chapters for which they are relevant.

Field assessment of crop residues for allelopathic effects on both crops and weeds

Several wild accessions of modem day crops are found to possess allelopathic traits that impart in them resistance against weeds and pests (Hoult & Lovett, 1993). To achieve consistent results in the field from the use of crop residues, it is important to understand the mechanism of allelopathy (Kumar *et al.,* 2009). Field trials investigating crop allelopathy of rice *(Oryza sativa)* cultivars showed that crop allelopathy does not kill weeds (Olofsdotter *et al.*, 1999; Olofsdotter, 2001), confirming that crop allelopathy is merely relevant for weed suppression.

Crop allelopathy is currently understood as an interaction between a crop and a weed that is taking place in an environment that can significantly influence the whole process. Manipulation of this environment is mediated by several input production factors, and special adaptations might be needed for successful application of crop allelopathy (Belz, 2007). The trend towards conservation tillage, a widening range of crop rotation options and diverse production practices in the Western Cape Province, has highlighted the potential exploitation of allelopathy for weed suppressions in cropping systems. Furthermore, the utilisation of allelopathy for weed management is likely to be most beneficial where other options have become limiting due to herbicide resistance and high control costs (Jones *et al.,* 1999). Both the latter factors are serious constraints in the wheat producing areas of the Western Cape Province.

Most plants apparently produce secondary metabolites that are phytotoxic to some degree, and in a small number of cases their release into the environment and their capability of causing allelopathic effects towards a number of noxious weeds have been demonstrated. Allelopathy is particularly relevant for weed management strategies applied in minimum and no-till cropping systems (Jones *et al.,* 1999), because weed control in such systems is particularly problematic and basically limited to the use of herbicides. The incidence of growth inhibition of certain weeds and the induction of phytotoxic symptoms by plants and their residues is well documented for many crops, including all major grain crops such as rice, rye, barley, sorghum, and wheat (Belz, 2004). Wheat straw has been found to be an excellent mulch crop in no-till farming due to the presence of triterpenoids and other phenolic acids (Singh *et al.,* 2001).

Crop allelopathy can be exploited for weed management by the release of allelochemicals from intact roots of living plants and/or through decomposition of plant residues (Batish *et al.,* 2002; Belz, 2004; Khanh *et al.,* 2005; Qasem & Hill, 1989). Chou (1999) found that allelochemicals can be released either through leaching from leaves, decomposition of residues, volatilisation or by root exudation. Strategies for the implementation of crop residue allelopathy, entails the application of phytotoxic residues or mulches primarily generated by intercropping of allelopathic cover, smother, rotational, or companion crops (Wu *et al.*, 1999), because phytotoxins are released by intact roots of living crop plants (Weston, 1996). Upon release into crop environment the nature and concentration of allelochemicals may change because of complex environmental conditions and microbial action (Batish *et al.,* 2001). Crop residues can, therefore, be very useful in maintaining the sustainability of agroecosystems, provided they are efficiently managed (Batish *et al.,* 2002). At present however, the evidence is that the nature of crop allelopathy does not allow for a sole reliance on this approach and, thus, planting a certain allelopathic cultivar will be just a component of an integrated weed management system (Wu *et al.,* 1999).

Apart from allelopathic effects, crop residues can exert an effect on weed germination and establishment through other mechanisms. Release of nutrients from the residues can stimulate weed germination (Teasdale & Pillai, 2005), whereas temporary immobilisation of nutrients from the soil upon decomposition of residues with high C:N ratios, can have the opposite effect (Liebman & Mohler, 2001). Most reports dealing with residue-mediated inhibitory effects on receptor plants mention that plant residues decomposing in soil exhibit a progressive decline in phytotoxicity with the most severe inhibition occurring at the early stages (An *et al.,* 2001; Xuan *et al.,* 2005). Weed suppressive effects of crop residues have been attributed to different mechanisms, including initial low nitrogen (N) availability following cover crop incorporation (Dyck & Liebman, 1994; Kumar *et al.,* 2008), mulch effects of a physical nature (Mohler & Teasdale, 1993), stimulation of pathogens or predators of weed seeds (Gallandt *et al.,* 2005), and allelopathy (Weston, 1996).

The availability of soil nitrate had no influence on the degree of phytotoxicity of any stubble type (Purvis, 1990). Soil nutrient status and nitrogen nutrition in particular did not appear to account for the differential effects observed in the field experiments. Under field conditions, the effective concentration of stubble-derived chemicals at any point in time is greatly influenced by environmental factors (Purvis, 1990). For this reason, high levels of allelochemicals occur only sporadically in soils. However, if they are present at a sensitive physiological stage of plant development, such as seedling emergence, they can exert long-lasting detrimental effects with respect to crop productivity. It is postulated that soil levels of stubble-derived chemicals may be high in one year and low in another, owing to differences in rainfall and temperature between these years (Purvis, 1990). This suggests a far greater likelihood of phytotoxicity if stubble has undergone little weathering or decomposition prior to sowing (Purvis, 1990).

Available evidence revealed that crop cultivars differ significantly in their abilities to suppress certain weed species and indicates possible development of crop cultivars able to inhibit growth of the principal weeds in a given area through allelopathic action and thus decrease the need for synthetic herbicides (Wu *et al.,* 1999). Many studies have clearly demonstrated genetic variability of the allelopathic character within crops (Wu *et al.,* 1999), which may be considered as an important genetic reservoir for selection of allelopathic cultivars. As was observed for several genetic traits, allelopathy is interwoven with environmental conditions (Olofsdotter *et al.*, 2002; Weston & Duke, 2003). Thus, in a wide range of environments, the allelopathic potential of a certain cultivar may differ considerably. A clear understanding of such genotype-environmental interactions is required if allelopathy is to become a reliable option for weed management (Belz, 2004).

Greenhouse and laboratory assessment of rotational crops for allelopathic potential that affects both crops and weeds

The allelopathic phenomenon encompasses both detrimental and beneficial interactions between plants through chemicals released by the donor (Xuan & Tsuzuki, 2002). Belz (2004) suggested that crop allelopathy can be exploited for weed management through the release of allelochemicals from intact roots of living plants or decomposition of plant residues and that in annual crops, root exudation of the phytotoxins by the crop is the preferred mechanism.

Kumar *et al.* (2009) suggested that one approach to understanding the allelopathic effects of crop residues is to separate soil effects occurring during the growth of crops from their residue effects. Another approach is to determine which parts of the cover crop—root, shoot, or root plus shoot—has the most suppressive effects on emergence and growth. Nevertheless, Olofsdotter *et al.* (1995) and Wu *et al.* (2000b; 2001) cautioned that an essential need in studying crop allelopathy is simulation of the natural release of allelochemicals so that chemical interference from living donor plants on living receiver plants can be measured.

The complicated nature of interference among plants makes it difficult to separate its components in natural environments (Qasem & Hill, 1989). Therefore, the relative importance of competition and allelopathy as mechanisms of plant interference is generally unknown (Hoffman *et al.*, 1996). Furthermore, the interaction of allelochemicals with soil components upon release from the plant is important in determining whether inhibition of the target plant is likely to occur in the field (Blum, 1996).

The presence of white goosefoot *(Chenopodium album)* residual material in soil caused growth reduction of wheat, lettuce, lucerne, and various other crop species (Reinhardt *et al.,* 1994). Furthermore, white goosefoot residues in the soil have been found to be phytotoxic and to affect the nutrient uptake process in maize and soybean. A better understanding of toxic weed root exudates that inhibit crops will lead to more effective decision making in crop rotation systems (Rice, 1984).

Rye (*Secale cereale* L.) root residues were found to be more suppressive than shoot tissues on growth and emergence of barnyard grass (*Echinochloa crus-galli* L. Beauv.) and growth of sicklepod (*Senna obtusifolia* L. Irwin and Barneby) (Brecke and Shilling 1996; Hoffman *et al.,* 1996). Aqueous shoot extracts of buckwheat stimulated Powell amaranth (*Amaranthus powellii* S. Wats.) germination slightly, but inhibited radicle growth (Kumar *et al.,* 2009).

Furthermore, allelopathic inhibition is typically the result of the combined action of a group of allelochemicals (Einhellig, 1996). Allelochemicals can be bound to soil organic matter or clay and become inactive (Daldon, *et al.,* 1983). These compounds affect soil micro-organisms in ways that significantly alter the ecology of the field where the allelopathic plant and their residues are present (Mamolos & Kalburtji, 2001).

Geographical differentiation and genetic variation of *Lolium* spp in the Western Cape: identification of the hybrid *Lolium multiflorum x perenne* and isolation of the pathogen *Fusarium pseudograminearum*

Widespread repeated use of synthetic herbicides has produced biotypes of annual ryegrass resistant to major herbicide classes (Wu *et al.,* 2003). Mimic weeds such as *Lolium* spp (rye grass) has convergently evolved with cereal crops as a result of unconscious selection by farmers and cannot survive without the agricultural practices to which they have become adapted (Spahillari *et al.,* 1999). Rye grass has been disseminated throughout the world with traditional wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) cultivation and is expected to have complex evolutionary patterns (Holm *et al.,* 1977). Weed species with a high level of genetic diversity, like rye grass, are considered to show significant potential for weed adaptation and decrease the efficacy of weed control.

According to O'Hanlon *et al.* (2000), there is a widespread concern that weed species with higher levels of genetic diversity will exhibit considerable potential for adaptation and, therefore, may be able to reduce the effectiveness of weed control. Weeds have genetic traits that give them remarkable plasticity, allowing them to adapt, regenerate, survive, and thrive in a multitude of ecosystems (Chao *et al.,* 2005). Many agronomic weeds are close relatives of crop plants and studies on the sequencing of a weed genome are likely to provide clues concerning weed phenotypes and their underlying gene networks (Broz & Vivanco, 2009).

A specie's ability to adapt to changing environmental conditions is found in the genetic diversity of its populations. Success in weed populations facing changing agricultural ecosystems often correlates with an abundance of genetic polymorphisms within those populations (Jasieniuk & Maxwell, 2001). Through the process of mutation and selection, however, weeds evolve resistance to herbicides when they are used repeatedly (Tranel & Trucco, 2009). Rigid ryegrass (*L. rigidum*) (Monaghan, 1980) was regarded by Tranel and Trucco (2009) to be the most important weed in terms of it having evolved resistance to multiple herbicides.

Perennial ryegrass (*L. perenne* L.) (Charmet & Balfourier, 1994) is native to most of Europe and parts of the Mediterranean and Middle East areas, whereas rigid rye grass is distributed all around the Mediterranean. The genus *Lolium* consists of two groups of species, which are outbreeding and inbreeding, respectively (Senda *et al*., 2005). The genetic diversity of outbreeding rye grass has been studied in relation to the characterisation of genetic resources of Italian ryegrass (*L. multiflorum* Lam.) (Charmet & Balfourier, 1994) and perennial ryegrass. Analysis of the frequency and distribution of genetic variation in natural populations of perennial ryegrass has supported the view that its centre of origin is the Fertile Crescent (Middle East) and that its distribution expanded following a clinical geographical pattern (Senda *et al*., 2005). Balfourier *et al*. (2000) reported that despite the weak genetic differentiation, significant patterns of geographical variation with respect to diversity indices and allele frequencies have been observed in perennial rye grass. In contrast, no spatial organisation of diversity has been detected in rigid rye grass (Balfourier *et al*., 2000).

Allelopathic root exudates of the weed *Lolium multiflorum x perenne* and crops influence plant growth and changes in the soil microbial community

Several studies have shown that some crop cultivars are allelopathic and that their inhibitory effects on weeds can cause significant suppression of the latter plants' growth under field conditions (Olofsdotter *et al.,* 1999; Wu *et al.,* 1999).

Alsaadawi *et al.* (2005) concluded that sorghum cultivars differ in allelopathic potential and that the exploitation of cultivars with higher allelopathic capacity would be of value for weed control, particularly in no-tillage cropping systems. Several rice cultivars identified in the individual screenings of weeds of rice were successful in substantial root growth inhibition of more than one weed type (Seal *et al.,* 2005). Belz (2007) discussed breeding efforts in wheat *(Triticum aestivum)* and barley *(Hordeum vulgare)* which showed that early vigour and allelopathy against perennial ryegrass were significantly related to field weed suppression, whereby the relative importance proved to be cultivar and crop specific.

Plant roots exude a wide variety of metabolites including carbohydrates proteins, vitamins, amino acids and other organic compounds (Kong *et al.,* 2008). Amongst the latter, in particular those root exudate components with low molecular weight, may act as allelochemicals and mediate interactions between plants and other organisms in the rhizosphere (Bertin *et al.,* 2003). Because the rhizosphere is the densely populated area of the soil where plant roots must compete with invading root systems of neighbouring plants and with soil-borne micro-organisms for space, water and mineral nutrients, interactions within the rhizosphere are based on complex exchanges involving a multitude of biotic and abiotic factors. However, below-ground biological interactions that are driven by root exudates are probably more complex than above-ground interactions (Lin *et al.,* 2007).

Micro-organisms have a profound effect on the allelopathic activity by altering and/or transforming the amount of allelochemicals, particularly the phenolic acids in the soil, depending upon the available carbon source and other environmental factors (Singh *et al.,* 2001). The microbes may metabolise the released phenolic acids by addition or deletion of side groups, polymerisation, production of other organic molecules and/or incorporation of carbon from other phenolic acids into microbial biomass (Blum, 1996). Furthermore, in the soil the preferential utilisation of carbon sources may also affect the plant-microbe soil system and the allelopathic phenomenon (Singh *et al.,* 2001).

The term allelopathy has been broadened, according to Kazinczi *et al.* (2005), to include not only plant-to-plant, but also plant-to-micro-organism interactions. Most of the natural products involved with allelopathy are compounds of secondary metabolism that are synthesised by plants and micro-organisms (Pacheco & Pohlan, 2007). According to Duke *et al.* (2000) the natural plant products from higher plants and micro-organisms are biodegradable and eco-friendly, and some of these compounds can be relied upon to enhance crop productivity in a sustainable way. Such products, termed allelopathic compounds, have been shown to play a role in allelopathy, defined here as inhibitory effects of secondary metabolites against either competitors or predators (Leflaive & Ten-Hage, 2007). Belz *et al.* (2009) reported on the degradation of parthenin, an allelopathic compound in the invasive species *Parthenium hysterophorus* L., which is most likely governed by physico-chemical processes combined with microbial activity. Ehrenfeld (2006) reported that allelochemicals are widespread in invasive species and can affect soil microbial communities and microbially-mediated ecosystem processes.

Micro-organisms have a profound effect on allelopathic activity by altering and/or transforming the amount of allelochemicals (Singh *et al.,* 2001). On the other hand, allelochemicals may influence the growth of micro-organisms positively or negatively thereby indirectly interfering with the availability of nutrients, particularly nitrogen and phosphorus, in the soil (Wardle & Nilsson, 1997). Furthermore, microbial communities provide useful data for studying impacts of environmental events. Micro-organisms are present in virtually all environments and are typically the first organisms to react to chemical and physical changes in the environment. Allelopathy can be better understood in terms of soil microbial ecology when the roles of soil micro-organisms in chemically-mediated interactions between plants are evaluated (Inderjit, 2005).

CHAPTER 2

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Field assessment of crop residues for allelopathic effects on both crops and weeds

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INTRODUCTION

In South Africa's south western corner, the widespread use of herbicides on crop fields has led to new weed problems in the form of shifts in the dominance of species' in weed communities and the increased evolution of herbicide-resistant weeds. Most proven cases of herbicide resistance in South Africa occur in the orchards, vineyards, and wheat fields of the Western Cape Province (Pieterse & Cairns, 2009). The overuse of synthetic agrochemicals for pest and weed control has increased environmental pollution, unsafe agricultural products, and human health concerns (Khanh *et al.,* 2005). Therefore, system-oriented approaches to weed management that make better use of alternative weed management tactics are being promoted (Liebman and Davis, 2000; Barberi, 2002). Weeds are an important constraint in agricultural production systems (Oerke, 2006) because they act at the same trophic level as the crop, capturing part of the available resources that are essential for plant growth (Bastiaans, 2008). For these reasons, there is increasing interest in integrated weed management strategies based on a wide range of control options. One of these options is the inherent ability of many crops to suppress weeds through a combination of high early vigour (competition) and allelopathic activity to further reduce weed interference (Bertholdsson, 2005).

The International Allelopathy Society (IAS) has defined allelopathy as follows: ‘allelopathy refers to any process involving secondary metabolites produced by plants, microorganisms and viruses that influence the growth and development of agricultural and biological systems’ (Kruidhof, 2008). Belz (2007) reported that allelopathy can be an important component of crop/weed interference. The trend towards conservation tillage and widening range of crop rotation options and diverse production practices in the Western Cape Province has highlighted the potential exploitation of allelopathy to suppress weeds in cropping systems and is likely to be most beneficial where other options have become limiting due to herbicide resistance and high control costs (Jones *et al.,* 1999).

Crop allelopathy controls weeds by the release of allelochemicals from intact roots of living plants and/or through decomposition of phytotoxic plant residues (Qasem and Hill, 1989; Weston, 1996; Batish *et al.,* 2002; Belz, 2004; Khanh *et al.,* 2005). The incidence of growth inhibition of certain weeds and the induction of phytotoxic symptoms by plants and their residues is well documented for many crops, including all major grain crops such as rice (*Oryza sativa*), rye (*Secale cereale*), barley, sorghum (*Sorghum bicolor*), and wheat (*Triticum aestivum*) (Belz, 2004).

Crop residues can interfere with weed development and growth through alteration of soil physical, chemical, and biological characteristics. In the case of crop residues, there are two possible sources of allelochemicals; the compounds can be released directly from crop litter or they can be produced by microorganisms that use plant residues as a substrate (Kruidhof, 2008). Retention of crop residues in conservation tillage systems is recognised as also providing several other benefits including improved soil conservation and soil structure, as well as increased water infiltration and reduced costs for fuel and labour (Jones *et al.,* 1999).

Crop residues can also affect the physical properties of the soil. Residues conserve moisture (Liebl *et al.,* 1992; Teasdale & Mohler, 1993). Residues left on the soil surface can lead to decreased soil temperature fluctuations and reduced light penetration, which can both have an inhibitory effect on weed germination (Teasdale & Mohler, 1993). Furthermore, in some cases soil microbial populations, including soilborne pathogens, are stimulated after soil amendment with fresh plant material (Dabney *et al.,* 1996; Conklin *et al.,* 2002; Manici *et al.,* 2004).

Although residue management seems a key factor in residue-mediated weed suppression, very few studies have systematically compared the influence of different residue management methods on germination and establishment of crop and weed species (Kruidhof, 2008). Allelopathy is particularly relevant for weed management strategies applied in minimum and no-till cropping systems (Jones *et al.,* 1999), because weed control in such systems is particularly problematic and basically limited to the use of herbicides.

The inclusive definition for allelopathy mentioned above recognises that compounds are involved in the defense against multiple biological threats, including competition by other plants, herbivores and disease (Macias *et al.,* 2007). Manipulation of the allelopathic environment is mediated by several input production factors, and special adaptations might be needed for successful application of crop allelopathy (Belz, 2007). Duke *et al.* (2001) and Scheffler *et al.* (2001) proposed adaptations for successful application of allelopathy in terms of genetic approaches as it would enhance the weed-suppressing capacity of crop cultivars.

To achieve consistent results in the field from the use of crop residues, it is important to understand the mechanism of allelopathy (Diab & Sullivan, 2003). Field trials investigating crop allelopathy of rice cultivars showed that crop allelopathy does not kill weeds (Olofsdotter *et al.,* 1999; Olofsdotter, 2001), confirming that crop allelopathy may suppress but not eliminate weeds. Similar to many plant characteristics, allelopathy is influenced by environmental conditions (Olofsdotter, 2002; Weston & Duke, 2003). Thus, in a wide range of environments, the allelopathic potential of a certain cultivar may differ considerably. A clear understanding of such genotype-environmental interactions is required if allelopathy is to become a reliable option for weed management (Belz, 2004).

Furthermore, no information is available on the role of allelopathy in crop rotation systems in the Western Cape Province, where 750 000 ha are subjected to crop rotation. Of this area, more than 200 000 ha are under threat from invasive herbicide-resistant rye grass weed type. The objective of the present studies was to explore the possibility of using allelopathic properties of rotational crop residues for weed suppression (specifically suppression of herbicide-resistant rye grass weed type) to determine whether crop and weed residues left in the field release phytotoxins that affect the growth and yield of rotational crops and weeds.

MATERIALS AND METHODS

The study was conducted at the Tygerhoek Research Farm (19°54'E, 34°08'S) near Riviersonderend, South Africa. The main crop produced in this area is wheat in rotation with barley, canola, lupine, medic, and lucerne. The average annual rainfall at Tygerhoek is 443 mm (Appendix A, Table A1) and the long-term mean daily maximum and minimum temperatures are 22.4 °C and 10.2 °C, respectively. At this locality the stony loam soils are weakly developed residual (pH 5.1) of Mispah (Entisol) type (Soil Classification Working Group, 1991) containing 22 % clay and 1.6 % carbon. Total soil cations at this locality is 8.5 cmol(+) kg^{-1} and resistance of 370 Ohms. The research approach was similar in concept to that followed by Qasem and Hill (1989), Batish *et al.* (2002) and Bruce *et al.* (2005).

Experiment 1a-d

Dried plant material was collected following harvest in 2002 from the following crops: barley (*Hordeum vulgare* L. v. Clipper), canola (*Brassica napus* L. v. ATR Hyden), wheat (*Triticum aestivum* v. SST 88), lupine (*Lupinus angustifolius* L. v. Tanjil), lucerne (*Medicago sativa* L. v. SA standard), medic (*Medicago truncatula* Gaertn. v. Parabinga) and rye grass (*Lolium multiflorum* Lam. v. Energa). Stubble left on the soil surface after the harvesting process was collected manually and each stored separately for three months in a shed as plant residues for Exp 1a in 2003. Residues for use in Exp 1b, 1c and, 1d were produced in the years 2003, 2004 and 2005, respectively. Over this 4-yr period, each trial was planted in the same field, but each year on a different fallow site in close proximity to where the previous plantings were done. During the period that fallow sites were not in use, they were kept weed free, by rotating the use of herbicides glyphosate (Mamba™) and diquat/paraquat (Preeglone™), but plant material from weeds that did escape control was removed by hand from the trial site so as to leave a seedbed free of any plant residues for at least a year.

In each of the four years from 2003 to 2006 liming at a rate of 400 kg ha^{-1} was done six months before planting, based on soil analyses and aiming for a soil pH of 5.5. This was followed with chisel cultivation for incorporating the lime about 10 cm deep. Two months before planting the seedbed was prepared with a second chisel cultivation to leave a smooth seedbed, followed by uniform scattering of a quantity of plant residues equivalent to five tons per hectare, which is typically produced in the region under field conditions for barley and wheat and left on the field after harvesting. Residues were scattered per plot according to the lay-out in Table 1 (Appendix A, Figure A1). For experimental purposes, the same amount of plant residues was used for each treatment.

Table 1 Schematic representation of experimental design at Tygerhoek

		Plant residues (donors)							
	Treatment number	Barley 1	Canola 2	Wheat 3	Lupine 4	Lucerne 5	Medic 6	Rye grass 7	Control 8
Plant species drilled	1 Barley	Barley	Barley	Barley	Barley	Barley	Barley	Barley	Barley
	2 Canola	Canola	Canola	Canola	Canola	Canola	Canola	Canola	Canola
	3 Wheat	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat
	4 Lupine	Lupine	Lupine	Lupine	Lupine	Lupine	Lupine	Lupine	Lupine
	5 Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne
	6 Medic	Medic	Medic	Medic	Medic	Medic	Medic	Medic	Medic
	7 Rye grass	Rye grass	Rye grass	Rye grass	Rye grass	Rye grass	Rye grass	Rye grass	Rye grass

To prevent residues being blown away by wind, plots were covered with bird netting. The amount of residues applied in this way was 9 kg per plot (3 m x 6 m). Because plant residues were not incorporated into the soil it was assumed that possible confounding effects of a nitrogen-negative period could be avoided or at least restricted to negligible effect levels. Furthermore, fertilisation (in particular nitrogen) application was done in order to negate growth differences due to nutrients that might be released from the plant residues.

Plots were arranged in a randomised complete block design with three replicates, and were planted to barley, canola, wheat, lupines, lucerne, medic or rye grass (Table 1) in May each year from 2003 to 2006 as this is the growing season in the winter rainfall area for the southern hemisphere. Control plots received no plant residues before planting. Planting was done with a no-till ‘star wheel’ grain drill. Therefore, each crop was planted into seven different crop residues. Plots planted to lupine, lucerne, and medic received 10 kg P ha^{-1} at planting whereas 20 kg N ha^{-1} was applied to all other plots. Four weeks after planting, barley, wheat, canola, and rye grass plots received 30 kg N ha^{-1} and 15 kg S ha^{-1}. A further top dressing of 30 kg N ha^{-1} was applied to wheat, canola, and rye grass plots at 10 weeks after planting. Weeds were controlled with iodosulfuron at a rate of 200 g ai ha^{-1} in wheat and barley plots. In all other plots, grass weeds were controlled with cycloxydim at a rate of 300 ml ai ha^{-1} at six weeks after planting. Plant height of all the crops was measured with a stainless steel ruler of 1000 mm length, from the base of the crop stem at the soil surface to the highest growth point of five plants per plot at four weeks, eight weeks and at maturity. Plants per m^2 and the number of tillers were determined at harvest. For barley, seed plumpness and percentage seed nitrogen were measured; for wheat seed hectolitre mass and percentage seed protein were determined. Harvesting was done with a small plot combine. Grain mass per plot was determined and yield expressed on a per hectare basis.

