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 aforementioned 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

[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).

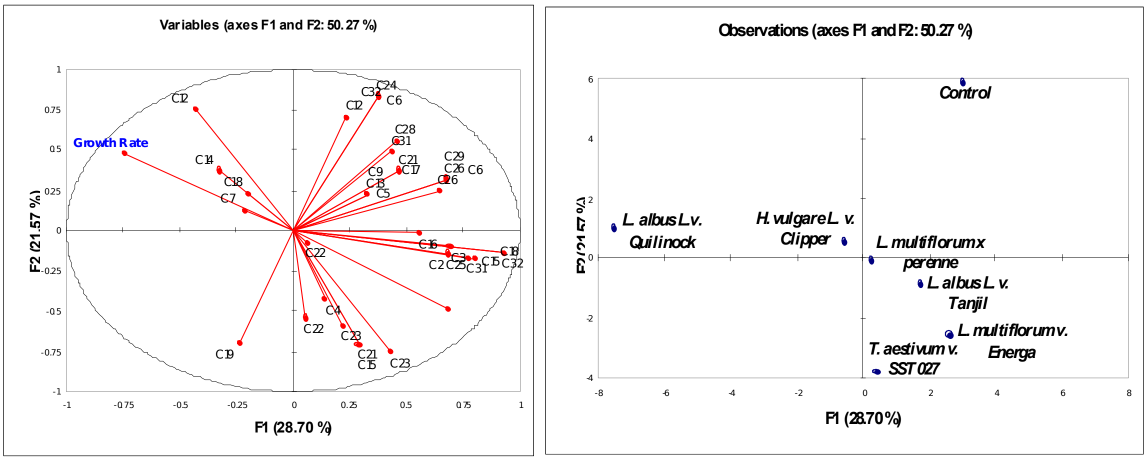

Figure 1a Score plot (left) and loading plot (right) of barley v. Clipper grown on Langgewens soil, and its association with carbon source utilisation

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).

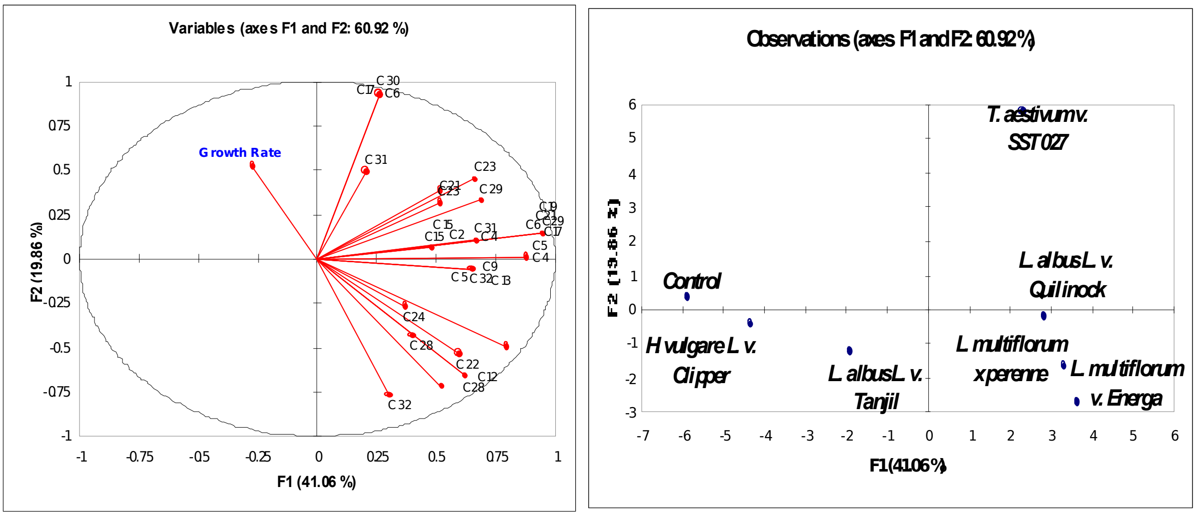

Figure 1b Score plot (left) and loading plot (right) of barley v. Clipper grown on Tygerhoek soil, and its association with carbon source utilisation

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).

