

Full Length Research Paper

Assessment of the allelopathic effects of seeds and seedlings of rotational crops and ryegrass

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Allelopathic effects were evaluated for a series of rotational crops, namely barley, canola, wheat, lupine, medic, lucerne (alfalfa) and ryegrass in laboratory studies. This followed on a field assessment of these crops to identify inter-species phytotoxic effects. Two different experiments to determine phytotoxicity effects of seed and seedling leachates were conducted under controlled conditions. The first experiment was set up in the laboratory to observe the mutual effect of seed leachates from the plant series. 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 other species. The phytotoxic activity observed for lupine and medic under controlled conditions, corresponds to results obtained in the field and confirms that these leguminous crops could be considered as living or terminated mulches for weed suppression. Crop mixtures containing legumes may provide weed suppression which would reduce dependency on herbicides that are associated with the development of weed resistance.

Key words: Cumulative germination, inhibition, stimulation, phytotoxic leachates.

INTRODUCTION

Plants can defend themselves against a wide array of enemies, from microbes to large animals, yet there is great variability in the effectiveness of such defences, both within and between species (Todesco et al., 2010). When competing against neighbouring plants, defence is by way of chemical interference, which was described by Hoffman et al. (1996) as a significant co-evolutionary force in plant communities when chemicals released from a plant or its litter affects other plants. This phenomenon, better known as allelopathy, encompasses

both detrimental and beneficial interactions between plants through chemicals released by the donor (Xuan and Tsuzuki, 2002) and may be much more important as a mechanism in recipient than in origin plant communities where it maintains climax vegetation (Hierro and Callaway, 2003).

According to Kato-Noguchi (2000), chemicals with allelopathic activity are present in many plants and in many organs, including leaves, flowers, fruits and, buds. These allelochemicals are of varied chemical nature,

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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. According to Kong et al. (2008) allelopathy usually occurs at early growth stages of a plant species when allelochemicals are released from the root system. Overall plants exude up to 20% of fixed carbon and 15% of nitrogen (Venturi and Keel 2016) which includes an array of simple molecules, such as sugars, organic acids and secondary metabolites, as well as complex polymers, such as mucilage (Sasse et al., 2018).

The persistent use of herbicides in crop fields has created shifts in weed populations and the evolution of severe herbicide resistance. Due to this phenomenon, there is interest in the exploitation of allelopathic activity as it is possible to utilise it as a cost-effective alternative to external inputs and thus contribute to sustainable agriculture (Wu et al., 1999). Kong et al. (2008) reported that rice seedlings release allelochemicals from its roots to paddy soils at early growth stages to inhibit neighbouring weeds. Since plant roots exude an enormous variety of potentially valuable low molecular weight compounds into the rhizosphere, individual components of rice root exudates, such as allelochemicals, can modify the soil microbial community. Although every plant produces exudates, the amount and composition of root exudates varies (Sasse et al., 2018). In addition, plant exudation is defined by the genotype of the host, it changes with plant developmental stage and it is modulated by abiotic stresses. Smith et al. (2001) emphasized that typical field studies cannot separate the effects of competition from allelopathy since they happen simultaneously between roots and shoots.

Loddo et al. (2014) regarded germination as a crucial moment in the life cycle of annual weeds, as plants can only rely upon seed resources to complete emergence and the early growth phase. However, the degree of weed seed germination inhibition and growth suppression which can be attributed to crop allelopathy is valuable and highly important (Asghari and Tewari, 2007). For instance, aqueous shoot extracts of buckwheat (*Fagopyrum esculentum* Moench) 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 soils that was not amended, showed no effect. 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 (*L. sativa* L.). By contrast, root extracts had no inhibitory effects on germination of these two species, but suppressed root growth.

Smith et al. (2001) cautioned that artificial environments must be devised that remove any possibility of competition while allowing chemical exchange to take place in studies on root leachates. In addition, Loddo et al. (2014) inferred that there is a general demand for more effective, efficient and sustainable integrated weed management (IWM) strategies to decrease dependency on herbicide use, but at the same time, guarantee adequate weed control and satisfactory yields to comply with worldwide increasing food demand. Acquiring thorough knowledge about weed biology and ecology represents a fundamental step in the development of IWM strategies that can meet these important challenges (Loddo et al., 2014).