Experiment 2

In the 2006 and 2007 winter rainfall seasons, in order to gather data that were more representative of local production practices, it was decided to plant all crops into plant residues left over from the 2005 and 2006 growing seasons (Exp 1c and 1d in 2005 and 2006), respectively. Apart from allelopathic effects, decomposing residues were expected to also release nutrients into the soil. Together with wheat and barley, it was decided that since lupine had suppressed grass weeds the most in Experiment 1, two cultivars should be evaluated as well as the weed type of *Lolium* spp, which was identified by the Compton Herbarium at Kirstenbosch Botanical Gardens as *L. multiflorum x perenne.* For commercial reasons, wheat v. SST 88 was replaced by v. SST 027 to ensure seed availability.

Crop planting in the 2006 and 2007 winter growing seasons was done at a 90º angle across the 2005 and 2006 plots of Experiment 1, respectively (Appendix A, Figure A2). Planting was done with a no-till 'star wheel' grain drill. Plots were 3 m x 3 m arranged in a randomised complete block design with three replicates and planted to barley, wheat, lupine v. Tanjil and v. Quilinock, rye grass, and rye grass weed type in May of each year. Plots were planted with row spacing of 17 cm and at seeding rates recommended for the area. All plant residues were manually removed from control plots. In terms of crop production practices, plots were handled in the same way as those in Experiment 1.

Prior to planting, counts of all weeds occurring on plots were done using a 0.25 m^2 steel grid at two positions spaced 1 m apart in the centre of each plot. In addition, weed population counts were done across all plots in June, August, and October to assess residue-mediated effects on weed seedling establishment for different residue treatments. Weed data expressed per m^2 were aggregated because non-destructive weed counts were done over the four sampling times. As density is a measure of weed severity, relative density values were calculated for each species. Relative density is the number of seedlings of a species expressed as percentage of total weed seedlings and was described by Cousens (1985) as a more appropriate representation of weed data than total weed counts.

Data Analyses

Data were subjected to ANOVA (SAS, 2000). Analyses of field data sets for Experiment 1 from 2003 until 2006 were done on data averaged over years, because the year by treatment interaction was not statistically significant, indicating that treatment effects were consistent over years, thus only the treatment main effect will be discussed. Analysis of variance was performed separately for the 2006 and 2007 experiments using the General Linear Model procedures of SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC, USA 2000). Results of the 2006 and 2007 experiments were also combined and investigated in a single analysis of variance (John and Quenouille, 1977) after testing that experiments are of comparable precision by means of Levene's test for homogeneity of variance (Levene, 1960). For crop stand the requirement of homogeneity of experiment variance was not met, therefore a weighted analysis was performed. The Shapiro-Wilk test was performed to test for normality (Shapiro, 1965). Data for crop stand was square root-transformed to improve assumptions of normality. Student's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all tests.

RESULTS

Experiment 1a-d

Both barley and rye grass residues reduced wheat grain yield (Table 2). Wheat residue significantly increased lupine yield above that attained with the no-residue control treatment. Treatment with wheat and rye grass plant residues increased rye grass yield significantly compared with the control.

Table 2 Effects of plant residues on yield for the various plant species in Exp 1

Plant residues	Barley yield (t ha^{-1})	Wheat yield (t ha^{-1})	Lupine yield (t ha^{-1})	Lucerne yield (t ha^{-1})	Medic yield (t ha^{-1})	Rye grass yield (t ha^{-1})
Barley	3.09a	2.94c	1.33ab	3.39ab	1.42bc	3.72c
Canola	3.14a	3.37b	1.27ab	3.28ab	1.68ab	3.73c
Wheat	3.35a	3.79ab	1.58a	3.62a	1.3bc	4.69ab
Lupine	3.1a	3.46b	1.02b	3.56ab	1.92a	3.59c
Lucerne	3.03a	3.98a	1.07b	3.51ab	1.32bc	4.03bc
Medic	3.11a	3.53b	1.13b	3.32ab	1.12c	3.78c
Rye grass	3.05a	2.84c	1.16b	2.74b	1.14c	4.98a
Control	3.19a	3.58ab	1.09b	3.3ab	1.56abc	3.68c
LSD ($P \leq 0.05$)	0.53	0.42	0.36	0.88	0.5	0.91

*Means followed by the same letter are not significantly different at the 0.05 probability level

Plant height of barley exposed to wheat or medic crop residues was significantly higher than the control. At harvest, plant residues from lucerne were associated with a significant increase in barley tillers above that attained in the control treatment. Barley plant residues caused a significant reduction in wheat seed hectolitre mass (data not presented).

Experiment 2

Barley

Compared with the control canola and lucerne residues had an inhibitory effect on the number of barley tillers (Table 3). This was also evident in barley yield, which was significantly reduced by canola and lucerne crop residues.

Table 3 Effects of retained plant residues in the 2006 and 2007 growing seasons on barley v. Clipper plant height, plant number, tillers, seed plumpness, percentage seed nitrogen and yield

Plant residues	Barley plant height (mm)	Barley plant number per m^2 at harvest	Barley tillers	Barley seed plumpness	Barley seed nitrogen (%N)	Barley yield (t ha^{-1})
Barley	761a*	69a	9.5ab	73.2a	2.33a	1.88bcd
Canola	805a	65a	7.3b	74.6a	2.34a	1.48cd
Wheat	805a	70a	10.8a	77.4a	2.32a	2.48a
Lupine	771a	75a	10.8a	80.1a	2.36a	2.42ab
Lucerne	760a	63a	7.3b	73.8a	2.44a	1.36d
Medic	782a	69a	8.8ab	79.7a	2.46a	2.30ab
Rye grass	784a	75a	8.8ab	80.9a	2.32a	2.00abc
Control	801a	72a	11a	79.8a	2.38a	2.21ab
LSD ($P\leq0.05$)	67.4	10	2.4	10.6	0.14	0.56

*Means followed by the same letter are not significantly different at the 0.05 probability level

Wheat

No significant differences compared to the control were observed for wheat (Table 4).

Table 4 Effects of retained plant residues in the 2006 and 2007 growing seasons on wheat v. SST 027 plant height, plant number, tillers, seed hectolitre mass, percentage seed protein and yield

Plant residues	Wheat plant height in mm at 16 wks	Wheat plant number per m^2 at harvest	Wheat tillers	Wheat seed hectolitre mass	Wheat seed % protein	Wheat yield (t ha^{-1})
Barley	987ab*	72ab	5a	68.4a	11.7a	2.64ab
Canola	976abc	70ab	5a	68.8a	12.0a	2.40b
Wheat	961bc	77ab	5a	69.7a	11.8a	3.02ab
Lupine	977abc	79a	5a	70.2a	12.2a	3.32a
Lucerne	938c	66b	4a	68.8a	12.4a	2.27b
Medic	1007a	73ab	5a	69.3a	12.4a	2.89ab
Rye grass	956bc	70ab	5a	68.7a	11.5a	2.53ab
Control	973abc	71ab	5a	70.0a	12.2a	2.77ab
LSD ($P\leq0.05$)	44	12	1	1.9	1.1	0.42

*Means followed by the same letter are not significantly different at the 0.05 probability level

Lupine v. Tanjil

Barley crop residues increased lupine (v. Tanjil) pod number per plant significantly above that attained with the control treatment (Table 5).

Table 5 Effects of plant residues in the 2006 and 2007 growing seasons on lupine v. Tanjil plant height, plant number, pod number per plant and yield

Plant residues	Lupine v. Tanjil plant height at 16 wks (mm)	Lupine v. Tanjil plant number per m^2 at harvest	Lupine v. Tanjil pod number per plant	Lupine v. Tanjil yield (t ha^{-1})
Barley	582a*	57a	7a	0.65ab
Canola	528a	46ab	6ab	0.71ab
Wheat	561a	45b	6ab	0.69ab
Lupine	509a	49ab	3cd	0.50bc
Lucerne	507a	48ab	2d	0.41c
Medic	514a	49ab	4cd	0.57bc
Rye grass	522a	44b	6ab	0.86a
Control	534a	52a	5bc	0.73ab
LSD ($P \leq 0.05$)	78	6	2	0.24

*Means followed by the same letter are not significantly different at the 0.05 probability level

Lupine v. Quilinock

Lucerne residue inhibited lupine (v. Quilinock) pod number significantly more than that attained with the control treatment (Table 6). Lupine crop residues, similar to canola, reduced lupine (v. Quilinock) pod number per plant, significantly more than with the control treatment. Lucerne crop residues, similar to canola and medic, also reduced lupine (v. Quilinock) yield significantly more than the control treatment.

Table 6 Effects of plant residues in the 2006 and 2007 growing seasons on lupine v. Quilinock plant height, plant number, pod number per plant and yield

Plant residues	Lupine v. Quilinock plant height at 16 wks (mm)	Lupine v. Quilinock plant number per m^2 at harvest	Lupine v. Quilinock pod number per plant	Lupine v. Quilinock yield (t ha^{-1})
Barley	596a*	52a	6a	0.65ab
Canola	544a	47a	4cd	0.71ab
Wheat	561a	48a	5ab	0.69ab
Lupine	532a	50a	3d	0.50bc
Lucerne	527a	48a	2e	0.41c
Medic	524a	46a	4bc	0.57bc
Rye grass	516a	46a	6a	0.86a
Control	538a	55a	5ab	0.73ab
LSD ($P \leq 0.05$)	85	10	1	0.24

*Means followed by the same letter are not significantly different at the 0.05 probability level

Rye grass

Medic, lucerne and canola crop residues inhibited rye grass significantly more than the control with regard to plant height at 16 weeks (Table 7).

Table 7 Effects of retained plant residues in the 2006 and 2007 growing seasons on rye grass v. Energa plant height, plant number, tillers and yield

Plant residues	Rye grass plant height at 16 wks (mm)	Rye grass plant number per m^2 at harvest	Rye grass tillers	Rye grass yield (t ha^{-1})
Barley	796abc*	80a	4ab	2.94a
Canola	698cd	76a	2b	2.97a
Wheat	773bcd	80a	5a	3.15a
Lupine	778bcd	76a	3ab	2.94a
Lucerne	699cd	78a	3ab	3.00a
Medic	690d	77a	3ab	2.76a
Rye grass	878a	84a	3ab	3.18a
Control	810ab	81a	4ab	3.24a
LSD ($P \leq 0.05$)	98	8	2	0.51

*Means followed by the same letter are not significantly different at the 0.05 probability level

Rye grass weed type

At 16 weeks after planting, crop residues of canola and medic had reduced rye grass weed type plant height significantly from that attained with the control treatment (Table 8). Medic and barley had reduced rye grass weed type plant number per m^2. This significant growth-inhibiting effect from barley crop residues on rye grass weed type was also evident in yield.

Table 8 Effects of retained plant residues in the 2006 and 2007 growing seasons on rye grass weed type plant height, plant number, tillers and yield

Plant residues	Rye grass weed type plant height at 16 wks (mm)	Rye grass weed type plant number per m^2 at harvest	Rye grass weed type tillers	Rye grass weed type yield (t ha^{-1})
Barley	646b*	74b	5a	2.61c

Canola	519c	84a	5a	3.09a
Wheat	645b	79ab	4a	2.79abc
Lupine	613bc	83a	4a	3.00abc
Lucerne	687ab	81ab	3a	2.91abc
Medic	546c	75b	5a	2.70bc
Rye grass	769a	80ab	3a	2.76abc
Control	693ab	84a	4a	3.03ab
LSD (P≤0.05)	96	7	2	0.39

*Means followed by the same letter are not significantly different at the 0.05 probability level

Relative Weed Density

A total of 39 weed species emerged across the trial area (Table 9). Control plots were dominated by broadleaf weeds (88.5 %) while grass weeds accounted for 11.5 % of weed seedlings. The number of weeds did not stay constant, but changed throughout the growing season as later emerging weeds appeared. The highest incidence of grass weeds occurred in barley and wheat plots at 25.7 % and 22.9 %, respectively. In contrast, plots planted to both lupines v. Tanjil and v. Quilinock, showed a reduction in grass weeds to 8.1 % and 10.1 %, respectively. The highest incidence of broadleaf weeds occurred in rye grass and rye grass weed type plots at 97.2 % and 95.9 %, respectively.

Table 9 Average relative weed density (%) at Tygerhoek for the 2006 and 2007 growing seasons, with totals for broadleaf and grass weeds indicated in the same row

	Barley v. Clipper	Wheat v. SST 027	Lupine v. Tanjil	Lupine v. Quilinock	Rye grass v. Energa	Rye grass weed type	Control
Broadleaf weeds - total %	74.3	77.3	92.2	90.2	97.2	95.9	88.5
Arctotheca calendula	1.4	0.3	0	0	0.6	0	0.4
Anagallis arvensis	3.2	3.4	0	0	0.7	0.7	0.5

Bidens pilosa	0	0.9	0.5	0.7	3	2.1	0.5
Capsella bursa-pastoris	3.2	2.1	1.1	0.5	0.3	0	0
Chenopodium album	0	0.1	0	0	0	0	5
Chenopodium carinatum	0	0.4	0	0	0	0	0.4
Chenopodium multifidium	0	0	0	0	0	0	0.3
Conyza albida	0	1.1	0.2	0.1	0.6	0.6	2.6
Coronopus didymus	0	0	0.4	0.5	0.7	0.6	0
Corrigiola litoralis	0	0	0	0.2	0	0	0
Cotula australis	2.1	1.8	0.4	0.5	5.7	2.3	2.3
Crassula thunbergiana	5.6	4.5	0.9	0.9	7.9	7.3	2.3
Daucus carota	1.3	1.9	1.1	0.8	0.4	0	0
Echium plantagineum	0	0.6	0	0	0	0	2
Emex australis	2	2.1	0	0	0.6	3.7	1.1
Erodium moschatum	3.1	3.5	0	1	1.7	2.8	8.6
Fumaria muralis	8.3	6	1.3	2.2	4	4.6	10.7
Gnaphalium subfalcatum	0	0	0.4	0.1	1.8	0	0.1
Lactuca serriola	0	1.1	1.1	1.3	0	0	0.7
Lepidium africanum	1.5	2.8	1	1.1	0.2	0.7	3
Linaria spuria	0	0.5	0.3	0.3	0.7	0	0.2
Lobelia erinus	0	0.9	0	0	0	0.5	0
Oenothera parodiana	3.4	1.6	0	0	2	2.7	0.2
Oxalis spp	1	1.8	0.4	1	2.7	2.1	4
Pichris echioides	0	0	1.2	0.9	0	0	0
Plantago lanceolata	0	1.4	37.6	27.5	0	0	7.6
Polycarpon tetraphyllum	8.6	6.4	19.5	18.3	5	11.2	7.2
Polygonum aviculare	0.4	5.6	17.3	23.4	24.1	18.5	14.2
Raphanus raphanistrum	4.9	3.8	0.9	1.5	1.9	0	0.6
Senecio pterophorus	0	0	0	0	0	0	0
Sonchus asper	1.2	2	5.3	4.2	1.1	0.5	2.1
Spergula arvensis	1.8	2	0	0	0	0	0.1
Stellaria media	21.3	18.7	1.3	3.2	31.5	35	11.8
Grass weeds – total %	25.7	22.9	8.1	10.1	3.2	4.1	11.5
Bromus diandrus	1.2	0.4	0	0	2.2	2.4	0.2
Digitaria sanguinalis	0	0.2	0	0	0	0.7	0.1
Isolepis antarctica	3.9	3.2	1.4	1.8	0	0	1
Juncus bufonius	1.2	1.1	0	0	0	0	0
Lolium multiflorum x perenne	7.8	8.7	3.9	4.5	0	0	8.2
Poa annua	11.6	9.3	2.8	3.8	1	1	2

Stellaria media had the highest relative density index and was the most prevalent emerging weed and hence, was the most important weed in terms of frequency in barley, wheat, rye grass, and rye grass weed type plots (Table 9). *Plantago lanceolata* had the highest relative density index and was the most important weed in terms of frequency in plots planted to both lupine varieties namely; v. Tanjil and v. Quilinock**.**

DISCUSSION

In Exp 1, the significant reduction in wheat hectolitre mass caused by barley residues and the significant reduction in wheat yield in the presence of residues of both barley and rye grass were probably due to allelopathic effects which are dependent on climatic and edaphic factors in the field and which should be replicated under controlled conditions for confirmation. Similarly, barley also reduced the yield of the rye grass in both Exp 1 and 2. Furthermore, plant height of this weed was reduced by canola and medic residues. In contrast, residues from the leguminous crops (lupine and medic) increased wheat growth with regard to plant number per m^2, yield, and plant height. Although allelopathic effects can be stimulatory (Belz, 2004) it must be considered that the N fixing ability of the leguminous crops could have had a subsequent beneficial effect on wheat.

The inhibitory effects of lucerne crop residues on the number of barley tillers and yield, and on plant height and yield of wheat is in accordance with those effects reported by Xuan and Tsuzuki (2002) and Xuan *et al.* (2005). Kruidhof (2008) also reported strong inhibitory effects by lucerne on seedling establishment. It was also reported by Kruidhof (2008) that lucerne plants contain water-soluble allelochemicals that are released into the soil environment from fresh leaf, stem, and crown tissues, as well as from dry hay, old roots and seeds.

A study in which sampling of lucerne plants as a mulch was spread over a long period showed that the immature lucerne residues contained more allelochemicals than older residues (Guenzi *et al.,* 1964). In the present study, effects of lucerne were probably more pronounced compared with other treatments of crop residues because although lucerne was dormant in the following winter growing season when Exp 2 was conducted, green plant material was still present as this perennial crop could not be controlled effectively in the field.

However, the results for barley from Exp 1 and 2 with regard to lucerne residues are contrasting as it increased barley tillers in Exp 1 while inhibiting it in Exp 2, but Xuan and Tsuzuki (2002) and Bertholdsson (2004) reported that between and within crop species there is large genetic variation in the allelochemical content of plant tissue. Also, various studies have shown that concentrations of allelochemicals in plants are not stable. The level of allelochemicals in a plant are influenced by abiotic and biotic stresses in combination with age or growth stage (Mwaja *et al.,* 1995; Reberg Horton *et al.,* 2005).

Kruidhof (2008) described a transition from inhibitory to stimulatory effects of crop residues over time. Low concentrations of allelochemicals can stimulate plant growth (Lovett *et al.,* 1989; Belz, 2004; Belz, 2007) and increased growth has also been associated with increased nitrate levels in residue-amended soil (Henson, 1970). Therefore, the increased growth observed in the present study may indicate that there was a positive nutrient effect in conjunction with growth-promoting allelopathic activity from the crop residues. This is congruent with most findings in allelopathy research that decomposing plant residues in soil exhibit the greatest inhibition at the early stages of decomposition and that phytotoxicity declines as decomposition proceeds (An *et al.,* 2001; Xuan *et al.,* 2005). The nature and strength of inhibitory allelopathic effects appear to be dependent on interactions between soil factors and crop residues and the allelochemicals they produce (Kumar *et al.,* 2009).

With respect to weeds, cover crop residues have been reported to negatively affect germination and establishment of weed seeds (Weston, 1996). Especially leguminous cover crops that contain high levels of allelochemicals seem well-suited for residue-mediated weed suppression. In combination with this, the physical effects (light interception) of the residue may also contribute to reduced weed emergence, as is conceivably the case in the field where an average of 5 t ha^{-1} crop residues from barley and wheat can be deposited on the soil surface. The possible positive effects of this organic mulch on soil moisture conservation must also be taken into consideration. In contrast, suppression of growth of Powell Amaranth (*Amaranthus powellii*) appears to be associated primarily with lower N availability in soils grown to certain crops (Kumar *et al.,* 2009). However, the impact of crop residues on weed management was not so much an absence of weeds, but rather delayed emergence and growth retardation, which could have been due to physical properties of the mulch, such as the prevention of light penetration, temperature changes and/or the physical obstruction of weed seedlings. Results from Exp 1 for medic on the suppression of rye grass weed type promise practical application under field conditions because of the crop’s spreading growth habit which could be effective for the establishment of effective organic mulches. According to results in Experiments 1 & 2, a mulch of this nature may suppress weeds without affecting wheat yield.

On plots planted to lupine (v. Quilinock) there was a reduction in total grass weeds to 8.1% and 10.1%, respectively (Table 8) when compared to control plots. As cycloxydim was applied across all lupine plots, including control plots, it should be taken into consideration that it is a more effective herbicide for grass control in lupine than iodosulfuron is in wheat. In the case of rye grass weed type, however, both lupine cultivars suppressed the weed to only 3.9% and 4.5%, respectively. Furthermore, a suppressive plant competition effect from broadleaf weeds on the grass weeds cannot be excluded. An early flush of emergence from a huge seed bank plus high growth rates probably benefited the dominance of broadleaved weeds. Lupine contain quinolizidine alkaloids that act as herbivore deterrents (Vilarino *et al.,* 2005), but these compounds have also been suggested to influence plant-plant interactions (Wink, 1983). In ascribing allelochemical-mediated effects under field conditions one has to be mindful of the fact that persistence of allelochemicals is largely influenced by soil type and weather conditions (Levitt *et al.,* 1984). Therefore any hypothesis based on crop residues imparting positive weed suppressive effects through the release of allelochemicals into the environment should be mindful of the fact that the practice is likely to be exposed to the vagaries of climatic (Bruce *et al.,* 2005) and edaphic factors, as well as likely being crop and weed-specific. Therefore, this field investigation warrants further investigation that ought to also involve work done under controlled conditions.

CONCLUSION

The optimal residue management strategy for weed suppression depends both on the nature (fine residues like those from medic are more effective as opposed to coarse residues of lupine) and amount (less residues leads to less weed control) of crop species' residues as well as on the target weed species. N-fixing leguminous crops such as medic and lupine had a stimulatory effect on wheat growth and yield and medic suppressed the important rye grass weed type. Lupine gave suppression of grass weeds, giving the mulches of both leguminous crops an added benefit and their inclusion and growing in crop rotation systems with wheat and barley as main crops, more importance. However, regarding weed suppression due to allelopathic effects from crop residues, the variability in effects ascribed to variable soil and climatic factors might argue against the practice being accepted as an effective stand-alone weed control option in the foreseeable future. Partial acceptance will likely be a compromise of combining the continued limited use of herbicides with leguminous crop residues for weed control.

CHAPTER 3

Greenhouse and laboratory assessment of rotational crops for allelopathic potential that affects both crops and weeds

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INTRODUCTION

Chemical interference was described by Hoffman *et al.* (1996) as a significant co-evolutionary force in plant communities, but it may be much more important as a mechanism in recipient than in origin communities (Hierro & Callaway, 2003). Alterations in the environment by various plant interference mechanisms can differentially affect neighbouring plant species. Allelopathy is defined as any direct or indirect, inhibitory or stimulative, effect by one plant (including micro-organisms) on another through the production of a chemical compound(s) (Rice, 1984). The phenomenon encompasses both detrimental and beneficial interactions between plants through chemicals released by the donor (Xuan & Tsuzuki, 2002).

According to Kato-Noguchi (2000), chemicals with allelopathic activity are present in many plants and in many organs, including leaves, flowers, fruits and buds. They are of varied chemical nature, e.g., phenolics, terpenes, alkaloids, flavonoids, etc. (Gupta, 2005). In agricultural ecosystems it is one of the important mechanisms of interference, affecting crop performance (Batish *et al.,* 2002). Allelochemicals appear to affect all aspects of crop development including germination, radicle and plumule (coleoptile in monocots) growth, seedling growth, metabolism, plant growth, flowering and fructification. Belz (2004) suggested that crop allelopathy can be exploited for weed management through the release of allelochemicals from intact roots of living plants or decomposition of plant residues and that in annual crops, root exudation of the phytotoxins by the crop is the preferred mechanism.

Kumar *et al.* (2009) suggested that one approach to understanding the allelopathic effects of crop residues is to separate soil effects occurring during the growth of crops from their residue effects. Another approach is to determine which parts of the cover crop-root, shoot, or root plus shoot-has the most suppressive effects on emergence and growth. Nevertheless, Olofsdotter *et al.* (1995) and Wu *et al.* (2000; 2001) cautioned that an essential need in studying crop allelopathy is simulation of the natural release of allelochemicals so that chemical interference from living donor plants on living receiver plants can be measured.

The complicated nature of interference among plants makes it difficult to separate its components in natural environments (Oasem & Hill, 1989). Therefore, the relative importance of competition and allelopathy as mechanisms of plant interference is generally unknown (Hoffman *et al.*, 1996). Furthermore, the interaction of allelochemicals with soil components upon release from the plant is important in determining whether inhibition of the target plant is likely to occur in the field (Blum, 1996).

Separation of allelopathic effects from those of competition is a major experimental challenge (Oasem & Hill, 1989), but many research reports proved its feasibility. In a study carried out by Caussanel *et al.* (1977) it was shown that root exudates of *C. album* (white goosefoot) retarded the radicle growth of *Zea mays* (maize) in culture solution. An aqueous extract of the weed also inhibited the growth of maize roots. Further studies carried out by Caussanel (1979), showed that white goosefoot exerted an inhibitory influence on maize growth. He demonstrated that the effect could not be attributed to competition alone. Bhatia *et al.* (1984) also reported an inhibitory effect of white goosefoot on *Triticum aestivum* L. (wheat) seedlings. Chemical effects of white goosefoot seeds on germination were reported by Stefureac and Fratilescue-Sesan (1979) who found that seeds of white goosefoot placed in Petri-dishes with seeds of meadow fescue, wheat (cv. Dacia) or *Medicago sativa* (lucerne) inhibited the germination of all three species.