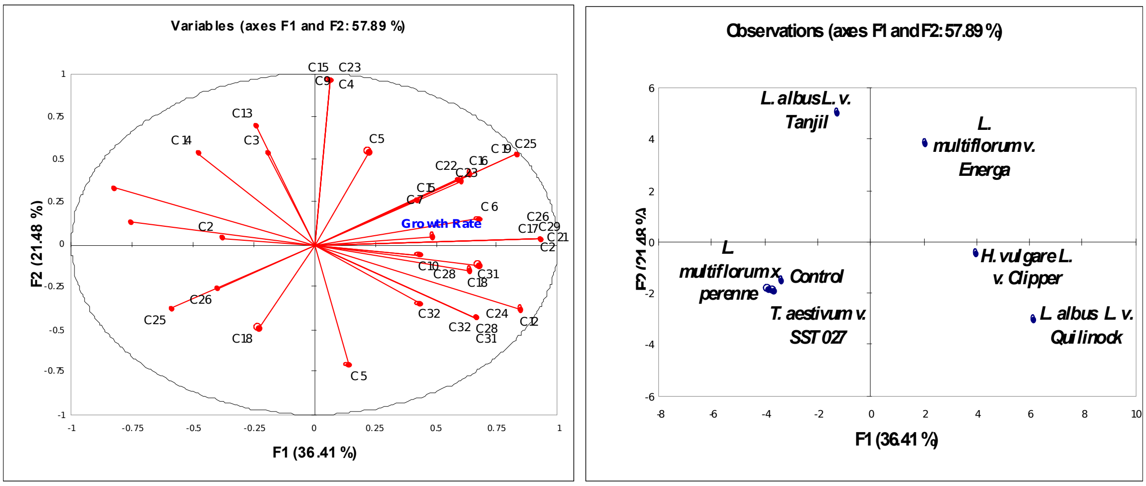

Figure 2a Score plot (left) and loading plot (right) of wheat v. SST 027 grown on Langgewens soil, and its association with carbon source utilisation

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.

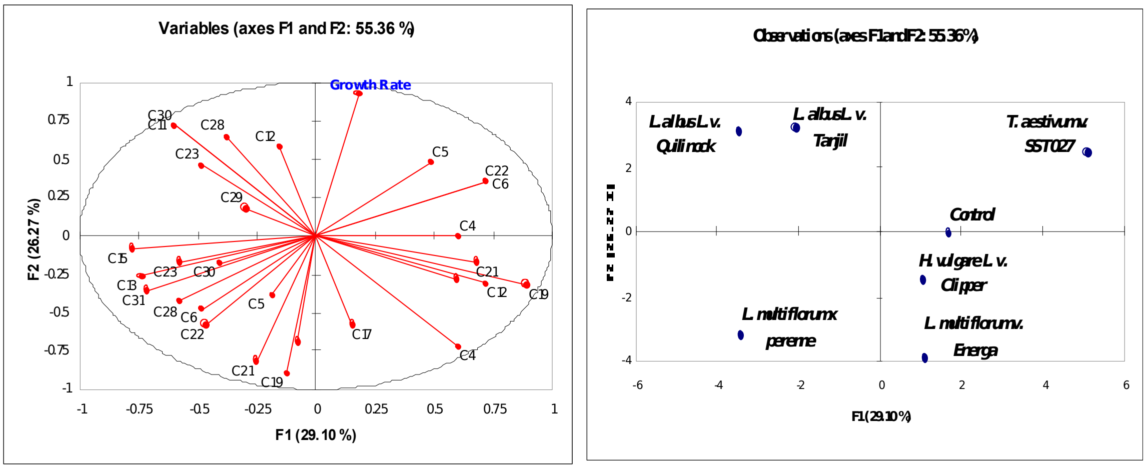

Figure 2b Score plot (left) and loading plot (right) of wheat v. SST 027 grown on Tygerhoek soil, and its association with carbon source utilisation

