



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, Olofsson *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 Fratilesco-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

| Plant type | Seed leachate | |
|-----------------------|----------------------------|-------------------------------------|
| | 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 ($P \leq 0.05$) | 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 ($P \leq 0.05$) | 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

| Plant type | Seed leachate | |
|-----------------------|---------------------------|-------------------------------------|
| | 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 ($P \leq 0.05$) | 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

| Plant type | Seed leachate | |
|------------|----------------------------|-------------------------------------|
| | 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 ($P \leq 0.05$) | 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

| Plant type | Seed leachate | |
|-----------------------|-----------------------------|-------------------------------------|
| | 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 ($P \leq 0.05$) | 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

| Plant type | Seed leachate | |
|------------|---------------------------|-------------------------------------|
| | 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

| Plant type | Seed leachate | |
|--------------|-------------------------------|-------------------------------------|
| | 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

| Plant type | Seedling leachate | |
|------------|----------------------------|-------------------------------------|
| | 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 ($P \leq 0.05$) | 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

| Plant type | Seedling leachate | |
|-----------------------|----------------------------|-------------------------------------|
| | 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 ($P \leq 0.05$) | 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

| Plant type | Seedling leachate | |
|------------|---------------------------|-------------------------------------|
| | 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

| Plant type | Seedling leachate | |
|--------------|----------------------------|-------------------------------------|
| | 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

| Plant type | Seedling leachate | |
|------------|-----------------------------|-------------------------------------|
| | 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

| Plant type | Seedling leachate | |
|--------------|---------------------------|-------------------------------------|
| | 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

| Plant type | Seedling leachate | |
|------------|-------------------------------|-------------------------------------|
| | 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

| Plant type | Root leachate | | |
|--------------|-----------------------------------|-----------------------------------|---------------------|
| | 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

| Plant type | Root leachate | | |
|--------------|----------------------------------|----------------------------------|--------------------|
| | 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

| Plant type | Root leachate | | |
|--------------|------------------------------------|------------------------------------|----------------------|
| | 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

| Plant type | Root leachate | | |
|--------------|----------------------------------|----------------------------------|--------------------|
| | 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

| Plant type | Root leachate | | |
|--------------|--------------------------------------|--------------------------------------|------------------------|
| | 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

| Plant type | Above-ground leachate | | |
|--------------|-----------------------------------|-----------------------------------|---------------------|
| | 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

| Plant type | Above-ground leachate | | |
|------------|----------------------------------|----------------------------------|--------------------|
| | 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

| Plant type | Above-ground leachate | | |
|--------------|-----------------------------------|-----------------------------------|---------------------|
| | 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

| Plant type | Above-ground leachate | | |
|------------|------------------------------------|------------------------------------|----------------------|
| | 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

| Plant type | Above-ground leachate | | |
|--------------|----------------------------------|----------------------------------|--------------------|
| | 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

| Plant type | Above-ground leachate | | |
|--------------|--------------------------------------|--------------------------------------|------------------------|
| | 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.