Quasem and Hill (1989) successfully segregated competitive and allelopathic effects of white goosefoot on tomato. Reinhardt *et al.* (1997) reported that white goosefoot caused inhibition of maize and soybean root growth. The presence of white goosefoot residual material in soil caused growth reduction of wheat, *Lactuca sativa* L. (lettuce), lucerne, and various other crop species (Reinhardt *et al.,* 1994). Furthermore, white goosefoot residues in the soil have been found to be phytotoxic and to affect the nutrient uptake process in maize and soybean (Reinhardt *et al.,* 1994). A better understanding of toxic weed root exudates that inhibit crop growth will lead to more effective decision-making in crop rotation systems (Rice, 1984).

Kumar *et al.* (2009) noted that for most plant species, shoot extracts were more effective than root extracts in inhibiting seed germination and growth of downy broom. Kumar *et al.* (2009) reported that shoot extracts of two goldenrod species (*Euthamia graminifolia* L. Nutt. and *Solidago canadensis* L.) had inhibitory effects on both germination and growth of radish (*Raphanus sativus* L.) and lettuce. In contrast, root extracts had no inhibitory effects on germination of these two species, but suppressed root growth. On the other hand, rye (*Secale cereale* L.) root residues were found to be more suppressive than shoot tissues on growth and emergence of barnyardgrass (*Echinochloa crus-galli* L. Beauv.) and growth of sicklepod (*Senna obtusifolia* L. Irwin and Barneby) (Brecke & Shilling 1996; Hoffman *et al.,* 1996). Aqueous shoot extracts of buckwheat stimulated Powell amaranth (*Amaranthus powellii* S. Wats.) germination slightly, but inhibited radicle growth (Kumar *et al.,* 2009). Aqueous soil extracts from buckwheat-amended soil inhibited germination of Powell amaranth whilst extracts from unamended soil showed no effect.

According to Hoffman *et al.* (1996) competitive hierarchies often form during early stages of plant growth, and therefore interference should be measured between germinating seeds and between seedlings. Typical field studies cannot separate the effects of competition from allelopathy since they happen simultaneously between roots and shoots. In view of this, artificial environments must be devised that remove any possibility of competition while allowing chemical exchange to take place (Smith *et al.,* 2001). Therefore, the primary objectives of this research were to evaluate the possible role of allelopathy from seeds, seedlings, roots and above-ground plant material, under controlled conditions.

MATERIALS AND METHODS

The plant series used in the laboratory and green house, consisted of the rotational crops barley (*Hordeum vulgare* L. v. Clipper), canola (*Brassica napus* L. v. ATR Hyden), wheat (*T. aestivum* v. SST 88), lupine (*Lupinus angustifolius* L. v. Tanjil), lucerne (*M. sativa* L. v. SA standard), medic (*M. truncatula* Gaertn. v. Parabinga) and rye grass (*Lolium multiflorum* Lam. v. Energa) in a lay-out for Experiments 1-4 as represented in Table 1 (Appendix A, Figure A3, A4 & A5).

Table 1 Schematic representation of experimental design for Experiments 1-4

		Plant donors							
	Treatment number	Barley 1	Canola 2	Wheat 3	Lupine 4	Lucerne 5	Medic 6	Rye grass 7	Control 8
Plant acceptors	1 Barley	Barley	Barley	Barley	Barley	Barley	Barley	Barley	Barley
	2 Canola	Canola	Canola	Canola	Canola	Canola	Canola	Canola	Canola
	3 Wheat	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat
	4 Lupine	Lupine	Lupine	Lupine	Lupine	Lupine	Lupine	Lupine	Lupine
	5 Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne
	6 Medic	Medic	Medic	Medic	Medic	Medic	Medic	Medic	Medic
	7 Rye grass	Rye grass	Rye grass	Rye grass	Rye grass	Rye grass	Rye grass	Rye grass	Rye grass

The research approach for Experiment 1 and 2, although similar in concept to that followed by Hoffman *et al.* (1996) and Kato-Noguchi (2000) for assessing whether crop seeds and seedlings release phytotoxins that affect the germination and development of radicles of rotational crops, was different in terms of both experimental method and plant series investigated.

For Experiment 3 and 4, research methods were similar to those followed by Reinhardt *et al.* (1994), Hoffman *et al.* (1996) and Smith *et al.* (2001), for assessing whether crop root exudates and above-ground plant material release phytotoxins that affect the growth and yield of rotational crops. The nature and extent of experiments conducted for this study which was done under controlled conditions, had a similar lay out (Table 1) to Exp 1 in Chapter 2, and therefore a dilution series was not considered, as it replicated treatments from the field experiment in order to compare and explain field data.

Experiment 1: The first experiment was set up in the laboratory to observe the mutual effect of seed leachates from the plant series. Ten seeds of each plant type were placed in Petri-dishes in combinations with ten seeds of each of the other species in the series. Seeds were placed on filter paper in 9.5 cm diameter Petri-dishes and moistened with 5 ml distilled water. The lay-out was done according to a Randomised Block design with ten replicates, equalling 100 seeds per species. Control Petri-dishes contained only one seed type (not in combinations). Petri-dishes were sealed with Parafilm® and placed in an incubator set at 12h/12h day/night cycle and a temperature range of 25/15 °C. Germination was determined after 7 and 14 days of incubation, by counting the number of germinated seeds and measuring the length of the radicle. A seed was regarded as germinated when the radicle was at least 2 mm long, and was then removed from the Petri-dish.

Experiment 2: The second experiment was conducted in the laboratory to study the effect of seedling leachates from all the plants in the series on germination and early development of all the other species. One hundred seeds of each plant type in the series were germinated in Petri-dishes. The seedlings were allowed to develop until they reached a length of roughly 50 mm, after which ten seedlings from each species were placed in a 4 cm porcelain Buchner funnel and washed with 5 ml distilled water to yield a leachate. This leachate was funnelled into 9.5 cm diameter Petri-dishes into which 10 seeds from each plant type had been placed on Whatman 9 cm filter paper according to a Randomised Block design with ten replicates, equalling 100 seeds per species. Control treatments were treated with distilled water only. Petri-dishes were sealed with Parafilm® and incubated at a 12h/12h day/night cycle with a temperature range of 25/15 °C. Germination was determined after 7 and 14 days of incubation, by counting the number of germinated seeds and measuring the length of the radicle.

Experiment 3: This experiment was conducted in the greenhouse to determine the effects of root exudates from each plant in the series on the growth of themselves and all other species. Ten crop seeds of each plant type were planted in separate donor pots filled with 6 kg of leached river sand, and thinned to five plants of similar size one week after emergence. Treatments in the greenhouse were replicated three times in a Randomised Block design. Pots were over-irrigated twice a week, from the first week after planting with 100 ml water to provide for sufficient drainage per pot. At the time of planting this was 150 ml water (100 ml drainage), reaching 900 ml per pot twice a week (300 ml drainage), as plants matured. All water leached from the same plant type was collected in the same container and used as root leachate on acceptor pots in which five plants were grown in the same growth medium. No planting was done in control pots, but the leachate was collected for use as control treatment.

Of the leachate collected, 100 ml was used twice a week at planting and increasing to 300 ml at maturity, to irrigate the acceptor (same as donor) species as well as each of the other plant types. The first irrigation occurred at the time of planting, and thereafter twice a week for five weeks after emergence. Once a week, Multifeed was applied as balanced plant nutrition at a concentration of 1g ℓ^{-1}, to each pot by using a volume of 50 ml at the time of planting and reaching 200 ml at five weeks.

Experiment 4: The fourth experiment was conducted in the greenhouse to study the effects of above-ground plant residue leachates from the plant series on the growth of the plant series itself. Plant material from each plant species was collected in the field and air-dried, after which it was ground to a coarse powder. This substratum was mixed shallowly into pots filled with 6 kg of leached river sand, at a rate of 15 g per pot (equivalent to 5 t plant residues per hectare), in which the donor plant itself, as well as all the other plant types, were planted separately (five plants per pot). Treatments in the greenhouse were replicated three times in a Randomised Block design. Since chemical products of the decomposition process are soluble in a weak carbonic acid solution, the surface irrigation would have leached allelochemicals into the soil, resulting in their absorption by the plant. This leachate from five donor plants was used to treat five acceptor plants planted in the same growth medium, but without residues mixed into pots. At the time of planting this was 50 ml leachate, reaching 600 ml per pot per week, as plants matured. Once a week, Multifeed was applied as balanced plant nutrition at a concentration of 1g ℓ^{-1}, to each pot by using a volume of 50 ml at the time of planting and reaching 200 ml at five weeks.

In the greenhouse, plant height was determined for all plants on a weekly basis, starting at one week after emergence. After five weeks all plants were cut off at ground level. Thereafter, all the above-ground plant parts were dried at 60°C for 72 hours and dry mass recorded.

All data were analysed statistically (ANOVA) with the statistical program SAS. Least significant differences were used to identify significant differences between means at the 5% level of probability.

RESULTS

Experiment 1

Seed leachate: laboratory

Barley

No significant differences between seed leachate treatments were recorded in barley radicle length (Table 2). At 14 days, leachates from wheat and medic seeds had reduced barley cumulative germination significantly from that attained in the control treatment.

Table 2 Effects of seed leachates on barley radicle length and germination

	Seed leachate	
Plant type	Barley radicle length (mm)	Cumulative germination % at 14 days
Barley	26.4a	77ab
Canola	25.2a	97a
Wheat	23.5a	67 b
Lupine	13a	73ab
Lucerne	12.2a	90ab
Medic	13a	70 b
Rye grass	25.7a	80ab
Control	21.6a	97a
LSD (P≤0.05)	18.1	25

*Means followed by the same letter are not significantly different at the 0.05 probability level

Canola

Canola radicle length was significantly reduced by leachates from barley, lupine and lucerne seeds (Table 3). At 14 days, leachates from lupine seeds had reduced canola cumulative germination significantly from that attained at the control treatment.

Table 3 Effects of seed leachates on canola radicle length and germination

	Seed leachate

Plant type	Canola radicle length (mm)	Cumulative germination % at 14 days
Barley	10.5 c	73ab
Canola	22.4a	97a
Wheat	12.4abc	70ab
Lupine	5.8 c	60 b
Lucerne	10.8 bc	100a
Medic	22.3ab	93a
Rye grass	23.4a	90ab
Control	23.7a	93a
LSD (P≤0.05)	11.5	33

*Means followed by the same letter are not significantly different at the 0.05 probability level

Wheat

The radicle length of wheat was significantly reduced by leachates from barley, wheat and lupine (Table 4). Lupine seed leachates also significantly reduced wheat cumulative germination.

Table 4 Effects of seed leachates on wheat radicle length and germination

	Seed leachate	
Plant type	Wheat radicle length (mm)	Cumulative germination % at 14 days
Barley	8.5 bc	53 bc
Canola	19.5ab	70ab
Wheat	9.5 bc	93a
Lupine	5 c	27 c
Lucerne	20.2ab	83ab
Medic	15.6abc	83ab
Rye grass	24.8a	93a
Control	27.9a	87ab
LSD (P≤0.05)	12.5	38

*Means followed by the same letter are not significantly different at the 0.05 probability level

Lupine

No significant differences between seed leachate treatments were observed in lupine radicle length (Table 5). Barley seed leachate had reduced lupine cumulative germination significantly from that attained at the control treatment.

Table 5 Effects of seed leachates on lupine radicle length and germination

	Seed leachate	
Plant type	Lupine radicle length (mm)	Cumulative germination % at 14 days

Barley	2.8 b	13 b
Canola	6.8ab	53a
Wheat	8.9ab	40ab
Lupine	11.9a	70a
Lucerne	9.6ab	43ab
Medic	13.5a	63a
Rye grass	9.1ab	47ab
Control	8.4ab	53a
LSD (P≤0.05)	9	40

*Means followed by the same letter are not significantly different at the 0.05 probability level

Lucerne

The radicle length of lucerne was significantly inhibited by seed leachates from barley and lupine (Table 6). Lupine seed leachate had reduced lucern cumulative germination significantly from that attained at the control treatment.

Table 6 Effects of seed leachates on lucerne radicle length and germination

	Seed leachate	
Plant type	Lucerne radicle length (mm)	Cumulative germination % at 14 days
Barley	3.7 bc	33 c
Canola	20.8ab	87a
Wheat	11.9abc	47 bc
Lupine	0 c	0 d
Lucerne	17.1abc	73ab
Medic	20.0ab	57abc
Rye grass	20.6ab	83a
Control	25.2a	43 bc
LSD (P≤0.05)	17.4	31

*Means followed by the same letter are not significantly different at the 0.05 probability level

Medic

Significant differences in medic radicle length were observed when seeds were treated with barley and lupine seed leachate (Table 7). No differences in cumulative germination were noted.

Table 7 Effects of seed leachates on medic radicle length and dry mass

	Seed leachate	
Plant type	Medic radicle length (mm)	Cumulative germination % at 14 days
Barley	13.4 b	57a

Canola	31.7a	73a
Wheat	19ab	77a
Lupine	12.6 b	50a
Lucerne	17ab	60a
Medic	19.7ab	93a
Rye grass	31.8a	70a
Control	31.6a	77a
LSD (P≤0.05)	16.1	44

*Means followed by the same letter are not significantly different at the 0.05 probability level

Rye grass

The radicle length of rye grass was significantly inhibited by seed leachates from barley, wheat and lupine (Table 8). This growth-inhibiting effect from barley and lupine seed leachates, was also evident in rye grass cumulative germination percentage.

Table 8 Effects of seed leachates on rye grass radicle length and dry mass

	Seed leachate	
Plant type	Rye grass radicle length (mm)	Cumulative germination % at 14 days
Barley	12.4 cd	50 bc
Canola	33.8ab	87a
Wheat	15.8 bcd	73ab
Lupine	1.5 d	17 c
Lucerne	25.1abc	90a
Medic	24.0abc	97a
Rye grass	28.2abc	90a
Control	36.8a	97a
LSD (P≤0.05)	19.5	34

*Means followed by the same letter are not significantly different at the 0.05 probability level

Experiment 2

Seedling leachate: laboratory

Barley

No significant differences between seedling leachate treatments were recorded in barley radicle length or cumulative germination at 14 days (Table 9).

Table 9 Effects of seedling leachates on barley radicle length and germination

	Seedling leachate	
Plant type	Barley radicle length (mm)	Cumulative germination % at 14 days
Barley	35a	73a

Canola	30.5a	90a
Wheat	27.3a	77a
Lupine	36.7a	97a
Lucerne	29.9a	83a
Medic	32.7a	73a
Rye grass	33a	90a
Control	40.4a	100a
LSD (P≤0.05)	23.4	28

*Means followed by the same letter are not significantly different at the 0.05 probability level

Canola

No significant differences between seedling leachate treatments were recorded in canola radicle length or cumulative germination (Table 10).

Table 10 Effects seedling leachates on canola radicle length and germination

	Seedling leachate	
Plant type	Canola radicle length (mm)	Cumulative germination % at 14 days
Barley	26a	87a
Canola	22a	83a
Wheat	26a	90a
Lupine	22.8a	87a
Lucerne	19.2a	80a
Medic	21.7a	93a
Rye grass	22.3a	87a
Control	19.5a	73a
LSD (P≤0.05)	15.3	22

*Means followed by the same letter are not significantly different at the 0.05 probability level

Wheat

No significant differences between seedling leachate treatments were observed in wheat cumulative germination (Table 11). After treatment with canola seedling leachates, wheat radicle length was significantly shorter than the control.

Table 11 Effects of seedling leachates on wheat radicle length and germination

	Seedling leachate	
Plant type	Wheat radicle length (mm)	Cumulative germination % at 14 days
Barley	33abc	80a
Canola	25.7 c	83a
Wheat	44.6a	77a
Lupine	31 bc	73a

Lucerne	40.6ab	83a
Medic	35.8abc	87a
Rye grass	41.2ab	70a
Control	41.4ab	87a
LSD (P≤0.05)	13.5	19

*Means followed by the same letter are not significantly different at the 0.05 probability level

Lupine

No significant differences between seedling leachate treatments were recorded in lupine radicle length (Table 12). The cumulative germination of lupine, treated with lucerne seedling leachates, was significantly less than the control.

Table 12 Effects of seedling leachates on lupine radicle length and germination

	Seedling leachate	
Plant type	Lupine radicle length (mm)	Cumulative germination % at 14 days
Barley	21.9a	80ab
Canola	15.2a	90a
Wheat	26.3a	87a
Lupine	23a	90a
Lucerne	12.9a	57 b
Medic	16.5a	77ab
Rye grass	24a	77ab
Control	27.7a	93a
LSD (P≤0.05)	14.8	25

*Means followed by the same letter are not significantly different at the 0.05 probability level

Lucerne

No significant differences between seedling leachate treatments were observed in percentage lucerne cumulative germination (Table 13). Rye grass seedling leachate stimulated the growth of lucerne seedlings significantly, as compared to the control, with regard to radicle length.

Table 13 Effects of seedling leachates on lucerne radicle length and germination

	Seedling leachate	
Plant type	Lucerne radicle length (mm)	Cumulative germination % at 14 days
Barley	18.3abc	63a
Canola	21.4ab	80a
Wheat	20.1abc	70a
Lupine	14.7 bc	63a

Lucerne	12.3 c	63a
Medic	22.8ab	70a
Rye grass	26.4a	57a
Control	14.7 bc	73a
LSD (P≤0.05)	8.7	38

*Means followed by the same letter are not significantly different at the 0.05 probability level

Medic

No significant differences in medic radicle length were observed when treated with seedling leachates (Table 14). The cumulative germination of medic, treated with lupine seedling leachates, was significantly less than the control.

Table 14 Effects of seedling leachates on medic radicle length and dry mass

	Seedling leachate	
Plant type	Medic radicle length (mm)	Cumulative germination % at 14 days
Barley	17.0ab	73ab
Canola	27.8a	70ab
Wheat	25.8ab	73ab
Lupine	15.6 b	63 b
Lucerne	18.5ab	73ab
Medic	19.2ab	77a
Rye grass	26.8a	70ab
Control	24.5ab	77a
LSD (P≤0.05)	10.9	13

*Means followed by the same letter are not significantly different at the 0.05 probability level

Rye grass

No significant differences between seedling leachate treatments were observed in rye grass cumulative germination percentage (Table 15). Seedling leachate from lupine, had significantly inhibited rye grass radicle length.

Table 15 Effects of seedling leachates on rye grass radicle length and dry mass

	Seedling leachate	
Plant type	Rye grass radicle length (mm)	Cumulative germination % at 14 days
Barley	35.6ab	83a
Canola	34.7ab	83a
Wheat	47.4a	87a
Lupine	31.6 b	87a
Lucerne	36.2ab	83a
Medic	39.1ab	80a
Rye grass	46.4a	93a

Control	46.1a	90a
LSD (P≤0.05)	13.9	17

*Means followed by the same letter are not significantly different at the 0.05 probability level

Experiment 3

Root exudates: greenhouse

Barley

At three weeks after planting, leachate from the root systems of lucerne and medic had reduced barley height significantly from that attained at the control treatment (Table 16). This significant growth-inhibiting effect from lucerne and medic on barley, along with lupine, was also evident at five weeks after planting. The dry mass of barley, treated with wheat, lupine and lucerne root leachates, was significantly less than barley treated with control leachate.

Table 16 Effects of root exudates on barley plant height and dry mass

	Root leachate		
Plant type	Barley plant height at 3 wks (cm)	Barley plant height at 5 wks (cm)	Barley dry mass (g)
Barley	38.6a	46.9ab	0.75a
Canola	37.5ab	47.2a	0.61 bcd
Wheat	37.6ab	43.9 bc	0.58 cd
Lupine	35.7 bc	42.6 c	0.56 d
Lucerne	31.2 d	38.6 d	0.54 d
Medic	34.3 c	42.3 c	0.63 bcd
Rye grass	37.3ab	45.3abc	0.67abc
Control	38.1ab	46.6ab	0.69ab
LSD (P≤0.05)	2.4	3.2	0.11

*Means followed by the same letter are not significantly different at the 0.05 probability level

Canola

After treatment with barley, canola, lucerne, medic and rye grass root leachates, canola plant height was significantly greater at five weeks after planting (Table 17). No significant differences between root leachate treatments were recorded in canola dry mass.

Table 17 Effects of root exudates on canola plant height and dry mass

	Root leachate

Plant type	Canola plant height at 3 wks (cm)	Canola plant height at 5 wks (cm)	Canola dry mass (g)
Barley	12.5a	21.9a	0.67ab
Canola	13.7a	20.5ab	0.70ab
Wheat	12.3a	18.8 bc	0.60 b
Lupine	12.9a	19.1 bc	0.63 b
Lucerne	12.4a	20.3ab	0.65 b
Medic	13.1a	21.7a	0.71ab
Rye grass	13.1a	21.7a	0.77a
Control	12.7a	18.1 c	0.67ab
LSD (P≤0.05)	1.7	2.1	0.12

*Means followed by the same letter are not significantly different at the 0.05 probability level

Wheat

No significant differences between root leachate treatments were recorded in wheat dry mass (Table 18). Rye grass root leachates increased wheat plant height significantly at three and five weeks after planting.

Table 18 Effects of root exudates on wheat plant height and dry mass

	Root leachate		
Plant type	Wheat plant height at 3 wks (cm)	Wheat plant height at 5 wks (cm)	Wheat dry mass (g)
Barley	34.6ab	45.0ab	0.96ab
Canola	33.2 bc	45.7ab	0.87ab
Wheat	34.1 bc	44.3ab	0.93ab
Lupine	32.1 c	42.8 b	0.77 b
Lucerne	32.5 bc	43.5ab	0.87ab
Medic	34.7ab	44.9ab	1.00a
Rye grass	36.4a	45.8a	0.97a
Control	33.5 bc	43.1ab	0.89ab
LSD (P≤0.05)	2.3	2.9	0.19

*Means followed by the same letter are not significantly different at the 0.05 probability level

Lupine

No significant differences between root leachate treatments were recorded in lupine dry mass (Table 19). Root leachates from barley increased lupine plant height significantly at three weeks after planting from that attained at the control. At five weeks after planting, a growth-stimulating effect from barley, medic and rye grass root leachates was evident in lupine plant height.

Table 19 Effects of root exudates on lupine plant height and dry mass

	Root leachate

Plant type	Lupine plant height at 3 wks (cm)	Lupine plant height at 5 wks (cm)	Lupine dry mass (g)
Barley	18.6a	29.9ab	0.8ab
Canola	17.3ab	28.3abc	0.82ab
Wheat	17.7ab	28.1 bc	0.86a
Lupine	16.2 bc	27.1 bc	0.87a
Lucerne	14.1 c	25.7 c	0.73 b
Medic	16.3abc	31.3a	0.87a
Rye grass	17.1ab	29.7ab	0.84a
Control	15.5 bc	25.9 c	0.82ab
LSD (P≤0.05)	5.5	3.2	0.1

*Means followed by the same letter are not significantly different at the 0.05 probability level

Lucerne

No significant differences between root leachate treatments were observed at five weeks after planting or in lucerne dry mass (Table 20). Barley root leachate significantly increased lucerne shoot length at three weeks after planting.

Table 20 Effects of root exudates on lucerne shoot length and dry mass

	Root leachate		
Plant type	Lucerne shoot length at 3 wks (cm)	Lucerne shoot length at 5 wks (cm)	Lucerne dry mass (g)
Barley	11.7a	26.3a	0.31a
Canola	9.5 b	20.7abc	0.24a
Wheat	8.6 b	18.1 bc	0.20a
Lupine	9.8ab	19.5abc	0.30a
Lucerne	7.9 b	17.5 c	0.32a
Medic	9.5 b	24.9ab	0.32a
Rye grass	9.8ab	23.7abc	0.25a
Control	8.5 b	21.1abc	0.30a
LSD (P≤0.05)	2.1	7.1	0.18

*Means followed by the same letter are not significantly different at the 0.05 probability level

Medic

Treatment with lupine root leachate significantly inhibited both shoot length of medic at three weeks and cumulative germination percentage (Table 21). At five weeks after planting, wheat and lupine root leachates inhibited medic shoot length significantly from that attained at the control. The dry mass of medic treated with lupine root leachates was significantly lower than the control, but in contrast to this, it was significantly increased by lucerne root leachates.

Table 21 Effects of root exudates on medic shoot length and dry mass

	Root leachate		
Plant type	Medic shoot length at 3 wks (cm)	Medic shoot length at 5 wks (cm)	Medic dry mass (g)
Barley	7.5ab	15.5ab	0.40 b
Canola	7.3ab	12.5 bc	0.42ab
Wheat	6.1 bc	10.6 cd	0.41 b
Lupine	3.7 d	7.9 d	0.20 c
Lucerne	6.5abc	15.2ab	0.59a
Medic	5.5 c	14.6abc	0.35 bc
Rye grass	8.0a	17.5a	0.46ab
Control	6.4abc	15.2ab	0.41 b
LSD (P≤0.05)	1.7	4	0.17

*Means followed by the same letter are not significantly different at the 0.05 probability level

Rye grass

Lucerne root leachates significantly inhibited rye grass plant height at three weeks after planting (Table 22). No significant differences between root leachate treatments were recorded in rye grass plant height at five weeks. The dry mass of rye grass treated with wheat and lupine root leachates was significantly higher than the control. In contrast to this, rye grass root leachate, significantly reduced rye grass dry mass.