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

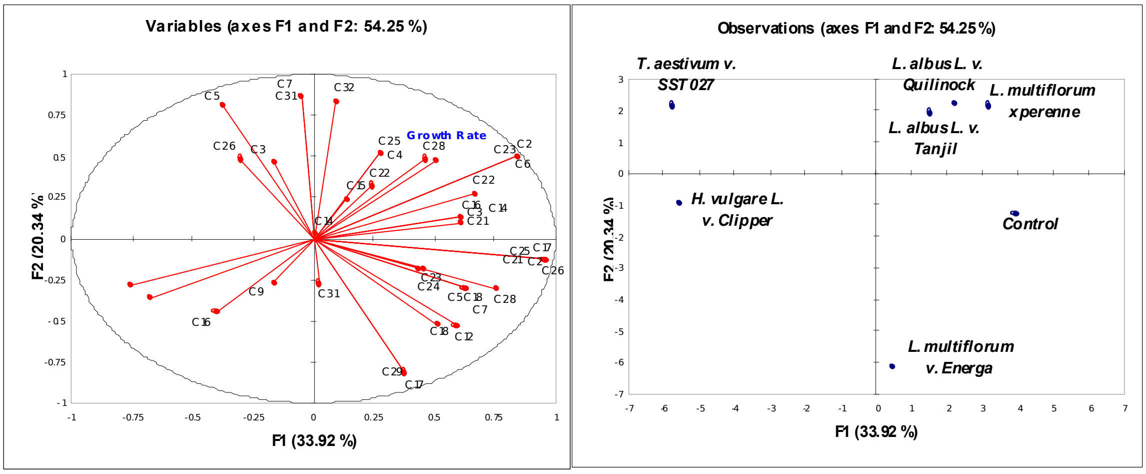

Figure 3a Score plot (left) and loading plot (right) of lupine v. Tanjil grown on Langgewens soil and its association with carbon source utilisation

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).

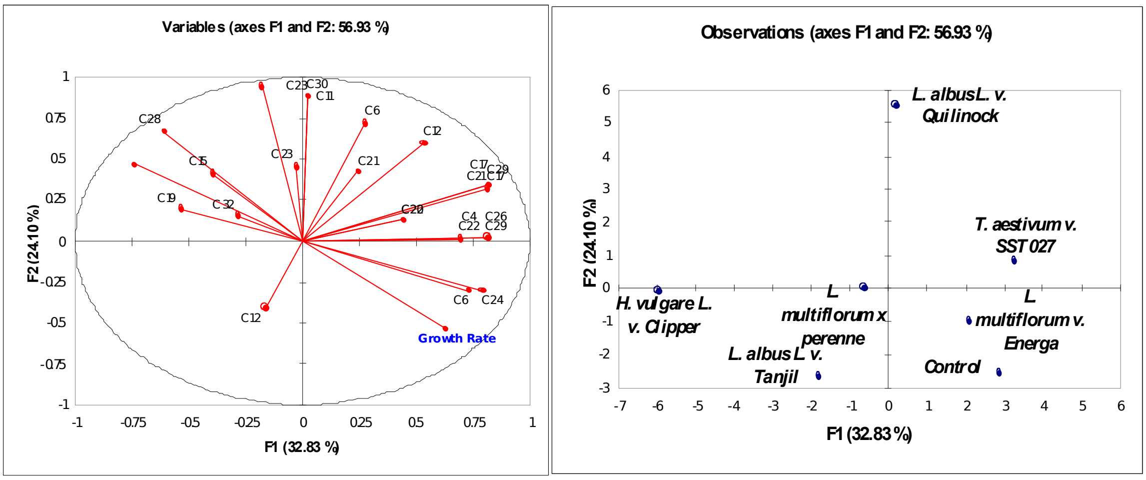

Figure 3b Score plot (left) and loading plot (right) of lupine v. Tanjil grown on Tygerhoek soil, and its association with carbon source utilisation

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).