It was reported by Belel and Belel (2015) that certain weed species tend to influence the performance of cultivated crops by exerting positive or negative effects on their germination and subsequent growth. Even though perennial ryegrass has been noted in studies for its ability to exhibit allelopathic potential in field and laboratory settings (Weston, 1990), none has considered the mutual role of leachates, which has been proven to contain allelochemicals, from seeds and seedlings of ryegrass and rotational crops grown in the Western Cape Province of South Africa. Therefore, the primary objective of this research was to evaluate the mutual effects of leachates exuded from both seeds and seedlings of ryegrass and selected rotational crop species for effective ryegrass management and less reliance on herbicides.

MATERIALS AND METHODS

Laboratory experiments were conducted to determine the mutual impact of seed and seedling leachates from seven seed types. One month prior to commencement of the experiment, mature, commercial quality seeds of each type were sourced from Agricol® seed company (<https://www.agricol.co.za/>) and stored at room temperature (18-22°C). The research approach for seed and seedling leachates was similar in concept to that followed by Kato-Noguchi (2000) and Ma et al. (2012) for assessing whether crop seeds and seedlings release phytotoxins that affect both the germination and development of radicles of selected rotational crops.

Seeds used in the laboratory were representative of the rotational crops grown in the Western Cape's grain production area. Seed viability for all seed types was above 95% according to tetrazolium chloride tests (International Seed Testing Association, 1985). These included barley (*Hordeum vulgare* L. v. Clipper), canola (*Brassica napus* L. v. ATR Hyden), wheat (*Triticum aestivum* v. SST 88), lupine (*Lupinus albus* L. v. Tanjil), lucerne (alfalfa) (*Medicago sativa* L. v. SA standard), medic (*Medicago truncatula* Gaertn. v. Parabinga) and ryegrass pasture type (*Lolium multiflorum* Lam. v. Energa). Ryegrass pasture type seed was used to ensure germination consistency among seed, as well as a stable seed source.



Figure 1. Illustration of the lay-out of Petri dishes in the incubator for germination studies on the effects of leachates from seeds of selected rotational crops and ryegrass.

Seed leachates

The first experiment was set up in the laboratory to observe the mutual effects of seed leachates from the plant series (Figure 1). 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; thus 20 seeds in total for each treatment combination. Seeds were placed on brown germination paper which lined 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® to maintain moisture levels and incubated at an alternating temperature range of 25/15°C which corresponds with conditions at crop planting in late autumn. The photoperiod was set at a 12 h/12 h day/night cycle, while the light phase from white fluorescent lamps coincided with the higher temperature. 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 subsequently removed from the Petri-dish before it was resealed with Parafilm® and returned to the incubator. Upon termination of the experiment at 14 days, viability of non-germinated seeds was tested with a 0.4% tetrazolium chloride solution. Seeds showing a pink to reddish colour after 4 h were considered viable (International Seed Testing Association, 1985). Germination percentages were based on the total number of viable seeds placed in Petri-dishes for each seed type.

Seedling leachates

The second experiment was conducted in the laboratory to determine the mutual effect of seedling leachates from all the plants in the series on germination and early development (Figure 2). One

hundred seeds of each plant type in the series were germinated in Petri-dishes as described above for the seed leachate experiment. The seedlings were allowed to develop until they reached a length of roughly 50 mm, after which seedlings from each species were placed in a 4 cm porcelain Büchner funnel and washed for 10 s with 5 ml distilled water to yield a leachate. This leachate was funnelled into 9.5 cm diameter Petri-dishes lined with brown germination paper onto which 10 seeds from each plant type had been evenly placed according to a randomised block design with ten replicates, equalling 100 seeds per species. Control treatments were treated with distilled water only. Henceforth, the experiment was conducted exactly as described above, from the sealing of Petri-dishes with Parafilm® for the seed leachate experiment. All data were statistically analysed (ANOVA) with the statistical program SAS, but this was preceded by exploring outliers and tests for normality and homogeneity of variances. Least significant differences were used to identify significant differences between means at the 5% level of probability.

RESULTS

Overall for all seed types, barley and lupine seed leachates showed the most activity on the plant series tested. Results showed that barley or lupine seed leachates significantly inhibited both radicle lengths and cumulative germination of canola, wheat, lucerne, medic and ryegrass.

Barley

Upon termination of the experiment at 14 days, seed



Figure 2. Illustration of the set-up for funnelling leachates from a Büchner funnel to a Petri-dish for germination studies on the effects of leachates from seedlings of selected rotational crops and ryegrass.

leachates from both wheat and medic had significantly reduced cumulative germination of barley which attained 69 and 72% respectively, compared to the control (Table 1). No significant interactions were observed for seedling leachates.