Table 22 Effects of root exudates on rye grass plant height and dry mass

	Root leachate		
Plant type	Rye grass plant height at 3 wks (cm)	Rye grass plant height at 5 wks (cm)	Rye grass dry mass (g)
Barley	30.8a	39.2a	0.72 b
Canola	30.3a	38.8a	0.78 b
Wheat	29.7a	37.8a	0.97a
Lupine	29.6ab	38.2a	0.97a
Lucerne	26.1 b	36.5a	0.86ab
Medic	27.4ab	38.7a	0.71 b
Rye grass	29.8a	36.4a	0.56 c
Control	30.9a	40.5a	0.78 b
LSD (P≤0.05)	3.5	4.4	0.15

*Means followed by the same letter are not significantly different at the 0.05 probability level

Experiment 4

Above-ground plant residue leachate: greenhouse

Barley

Leachates from medic plant residues increased barley plant height significantly at three weeks after planting (Table 23). At five weeks after planting, leachate from lucerne had stimulated barley height significantly from that attained at the control treatment. The dry mass of barley treated with wheat plant residue leachate was significantly greater than the control. In contrast to this, the dry mass of barley treated with medic residues, were significantly reduced.

Table 23 Effects of above-ground leachates on barley plant height and dry mass

	Above-ground leachate		
Plant type	Barley plant height at 3 wks (cm)	Barley plant height at 5 wks (cm)	Barley dry mass (g)
Barley	25 c	36.9 b	2.09 bc
Canola	27.7abc	38ab	1.56 bc
Wheat	29.3abc	41.6ab	3.97a
Lupine	30.3abc	42.2ab	2.35 bc
Lucerne	33.7ab	46.9a	1.44 bc
Medic	34.4a	44.8ab	1.36 c
Rye grass	32.8ab	41.1ab	1.69 bc
Control	26.4 bc	36.7 b	2.42 b
LSD (P≤0.05)	7.7	9.4	1.04

*Means followed by the same letter are not significantly different at the 0.05 probability level

Canola

Above-ground leachates from lucerne, medic and rye grass increased canola plant height significantly from that attained with the control at three and five weeks after planting (Table 24). The dry mass of canola treated with wheat above-ground leachates was significantly higher than the control.

Table 24 Effects of above-ground leachates on canola plant height and dry mass

	Above-ground leachate

Plant type	Canola plant height at 3 wks (cm)	Canola plant height at 5 wks (cm)	Canola dry mass (g)
Barley	5.5 d	12.6 c	2.52 b
Canola	7.0 bcd	14.2 bc	2.23 bc
Wheat	7.1 bcd	14.8abc	4.34a
Lupine	5.7 cd	15.2abc	2.19 bc
Lucerne	9.3a	17.2ab	1.35 c
Medic	8.1ab	17.4a	1.41 bc
Rye grass	7.7abc	15.7ab	1.58 bc
Control	5.5 d	12.3 c	1.77 bc
LSD (P≤0.05)	2.1	3.1	2.82

Wheat

Lucerne, medic and rye grass above-ground leachates increased wheat plant height significantly more than that attained at the control at three weeks after planting (Table 25). At five weeks after planting, leachate from barley, canola, wheat, lupine and lucerne had inhibited wheat height significantly from that attained at the control treatment. The dry mass of wheat treated with barley, canola, lucerne, medic and rye grass above-ground leachates, was significantly less than the control.

Table 25 Effects of above-ground leachates on wheat plant height and dry mass

	Above-ground leachate		
Plant type	Wheat plant height at 3 wks (cm)	Wheat plant height at 5 wks (cm)	Wheat dry mass (g)
Barley	29.1 bc	33.4 c	2.67 cd
Canola	26.9 bc	37.6 c	1.89 de
Wheat	28.9 bc	37.6 c	4.67a
Lupine	28.8 bc	36.3 c	3.46 bc
Lucerne	30.3 b	36.5 c	0.93 e
Medic	37.0a	46.5a	1.01 e
Rye grass	34.0a	38.7 bc	1.8 de

Control	26.5 c	44.6ab	3.94ab
LSD (P≤0.05)	3.4	6.4	1.06

*Means followed by the same letter are not significantly different at the 0.05 probability level

Lupine

No significant differences between treatments were recorded in both lupine plant heights at three and five weeks, or dry mass (Table 26).

Table 26 Effects of above-ground plant residue leachates on lupine plant height and dry mass

	Above-ground leachate		
Plant type	Lupine plant height at 3 wks (cm)	Lupine plant height at 5 wks (cm)	Lupine dry mass (g)
Barley	17.8ab	24.1ab	2.61ab
Canola	9.0 b	16.2 b	2.31abc
Wheat	19.1a	31.8a	3.48a
Lupine	9.8 b	16.0 b	1.77 bc
Lucerne	20.6a	29.4ab	0.87 c
Medic	14.7ab	27.4ab	1.07 bc
Rye grass	14.9ab	25.8ab	2.06abc
Control	12.7ab	22.1ab	1.90abc
LSD (P≤0.05)	9	14.3	1.64

*Means followed by the same letter are not significantly different at the 0.05 probability level

Lucerne

At three weeks after planting, above-ground leachates from barley, lucerne, medic and rye grass had increased lucerne shoot length significantly from that attained at the control treatment (Table 27). Only medic leachates increased lucerne shoot length significantly from that attained at the control treatment, at five weeks after planting. This growth-stimulating effect was also evident in lucerne dry mass after treatment with barley, canola, wheat, lupine, medic and rye grass leachates.

Table 27 Effects of above-ground leachates on lucerne shoot length and dry mass

	Above-ground leachate		
Plant type	Lucerne shoot length at 3 wks (cm)	Lucerne shoot length at 5 wks (cm)	Lucerne dry mass (g)
Barley	9.0a	25.7ab	3.33 bc
Canola	8.2ab	23.3ab	2.62 cd
Wheat	8.4ab	22.4ab	4.81a

Lupine	4.7 c	16.9 b	4.21ab
Lucerne	10.3a	22.8ab	1.67 de
Medic	9.2a	28.7a	2.12 d
Rye grass	10a	22.5ab	3.11 c
Control	5.5 bc	17.3 b	0.74 e
LSD (P≤0.05)	3.4	10	0.96

*Means followed by the same letter are not significantly different at the 0.05 probability level

Medic

At three weeks after planting, leachates from lucerne and medic had stimulated medic shoot length significantly from that attained at the control treatment (Table 28). This growth stimulating effect from lucerne leachates, was also evident at five weeks after planting. The dry mass of medic, treated with barley, canola, wheat, lupine and rye grass leachates, was significantly greater than the control.

Table 28 Effects of above-ground leachates on medic shoot length and dry mass

	Above-ground leachate		
Plant type	Medic shoot length at 3 wks (cm)	Medic shoot length at 5 wks (cm)	Medic dry mass (g)
Barley	5.8ab	11.9ab	3.52 b
Canola	5.8ab	10.4 b	3.09 bc
Wheat	5.4ab	11.5ab	6.10a
Lupine	5.1ab	10.4 b	3.08 bc
Lucerne	7.1a	15.1a	1.69 cd
Medic	7.2a	13.5ab	2.23 bcd
Rye grass	6.1ab	11.7ab	2.66 bc
Control	3.7 b	10.7 b	0.73 d
LSD (P≤0.05)	2.6	4.1	1.59

*Means followed by the same letter are not significantly different at the 0.05 probability level

Rye grass

Above-ground leachate from lucerne increased rye grass plant height significantly from that attained at the control at three weeks after planting (Table 29). No significant differences between above ground leachates treatments were observed in rye grass plant height at five weeks. The dry mass of rye grass treated with wheat above ground leachates was significantly higher than the control.

Table 29 Effects of above-ground leachates on rye grass plant height and dry mass

	Above-ground leachate		
Plant type	Rye grass plant height at 3 wks (cm)	Rye grass plant height at 5 wks (cm)	Rye grass dry mass (g)
Barley	22.5ab	33.3ab	2.7 b
Canola	21.1 b	30.1 b	2.29 bc
Wheat	24.9ab	34.3ab	4.27a
Lupine	21.7ab	35.2ab	2.43 b
Lucerne	28.3a	38.3a	1.57 cd
Medic	24.7ab	33.8ab	1.47 d
Rye grass	23.1ab	33.0ab	1.99 bcd
Control	20.7 b	35.5ab	1.98 bcd
LSD (P≤0.05)	2.8	5.6	0.78

*Means followed by the same letter are not significantly different at the 0.05 probability level

The methodology followed in Experiment 2, is being suggested as a bioassay to study the effects of seedling leachates on the germination process of crop seeds. Compared to existing procedure that screen for potential seedling allelopathy under laboratory conditions, the advantages of this method are: a) it can be applied to most grain and leguminous crops; b) the possibility of measuring several response parameters on roots or shoots; c) it is suitable for testing early stages of plant development within a short time of less than a week for donor and receiver germination, totalling roughly two weeks for a data set, and d) the possibility of testing various donor densities, easy handling and low costs of material. In addition, testing of the dose-response method as part of the protocol gives it a wider applicability. However, the dose-response design requires high rates of germination of donor plants especially for the higher densities, which can be a problem for poorly germinating cultivars and/or small quantities of available seeds (Belz, 2004). The assay is, however, reliable, simple, and fast, and facilitates high-throughput screening to screen and select for allelopathic traits in several grain crops.

DISCUSSION

Although results from seed and seedling leachates do not have obvious practical relevance, it was suggested by Hoffman *et al.* (1996) that competitive hierarchies often form during early stages of plant growth, including between germinating seeds and between seedlings. For this reason and to obtain comprehensive data from all plant parts, results from seeds and seedling leachates indicated allelopathic activity for crop species.

Barley

Cumulative germination of barley was inhibited 31% and 28% by wheat and medic seed leachates, respectively. Plant height of barley at 5 weeks after planting was inhibited by root leachates from lupine (9%), lucerne (17%) and medic (9%). The dry mass of barley was reduced after treatment with root leachates from wheat (16%), lupine (19%) and lucerne (22%). This finding is in accordance with those of Xuan *et al.* (2005), who also reported plant inhibition by lucerne.

Canola

Canola radicle length was reduced by lupine (76%) and lucerne (54%) seed leachate, respectively. After treatment with lucerne (12%) and medic (20%) root leachates, canola plant height was greater at five weeks after planting. Ground lucerne (40%) and medic (41%) residues stimulated canola with regard to plant height at both three and five weeks after planting.

The effects of lupine, lucerne and medic on barley, canola and wheat are generally similar to those reported by Xuan and Tsuzuki (2002). Many reports have indicated that lucerne (*M. sativa* L.) plants contain water-soluble allelochemicals that are released into the soil environment from fresh leaf, stem and crown tissues, as well as from dry hay, old roots and seeds.

Wheat

The radicle length of wheat was reduced by seed leachates from barley, wheat and lupine. Lupine seed leachates also reduced wheat cumulative germination by 77%. Ben-Hammouda *et al.,* (2001) reported that the allelopathic potential of barley increased near physiological maturity. Leaves and roots were the most phytotoxic barley plant parts for durum and bread wheats, respectively. Laboratory experiments (Qasem, 1994) showed that aqueous extracts of many weed species inhibited germination, coleoptile length, root length, and shoot and root dry weight of wheat and barley seedlings grown in Petri-dishes. Extracts of the fresh materials were inhibitory to cereal seedlings compared to extracts from the dried materials.

Rye grass root leachates increased wheat plant height by 9% at three weeks after planting. This growth stimulating effect by rye grass root leachates on wheat plant height was also evident at five weeks after planting. At five weeks after planting, leachate from barley, canola, wheat, lupine and lucerne had inhibited wheat height. The dry mass of wheat treated with lucerne (76%), medic (74%) and rye grass (54%) above-ground leachates, was less than the control. A transition from stimulatory to inhibitory effects over time was observed for rye grass root leachates and above-ground residues. According to Kruidhof (2008) there are two possible explanations for this. Firstly, it is widely recognised that low concentrations of allelochemicals can be stimulating to weed germination and early growth (Lovett *et al.,* 1989; Belz, 2004). Secondly, the observed stimulation could be a response to increased nutrient and especially nitrate levels in the residue-amended soil, because nitrate stimulates weed seed germination (Bouwmeester & Karssen, 1993).

Results indicating inhibition of wheat growth by leachates from wheat seeds correspond with those by McCalla and Norstadt (1974), who also showed that the water soluble substances in wheat residues reduced germination and growth of wheat seedlings. Furthermore, in pot experiments, Sozeri and Ayhan (1998) found that mixing wheat straw with soil decreased germination of wheat seeds, and increased seedling mortality.

Lupine

Barley seed leachate reduced lupine cumulative germination (75%) from that attained at the control treatment. In contrast, root leachates from barley increased lupine plant height (15%) at five weeks.

Lucerne

Lupine seed leachate had reduced lucerne cumulative germination by 100%. In contrast, canola (102%) and rye grass (93%) seedling leachate stimulated the growth of lucerne seedlings with regard to radicle length. A growth-stimulating effect was evident in lucerne dry mass after treatment with barley, lupine and rye grass residue leachates.

The influence of rye grass on wheat and lucerne contrasted with findings of Breland (1996), who investigated phytotoxicity after spring grain on a loam soil was undersown with Italian ryegrass (*L. multiflorum*), following on clover (*Trifolium repens*) or no cover crop in the previous year. The ryegrass incorporated by spring rotary tillage reduced radish germination up to 45%. Germination values, in response to leachates from fresh ryegrass, were 64%. At double the amount of crop residues, the corresponding value was 27%.

Lucerne produces allelopathic saponins which might be the major cause of yield reduction in subsequent crops (Hall & Henderlong, 1989). Hall and Henderlong (1989) indicated that the water soluble fraction from lucerne shoots have the characteristics of phenolic compounds. Among several phenolic compounds assayed for their phytotoxicity on root and shoot growth of lucerne, coumarin and t-cinnamic were most inhibitory. Most parts of lucerne plants contain autotoxic substances that inhibit seed germination and early seedling growth. Chung *et al.* (2000) reported that chlorogenic acid occurs in relatively large amounts (0.39 mg g^{-1}) in lucerne aqueous extracts as compared to salicylic acid (0.03 mg g^{-1}), and bioassays suggest that chlorogenic acid is involved in lucerne autotoxicity.

Medic

The radicle length of medic was inhibited by lupine seed (60%) and seedling (18%) leachates as was cumulative germination. Treatment with lupine root leachate inhibited radicle length (51%) of medic at five weeks, cumulative germination percentage and reduced medic dry mass. In contrast, medic dry mass was increased (44%) by lucerne root leachates. At both three and five weeks after planting, above-ground leachates from lucerne had stimulated medic shoot length. The dry mass of medic, treated with lupine above-ground leachates, was greater than the control.

Rye grass

The radicle length of rye grass was inhibited by seed leachates from barley (66%) wheat (57%) and lupine (96%). This growth-inhibiting effect from lupine seed and seedling leachates, was also evident in rye grass cumulative germination percentage. These findings on wheat are in accordance with those by Wu *et al.*

(2000a), who evaluated 92 wheat cultivars for their allelopathic activity on the inhibition of root growth of annual ryegrass. They found significant differences between wheat cultivars in their allelopathic potential at the seedling stage on the inhibition of root elongation of annual ryegrass, with percentage inhibition ranging from 24 to 91 percent.

However, the dry mass of rye grass treated with wheat (24%) and lupine (24%) root leachates was higher than the control, as was dry mass yield of rye grass treated with wheat above-ground leachates. Although the pasture type of rye grass (*L. multiflorum* Lam. v. Energa) was used under controlled conditions in order to ensure one seed source and consistent germination, results from the field experiment suggest similar responses for this species and the weed type hybrid (*L. multiflorum x perenne*).

Results from the dry mass of rye grass, which was reduced by medic, correspond with those of Fourie (2005) who reported that ‘Paraggio’ medic as a cover crop had a significant negative impact on weed growth during winter. It was speculated that effectively suppressing the winter growing weeds may result in a reduction in the dosage of herbicide applied, and it may minimise the negative effects caused by weeds, such as the harbouring of nematodes and insects during winter (Fourie *et al.,* 2005). However, such a practice is likely to be exposed to the vagaries of environmental factors, as well as likely being crop and weed-specific.

In contrast, Hoffman *et al.* (1996) found that rye root residues had more suppressive effects on both emergence and growth of barnyardgrass than did shoot tissues. Inhibitory effects of both root and shoot extracts of buckwheat on germination of downy brome, although low, (17 to 22%) were similar (Machado, 2007).

Vanillic and o-coumaric acids along with scopoletin may be responsible for the allelopathic effects of barley and wheat (Baghestani *et al.,* 1999). Baghestani *et al.* (1999) recommended that an increase in these three allelochemicals may be considered in any cereal breeding programme.

CONCLUSION

The allelopathic activity observed for lupine and medic under controlled conditions, corresponds to results obtained in the field and confirms that these leguminous crops should be used prominently, although medic is already planted extensively as rotational crop in the Swartland region. In the long rotation systems of the Overberg region, lupine should be used more frequently in the crop rotation systems used between lucerne plantings. Further studies on the use of crop mulches that do not affect the crop they are used in, yet inhibit or suppress weeds, appear to be warranted. Crop mulches that can provide weed control could reduce dependency on herbicides, in particular those products which are associated with the development of weed resistance. In the case of the mulch being a leguminous plant, the better known attribute of nitrogen fixation will also be achieved.

CHAPTER 4

Geographical differentiation and genetic variation of *Lolium* spp in the Western Cape: identification of the hybrid *Lolium multiflorum x perenne* and isolation of the pathogen *Fusarium pseudograminearum*

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INTRODUCTION

While conducting research on rye grass v. Energa described in Chapter 2 Exp 1 and all experiments conducted in Chapter 3, it became increasingly evident that identification of the different *Lolium* spp is problematic. Since representative rye grass weed seeds, as opposed to rye grass pasture type v. Energa used in Chapter 2 & 3, were required for further representative research, it was decided to do relatively quick, but extensive tests in order to gather background information and establish a reliable seed source for research conducted in Chapter 2 Exp 2 and Chapter 5. A random site adjacent to a wheat field was identified at Hermon (18°97’E, 33°43’S, Western Cape) and rye grass samples were collected, prepared and sent for morphological identification. When results from the Compton Herbarium at Kirstenbosch indicated a rye grass hybrid type, the study described in this chapter, followed. As it was a hybrid never before described in South Africa, it was decided that various aspects should be covered by research collaborators to gain as much information as possible in a relatively short period of time.

Economically, there is no doubt that herbicides and herbicide-resistant crops have drastically improved agricultural efficiency and yields. However, the broad application and/or sometimes the misuse of herbicides has also created problems. The major problem is the evolution of weeds with resistance to herbicides which refers to the capacity of a plant to grow and reproduce under the dose of herbicide that is normally lethal to the species (Yuan *et al.*, 2007). Weed resistance to herbicides presents one of the greatest current economic challenges to agriculture (Baucom, 2009) with more than 346 biotypes of weed known to be resistant to herbicides (Heap, 2010). A species' ability to adapt to changing environmental conditions is found in the genetic diversity of its populations. Success in weed populations facing changing agricultural ecosystems often correlates with an abundance of genetic polymorphisms within those populations (Jasieniuk & Maxwell, 2001). Through the process of mutation and selection, however, weeds evolve resistance to herbicides when they are used repeatedly (Tranel & Trucco, 2009). *L. rigidum* (rigid ryegrass) (Monaghan, 1980) was regarded by Tranel and Trucco (2009) to be the most important weed in terms of it having evolved resistance to multiple herbicides.

According to O'Hanlon *et al.* (2000), there is a widespread concern that weed species with higher levels of genetic diversity will exhibit considerable potential for adaptation and, therefore, may be able to reduce the effectiveness of weed control. Weeds have genetic traits that give them remarkable plasticity, allowing them to adapt, regenerate, survive, and thrive in a multitude of ecosystems (Chao *et al.,* 2005). Many agronomic weeds are close relatives of crop plants and studies on the sequencing of a weed genome are likely to provide clues concerning weed phenotypes and their underlying gene networks (Broz & Vivanco, 2009).

Molecular marker studies have revealed differences in population structure and diversity between the native and introduced types for many invasive weed species (Lee, 2002; Bossdorf *et al.,* 2005). The development of polymerase chain reaction (PCR)-based techniques to assess genetic diversity has since proven to be a quick, easy, and cost-effective way of performing genetic analysis. In cases where genomic sequence data is available, it is possible to work with microsatellite markers (SSRs - simple sequence repeats) as these tend to give a single unique PCR product and in many cases have numerous alleles, making the evaluation of genotypes much more informative. For rye grass a large number of SSRs have been characterised and published (Gill *et al.,* 2006, Hirata *et al*., 2006, Jones *et al.,* 2002, Mian *et al*., 2005, Saha *et al*., 2004, Saha *et al*., 2005, Senda *et al*., 2005, Studer *et al.,* 2006), and these SSRs provided the basis for the analysis required in the present study.

L. perenne L. (perennial ryegrass) (Charmet & Balfourier, 1994) is native to most of Europe and parts of the Mediterranean and Middle East areas, whereas rigid rye grass is distributed all around the Mediterranean. The genus *Lolium* consists of two groups of species, which are outbreeding and inbreeding, respectively (Senda *et al*., 2005). *L. temulentum* L. (darnel) is an inbreeding species and regarded as a mimic weed and has convergently evolved with cereal crops as a result of unconscious selection by farmers (Senda *et al.,* 2005). The genetic diversity of outbreeding rye grass has been studied in relation to the characterisation of genetic resources of *L. multiflorum* Lam. (Italian ryegrass) (Charmet & Balfourier, 1994) and perennial ryegrass. Analysis of the frequency and distribution of genetic variation in natural populations of perennial ryegrass has supported the view that its centre of origin is the Fertile Crescent (Middle East) and that its distribution expanded following a clinical geographical pattern (Senda *et al*., 2005). Both perennial and rigid rye grass are wind-pollinated, self-incompatible outbreeding species (Balfourier *et al*., 2000). Balfourier *et al.* (2000) reported on the weak genetic differentiation, but significant patterns of geographical variation with respect to diversity indices and allele frequencies have been observed in perennial rye grass. In contrast, no spatial organisation of diversity has been detected in rigid rye grass (Balfourier *et al*., 2000).

Herbicide resistant rye grass is a serious problem in Western Cape grain producing areas as it is threatening more than 100 000 ha of productive grain fields. Wheat fields have become so heavily infested that economic grain production, will be impossible in certain areas in the foreseeable future, leading to huge production losses and less sustainable grain production. Knowledge about the genetic constitution of rye grass and its populations is increasingly becoming crucial, particularly given the extent of herbicide-resistance within the Western Cape. These data will in turn further enhance our understanding of the genetics and evolution of herbicide-resistant weeds. Descriptive studies of patterns of genetic diversity in weedy populations can be an extremely important tool for helping to minimise the evolvement of resistance to herbicides (Madhou *et al.*, 2005).

Crown rot, caused by *Fusarium pseudograminearum*, is one of the most important soilborne diseases of wheat in South Africa and also poses a major threat to barley and wheat production in the Western Cape Province. The disease can be significantly reduced by crop rotation with non-susceptible crops such as *Brassica napus* (canola), (*Lupinus angustifolius*) (lupine), annual *Medicago* spp (medic) and *Trifolium* spp (clover) (Lamprecht *et al*., 2006). However, it is known that grass weed infestation in the non-crop phase of the rotation can favour the disease, and grass weed control is therefore recommended as part of an integrated strategy to manage crown rot (Burgess *et al*., 2001). Currently there is no information available on grass weed hosts of crown rot in South Africa.

The aims of this study were to: 1) assess the distribution of genetic variability of rye grass; 2) determine its botanical classification by morphological analyses; 3) determine the presence of the crown rot pathogen of barley and wheat on rye grass; and 4) analyse soil samples from each collection point where rye grass were sampled to determine its preference for soil chemical properties.

MATERIALS AND METHODS

Four agricultural production areas of the Western Cape Province, as indicated in Figures 1 & 2, were included in this study, namely Malmesbury-

Moorreesburg (Swartland) - area A, Worcester-Robertson (Breede River Valley) - area B, Stellenbosch-Paarl (Winelands) - area C, and Caledon-Swellendam (Overberg) - area D. These areas were used for *Lolium* spp sampling in 2008 from August until October at 10 localities in each area. Two additional localities with known resistant and susceptible populations of rye grass were also sampled and designated F (Fairview Farm, multiple resistant) and G (Glencairn, susceptible).

Collection points

A simple random sampling strategy, using geographic coordinate points, was followed to ensure representative sample collection. To achieve this, the Random Geographic Coordinate Sampling function of the software program Survey Toolbox© was used to determine 40 randomly selected geographic coordinate points in the main agricultural production areas for grain, fruit, vineyards and mixed agricultural production in the Western Cape. ArcView 8.3 software was used for GIS manipulation of these collection points for easy reference during collection. A Magellan® SporTrak GPS system (with 3 meter accuracy) was utilised in the location of these randomly selected collection points.