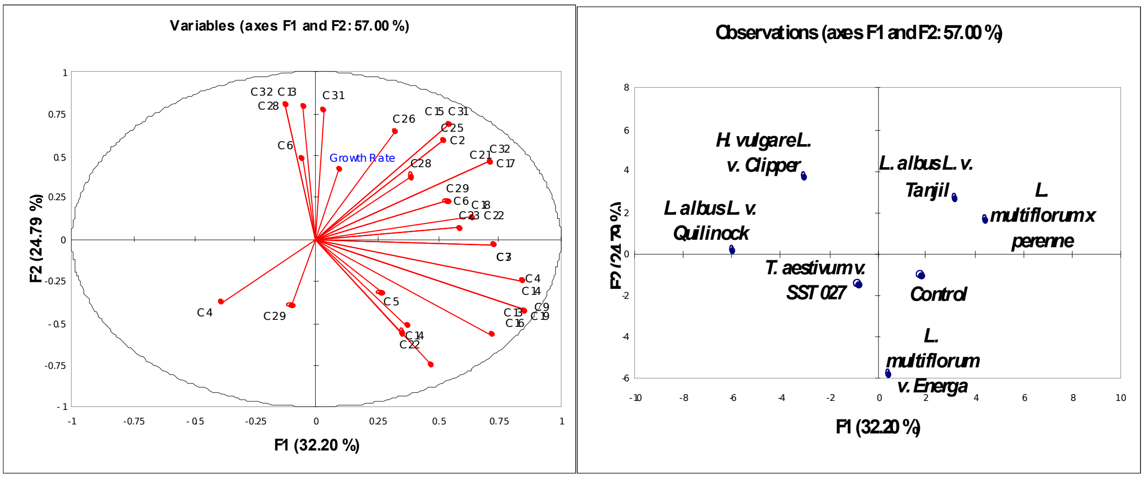

Figure 4a Score plot (left) and loading plot (right) of lupine v. Quilinock grown on Langgewens soil, and its association with carbon source utilisation

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).

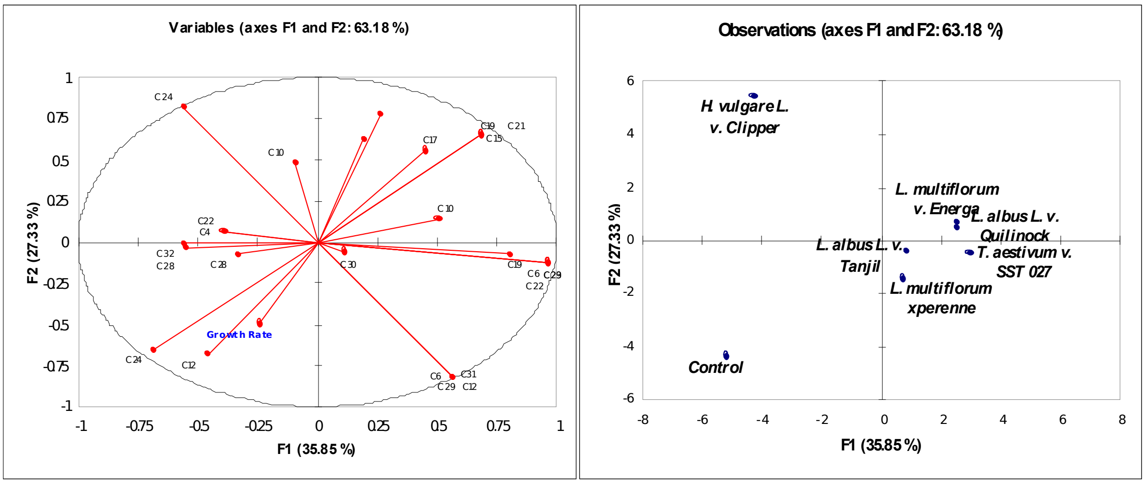

Figure 4b Score plot (left) and loading plot (right) of lupine v. Quilinock grown on Tygerhoek soil, and its association with carbon source utilisation

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.