Canola

Radicle length of canola was significantly reduced by seed leachates from barley (44%), lupine (24%) and lucerne (46%) respectively, compared to the control (Table 2). Lupine seed leachate had strong inhibitory effects on cumulative germination of canola which was reduced by 65% compared to the control.

Wheat

Radicle elongation of wheat was significantly reduced by phytotoxic seed leachates from barley (30%), wheat

(34%) and lupine (18%) compared to the control (Table 3). Also, cumulative germination of wheat was significantly inhibited by lupine seed leachate (31%). After treatment with seedling leachate of canola, strong inhibitory effects of radicle length of wheat (62%) was observed compared to the control.

Lupine

Seed leachate of barley inhibited both lupine radicle elongation and cumulative germination significantly by 33 and 25% respectively compared to the control (Table 4). After treatment with lucerne seedling leachate, the cumulative germination of lupine, was significantly less (61%) than the control.

Lucerne (alfalfa)

Lucerne radicle length was significantly inhibited by seed

Table 1. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Hordeum vulgare* L. v. Clipper.

Plant type	Seed leachate		Seedling leachate	
	Barley radicle length (mm)	Cumulative germination % at 14 days	Barley radicle length (mm)	Cumulative germination % at 14 days
Barley	26.4 ^a	77 ^{ab}	35 ^a	73 ^a
Canola	25.2 ^a	97 ^a	30.5 ^a	90 ^a
Wheat	23.5 ^a	67 ^b	27.3 ^a	77 ^a
Lupine	13 ^a	73 ^{ab}	36.7 ^a	97 ^a
Lucerne	12.2 ^a	90 ^{ab}	29.9 ^a	83 ^a
Medic	13 ^a	70 ^b	32.7 ^a	73 ^a
Ryegrass	25.7 ^a	80 ^{ab}	33 ^a	90 ^a
Control	21.6 ^a	97 ^a	40.4 ^a	100 ^a
LSD (P≤0.05)	NS	25	NS	NS

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

Table 2. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Brassica napus* L. v. ATR Hyden.

Plant type	Seed leachate		Seedling leachate	
	Canola radicle length (mm)	Cumulative germination % at 14 days	Canola radicle length (mm)	Cumulative germination % at 14 days
Barley	10.5 ^c	73 ^{ab}	26 ^a	87 ^a
Canola	22.4 ^a	97 ^a	22 ^a	83 ^a
Wheat	12.4 ^{abc}	70 ^{ab}	26 ^a	90 ^a
Lupine	5.8 ^c	60 ^b	22.8 ^a	87 ^a
Lucerne	10.8 ^{bc}	100 ^a	19.2 ^a	80 ^a
Medic	22.3 ^{ab}	93 ^a	21.7 ^a	93 ^a
Ryegrass	23.4 ^a	90 ^{ab}	22.3 ^a	87 ^a
Control	23.7 ^a	93 ^a	19.5 ^a	73 ^a
LSD (P≤0.05)	11.5	33	NS	NS

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

leachates from both barley (15%) and lupine (100%) compared to the control (Table 5). Lupine seed leachate also completely inhibited lucerne cumulative germination (100%). By contrast, both canola (98%) and ryegrass (93%) seed leachates showed strong stimulatory effects on lucerne seedlings with regard to radicle elongation (Table 5). This growth promoting activity of ryegrass was also evident in its seedling leachate which significantly stimulated (80%) the radicle length of lucerne (alfalfa) seedlings compared to the control.

Medic

The radicle length of medic was significantly inhibited by

seed leachates from both barley (42%) and lupine (40%) compared to the control (Table 6). This phytotoxic activity of lupine was also observed for seedling leachate which strongly inhibited both radicle elongation of medic (64%) and cumulative germination of medic (82%) compared to the control (Table 6).

Ryegrass

The radicle length of ryegrass was significantly inhibited by phytotoxic seed leachates from barley (34%), wheat (43%) and lupine (4%) compared to the control (Table 7). This growth-inhibiting effect from barley (52%) and lupine (18%) seed leachates, was also evident in cumulative

Table 3. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Triticum aestivum* v. SST 88.