Genetic analyses

The first specimen taken at each collection point was used for genetic analyses. Total DNA was extracted from leaves according to the modified CTAB protocol (Senda *et al*., 2004). DNA was prepared twice for experimental replication in each analysis. The SSR technique is a high-resolution genetic marker analysis used to assess genetic relationships in many species. The polymerase chain reaction (PCR) enables the development of powerful genetic markers for the measurement of genotype variation. By measuring genotype, rather than phenotype, genetic markers avoid complicating environmental effects and provide ideal tools for assessing genetic variation, identifying species and other locally adapted forms, as well as the definition of genetic relationships.

SSRs were analysed using an appropriate selection of the published primer pairs for *Lolium*, distributed across the genetic map to ensure a random selection of genetic markers. The SSRs were chosen from those, which were known to work across species, and to have the largest number of alleles. Primers were synthesised with fluorescent labels for subsequent analysis (Madhou *et al.*, 2005). Primer optimisation was undertaken to obtain conditions of selective PCR giving unique products for each primer set. Where appropriate, multiple reactions containing several sets of primers were used (Madhou *et al.*, 2005). When this was completed the analysis of a range of *Lolium* isolates was undertaken. Alleles were scored by analysis using the ABI Genetic Analyser, and scored using the GenoTyper^{TM} software.

SSR similarities between isolates were calculated by the simple matching coefficient, *m/n*, where m is the number of alleles matched and n is the total number of alleles. Cluster analysis was performed using the un-weighted pair-group method with arithmetic averages (UPGMA) (Senda *et al*., 2005). For each dendrogram, the correlation coefficient between the matrix of genetic similarities and the matrix of co-phenetic values was computed, and data produced by AFLP were compared using the Mantel test (Senda *et al*., 2005).

Morphological analyses

A second specimen of each sample was collected and morphologically analysed at the Compton Herbarium, Kirstenbosch Botanical Gardens, Cape Town, in order to identify the different species or hybrids.

Pathogenic analyses

A third specimen of each sample was collected and analysed for the soilborne pathogen crown rot at the Agricultural Research Council - Plant Protection Research Institute’s laboratory at Stellenbosch. The number of plants collected from each area for isolation of the fungus varied from three and five for areas F and G, respectively, to 50 each for areas A, B, C and D. The protocol described by Lamprecht *et al*. (2006) was used for the isolation and identification of crown rot.

Soil analyses

A soil sample was taken at each rye grass collection point and analysed at the Soil Science Laboratory at Elsenburg.

RESULTS AND DISCUSSION

Genetic analyses

SSRs use an appropriate selection of the published primer pairs for *Lolium*, but as these were only published for Italian -, perennial - and rigid rye grass, all specimens were categorised as one of these species. Therefore, no SSRs have been characterised and published for hybrids among rye grass species, creating contrasts in results between genetic and morphological analyses. However, evaluation of these two methods for identification of Italian rye grass revealed that 90% of specimens occurring as weeds were morphologically classified as a hybrid.

Huge genetic variation was detected between Italian rye grass weed populations with no identifiable alleles associated with herbicide resistance. This finding was complicated by the number of alleles per locus for grass species which is 8*n* as opposed to 2*n* for humans, and the occurrence of quantitative trait loci (http://wikipedia.org) which occurs in organisms displaying chemical resistance. Rigid rye grass showed similarity in genetic make-up in the eastern part of area D and perennial rye grass to a lesser extent in area B from samples collected at Robertson and Montagu, but there was no consistent correlation between geographical and genetic distance of specimen pairs.

Overall, SSRs indicated 47.6% of specimens as rigid rye grass, 42.9% as Italian rye grass and 9.5% as perennial rye grass (Figure 1 & Appendix A, Tables A2-A5). Genetic variation analyses indicated 38% of specimens as rigid rye grass from the areas A and D (Swartland-Overberg), while 9.5% classified as rigid rye grass was sampled in areas B and C (Breede River Valley-Winelands). Only four specimens (9.5%) were classified as perennial rye grass, of which three occurred in areas B and C and a single specimen in area D.

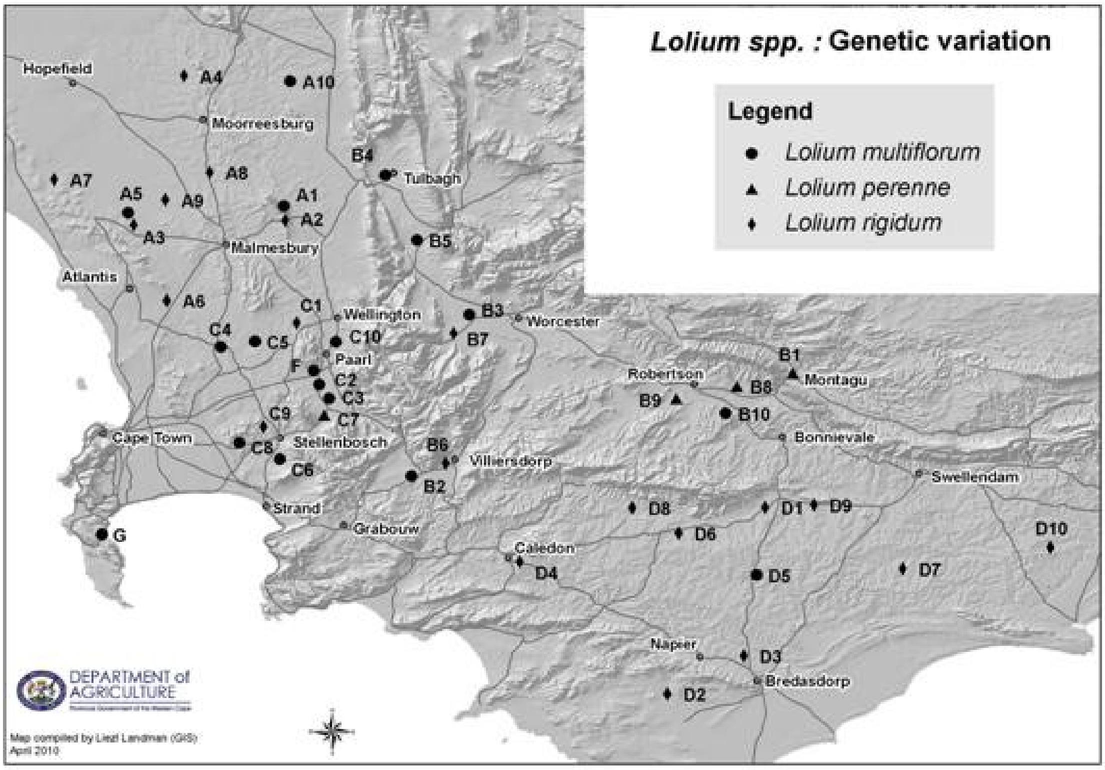

Figure 1 Distribution of rye grass based on genetic classification in the Western Cape

Morphological analyses

Morphologically, 50% of the total number of specimens was classified as rigid rye grass, 48% as the hybrid, namely *L. multiflorum x perenne* and 2% as perennial rye grass. Both the proven herbicide resistant (F) and susceptible specimens (G) were identified as the hybrid *L. multiflorum x perenne* (Figure 2 & Appendix A, Tables A2-A5). Although genetic analyses could only identify Italian rye grass, it would be safe to assume that if published primer pairs were available, many samples would be classified as *L. multiflorum x perenne*.

Of the specimens collected from the wheat, barley and sheep production areas of the Swartland and Overberg (areas A and D), 40.5% was morphologically identified as rigid rye grass, 7% as *L. multiflorum x perenne,* and 52.5% as Italian rye grass. Morphological analyses identified 40.5% of specimens sampled in areas B and C as *L. multiflorum x perenne,* 10% as rigid rye grass and 49.5% as Italian rye grass. Only one specimen (2%) which occurred in area B (Breede River valley) was classified as perennial rye grass. *L. multiflorum x perenne* displayed forked ears, indicating prolific seed production, in 8% of specimens. This characteristic could promote the proliferation of this weed to the extent that it may be a factor contributing to it becoming dominant in weed communities. Treier *et al.* (2009) reported that taxonomists have identified at least two forms of the allelopathic weed, *Centaurea maculosa,* in its region of origin. In addition to multiple flowering in a particular year, the tetraploid form of this weed is capable of producing multiple flowering stems with up to fifteen capitula each, whereas the diploid produces only one stem (Broz & Vivanco, 2009).

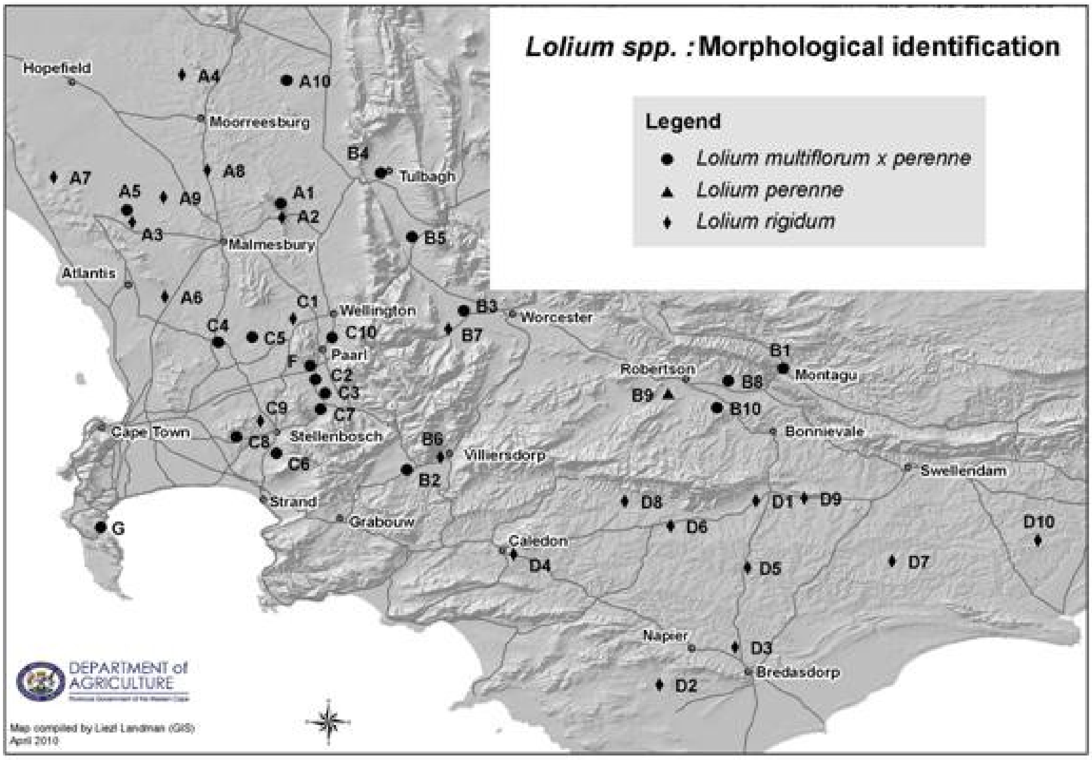

Figure 2 Distribution of rye grass based on morphological classification in the Western Cape

Comparing genetic and morphological maps

A comparison between the genetic and morphological maps revealed that 90% of specimens genetically classified as Italian rye grass were morphologically identified as the hybrid *L. multiflorum x perenne.* In three instances (7.5%), specimens genetically classified as perennial rye grass*,* were morphologically also analysed as *L. multiflorum x perenne* namely at B1 (20°1283'E, 33°7780'S), B8 (19°9901'E, 33°8102'S) and C7 (18°9705'E, 33°8803'S)*.* One specimen (2.5%) collected from B9 (19°8401'E, 33°8402'S) was genetically analysed as Italian rye grass, while it was morphologically classified as rigid rye grass (Appendix A, Tables A2-A5). As no published primer pairs exist for *L. multiflorum x perenne,* no identifiable alleles associated with herbicide resistance could be observed.

A case in point is the important and well-recognised component in the evolutionary history of *Amaranthus* spp of inter-specific hybridisation (Tranel & Trucco, 2009). Hybridisation has been proposed as a critical stimulus for invasiveness and is perhaps aiding in the evolution of adaptations critical for the success of *Amaranthus* spp as weeds. Although species interbreeding is most often maladaptive, it might represent an important route for the evolution of genotypes favoured under the intense selection pressure found in agricultural habitats (Tranel & Trucco, 2009). A clear example of this possibility is herbicide resistance evolution. A resistant individual resulting from a hybridisation event may be lacking in health, vigour, and fertility, but may represent the only viable genotype upon herbicide treatment (Tranel & Trucco, 2009). From a weed management perspective, however, the most significant aspect of invasiveness is the ability of a species to modify a given attribute over time and in response to selection. The evolution of herbicide resistance often forces dramatic changes in weed management practices (Tranel & Trucco, 2009).

Results from the current study on the variability and occurrence of hybrids in rye grass populations from the study area, is in accordance with reports by

Dinelli *et al.* (2002). This author reported high variability in Italian populations of rye grass and hybrid banding profiles from electrophoretic data with up to 24% of individuals which were placed in an intermediate position between rigid rye grass and Italian rye grass. In contrast, Australian populations were more homogeneous with 88% of individuals showing an ordination closely related to rigid rye grass. Furthermore, Italian populations of rye grass were heterogeneous, consisting of several genetically unique individuals which can readily hybridise (Dinelli *et al.,* 2002).

It should also be noted that darnel (*L. temulentum*) was not detected at any collection point, though it was supposed to occur in the sampling area. There could be a few reasons for this: a) not enough sampling points in the study area; b) it was replaced by the more aggressively invasive *L. multiflorum x perenne* and/or rigid rye grass and c) as crop production practices changed substantially over the last two decades in the study area, it was eradicated, because according to Spahillari *et al.* (1999), it cannot survive without the agricultural practices to which it has become adapted. These crop production practices include type of soil disturbance, seed drilling as opposed to sowing, shorter growth season crop cultivars allowing less time for the weed to reproduce, different times of nitrogen application and weed control with herbicides containing active ingredients from different chemical groups, effectively eradicating darnel.

Soil analyses

From Table 1 it is clear that the soils preferred by rye grass cover a wide range of fertility below and above optimum ranges for wheat. Soils range from very acidic pH to high pH or alkaline soils (with the mean being a little above optimum for wheat). The high upper value in the range for sodium values indicates very saline soils. On average the phosphorus content is high, indicating a preference for agricultural fields. Soil analyses emphasises the wide adaptability of rye grass which contributes to their success as invasive weeds.

Table 1 Properties of soils sampled at rye grass collection points

Soil Property	All soils (n = 42)		Optimum
	Mean	Range	range for wheat
pH (KCl)	6.0	4.2-7.9	4.5-5.5
Resistance Ω	740	40-3450	> 400
Calcium (cmol/kg)	8.7	1.17-61.45	1-10
Magnesium (cmol/kg)	2.3	0.27-5.99	0.3-3
Sodium (mg/kg)	128	10-1564	< 100
Potassium (mg/kg)	229	64-790	60-150
Total bases (cmol/kg)	12.5	2.56 – 65.98	2-20
Phosphorus (mg/kg)	104	12-518	60-100
Copper (mg/kg)	3.4	0.42-21.49	0.5-1
Zinc (mg/kg)	7.9	1.05-50.06	0.5-1
Manganese (mg/kg)	60.2	4.4-269.2	> 5
Boron (mg/kg)	0.4	0.07-1.3	0.3-0.8
Sulphur (mg/kg)	10.4	1.12-130	> 7
Carbon (%)	1.9	0.6-4.84	2-3

The only distinction in soil preference among rye grass samples could be drawn on clay content among the different soil samples analysed. In the Swartland (area A) and Overberg (area D), where rigid rye grass mainly occurred, the soil samples contained an average of 21% clay. Italian rye grass was predominant in the Breede River Valley (area B) and Winelands (area C), where the average clay content was 16%. However, since the latter two areas has a high incidence of irrigation, this could have contributed to the wide spread occurrence of Italian rye grass.

Pathogenic analyses

Crown rot was isolated from six localities namely, A2 (18°6734’E, 33°4008’S) on rigid rye grass A4 (18°6236’E, 33°0443’S) on rigid rye grass, A7 (18°3026’E, 33°3001’S) on rigid rye grass, B5 (19°2001’E, 33°4502’S) on *L. multiflorum x perenne*, C9 (18°8200’E, 33°9101’S) on rigid rye grass and D10 (20°7653’E, 34°2078’S) on rigid rye grass (Figure 3). To our knowledge and according to literature searches, this data on crown rot had not been published before, and this is the first report of crown rot on rye grass in South Africa.

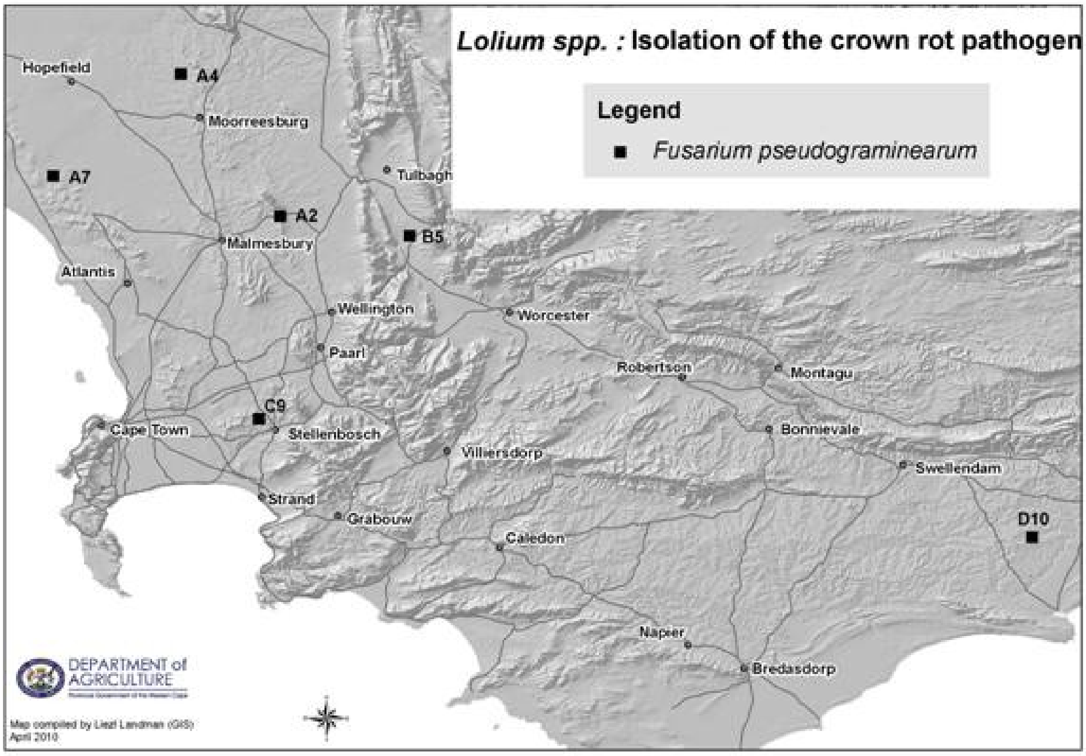

Figure 3 Collection points where *F. pseudograminearum* was isolated from rye grass in the Western Cape

Rye grass can therefore act as alternative hosts and as a source of inoculum of this important soilborne pathogen of barley and wheat in the Western Cape Province. This further complicates sustainable dry land crop production, since the build-up of herbicide resistant rye grass may lead to a higher incidence of crown rot on wheat and barley due to a higher disease pressure. However, it could also point to a possible biological control agent for rye grass.

Implications for invasive weed control

There is some evidence to suggest that environmental adaptation and evolution play an important role in the success of invasive weed species and ecological hypotheses of plant invasion have been developed based on this evidence (Callaway & Aschehoug, 2000). Furthermore, strong evidence points to the fact that allelopathy plays a role in the invasive success of many plant invaders and weeds (Ridenhour & Callaway, 2001; Bertin *et al.,* 2003;

Hierro & Callaway, 2003). Callaway and Aschehoug (2000) suggested that plants come to the new environment equipped with chemical or biochemical weapons that have a greater negative effect against plants in the invaded range than similar species in the native range. These weapons (allelochemicals) give the invader an advantage in the new environment, because they have putative strong phytotoxic effects on other plants. If plant invaders gain a competitive advantage through the use of novel weapons in the invaded range they will evolve to have greater concentrations of these weapons than populations in the native range (Callaway & Aschehoug, 2000). For the high population densities of rye grass occurring in the Western Cape Province, this implies that the weed gained a competitive advantage, not only by being better competitors than other plant species, but also by exuding allelopathic substances. Allelopathy has long been suspected to be important in both agronomic and native ecosystems (Weston & Duke, 2003) and could account for the aggressive behaviour of weeds (Locken & Kelsey, 1987).

In contrast, Moodie *et al.* (1997) suggested that it is more likely that the variation detected between weed populations may be a result of herbicide treatments giving rise to variations in phenotypes, which may not be due completely to herbicide resistance. Ash *et al.* (2003) reported that genetic diversity studies give an indication of underlying genetic diversity and an indication of divergent evolution. As highly diverse populations may harbour differing resistances and so may be more difficult to control as it may result in differential reactions and prevent uniform plant responses. Increased understanding of the meaning of identity values could lead to important evidence related to differential tolerance to herbicides in field conditions and to development and spread of resistance (Frigo *et al.,* 2009).

For the development of effective procedures to control rye grass, it is necessary to learn about their mechanisms of spread, for which an understanding of the plant’s genetic variation may be important. Data have indicated that there are distinct genetic groups within weedy rye grass populations of the Western Cape. Knowledge about this differentiation of rye grass could aid in the research approach on rye grass resistance and integrated control methods. In fact, knowledge of both genetic and morphological diversity may be important to guide the development of differential management of rye grass. Results from this study will further enhance our understanding of the genetics and evolution of herbicide-resistant rye grass and may lead to the development of specific and differential management strategies for weed control in each population.

In the Literature Review, Chapter 2 Exp 2 and in Chapter 5 the difference in responses by cultivars from the same crop is emphasized. As rigid, Italian, perennial and weed hybrid rye grass occurred in the study area, it could be speculated that their responses to herbicides may display plant differential effects. Practically speaking and with herbicides registered for grass control (graminicides) grouped as controlling either annual or perennial grass species, this means that the rye grass weed hybrid may have characteristics enabling it to be non-susceptible to herbicides registered as annual graminicides. In that case it cannot be regarded as weed resistance to herbicides but rather as non-susceptibility, because the weed has perennial characteristics. The implication of this is that different control strategies should be devised according to the prevalent species occurring in a particular field.

CONCLUSION

The wide genetic and morphological variation detected in rye grass is interpreted on the basis of high genotypic plasticity and hybridisation for producing *Lolium multiflorum x perenne*. High levels of heterozygosity would indicate that rye grass plant populations probably have substantial amounts of adaptive genetic variations to escape the effects of a control agent. It may also be the result of the differential selection pressure or of the heterogeneity of environmental factors. Effective localised control methods for the various species and hybrids in this genus should be prioritised to curb further development of herbicide resistance. Soils preferred by rye grass cover a wide range of fertility below and above optimum ranges for wheat and emphasises its wide adaptability and success as an invasive genus. Furthermore, rye grass can act as alternative hosts and a source of inoculum of the important soilborne pathogen crown rot of barley and wheat, underlining its importance as a production constraint in crop production.

CHAPTER 5

Allelopathic root exudates of the weed *Lolium multiflorum x perenne* and crops influence plant growth and changes in the soil microbial community

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INTRODUCTION

Plant interactions mediated through chemical substances are identified within the allelopathy phenomenon. The allelopathic process involves excretion of bioactive compounds from plants or micro-organisms that inhibit or stimulate physiological processes of neighbouring individuals belonging to either the same or different species (Kazinczi *et al.,* 2005; Weston, 2005; Gu *et al.,* 2008b). Allelopathic compounds can exert a harmful impact on the emergence of seedlings and their establishment as well as on the development of plants (Lipin´ska & Lipin´ski, 2009).

Several studies have shown that some crop cultivars are allelopathic and that their inhibitory effects on weeds can cause significant suppression of the latter plants' growth under field conditions (Olofsdotter *et al.,* 1999; Wu *et al.,* 1999). Alsaadawi *et al.* (2005) concluded that sorghum cultivars differ in allelopathic potential and that the exploitation of cultivars with higher allelopathic capacity would be of value for weed control, particularly in no-tillage cropping systems. Several rice cultivars identified in the individual screenings of weeds of rice were successful in substantial root growth inhibition of more than one weed type (Seal *et al.,* 2005). Belz (2007) discussed breeding efforts in wheat *(T. aestivum)* and barley *(H. vulgare)* which showed that early vigour and allelopathy against *L. perenne* L. (perennial ryegrass) were significantly related to field weed suppression, whereby the relative importance proved to be cultivar and crop specific.

These root exudates may have dramatic impacts on soil rhizospere ecology, including enhancement of certain soil microbial populations and dramatic reductions in others, leading to a shift in nutrient availability and uptake by plants within the ecosystem (Weston, 2005). Allelopathic rice releases allelochemicals from roots to soil at significant rates to interact with soil micro-organisms (Gu *et al.,* 2008b). Potent allelochemicals from the rice material and root exudates may modify soil micro-organisms to the crop's advantage (Kong, 2008). This author found that allelopathic rice releases allelochemicals from its roots to paddy soils at early growth stages to inhibit neighbouring weeds and it was shown that allelopathic rice can have a great impact on the population and community structure of soil microbes. Micro-organisms such as fungi, bacteria, viruses and nematodes are integral parts of agro-ecosystems. Some of them are harmful plant pathogens, whereas others are neutral or beneficial in their effects on plant growth (Huang & Chou, 2005).