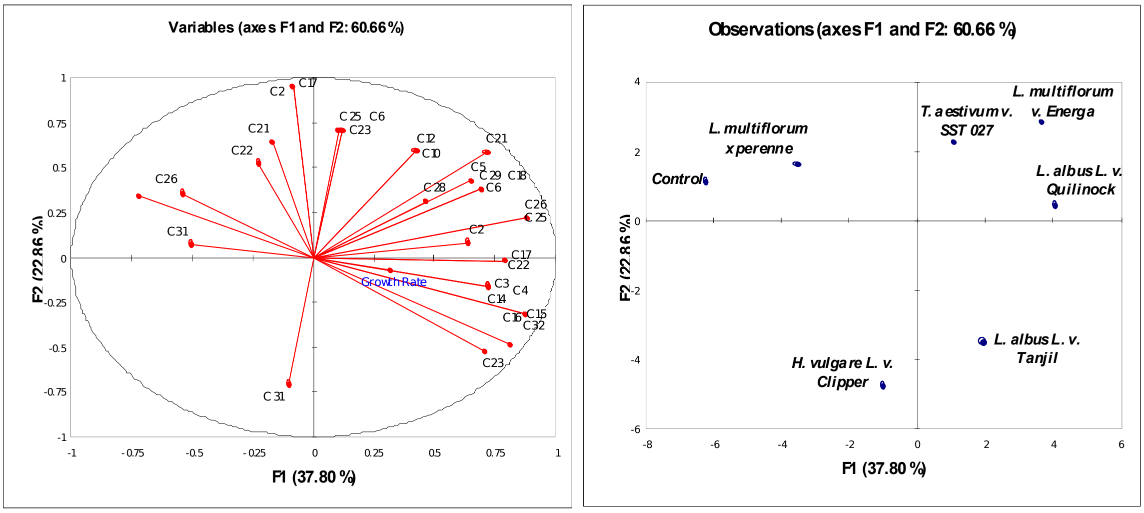

Figure 5a Score plot (left) and loading plot (right) of *L. multiflorum* v. Energa grown on Langgewens soil, and its association with carbon source utilisation

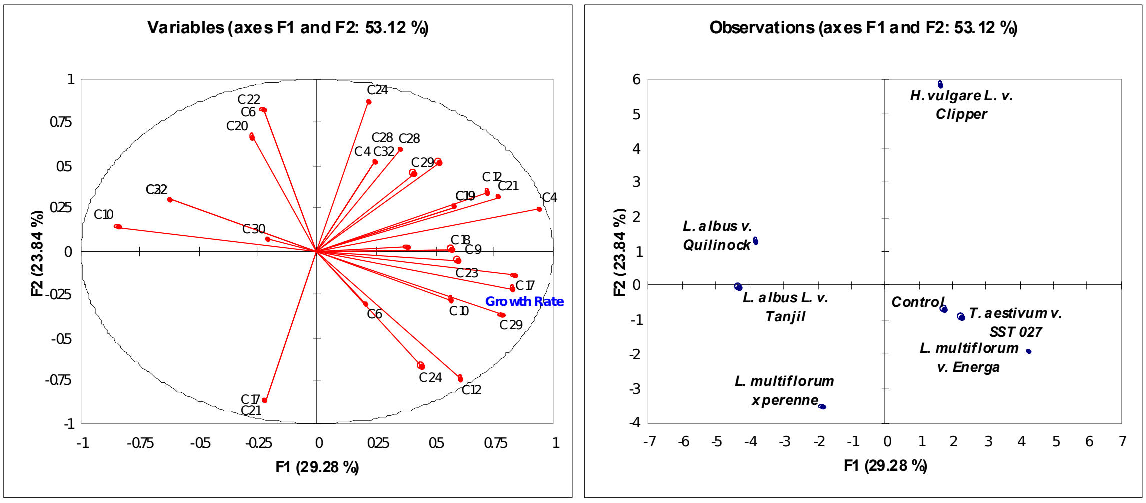

Figure 5b Score plot (left) and loading plot (right) of *L. multiflorum* v. Energa grown on Tygerhoek soil, and its association with carbon source utilisation

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).