Plant type	Seed leachate		Seedling leachate	
	Wheat radicle length (mm)	Cumulative germination % at 14 days	Wheat radicle length (mm)	Cumulative germination % at 14 days
Barley	8.5 ^{bc}	53 ^{bc}	33 ^{abc}	80 ^a
Canola	19.5 ^{ab}	70 ^{ab}	25.7 ^c	83 ^a
Wheat	9.5 ^{bc}	93 ^a	44.6 ^a	77 ^a
Lupine	5 ^c	27 ^c	31 ^{bc}	73 ^a
Lucerne	20.2 ^{ab}	83 ^{ab}	40.6 ^{ab}	83 ^a
Medic	15.6 ^{abc}	83 ^{ab}	35.8 ^{abc}	87 ^a
Ryegrass	24.8 ^a	93 ^a	41.2 ^{ab}	70 ^a
Control	27.9 ^a	87 ^{ab}	41.4 ^{ab}	87 ^a
LSD (P≤0.05)	12.5	38	13.5	NS

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

Table 4. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Lupinus albus* L. v. Tanjil.

Plant type	Seed leachate		Seedling leachate	
	Lupine radicle length (mm)	Cumulative germination % at 14 days	Lupine radicle length (mm)	Cumulative germination % at 14 days
Barley	2.8 ^b	13 ^b	21.9 ^a	80 ^{ab}
Canola	6.8 ^{ab}	53 ^a	15.2 ^a	90 ^a
Wheat	8.9 ^{ab}	40 ^{ab}	26.3 ^a	87 ^a
Lupine	11.9 ^a	70 ^a	23 ^a	90 ^a
Lucerne	9.6 ^{ab}	43 ^{ab}	12.9 ^a	57 ^b
Medic	13.5 ^a	63 ^a	16.5 ^a	77 ^{ab}
Ryegrass	9.1 ^{ab}	47 ^{ab}	24 ^a	77 ^{ab}
Control	8.4 ^{ab}	53 ^a	27.7 ^a	93 ^a
LSD (P≤0.05)	9	40	NS	25

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

germination percentages of ryegrass. Lupine seedling leachate significantly inhibited ryegrass radicle elongation by 68% compared to the control (Table 7).

DISCUSSION

The goals of this study were to evaluate the mutual effects of leachates exuded from both seeds and seedlings of ryegrass and selected rotational crop species. It was also envisaged that results would offer perspectives on possible phytotoxic reactions. In general, results from the current study are consistent with a field assessment by Ferreira and Reinhardt (2010) which

showed suppression of ryegrass weed type by selected leguminous crops. Furthermore, results confirm an observation by Baghestani et al. (1999) that phytotoxic effects on seed germination depend on the donor and receiver plants.

Barley

Cumulative germination of barley was inhibited by both wheat and medic seed leachates. In the reciprocal response reaction, barley seed leachates reduced canola, wheat, lucerne, medic and ryegrass radicle lengths as well as lupine and ryegrass cumulative

Table 5. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Medicago sativa* L. v. SA standard.

Plant type	Seed leachate		Seedling leachate	
	Lucerne radicle length (mm)	Cumulative germination % at 14 days	Lucerne radicle length (mm)	Cumulative germination % at 14 days
Barley	3.7 ^{bc}	33 ^c	18.3 ^{abc}	63 ^a
Canola	20.8 ^{ab}	87 ^a	21.4 ^{ab}	80 ^a
Wheat	11.9 ^{abc}	47 ^{bc}	20.1 ^{abc}	70 ^a
Lupine	0 ^c	0 ^d	14.7 ^{bc}	63 ^a
Lucerne	17.1 ^{abc}	73 ^{ab}	12.3 ^c	63 ^a
Medic	20.0 ^{ab}	57 ^{abc}	22.8 ^{ab}	70 ^a
Ryegrass	20.6 ^{ab}	83 ^a	26.4 ^a	57 ^a
Control	25.2 ^a	43 ^{bc}	14.7 ^{bc}	73 ^a
LSD (P≤0.05)	17.4	31	8.7	NS

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

Table 6. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Medicago truncatula* Gaertn. v. Parabinga.

Plant type	Seed leachate		Seedling leachate	
	Medics radicle length (mm)	Cumulative germination % at 14 days	Medics radicle length (mm)	Cumulative germination % at 14 days
Barley	13.4 ^b	57 ^a	17.0 ^{ab}	73 ^{ab}
Canola	31.7 ^a	73 ^a	27.8 ^a	70 ^{ab}
Wheat	19 ^{ab}	77 ^a	25.8 ^{ab}	73 ^{ab}
Lupine	12.6 ^b	50 ^a	15.6 ^b	63 ^b
Lucerne	17 ^{ab}	60 ^a	18.5 ^{ab}	73 ^{ab}
Medic	19.7 ^{ab}	93 ^a	19.2 ^{ab}	77 ^a
Ryegrass	31.8 ^a	70 ^a	26.8 ^a	70 ^{ab}
Control	31.6 ^a	77 ^a	24.5 ^{ab}	77 ^a
LSD (P≤0.05)	16.1	NS	10.9	13