According to Inderjit (2005), allelopathy methodology has been criticized due to the neglect of its effects on soil microbes. In addition, crop-microbe interactions mediated by allelochemicals in soil have yet not been clearly described (Kong, 2008). Findings made by Kong (2008) imply that soil microbial populations are affected by the compounds released by allelopathic rice varieties. Kong (2008) also confirmed that variation of the soil microbial populations and community structures could be distinguished by the allelopathic and non-allelopathic rice varieties tested. It was therefore decided to use the Biolog EcoPlate™ to determine physiological profiling of micro-organisms present in the rhizosphere of the tested plant species that were tested in the present study.

Following on results from Chapters 2 and 3 and because the allelopathic process involves excretion of bioactive compounds from plants or micro-organisms, it was decided to extend this research to include an additional lupine cultivar and both pasture and weed types of *Lolium* spp, as several studies have shown that some crop cultivars and weeds are allelopathic (Olofsdotter *et al.,* 1999; Wu *et al.,* 1999; Belz, 2004), with the objective of determining the interactions among allelopathic root leachates, from different crop cultivars and the weed type rye grass, their growth rate, and soil micro-organisms. Also assessed were the allelopathic effects of the afore-mentioned plant species on wheat and barley as representatives of main crops in rotational systems in the Western Cape.

MATERIALS AND METHODS

Pot experiment

The plant series used in a greenhouse study comprised the rotational crops barley (*H. vulgare* L. v. Clipper), wheat (*T. aestivum* v. SST 027), lupine (*Lupinus angustifolius* L. v. Tanjil and v. Quilinock), rye grass (*L. multiflorum* Lam. v. Energa) and the rye grass hybrid type *(L. multiflorum x perenne).*

The research approach was based on research methods followed by Reinhardt *et al.* (1994), Hoffman *et al.* (1996) and Smith *et al.* (2001) for assessing whether crop root exudates release phytotoxins that affect the growth and yield of rotational crops and weeds. The present study was however different in terms of both experimental method and plant series investigated.

According to Inderjit (2005), several climatic and edaphic factors affect the soil microflora; therefore, allelopathy should ideally be assessed in a range of soil types. For this reason, soil from two diverse localities, namely Langgewens (18°70'E, 33°27'S) and Tygerhoek (19°54'E, 34°08'S) (Appendix A, Table A7) research farms in the grain-producing area of the Western Cape Province, was collected for the greenhouse experiment. Soils from Langgewens are residual and of the Glenrosa type (Soil Classification Working Group, 1991). Tygerhoek soils are weakly developed residual soils and of Mispah type (Soil Classification Working Group, 1991). In the greenhouse, which was set at a constant temperature of 18 °C, natural lighting was used, simulating normal day length for the crop growth period from May to September (Southern Hemisphere).

Experimental design made provision for the establishment of "donor" plants in pots from which leachates were collected on a regular basis to treat "acceptor" plants grown in separate pots. Each pot was filled with 6 kg of top soil collected from either Langgewens or Tygerhoek. For both the "donor" and "acceptor" plant series, six crop seeds of each plant type were planted in potted soil. Seedlings were thinned to three plants of similar size one week after emergence. Once a week, 100 ml Multifeed[1] was

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[1] Plaaskem (Pty) Ltd, PO Box 14418, Witfield, 1448

applied as a balanced plant nutrition solution at a concentration of 1 g ℓ^{-1}, to each pot. Each pot was over-irrigated bi-weekly with 150 ml (100 ml drainage) tap water from the first week after planting to ensure drainage from pots, reaching 900 ml (300 ml drainage), as plants matured. In the case of the “donor” series all water leached from the same plant species was collected in one container, separately for each species and used as root leachate treatment. No planting was done in control pots, but the leachate was collected in the same way described above for use as control treatment. Treatments in the greenhouse were replicated three times in a randomised block design and the experiment was repeated once.

Of the leachate collected from the “donor" plant series, which served as sources of allelochemicals, 100 ml was transferred bi-weekly to the “acceptor” plant series. In this way the leachate from a particular species was applied to plants of the same type as well as to each of the other plant types. The first transfer of leachate took place at the time of planting, and thereafter bi-weekly up to sixteen weeks after emergence.

Microbial community analysis

To determine changes in microbial populations over the trial period, whole community metabolic analyses on all soil samples from the pot experiment were performed (Garland & Mills, 1991). The Biolog EcoPlate™ was developed specifically for microbial community analysis (www.biolog.com). In applied ecological research, the Biolog EcoPlate™ is used as both an assay of the stability of a normal population and to detect and assess changes based upon the variable introduced. The Biolog EcoPlate™ presents micro-organisms in the soil solution with 31 of the most preferred carbon sources (Appendix A, Table A6). The consumption of these carbon sources would be specific to a microbial community, presenting the observer with a physiological profile of the microbial community under observation. Any changes in the composition of this microbial community will thus be reflected in changes in the carbon source utilisation pattern. In this study we used the Biolog EcoPlate™ system to indicate a change in the microbial community in response to the plant root leachate added. It has to be considered that because micro-organisms are at the bottom of the food chain, changes in microbial communities are often a precursor to change in the health and viability of the environment as a whole (Garland & Mills, 1991).

Soil samples of 10 g each were taken at the onset of the experiment before filling of the pots to serve as reference point. After harvesting of plants, two soil samples (denoted by _1 and _2 in Tables A8 – A10, Appendix A) of 10 g were again taken from each treatment. All soil samples taken in this way were suspended in 90 ml sterile distilled water. After shaking for 10 minutes the sample was prolapsed and inoculated directly into Biolog EcoPlate™ (Biolog, Haywood, CA, USA) as a soil suspension and incubated at 22 °C in the dark. After 48 hours the microbial community-level physiological profile was assessed visually for colour development by noting “no change” and “change” (purple discolouration) compared to the control treatment. Utilisation of the carbon source in each well, indicated by a reduction of the tetrazolium dye, was then recorded as either negative (carbon source not used) or positive (carbon source used). The utilisation of a carbon source (positive reaction), was indicated by a colour change when compared to the control without any carbon source.

Plant and microbial data collection and statistical analysis

Plant height was determined for all acceptor plants on a weekly basis, starting from the first week after planting until plants were harvested at maturity. Plants were regarded as mature when the reproductive growth phase was completed at the onset of senescence as indicated by visible loss of chlorophyll, i.e. yellowing of leaves. Growth rate was measured and expressed as cm gained per day from the regression parameters of the fitted regression models. At maturity, tillers for Graminaceae species and pods for lupine, were counted per pot and seed mass determined. Data for all these parameters are not presented here. Because of differences in plant growth patterns between the two localities, data for each soil type were analysed separately. All data were averaged over the two sets of data for each locality and were analysed statistically (ANOVA) with the statistical program SAS. Least significant difference (LSD) values were used to differentiate between the effects of the donor plant series on the acceptor plant series at the 5% level of probability.

The carbon-source-use Biolog EcoPlate™ data, collected on the two sampling occasions were analysed using principal component analysis (PCA) to determine the effects of root leachate treatments on soil micro-organisms and plant growth rate. PCA was done with Pearson correlation matrix as input (Appendix A, Table A3 – A5).

RESULTS

Barley v. Clipper

The growth rate of barley grown on Langgewens soil and exposed to barley or lupine v. Tanjil root leachates was significantly greater than the control (zero root leachates) (Table 1). Barley grown on the same soil and treated with wheat, lupine v. Quilinock, *L. multiflorum* v. Energa or *L. multiflorum x perenne* root leachates had its growth rate reduced compared to the control (Table 1).

For barley, grown on Tygerhoek soil, no significant differences in growth rate were recorded following treatment with root leachates (Table 1).

Table 1 Effects of root leachates from the donor plant series on growth rate of barley v. Clipper on Langgewens or Tygerhoek soils

	Langgewens soil	Tygerhoek soil
Plant type	Growth rate X 10^{-2} cm day^{-1}	Growth rate X 10^{-2} cm day^{-1}
Barley v. Clipper	5.575a	3.932a
Wheat v. SST 027	4.405c	3.968a
Lupine v. Tanjil	5.931a	3.814a
Lupine v. Quilinock	4.153c	3.992a
L. multiflorum v. Energa	4.209c	3.648a
L. multiflorum x perenne	4.365c	3.633a
Control	4.996b	3.697a
LSD (P=0.05)	0.410	0.360

*Means followed by the same letter are not significantly different at the 0.05 probability level

In the score plot for barley grown on Langgewens soil, physiological profiles were observed which clustered together in the top left quadrant, showing a correlation with growth rate which had an association with carbon sources C7, C12, C14 and C18. The loading plot indicates that utilised carbon sources which clustered together in the top left quadrant followed treatments with root leachates from barley or lupine *v.* Quilinock (Figure 1a).

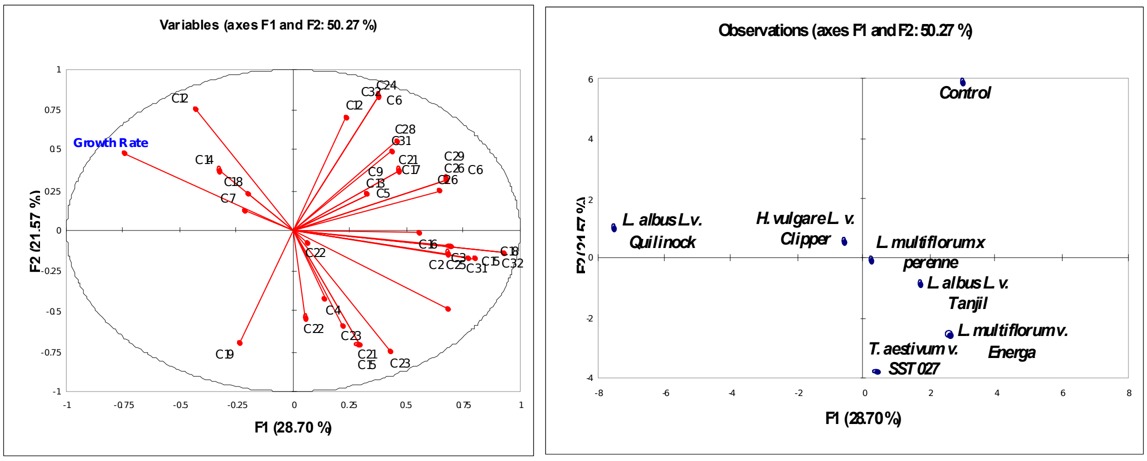

Figure 1a Score plot (left) and loading plot (right) of barley v. Clipper grown on Langgewens soil, and its association with carbon source utilisation

For barley grown on Tygerhoek soil, no carbon source utilisation was observed in the top left quadrant of the score plot in Figure 1b. Therefore, growth rate had no association with carbon sources and no correlation with control root leachates, which is evident in the top left quadrant of the loading plot (Figure 1b).

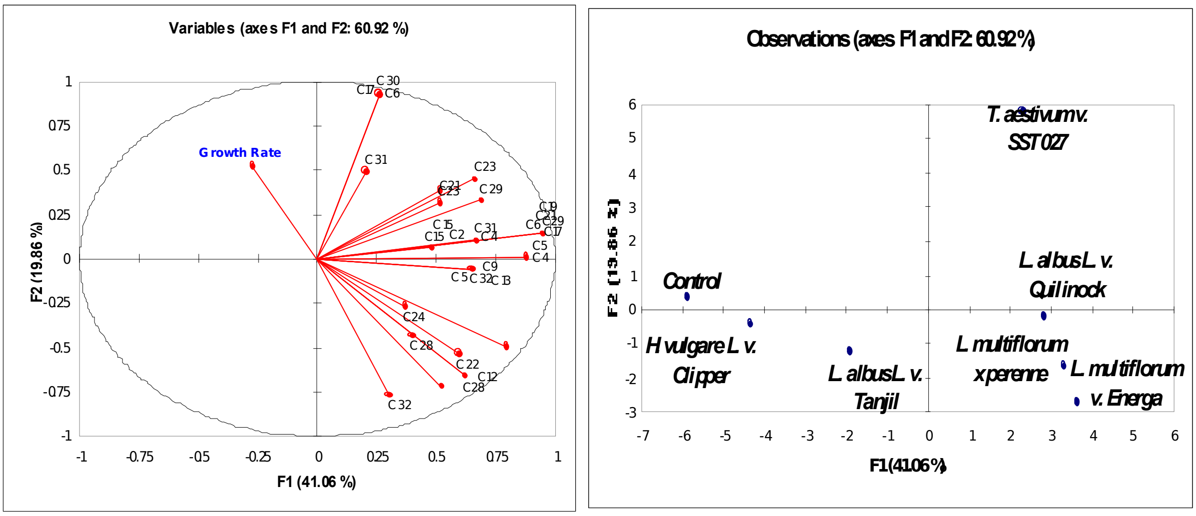

Figure 1b Score plot (left) and loading plot (right) of barley v. Clipper grown on Tygerhoek soil, and its association with carbon source utilisation

Wheat v. SST 027

Lupine v. Tanjil or v. Quilinock root leachates caused a significant increase from the control in wheat growth rate, when grown on Langgewens soil (Table 2). For wheat grown on Tygerhoek soil, no significant differences between treatments were recorded in growth rate (Table 2).

Table 2 Effects of root leachates from the donor plant series on growth rate of wheat v. SST on Langgewens or Tygerhoek soils

	Langgewens soil	Tygerhoek soil
Plant type	Growth rate X 10^{-2} cm day^{-1}	Growth rate X 10^{-2} cm day^{-1}
Barley v. Clipper	5.435ab	4.458a
Wheat v. SST 027	5.466ab	4.777a
Lupine v. Tanjil	5.813a	4.703ab
Lupine v. Quilinock	5.734a	4.641ab
L. multiflorum v. Energa	4.987bc	4.368b
L. multiflorum x perenne	4.765c	4.379b
Control	5.109bc	4.454ab
LSD (P=0.05)	0.500	0.340

*Means followed by the same letter are not significantly different at the 0.05 probability level

In the score plot of Figure 2a, the physiological profile for wheat grown on Langgewens soil, clustered in the top right quadrant which shows a correlation with growth rate and an association with a particular series of carbon sources. The top right quadrant of the loading plot reveals that this followed treatment with *L. multiflorum* v. Energa root leachates (Figure 2a).

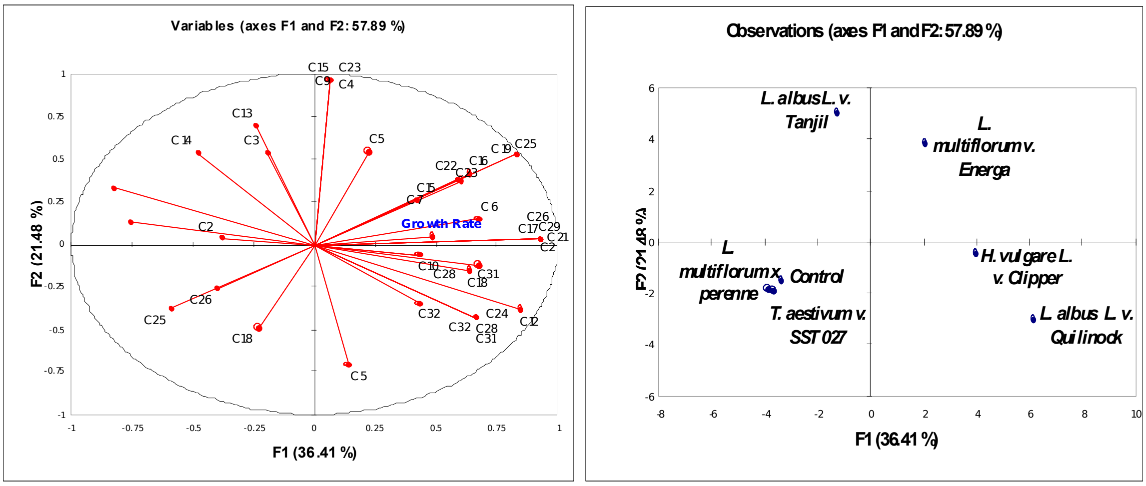

Figure 2a Score plot (left) and loading plot (right) of wheat v. SST 027 grown on Langgewens soil, and its association with carbon source utilisation

The score plot in Figure 2b indicates that a cluster of utilised carbon sources in the top right quadrant correlates with growth rate and is associated with carbon sources C5, C6 and C22. This followed treatment of wheat grown on Tygerhoek soil, with wheat root leachates, as revealed by the loading plot.

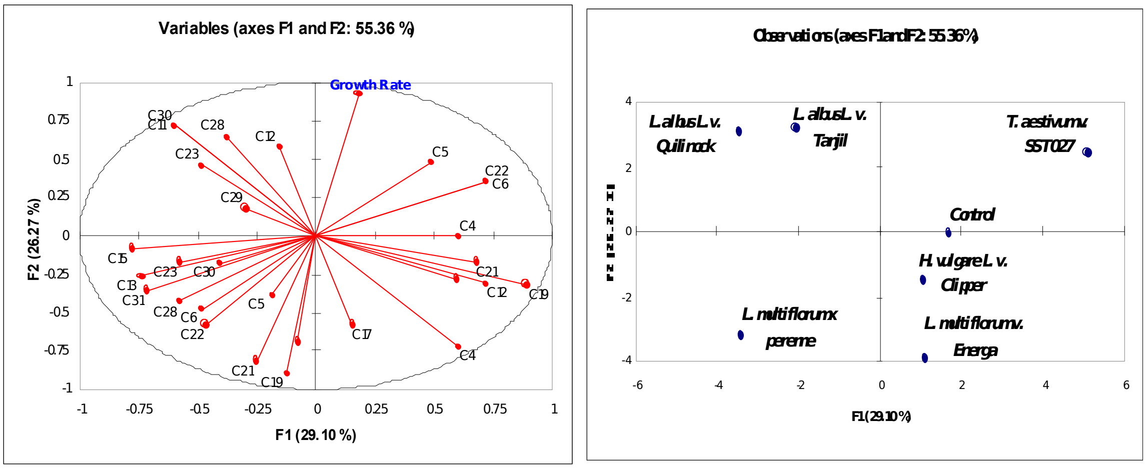

Figure 2b Score plot (left) and loading plot (right) of wheat v. SST 027 grown on Tygerhoek soil, and its association with carbon source utilisation

Lupine v. Tanjil

Lupine v. Tanjil, grown on Langgewens soil and exposed to lupine v. Quilinock root leachate, had a significantly faster growth rate than that attained in the control treatment (Table 3).

No significant differences in growth rate between treatments were recorded in lupine v. Tanjil grown on Tygerhoek soil (Table 3).

The score plot for Langgewens soil in Figure 3a indicates that the physiological profile which clustered together in the top right quadrant, has a correlation with growth rate and an association with a particular series of carbon sources. This corresponds with the physiological profile clustering together in the top right quadrant of the loading plot in Figure 3a, following treatment of lupine v. Tanjil, grown on Langgewens soil and treated with lupine v. Tanjil, lupine v. Quilinock or *L. multiflorum x perenne* root leachates.

Table 3 Effects of root leachates from the donor plant series on growth rate of lupine v. Tanjil on Langgewens or Tygerhoek soils

	Langgewens soil	Tygerhoek soil
Plant type	Growth rate X 10^{-2} cm day^{-1}	Growth rate X 10^{-2} cm day^{-1}
Barley v. Clipper	5.366b	4.483b
Wheat v. SST 027	4.789b	4.807ab
Lupine v. Tanjil	5.831ab	4.622ab
Lupine v. Quilinock	6.634a	4.918ab
L. multiflorum v. Energa	4.930b	4.965a
L. multiflorum x perenne	5.671ab	4.535ab
Control	5.482b	4.785ab
LSD (P=0.05)	1.100	0.480

*Means followed by the same letter are not significantly different at the 0.05 probability level

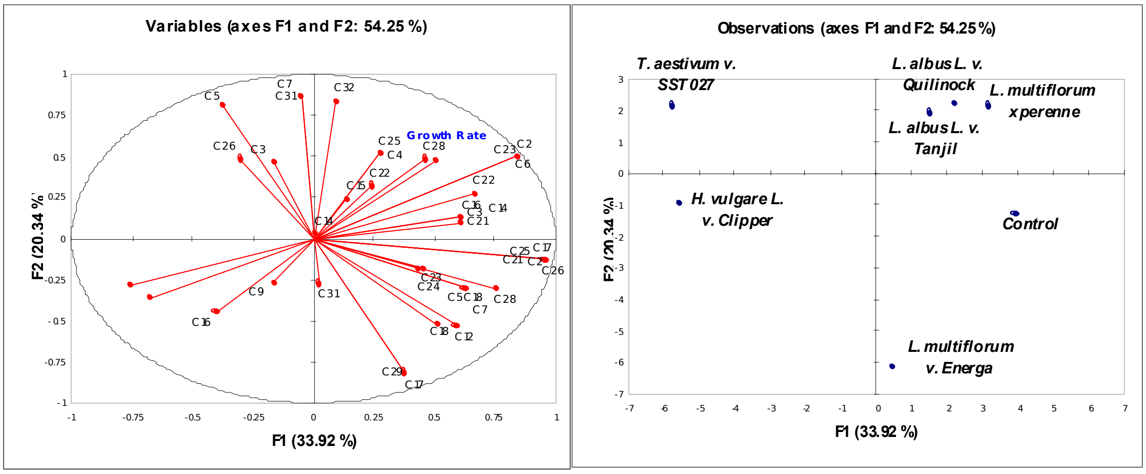

Figure 3a Score plot (left) and loading plot (right) of lupine v. Tanjil grown on Langgewens soil and its association with carbon source utilisation

The score plot for Tygerhoek soil reveals a physiological profile in Figure 3b, which clustered together in the bottom right quadrant; correlating with growth rate and associated with carbon sources C6 and C24. The bottom right quadrant of the loading plot indicates that microbes utilising those two carbon sources were affected by *L. multiflorum* v. Energa root leachates (Figure 3b).

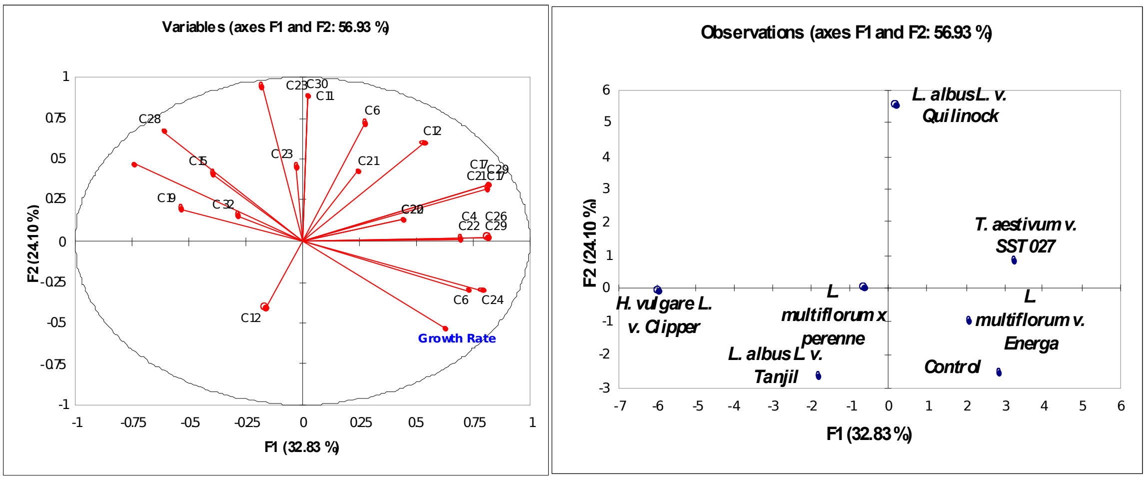

Figure 3b Score plot (left) and loading plot (right) of lupine v. Tanjil grown on Tygerhoek soil, and its association with carbon source utilisation

Lupine v. Quilinock

The growth rate of lupine v. Quilinock grown on Langgewens soil and exposed to barley, wheat or *L. multiflorum x perenne* root leachates, was significantly greater than the control (Table 4). There were no significant differences in the growth rate of lupine v. Quilinock on Tygerhoek soil.

Table 4 Effects of root leachates from the donor plant series on growth rate of lupine v. Quilinock on Langgewens or Tygerhoek soils

	Langgewens soil	Tygerhoek soil
Plant type	Growth rate X 10^{-2} cm day^{-1}	Growth rate X 10^{-2} cm day^{-1}
Barley v. Clipper	5.073ab	4.545b
Wheat v. SST 027	5.656a	4.489b
Lupine v. Tanjil	4.665bc	4.681ab
Lupine v. Quilinock	4.937bc	4.522b
L. multiflorum v. Energa	4.372c	4.486b
L. multiflorum x perenne	5.243ab	4.995a
Control	4.467c	4.792ab
LSD (P=0.05)	0.600	0.420

*Means followed by the same letter are not significantly different at the 0.05 probability level

The physiological profile in the score plot of Figure 4a, which clustered together in the top right quadrant, indicates a correlation with growth rate which had an association with a particular series of carbon sources. The loading plot indicates that treatment of lupine v. Quilinock grown on Langgewens soil, with root leachates from lupine v. Tanjil or *L. multiflorum x perenne,* resulted in this cluster of carbon source utilisation in the top right quadrant (Figure 4a).