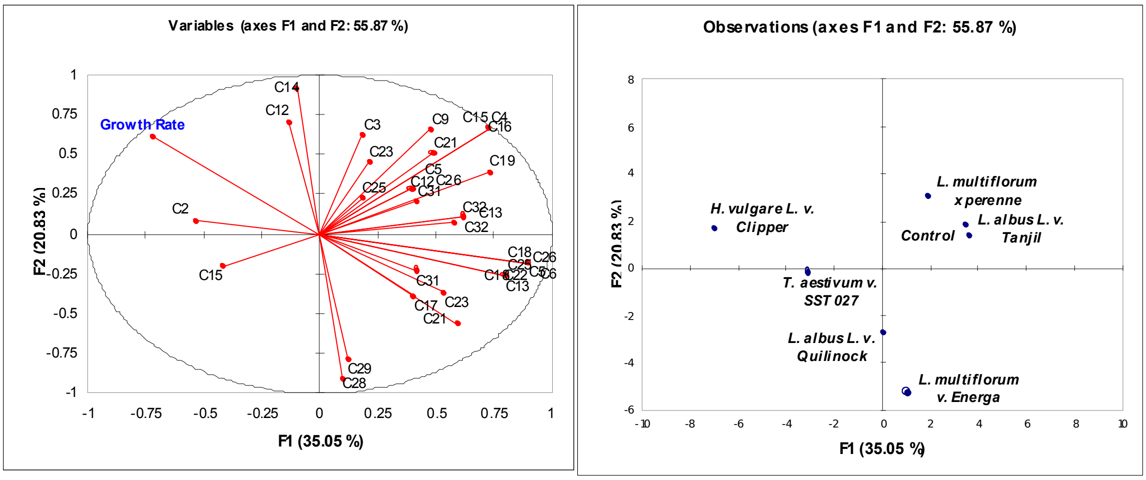

Figure 6a Score plot (left) and loading plot (right) of *L. multiflorum x perenne* grown on Langgewens soil, and its association with carbon source utilisation

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).

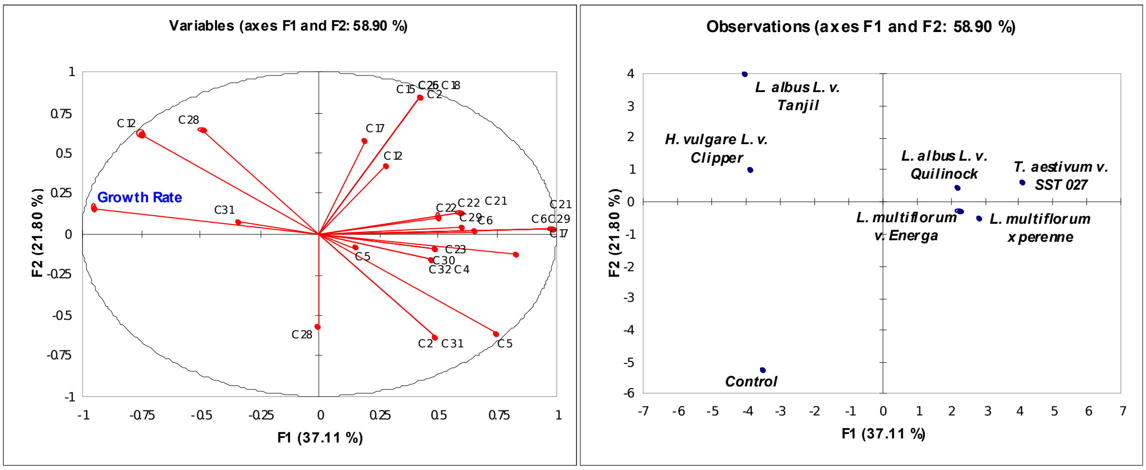

Figure 6b Score plot (left) and loading plot (right) of *L. multiflorum x perenne* grown on Tygerhoek soil, and its association with carbon source utilisation

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.