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

germination percentages. These results are in accordance with those of Xuan et al. (2005), who also reported plant inhibition by lucerne. However, Ben-Hammouda et al. (2001) reported that the allelopathic potential of barley increased near physiological maturity. According to Baghestani et al. (1999) vanillic and o-coumaric acids along with scopoletin may be responsible for the allelopathic effects of barley and wheat. However, Bouhaouel et al. (2016) reported that barley produces a complex mixture of allelochemicals that seem to be highly diversified. Moreover, barley genotypes exhibited differential allelopathic activity against weeds.

Canola

Canola radicle length was inhibited by barley, lupine and

lucerne seed leachates. Lupine seed leachate also reduced canola cumulative germination. In the reciprocal response reaction, canola seed leachate promoted lucerne radicle length, but by contrast reduced wheat radicle length. 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. However, when Mutlu and Atici (2009) evaluated the allelopathic potential of root and shoot extracts from *Nepeta meyeri* they found a general phytotoxic effect at all concentrations on seed germination of barley, while at lower concentrations wheat and canola seedling growth

Table 7. Effects of seed and seedling leachates exuded by selected rotational crops on the radicle length and cumulative germination of *Lolium multiflorum* Lam. v. Energa

Plant type	Seed leachate		Seedling leachate	
	Rye grass radicle length (mm)	Cumulative germination % at 14 days	Rye grass radicle length (mm)	Cumulative germination % at 14 days
Barley	12.4 ^{cd}	50 ^{bc}	35.6 ^{ab}	83 ^a
Canola	33.8 ^{ab}	87 ^a	34.7 ^{ab}	83 ^a
Wheat	15.8 ^{bcd}	73 ^{ab}	47.4 ^a	87 ^a
Lupine	1.5 ^d	17 ^c	31.6 ^b	87 ^a
Lucerne	25.1 ^{abc}	90 ^a	36.2 ^{ab}	83 ^a
Medic	24.0 ^{abc}	97 ^a	39.1 ^{ab}	80 ^a
Ryegrass	28.2 ^{abc}	90 ^a	46.4 ^a	93 ^a
Control	36.8 ^a	97 ^a	46.1 ^a	90 ^a
LSD (P≤0.05)	19.5	34	13.9	NS

Means followed by the same letter are not significantly different at the 0.05 probability level.
NS=non-significant.

was increased. This an example of the hormesis effect in that the same allelochemicals could have resulted in different growth responses, either positive or negative, from the species considered here. Furthermore, the span between stimulation and inhibition for allelochemicals can be small and hormetic effects (Calabrese, 2007) 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. In addition, plant genotype was considered by Asaduzzaman et al. (2014) who measured the allelopathic activity of canola on the reduction in ryegrass root and shoot growth. These differed significantly between canola genotypes in their ability to inhibit ryegrass root length.

Wheat

The radicle length of wheat was decreased when germinated with barley, wheat and lupine seed leachate and also canola seedling leachate. Lupine seed leachate also reduced wheat cumulative germination. In the reciprocal response reaction, wheat seed leachate inhibited ryegrass radicle length, but showed autotoxicity by reducing wheat radicle length. Current results on wheat radicle inhibition by wheat seed leachate, corresponds with results on varietal autotoxicity reported by Wu et al. (2007) who studied wheat varieties. Allelopathic studies by Bakhshayeshan-Agdam et al. (2015) indicated that the germination response among the crops tested for wheat and common bean were the most resistant to redroot pigweed (*Amaranthus retroflexus* L.) leachate treatments.

Lupine

Barley seed leachate reduced both lupine radicle length

and cumulative germination. In addition, cumulative germination of lupine was also inhibited by lucerne seedling leachate. In the reciprocal response reaction, lupine seed leachate inhibited both radicle length and cumulative germination of canola, wheat and lucerne. A report by Belel and Belel (2015) on a different leguminous crop, namely cowpea (*Vigna unguiculata* (L.) Walp) showed the toxic effect of nutgrass (*Cyperus tuberosus* Rottb.).