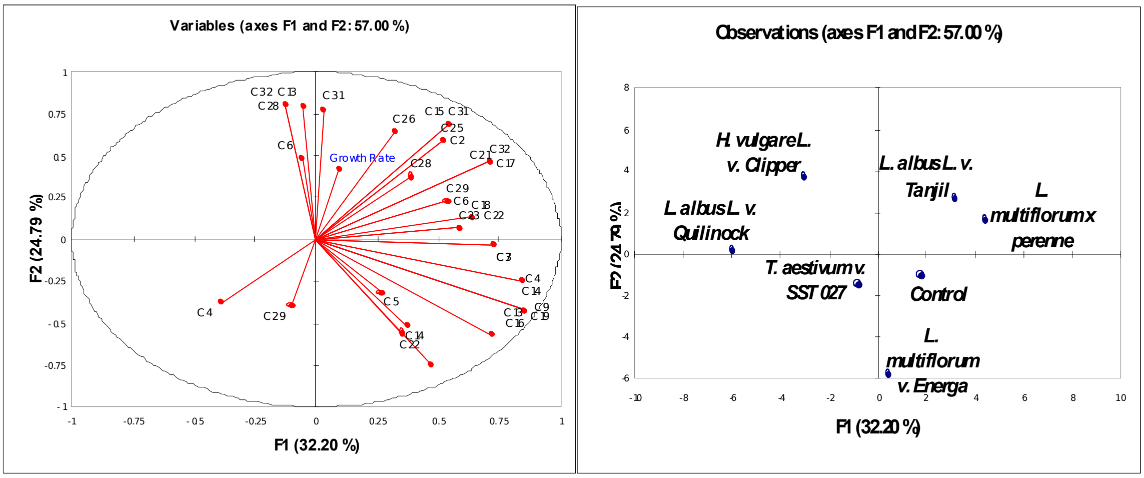

Figure 4a Score plot (left) and loading plot (right) of lupine v. Quilinock grown on Langgewens soil, and its association with carbon source utilisation

In the score plot of Figure 4b for lupine v. Quilinock grown on Tygerhoek soil and treated with lupine v. Quilinock or *L. multiflorum v.* Energa root leachates, a profile of carbon sources was observed as it clustered together in the bottom left quadrant, indicating a correlation with growth rate which had an association with carbon sources C12 and C24. However, the bottom left quadrant of the loading plot reveals that this treatment was control leachate (Figure 4b).

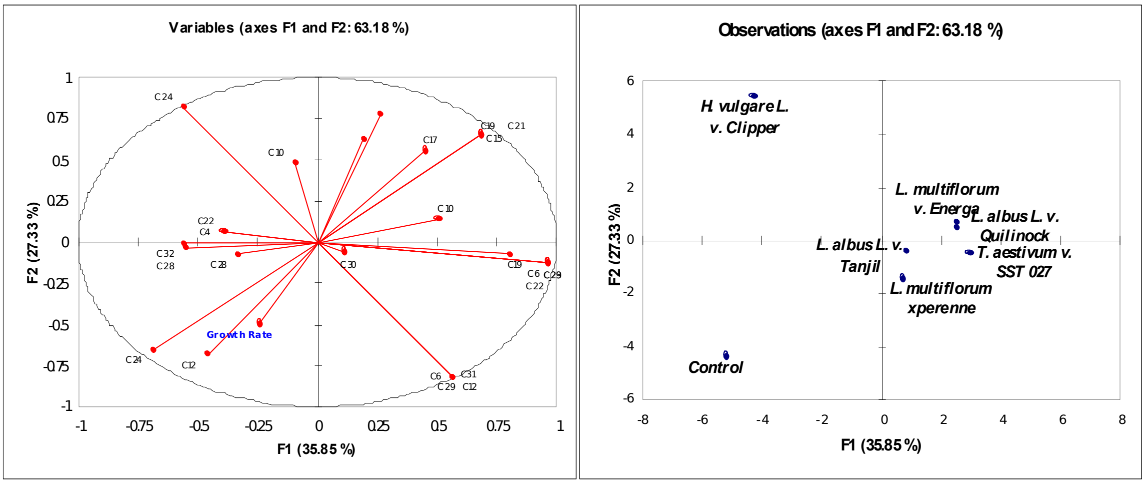

Figure 4b Score plot (left) and loading plot (right) of lupine v. Quilinock grown on Tygerhoek soil, and its association with carbon source utilisation

L. multiflorum v. Energa

Barley root leachate significantly inhibited the growth rate of *L. multiflorum* v. Energa grown on Langgewens soil (Table 5).

The growth rate of *L. multiflorum* v. Energa grown on Tygerhoek soil and treated with *L. multiflorum* v. Energa root leachate, was significantly faster than the control (Table 5).

Table 5 Effects of root leachates from the donor plant series on growth rate of *L. multiflorum* v. Energa on Langgewens or Tygerhoek soils

	Langgewens soil	Tygerhoek soil
Plant type	Growth rate X 10^{-2} cm day^{-1}	Growth rate X 10^{-2} cm day^{-1}
Barley v. Clipper	6.385c	5.009b
Wheat v. SST 027	6.940a	4.894bc
Lupine v. Tanjil	7.115a	4.570c
Lupine v. Quilinock	7.206a	4.637bc
L. multiflorum v. Energa	6.484bc	5.390a
L. multiflorum x perenne	6.445bc	5.002b
Control	6.848ab	4.902bc
LSD (P=0.05)	0.450	0.370

*Means followed by the same letter are not significantly different at the 0.05 probability level

In the score plot of Figure 5a, the physiological profile for *L. multiflorum* v. Energa grown on Langgewens soil, clustered in the bottom right quadrant which shows a correlation with growth rate and an association with a particular series of carbon sources. The bottom right quadrant of the loading plot reveals that this followed treatment with lupine v. Tanjil root leachates (Figure 5a).

The loading plot for Tygerhoek soil in Figure 5b indicates that utilised carbon sources which cluster together in the bottom right quadrant had a correlation with growth rate and an association with a particular series of carbon sources. A similar physiological profile clustered together in the bottom right quadrant of the score plot in Figure 5b, following treatment of *L. multiflorum* v. Energa grown on Tygerhoek soil and treated with wheat or *L. multiflorum x perenne* root leachates.

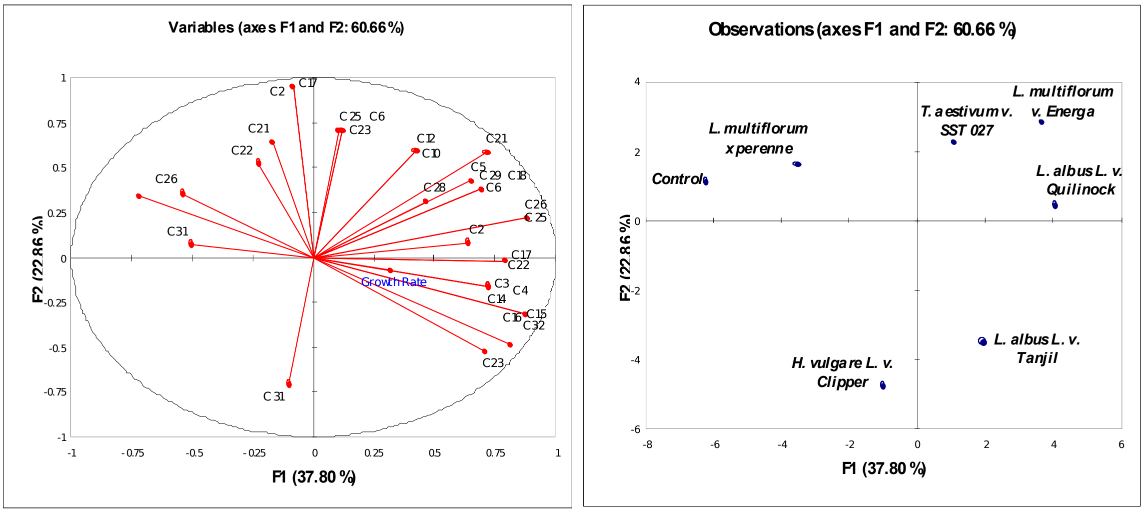

Figure 5a Score plot (left) and loading plot (right) of *L. multiflorum* v. Energa grown on Langgewens soil, and its association with carbon source utilisation

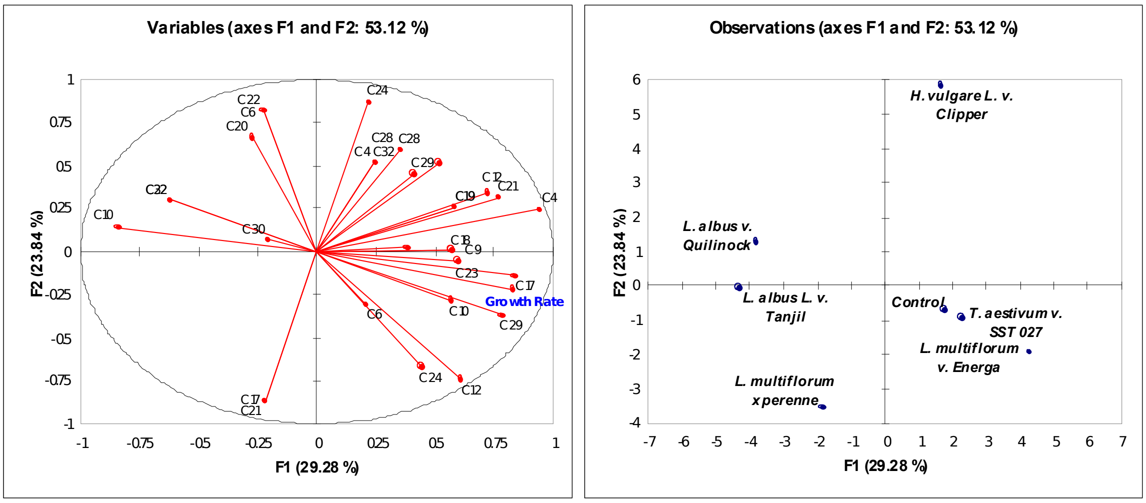

Figure 5b Score plot (left) and loading plot (right) of *L. multiflorum* v. Energa grown on Tygerhoek soil, and its association with carbon source utilisation

L. multiflorum x perenne

The growth rate of *L. multiflorum x perenne* grown on Langgewens soil and treated with barley root leachates, was highly significantly (P=0.01) faster, while wheat or *L. multiflorum x perenne* root leachates, was significantly (P=0.05) faster than the control (Table 6).

No significant differences between the control and other treatments were observed in the growth rate of *L. multiflorum x perenne* grown on Tygerhoek soil (Table 6).

Table 6 Effects of root leachates from the donor plant series on growth rate of *L. multiflorum x perenne* on Langgewens or Tygerhoek soils

	Langgewens soil	Tygerhoek soil
Plant type	Growth rate X 10^{-2} cm day^{-1}	Growth rate X 10^{-2} cm day^{-1}
Barley v. Clipper	3.331a	2.399a
Wheat v. SST 027	3.019b	2.240b
Lupine v. Tanjil	2.823c	2.289ab
Lupine v. Quilinock	2.883c	2.375a
L. multiflorum v. Energa	2.768c	2.294ab
L. multiflorum x perenne	3.132b	2.290ab
Control	2.829c	2.341ab
LSD (P=0.05)	0.130	0.110

*Means followed by the same letter are not significantly different at the 0.05 probability level

The score plot in Figure 6a reveals the profile of carbon sources utilised, which clustered together in the top left quadrant, correlating with growth rate and showing an association with carbon sources C2, C12 and C14. The top left quadrant of the loading plot indicates that *L. multiflorum x perenne* grown on Langgewens soil was treated with barley root leachates (Figure 6a).

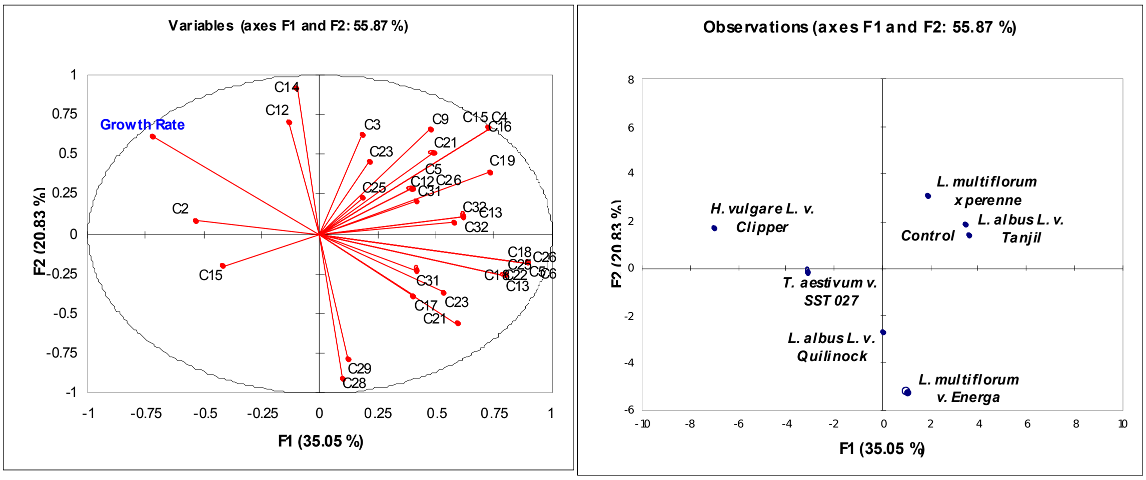

Figure 6a Score plot (left) and loading plot (right) of *L. multiflorum x perenne* grown on Langgewens soil, and its association with carbon source utilisation

A physiological profile in the score plot of Figure 6b was observed, which clustered together in the top left quadrant where growth rate had an association with carbon sources C12, C28 and C31. The loading plot indicates that treatment of *L. multiflorum x perenne* grown on Tygerhoek soil, with root leachates from barley and lupine v. Tanjil, resulted in this cluster of utilised carbon sources in the top left quadrant (Figure 6b).

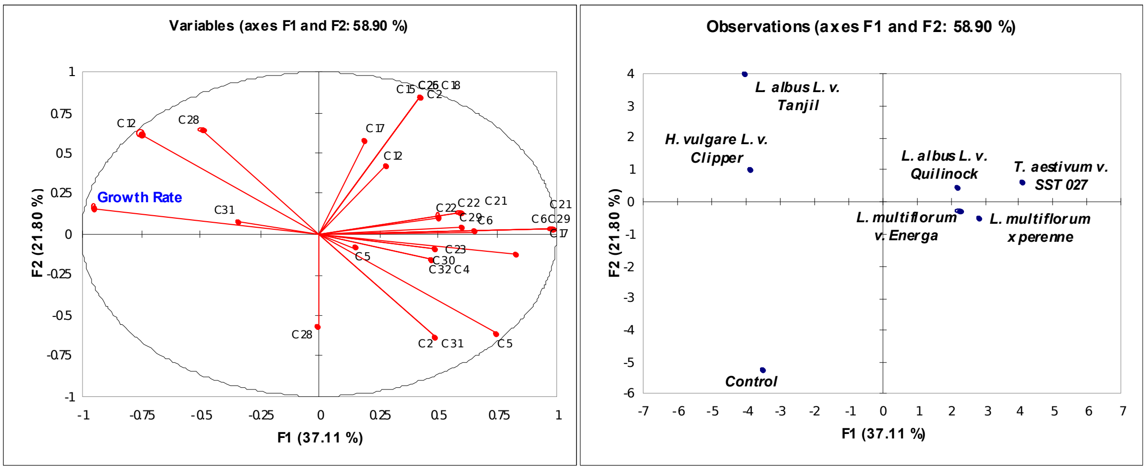

Figure 6b Score plot (left) and loading plot (right) of *L. multiflorum x perenne* grown on Tygerhoek soil, and its association with carbon source utilisation

DISCUSSION

Barley v. Clipper

The growth rate of barley was increased by root leachates from barley, and slowed by those from lupine v. Quilinock. Principal component analysis (PCA) indicated that soil micro-organisms responded differently to those treatments, which may or may not influence allelochemical bioactivity and/or plant growth. Previous reports by both Kruidhof (2008) and Lehle *et al.* (1983) also reported inhibitory effects by lupine on crop plants.

The inhibition and stimulation noted for barley growth is probably related to allelopathic agents in barley as reported by Lovett and Hoult (1995). The production of these allelochemicals in barley appeared to be highly responsive to stressful conditions (Belz, 2004). In the field this could happen due to *inter alia* climatic conditions, soil factors, competition and/or allelochemicals. Furthermore, the production of allelochemicals differs among cultivars as Belz (2007) discussed breeding efforts in barley which showed that early vigour and allelopathy proved to be cultivar specific.

Olofsdotter *et al.* (2002) suggested that different rice cultivars have different selectivity against weed species, indicating that several chemicals are involved in allelopathic action. Broadleaf and grass plants have differential sensitivity towards particular allelochemicals. It should be borne in mind that different rates of the same allelochemicals could have resulted in different growth responses from the species considered here. This dose-response phenomenon is termed hormesis and represents an evolutionarily conserved process of adaptive, potentially beneficial responses to low doses of a stressor agent (Calabrese, 2007). Dose-response studies showed that the occurrence and the magnitude of hormesis depended on concentration of the allelochemical, climatic conditions and the parameter measured (Belz, 2008). Furthermore, as mentioned earlier, the span between stimulation and inhibition for allelochemicals can be small and hormetic effects may occur in a natural setting if doses released are low (Belz, 2008). Under field conditions this equates to higher and lower doses as plant density varies.

Wheat v. SST 027

On Langgewens soil, the growth rate of wheat was stimulated by lupine v. Tanjil or lupine v. Quilinock. This significantly faster growth rate of wheat can most probably be attributed to the N fixing ability of lupine, as N compounds are known to stimulate growth of many plant species (Kumar *et al.,* 2009). Any combined chemical root exudates, including allelopathic effects of a stimulatory nature, could have been masked by the growth promoting effect of nitrogen that conceivably was added to the system by the legume.

An association with microbes utilising particular carbon sources was indicated by PCA, when treated with root leachates from *L. multiflorum* v. Energa or wheat, respectively. Root exudation serves as an important carbon and energy source for micro-organisms contained in the rhizosphere (Bertin *et al.,* 2003). Therefore, it is conceivable that soil microbial populations used particular carbon sources which influenced the growth rate of wheat grown on either Langgewens or Tygerhoek soils. Kong (2008) confirmed that variation of the soil microbial populations and community structures could be distinguished by the allelopathic and non-allelopathic crop varieties tested. Bacilio-Jimenez *et al.* (2003) showed that the components of rice root exudates could affect soil-borne microbes. Although the present study did not consider only the effects of allelochemicals contained in root leachates, but the combined effects of all solutes contained in them, it indicated that the effect on soil microbial population and community structure may be pronounced. This corresponds with the findings of Kong (2008) that the composition of soil microbes is defined at least in part by the nature and amount of chemicals contained in root exudates. Therefore, we contend that the growth rate of test plants in this study could be ascribed to the combination of compounds contributed by root exudates and soil microbial populations. Furthermore, differences in plant growth rate and responses in physiological profiles of micro-organisms observed on the two soils used in the study, suggest that location is an important factor governing plant-plant and plant-microbe interactions.

Lupine v. Tanjil

The faster growth rate of lupine v. Tanjil, grown on Langgewens soil when exposed to lupine v. Quilinock root leachate was probably associated with soil micro-organisms and not plant-derived allelopathic compounds. Nitrogen derived from N-fixing leguminous lupine is known to stimulate plant growth of many plant species (Kumar *et al.,* 2009) hence no inferences on possible stimulatory allelopathic effects would be appropriate, although stimulatory allelopathic effects have been reported (Belz, 2008).

Lupine v. Quilinock

The faster growth rate of lupine v. Quilinock grown on Langgewens soil, which was stimulated by root leachates from barley, wheat or *L. multiflorum x perenne,* is congruent with findings on stimulation by grass species of plant growth (Sarika *et al.,* 2008). Furthermore, PCA indicated that the effect of *L. multiflorum x perenne* on lupine v. Quilinock was probably related to soil micro-organisms, which corresponds generally with results reported by Qasem & Foy (2001) on the stimulation of crop growth by root exudates of certain weed species used by soil micro-organisms as food source.

L. multiflorum v. Energa

The slower growth rate of *L. multiflorum* v. Energa grown on Langgewens soil, which resulted from barley root leachate, confirms results by Baghestani *et al.* (1999) and Belz (2007) who also reported on inhibition of barley leachates. Ben-Hammouda *et al.* (2001) reported for barley that leaves and roots were the most phytotoxic parts reducing plant growth. However, the reported response varied depending on the source of allelochemical(s) (plant part) and the growth stage of the barley plant. Both positive and negative allelopathic effects by rigid rye grass on Italian rye grass was reported by San Emeterio *et al.* (2004), while Wu *et al.* (2003) reported inhibition of rigid rye grass by wheat.

PCA revealed that for Tygerhoek soil an association existed between soil micro-organisms and *L. multiflorum* v. Energa treated with wheat or *L. multiflorum x perenne* root leachates.

L. multiflorum x perenne

L. multiflorum x perenne showed positive responses to Graminaceae species in that wheat or *L. multiflorum x perenne* root leachates stimulated its growth rate when grown on Langgewens soil. The significantly faster growth rate of *L. multiflorum x perenne* on Langgewens soil treated with barley root leachates was revealed by PCA as a probable association with growth-promoting soil micro-organisms. In contrast, the non-significance observed for growth rate of this species on Tygerhoek soil, most probably indicates that either no growth-promoting or growth-inhibiting soil micro-organisms occurred, emphasising the importance of location in plant-microbe interactions.

Generally, the investigated plant species showed not only different plant-micro-organism associations, thus confirming results by Oberan *et al.* (2008) and Kong *et al.* (2008) who reported that different micro-organism associations exist among plant species, but results also pointed to the presence of different allelochemicals for each plant type. Kong *et al.* (2008) also reported that soil microbial populations were affected by the compounds released from allelopathic cultivars.

Comparisons between growth mediums of the leached sand in Chapter 3 and natural soil in Chapter 5 showed that results from Chapter 3 Exp 3 were similar in terms of the inhibition of barley by leguminous crop root leachates. Wheat was stimulated by lupine in the current study, probably because effects became more pronounced after 16 weeks as opposed to the five week duration for the study in Chapter 3 Exp 3. Lupine was stimulated in both studies, while barley root leachates inhibited rye grass v. Energa and stimulated rye grass weed type growth rate in both instances in the current study.

Gu *et al.* (2008a) and Kong *et al.* (2008) suggested that allelopathic crops and weeds could modify the microbial community structure in soil to their advantage through the release of allelochemicals. Own findings strengthen the significance of soil micro-organisms in chemical root exudates and allelochemical-mediated interactions between plants, whether to lessen or to magnify effects. It has been demonstrated that not only the originally exuded compounds but also their derivatives can have allelopathic activity (Belz, 2007).

Kato-Noguchi *et al.* (2009) speculated that the secretion of allelopathic compounds into the rhizospere may provide a competitive advantage for root establishment through local suppression of pathogenic soil micro-organisms and inhibition of the growth of competing plant species. El-Shatnawi & Makhadmeh (2001) suggested that rhizospere micro-organisms have positive or negative effects on plant growth and morphology by affecting the plant hormone balance, plant ensymatic activity, nutrient availability and toxicity, and competition with other plants. Plants can modify the rhizospere in other ways than through the release of allelochemicals, e.g. by causing changes in soil pH, nutrient and moisture levels and as a result can modify the local plant community.

CONCLUSION

Crop cultivars and weeds may modify the soil micro-organism populations to their advantage and to the disadvantage of other species by the release of root exudates that apparently differ in composition between plant species, thus confirming their allelopathic potential. Findings indicate that root exudates contained putative allelochemicals which influenced microbial community profiles. The effect on microbial communities varied with source of exudates and between soils. Changes in microbial community structure could affect plant growth through the promotion or suppression of harmful or beneficial microbes and the microbial production of allelochemicals. Further research is required to elucidate the allelochemicals involved and the link between them, microbial community structure, and plant growth.

CHAPTER 6

General discussion and conclusions

This study investigated the use of allelopathic properties from crop residues for the suppression of rye grass weed type *(Lolium multiflorum x perenne)*; evaluated the role of allelopathy from seeds, seedlings, roots and above-ground plant material of rotational crops; assessed the geographic distribution of genetic and morphological variability of rye grass and determined the interactions among allelopathic root leachates from rotational crops and rye grass on their growth rate and soil micro-organisms. With wheat fields so heavily infested, that economic grain production in certain areas will be impossible in the foreseeable future, this data will further enhance our understanding of herbicide-resistant rye grass and minimise the emergence of more species' with resistance to herbicides (Madhou *et al*., 2005) and promote weed control measures that are alternative to herbicides.

Crop residues

Crop residues from the leguminous crops (lupine and medic) increased wheat growth with regard to plant number per m^2, yield, and plant height. The inhibitory effects of lucerne crop residues on the number of barley tillers and yield, and on plant height and yield of wheat is in accordance with those effects reported by Xuan and Tsuzuki (2002) and Xuan *et al.* (2005). Xuan and Tsuzuki (2002) and Bertholdsson (2004) reported that between and within crop species there is large genetic variation in the allelochemical content of plant tissue. Also, various studies have shown that concentrations of allelochemicals in plants are not stable. The levels of allelochemicals in a plant are influenced by abiotic and biotic stresses in combination with age or growth stage (Mwaja *et al.,* 1995; Reberg Horton *et al.,* 2005). Kruidhof (2008) described a transition from inhibitory to stimulatory effects of crop residues over time. Low concentrations of allelochemicals can stimulate plant growth (Lovett *et al.,* 1989; Belz, 2004; Belz, 2007) and increased growth has also been associated with increased nitrate levels in residue-amended soil (Henson, 1970). Therefore, the increased growth observed in some instances in the present study may indicate that there was a positive nutrient effect in conjunction with growth-promoting allelopathic activity from the crop residues. Allelopathic crops when used in rotational sequence are helpful in reducing noxious weeds, improve soil quality and crop yield (Khanh *et al.,* 2005). According to results in Experiments 1 & 2 of this study, allelopathic crops of this nature may suppress weeds without affecting wheat yield. Khanh *et al.* (2005) reported that these crop plants, particularly the legumes (*Medicago* spp), can reduce weed infestation and increase rice yield by between 20 and 70%, and are suggested for use as natural herbicides. This is congruent with most findings in allelopathy research that decomposing plant residues in soil exhibit the greatest inhibition at the early stages of decomposition and that phytotoxicity declines as decomposition proceeds (An *et al.,* 2001; Xuan *et al.,* 2005). The nature and strength of inhibitory allelopathic effects appear to be dependent on interactions between soil factors and crop residues and the allelochemicals they produce (Kumar *et al.,* 2009). Furthermore, similar to many plant characteristics, allelopathy is influenced by environmental conditions (Olofsdotter, 2002; Weston & Duke, 2003), as was experienced in Experiment 1 of Chapter 2 where high rainfall conditions could have diluted allelochemicals.

Results from Experiment 1 in Chapter 2, which demonstrated the ability of medic to suppress the rye grass weed type promise practical application under field conditions because of the crop's spreading growth habit which could be effective for the establishment of an effective organic mulch. According to results in Experiments 1 & 2, a mulch of this nature may suppress weeds without affecting wheat yield. In the case of rye grass weed type, however, both lupine cultivars suppressed the weed to only 3.9 and 4.5%, respectively. Furthermore, a suppressive plant competition effect from broadleaf weeds on the grass weeds cannot be excluded. An early flush of emergence from a huge seed bank plus high growth rates probably benefited the dominance of broadleaf weeds. In ascribing allelochemical-mediated effects under field conditions one has to be mindful of the fact that persistence of allelochemicals is largely influenced by soil type and weather conditions (Levitt *et al.,* 1984). Therefore any hypothesis based on crop residues imparting positive weed suppressive effects through the release of allelochemicals into the environment should be mindful of the fact that the practice is likely to be exposed to the vagaries of climatic (Bruce *et al.,* 2005) and edaphic factors, as well as likely being crop and weed-specific. High rainfall conditions were recorded in Experiment 1 of Chapter 2 which diluted allelochemicals, while average rainfall in 2006 & 2007 for Experiment 2 of Chapter 2 (Appendix A, Table A1) resulted in pronounced allelopathic interactions.

The optimal residue management strategy for weed suppression depends both on the nature (fine residues like those from medic are more effective as opposed to coarse residues of lupine) and amount (less residues leads to less weed control) of crop species' residues, as well as on the target weed species. Lupine gave suppression of grass weeds, giving the mulches of both leguminous crops an added benefit and their inclusion and growing in crop rotation systems with wheat and barley as main crops, more importance. However, regarding weed suppression due to allelopathic effects from crop residues, the variability in effects ascribed to variable soil and climatic factors might argue against the practice being accepted as an effective stand-alone weed control option in the foreseeable future. Partial acceptance will likely be a compromise of combining the continued limited use of herbicides with leguminous crop residues for weed control.

Plant leachates

Studies under controlled conditions are generally in accordance with those in the field (Chapter 2). The allelopathic activity observed for lupine and medic under controlled conditions, confirms that these leguminous crops should be used more frequently in the crop rotation systems of the Western Cape. Medic is already planted extensively as rotational crop in the Swartland region, but in the long rotation systems of the Overberg region, lupine should be used more frequently in the crop rotation systems used between lucerne plantings. Lupine is preferred to medic, which is a winter growing legume, as the latter is not an option in the Overberg region due to year-round rainfall which makes lucerne cultivation possible to ensure adequate grazing for the large live-stock component in agricultural production systems. Crop mulches that can provide weed control could reduce dependency on herbicides, in particular those products which are associated with the development of weed resistance. However, such a practice is likely to be exposed to the vagaries of environmental factors, as well as likely being crop and weed-specific. Results from the dry mass of rye grass, which was reduced by medic, correspond with those of

Fourie (2005) who reported that 'Paraggio' medic as a cover crop in the vineyards of the Lower Orange River had a significant negative impact on weed growth during winter. It was speculated that effectively suppressing the winter growing weeds may result in a reduction in the dosage of herbicide applied in spring, and it may minimise the negative effects caused by weeds, such as the harbouring of nematodes and insects during winter (Fourie *et al.,* 2005). Unlike medic, lupine cultivation is problematic in that a good crop stand is seldom achieved and thus negating the beneficial weed suppressive effects observed under controlled conditions. In the case of the mulch being a leguminous plant, the added benefit of nitrogen fixation will also be achieved.

Geographical variation of rye grass hybrid type

A rye grass hybrid type (*L. multiflorum x perenne*) never described before, was identified in this study. Huge genetic variation was detected between Italian rye grass weed populations with no consistently identifiable alleles among individual plants and no consistent correlation between geographical and genetic distance of specimen pairs. As no published primer pairs exist for *L. multiflorum x perenne,* no identifiable alleles associated with herbicide resistance could be identified. Nevertheless, data has indicated that there are distinct genetic groups within weedy rye grass populations of the Western Cape. Knowledge about this differentiation of rye grass could aid in the research approach on rye grass resistance and integrated control methods. In fact, knowledge of both genetic and morphological diversity may be important to guide the development of differential management of rye grass. Results from this study will further enhance our understanding of the genetics and evolution of herbicide-resistant rye grass and may lead to the development of specific and differential management strategies for weed control in each population. Although species interbreeding is most often maladaptive, it might represent an important route for the evolution of genotypes favoured under the intense selection pressure found in agricultural habitats (Tranel & Trucco, 2009). Hybridisation has been proposed as a critical stimulus for weed aggressiveness and is perhaps aiding in the evolution of adaptations critical for the success of weeds (Tranel & Trucco, 2009).

As rigid, Italian, perennial and weed hybrid rye grass occurred in the study area, it could be speculated that their responses to herbicides may display plant differential effects. Practically speaking and with herbicides registered for grass control (graminicides) grouped as controlling either annual or perennial grass species, this means that the rye grass weed hybrid may have characteristics enabling it to be tolerant to herbicides registered as annual graminicides. In that case it cannot be regarded as weed resistance to herbicides but rather as non-susceptibility, because the weed has perennial characteristics. The implication of this is that different control strategies should be devised according to the prevalent species occurring in a particular field.

The wide genetic and morphological variation detected in rye grass is interpreted on the basis of high genotypic plasticity and hybridisation for producing *Lolium multiflorum x perenne*. High levels of heterozygosity would indicate that rye grass plant populations probably have substantial amounts of adaptive genetic variations to escape the effects of a control agent. It may also be the result of the differential selection pressure or of the heterogeneity of environmental factors. Effective localised control methods for the various species and hybrids in this genus should be prioritised to curb further development of herbicide resistance.

Soils preferred by rye grass cover a wide range of fertility below and above optimum ranges for wheat and emphasises its wide adaptability and success as an invasive genus. Data on crown rot occurring on rye grass in South Africa had not been published before. Rye grass can act as alternative hosts and as a source of inoculum of this important soilborne pathogen of barley and wheat in the Western Cape Province. This further complicates sustainable dry land crop production, since the build-up of herbicide resistant rye grass may lead to a higher incidence of crown rot on wheat and barley due to a higher disease pressure. However, it could also point to a possible biological control agent for rye grass.

Effects of root leachates on micro-organisms

Plant root exudation serves as an important carbon and energy source for micro-organisms contained in the rhizosphere (Bertin *et al.,* 2003). Therefore, it is conceivable that soil microbial populations used particular carbon sources which influenced the growth rate of wheat grown on either Langgewens or Tygerhoek soils. Although the present study did not consider only the effects of allelochemicals contained in root leachates, but the combined effects of all solutes contained in them, it indicated that the effect on soil microbial population and community structure may be pronounced. This corresponds with the findings of Kong (2008) that the composition of soil microbes is defined at least in part by the nature and amount of chemicals contained in root exudates. Therefore, we contend that the growth rate of test plants in this study could be ascribed to the combination of compounds contributed by root exudates and soil microbial populations. The significantly faster growth rate of *L. multiflorum x perenne* on Langgewens soil treated with barley root leachates was revealed by PCA as a probable association with growth-promoting soil micro-organisms. In contrast, the non-significance observed for growth rate of this species on Tygerhoek soil, most probably indicates that either no growth-promoting or growth-inhibiting soil micro-organisms occurred, emphasising the importance of location in plant-microbe interactions. Furthermore, differences in plant growth rate and responses in physiological profiles of micro-organisms observed on the two soils used in the study, suggest that location is an important factor governing plant-plant and plant-microbe interactions.

Generally, the investigated plant species showed not only different plant-micro-organism associations, thus confirming results by Oberan *et al.* (2008) and Kong *et al.* (2008) who reported that different micro-organism associations exist among plant species, but results also pointed to the presence of different allelochemicals for each plant type. Kong *et al.* (2008) also reported that soil microbial populations were affected by the compounds released from allelopathic cultivars.

Comparisons between growth mediums of the leached sand in Chapter 3 and natural soil in Chapter 5 showed that results from Chapter 3 Experiment 3 were similar in terms of the inhibition of barley by leguminous crop root leachates. Wheat was stimulated by lupine in the current study, probably because effects became more pronounced after 16 weeks as opposed to the five week duration for the study in Chapter 3 Experiment 3. Lupine was stimulated in both studies, while barley root leachates inhibited rye grass v. Energa and stimulated rye grass weed type growth rate in both instances in the current study.

Gu *et al.* (2008a) and Kong *et al.* (2008) suggested that allelopathic crops and weeds could modify the microbial community structure in the soil to their advantage through the release of allelochemicals. This study strengthens the significance of soil micro-organisms in chemical root exudates and allelochemical-mediated interactions between plants, whether to lessen or to magnify effects. It has been demonstrated that not only the originally exuded compounds but also their derivatives can have allelopathic activity (Belz, 2007). Crop cultivars and weeds may modify the soil micro-organism populations to their advantage and to the disadvantage of other species by the release of root exudates that apparently differ in composition between plant species, thus confirming their allelopathic potential. Findings indicate that root exudates contained putative allelochemicals which influenced microbial community profiles. The effect on microbial communities varied with source of exudates and between soils. Changes in microbial community structure could affect plant growth through the promotion or suppression of harmful or beneficial microbes and the microbial production of allelochemicals.

Lolium multiflorum x perenne

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SUMMARY

This study investigated the use of allelopathic properties from crop residues for the suppression of herbicide resistant rye grass *(Lolium multiflorum x perenne)*, the role of allelopathy from different plant parts, the geographical distribution of genetic and morphological variability of rye grass and the interactions among micro-organisms and allelopathic root leachates. With heavily infested wheat fields, this data will further enhance our understanding of rye grass and promote weed control measures that are alternatives to herbicides. In both Experiments 1 & 2 of the field trial, growth inhibitory or stimulatory effects were observed on crops exposed to the residues of other crops. Medic suppressed *L. multiflorum x perenne* whilst lupine suppressed grass weeds. Lupine seed leachate also reduced wheat cumulative germination. The radicle length of rye grass was inhibited by seed leachates from wheat and lupine. This growth-inhibiting effect from lupine seed and seedling leachates was also evident in rye grass radicle length and cumulative germination percentage. Morphologically, 50% of the total number of specimens was classified as rigid rye grass, 48% as the hybrid, namely *L. multiflorum x perenne* and 2% as perennial rye grass. *Fusarium pseudograminearum* (crown rot) was isolated from rye grass at six localities, indicating that this weed complex can act as alternative hosts and a source

of inoculum of this important soil-borne pathogen. On Langgewens soil, the growth rate of wheat was stimulated by lupine (v. Tanjil or v. Quilinock). The faster growth rate of rye grass on Langgewens soil treated with barley root leachates was revealed by Principal Component Analysis (PCA) as a probable association with growthpromoting soil micro-organisms. Results from the field for medic on the suppression of rye grass weed type growth promises practical application under field conditions because of the crop's preading growth habit which could be effective for the establishment of effective organic mulches. Studies under controlled conditions confirmed effects of leguminous crops in the field. The wide genetic and morphological variation detected in rye grass may be due to high genotypic plasticity and hybridisation for producing the weed type *L. multiflorum x perenne*. Effective localised control methods for the various species and hybrids in this genus should be prioritised to curb further development of herbicide resistance. Crop cultivars and weeds may modify the soil micro-organism populations to their advantage and to the disadvantage of other species by the release of root exudates that apparently differ in composition between plant species. The effect on microbial communities varied with source of exudates and between soils.

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Appendix A

Table A1 Total rainfall in mm per month at Tygerhoek over years for the period 2003-2007

	2003	2004	2005	2006	2007
January	58.4	324.4	236.1	77.0	4.3
February	477.7	0	840.9	4.0	13.5
March	0	14.4	22.4	36.0	11.6
April	32.0	29.8	22.4	79.0	21.6
May	36.8	1.8	78.6	44.4	31.2
June	14.6	16.6	2.5	24.2	22.5
July	11.2	17.6	3.3	83.8	36.1
August	16.2	6.6	26.3	83.3	11.7
September	24.0	65.6	0.6	8.4	9.5
October	25.2	73.6	1.4	16.2	28.9
November	4.2	233.2	54.2	18.1	205.7
December	281.0	236.4	7.2	17.3	73.1
Total annual rainfall	981.3	1020.0	1295.9	491.7	469.7

Figure A1 Crop residue plots in the field

Figure A2 Crops grown in residue plots in the field

Figure A3 Petri dishes with seeds to obtain leachates in the incubator

Figure A4 Arrangement of donor pots to obtain leachates in the greenhouse

Figure A5 Arrangement of acceptor pots for treatment with leachates in the greenhouse

Table A2 Genetic and morphological analyses of *Lolium* spp in Area A

	Italian rye grass	Rye grass hybrid type	Perennial rye grass		Rigid rye grass	
	Genetic analysis	Morphological analysis	Genetic analysis	Morphological analysis	Genetic analysis	Morphological analysis
A1	x	x				
A2					x	x
A3					x	x
A4					x	x
A5	x	x				
A6					x	x
A7					x	x
A8					x	x
A9					x	x
A10	x	x				

Table A3 Genetic and morphological analyses of *Lolium* spp in Area B

	Italian rye grass	Rye grass hybrid type	Perennial rye grass		Rigid rye grass	
	Genetic analysis	Morphological analysis	Genetic analysis	Morphological analysis	Genetic analysis	Morphological analysis
B1	x	x				
B2	x	x				
B3	x	x				
B4	x	x				
B5	x	x				
B6					x	x
B7					x	x
B8		x	x			
B9			x	x		
B10	x	x				

Table A4 Genetic and morphological analyses of *Lolium* spp in Area C

	Italian rye grass	Rye grass hybrid type	Perennial rye grass		Rigid rye grass	
	Genetic analysis	Morphological analysis	Genetic analysis	Morphological analysis	Genetic analysis	Morphological analysis
C1					x	x
C2	x	x				
C3	x	x				
C4	x	x				
C5	x	x				

C6	x	x				
C7		x	x			
C8	x	x				
C9					x	x
C10	x	x				

Table A5 Genetic and morphological analyses of *Lolium* spp in Area D

	Italian rye grass	Rye grass hybrid type	Perennial rye grass		Rigid rye grass	
	Genetic analysis	Morphological analysis	Genetic analysis	Morphological analysis	Genetic analysis	Morphological analysis
D1					x	x
D2					x	x
D3					x	x
D4					x	x
D5	x					x
D6					x	x
D7					x	x
D8					x	x
D9					x	x
D10					x	x

Table A6 Carbon sources used by the Biolog EcoPlate™ for micro-organism community analysis

Carbon source	No
Water	C1
ß-Methyl-D-Glucoside	C2
D-Galactonic Acid *y*-Lactone	C3
L-Arginine	C4
Pyruvic Acid Methyl Ester	C5
D-Xylose	C6
D-Galacturonic Acid	C7
L-Asparagine	C8
Tween 40	C9
I-Erythritol	C10
2-Hydroxy-Benzoic Acid	C11
L-Phenylalanine	C12
Tween 80	C13
D-Mannitol	C14
4-Hydroxy Benzoic Acid	C15
L-Serine	C16
α-Cyclodextrin	C17
N-Acetyl-D-Glucosamine	C18
y-Hydroxibutyric Acid	C19
L-Threonine	C20
Glycogen	C21
D-Glucosaminic Acid	C22
Itatonic Acid	C23
Glycyl-L-Glutamic Acid	C24
D-Cellobiose	C25
Glucose-1-Phosphate	C26
α-Ketobutyric Acid	C27
Phenylehthylamine	C28
α-D-Lactose	C29
D,L-α-Glycerol Phosphate	C30
D-Mallic Acid	C31
Putrecine	C32

Table A7 Soil analyses for soils collected at Langgewens or Tygerhoek

Locality	**Langgewens**		**Tygerhoek**	
Soil properties	Value	Unit	Value	Unit
pH	6.3		5.2	
Resistance	850	Ohms	460	Ohms
Texture	Sandy loam		Loam	
Acidity	0.89	cmol(+)/kg	0.71	cmol(+)/kg
Calcium	3.96	cmol(+)/kg	3.45	cmol(+)/kg
Magnesium	0.75	cmol(+)/kg	1.78	cmol(+)/kg
Potassium	220	mg/kg	305	mg/kg
Sodium	23	mg/kg	63	mg/kg

P (citric acid)	99	mg/kg	40	mg/kg
Total cations	5.38	cmol(+)/kg	6.99	cmol(+)/kg
Copper	1.63	mg/kg	1.26	mg/kg
Zinc	5.59	mg/kg	1.58	mg/kg
Manganese	191.3	mg/kg	120.20	mg/kg
Sulphur	3.61	mg/kg	9.84	mg/kg
Boron	0.32	mg/kg	1.49	mg/kg
Carbon	0.98	%	1.55	%

Table A8 Pearson correlation matrix used for principal component analysis (PCA) to determine the correlation among growth rate and effects of root leachate treatments on physiological profiling of soil micro-organisms for *H. vulgare* and *T. aestivum* for Langgewens and Tygerhoek soils

Carbon source	*H vulgare*		*T. aestivum*	
	Langewens	Tygerhoek	Langewens	Tygerhoek
C2_1	-0.639		-0.342	
C3_1	-0.329		0.072	
C4_1	-0.123	-0.144	0.054	0.126
C5_1	-0.099	0.328	-0.388	0.432
C6_1	0.118	0.445		0.636
C7_1	0.405		0.639	
C9_1	-0.099		0.054	
C11_1				0.547
C12_1	0.633			-0.031
C13_1	-0.099		-0.403	
C14_1	0.370		-0.019	
C15_1	-0.477	-0.342	0.054	
C16_1	-0.227		0.375	
C17_1		0.445		-0.461
C18_1	-0.083		-0.446	
C19_1	-0.462	-0.373	0.519	-0.194
C21_1	-0.477	-0.018		0.135
C22_1	-0.246			0.636
C23_1	-0.098	0.322	0.054	0.436
C25_1	-0.639		-0.074	
C26_1	-0.464		0.154	
C28_1		-0.468	0.119	0.336
C29_1		-0.012		0.347
C30_1		0.445		0.547
C31_1	-0.396	-0.274	0.547	
C32_1	0.118	-0.091	0.547	
C2_2		-0.342	0.196	
C4_2		-0.011		-0.547
C5_2		-0.011	-0.457	-0.513
C6_2	-0.350	-0.373	0.794	-0.432
C9_2		0.328		
C10_2			0.119	
C12_2	0.462	-0.325	0.515	0.461
C13_2		0.328		-0.314
C15_2	-0.699	-0.342	0.639	-0.132
C17_2	-0.033	-0.373	0.196	
C18_2	-0.699		-0.256	
C19_2				-0.786
C21_2	-0.033	-0.373	0.196	-0.692
C22_2	-0.478	-0.758	0.688	-0.477
C23_2	-0.633	-0.042	0.074	-0.152
C24_2	0.118	-0.511	0.547	
C25_2			0.519	
C26_2	-0.350		0.196	
C28_2	-0.222	-0.325	0.547	-0.323
C29_2	-0.350	-0.373	0.196	-0.314
C31_2	-0.911	-0.144	0.020	-0.636
C32_2	-0.699	0.328	0.141	

Table A9 Pearson correlation matrix used for principal component analysis (PCA) to determine the correlation among growth rate and the effects of root leachate treatments on physiological profiling of soil micro-organisms for *L. albus* v. Tanjil and *L. albus* v. Quilinock for Langgewens and Tygerhoek soils

Carbon source	*L. albus* v. Tanjil		*L. albus* v. Quilinock	
	Langgewens	Tygerhoek	Langgewens	Tygerhoek
C2_1	0.502			
C3_1	0.117			
C4_1		0.810	-0.020	0.359
C5_1	0.531			
C6_1	0.762	0.281	0.629	-0.086
C7_1	0.531			
C10_1				0.469
C11_1		-0.255		
C12_1	-0.256	0.037		0.888
C13_1			0.528	
C14_1	0.117		-0.152	
C15_1		-0.552		-0.339
C16_1	0.117			
C17_1	0.502	0.427		-0.045
C18_1	0.531			
C19_1		-0.190		0.120
C20_1		0.179		
C21_1	0.502	0.110		-0.339
C22_1	0.608	0.810	0.359	-0.086
C23_1	0.762	-0.255		-0.086
C24_1	-0.034	0.607		-0.229
C25_1	0.502			
C26_1	0.502	0.584		
C28_1	-0.103	-0.650	0.174	0.560
C29_1	-0.359	0.584	-0.321	-0.086
C31_1	0.430		-0.147	0.229
C32_1			0.174	
C2_2	0.762		0.597	
C3_2	-0.281		0.243	
C4_2	-0.174		-0.443	
C5_2	0.359		0.173	
C6_2		-0.380	0.317	0.229
C7_2	0.430		0.243	
C9_2	-0.793		0.071	
C10_2				-0.216
C12_2		-0.259		0.229
C13_2			0.071	
C14_2	-0.454		-0.443	
C15_2	0.117		0.038	
C16_2	-0.693		0.071	
C17_2	-0.359	0.110	0.261	
C18_2	-0.174		-0.367	
C19_2			0.071	-0.339
C21_2	0.052	-0.442	0.261	
C22_2	0.217	0.179	-0.092	0.359
C23_2	-0.079	-0.441	-0.092	
C24_2				0.339
C25_2	-0.174		0.597	
C26_2	-0.243		0.853	
C28_2	0.247		0.020	-0.085
C29_2		0.110	0.317	0.229
C30_2		-0.255		-0.281
C31_2	-0.161		0.038	
C32_2	0.410	-0.128	0.261	0.212

Table A10 Pearson correlation matrix used for principal component analysis (PCA) to determine the correlation among growth rate and the effects of root leachate treatments on physiological profiling of soil micro-organisms for *L. multiflorum* v. Energa and *L. multiflorum x perenne* for Langgewens and Tygerhoek soils

	L. multiflorum v. Energa		*L. multiflorum x perenne*	
Carbon source	Langgewens	Tygerhoek	Langgewens	Tygerhoek
C2_1	-0.042			-0.178
C4_1		0.618		-0.639
C5_1			-0.690	-0.345
C6_1	-0.422	-0.317	-0.690	-0.655
C9_1		0.453		
C10_1	-0.127	-0.786		
C12_1	-0.127	0.642	-0.186	0.843
C13_1			-0.783	
C15_1			0.304	-0.178
C17_1	-0.042	0.786	-0.483	-0.282
C18_1		0.453	-0.690	-0.178
C19_1		0.326		
C20_1		-0.232		
C21_1	-0.192	0.632	-0.483	-0.657
C22_1	-0.565	-0.317	-0.783	-0.621
C23_1	0.511	0.732	-0.498	-0.324
C24_1		0.153		
C25_1	0.511		-0.690	-0.178
C26-1	-0.783		-0.690	-0.178
C28-1	0.513	0.103	-0.583	0.450
C29_1		0.234	-0.437	-0.630
C30-1		-0.451		
C31-1	-0.142		-0.580	0.225
C32_1		-0.584	-0.362	-0.639
C2_2	-0.162		0.275	-0.450
C3_2	-0.096		0.255	
C4_2	-0.096	-0.142	-0.098	
C5-2	0.321		-0.186	-0.843
C6_2	0.050	0.203		-0.895
C9-2			-0.021	
C10_2		0.775		
C12_2		0.609	0.671	-0.138
C13_2			-0.362	
C14_2	-0.096		0.583	
C15_2	0.260		-0.098	
C16_2	0.260		-0.098	
C17_2	0.445	-0.153		-0.895
C18_2	0.050		-0.783	
C19_2		0.326	-0.321	
C21_2	0.199	-0.153	0.038	-0.895
C22_2	0.445	-0.584		-0.657
C23_2	0.084		0.352	
C24_2		0.624		
C25_2	0.599		-0.030	
C26_2	0.599		-0.186	
C28_2		-0.142		0.133
C29_2	0.050	0.509		-0.895
C30_2				-0.324
C31_2	0.422		-0.304	-0.450
C32_2	0.260	-0.142	-0.570	