Lucerne

While lucerne radicle length was reduced by barley seed leachate, lupine seed leachate showed complete inhibition of both its radicle growth and cumulative germination. By contrast, both canola and ryegrass seed leachates stimulated the growth of lucerne seedlings with regard to radicle length. This activity of ryegrass was also evident in its seedling leachate which increased radicle length of lucerne seedlings. In the reciprocal response reaction, lucerne seedling leachate showed inhibition of both canola radicle length and lupine cumulative germination.

Bakhshayeshan-Agdam et al. (2015) reported slight germination in the presence of redroot pigweed (*Amaranthus retroflexus* L.) allelochemicals, but seedling growth was completely inhibited. It was concluded that of the four crops tested, lucerne germination was the most sensitive to the leachate treatments. Studies by Gholami et al. (2014) indicated that lucerne produces allelopathic triterpene saponins and flavonoids which might be the major cause of yield reduction in subsequent crops. Chung et al. (2000) showed that chlorogenic acid occurs in relatively large amounts in lucerne aqueous extracts as compared to salicylic acid, and bioassays suggest that chlorogenic acid is involved in lucerne autotoxicity.

Medic

The radicle length of medic was inhibited not only by barley and lupine seed leachates, but also by lupine seedling leachate. Additionally, activity of lupine seedling leachate was also evident in reduced medic cumulative germination. In the response reaction, medic seed leachate showed reduced barley cumulative germination.

Ryegrass

The radicle length of ryegrass was inhibited by seed leachates from barley, wheat and lupine. This growth-inhibiting effect by both barley and lupine seed and seedling leachates was also evident in ryegrass cumulative germination percentages. Further proof of this activity by lupine seedling leachate was also evident in ryegrass radicle length. In the reciprocal response reaction, both ryegrass seed and seedling leachates showed a stimulatory reaction on lucerne radicle elongation.

These findings on wheat are in accordance with those by Wu et al. (2000), 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%. Although no growth-inhibiting effect from canola seed or seedling leachates on ryegrass was observed in this study, the varietal responses by wheat (Wu et al. 2000), was also confirmed for canola in a report by Asaduzzaman et al. (2014) as it showed considerable genetic variation among canola genotypes for their allelopathic effects on ryegrass. It was concluded that highly allelopathic canola genotypes could potentially suppress ryegrass in integrated weed management programmes.

Allelopathic compounds affect several metabolic and physiological processes in seeds that are known to retard germination and impair seedling growth (Mathiassen et al., 2006). Moreover, Shui et al. (2010) reported that for both perennial ryegrass (*L. perenne* L.) and lucerne (*M. sativa* L.) seedling growth was more sensitive than seed germination as an indicator of allelopathic effect. Contrary to this, results from this study indicate more sensitivity by seed germination than seedling growth and depend on the specific acceptor species and donor leachate.

Given the synchronous germination of ryegrass weed type and rotational crops in the field, this data confirms earlier findings by Ferreira and Reinhardt (2010). Ryegrass typically germinates in autumn and its growth habit and life cycle coincide with that of most rotational crops tested in this study. Chemical control of ryegrass has become very problematic due to the increasing

incidence of herbicide resistance. Moreover, in some countries legislations have required the agricultural industry to become less dependent on pesticide use by following integrated weed management principles (Barzman et al., 2015). This would surely aid the move to more sustainable systems by reducing, or in future, even eliminate the use of herbicides by innovative approaches (Peigné et al., 2015). Since results from the present study suggest that wheat, barley and lupine strongly inhibited ryegrass radicle elongation with the two latter named crops showing the most activity, one possible option in the field for weed suppression might entail high barley or lupine seeding densities. Though, from practical experience in the field, this would not be a feasible weed management option. This is due to the fact that mono cropping requires many inputs in terms of agrochemicals. In addition, final lupine plant population in the field are seldom above 50 plants m⁻² which is due to the soil and climatic factors of the Western Cape, South Africa. Under these field conditions adequate ryegrass suppression would theoretically only be achieved at a final lupine plant population of 100 plants m⁻². Since this is not feasible, the only realistic option would be a smother crop mixture with a monocotyledonous species such as barley, black oats or rye in combination with lupine as the leguminous component.

Conclusion

For effective ryegrass management with less reliance on herbicides, results from the present study showed that leguminous crops should play a more prominent role in crop rotation systems. Leguminous crops should not only be considered as rotational crops, but also used in intercropping where living mulches are used for weed suppression. However, under field conditions, such a practice is likely to be exposed to the vagaries of environmental factors, as well as likely being crop cultivar and weed-specific. Nevertheless, by promoting diversity in weed management tools, this would ensure sustainability and reduce the possibility of the development of weed resistance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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