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Phytotoxic potential of Shepherd's purse on annual ryegrass and wild radish

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ABSTRACT

Control options for weeds such as annual ryegrass in winter wheat crops are limited due to the development of herbicide resistance. This study reports the potential of shepherd's purse (*Capsella bursa-pastoris*) as a source for novel compounds for weed control. The extract of shepherd's purse was tested for phytotoxicity against annual ryegrass and wild radish. All concentrations of 5 % and above significantly reduced the growth of both test species. The stability of the extract was also tested, and there was no significant change in phytotoxicity of the extract over 256 days. The negative effect of shepherd's purse extract was also observed (in trays filled with soil) on annual ryegrass root growth. The 400 % extract inhibited shoot length and weight by > 70 % compared to the control.

In addition to biological studies, the chemical components of shepherd's purse extract were also investigated. Bioassay guided fractionation determined that the ethyl acetate fraction was most phytotoxic. All fractions were analysed by GC/MS and tentative identifications of detected compounds were conducted and the overall phytotoxicity of extract was also determined.

Key words: Annual ryegrass, bioassay guided isolation, *Capsella bursa-pastoris*, extract stability, GC/MS, wild radish

INTRODUCTION

Annual ryegrass (ARG) is the most important weed infesting wheat crop in southern Australia, reducing both crop yield and crop quality. Control options for this weed are limited due to the development of herbicide resistance in ARG. Some ARG biotypes are resistant to several major herbicide groups (25). This has prompted research to discover novel natural plant compounds with herbicidal properties.

In one such research project, Haig *et al.* (12) screened over 130 crop, weed and native plant species for their phytotoxicity against ARG. Many grasses, trees and herbaceous perennial plants with suspected allelopathic or pharmaceutical properties were selected. Shepherd's purse (*Capsella bursa-pastoris*) ranked in the top three most phytotoxic species and was thus selected for further analysis.

Despite the large volume of information available about the medicinal qualities of shepherd's purse, less is known about its phytotoxic potential. Duke (7) lists the chemical

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components of shepherd's purse, however, it does not distinguish between nutritional and phytotoxic compounds. Belonging to the Brassicaceae family, shepherd's purse contains glycosinolates and myrosinase, which breaks down glycosides into more biologically toxic compounds such as thiocyanates and nitriles (30). Many of these compounds have high biological activities (3,27) and the potential use of such compounds from Brassicaceae as cover crops and bioherbicides has been reviewed (3,13). Isothiocyanates (ITCs) are commonly reported to be fungitoxic (20,22), insecticidal (24), nematicidal (17) and phytotoxic (3,21,23).

This study aimed to determine the broader weed control potential of shepherd's purse against weeds typically found in winter wheat crops. The stability of the extract over long time was also examined. Besides examining the biological/agronomic aspects of shepherd's purse phytotoxicity, this study also focussed on the chemical nature of this phenomenon. Using bioassay-guided fractionation, the most phytotoxic fraction was identified. All fractions were analysed via GC/MS to elucidate their chemical composition.

MATERIALS AND METHODS

Preparation of Extract

Whole shepherd's purse (*Capsella bursa-pastoris*) plants were collected from the Riverina, NSW, dried for 72 h at 40°C and then chopped into a fine chaff using a 40 mesh screen. Ten g ground plant material was added to 100 mL distilled water in a 250 mL Schott bottle, wrapped in aluminium foil and incubated at 20°C for 72 h. The extract was then filtered through two layers of cheesecloth and centrifuged for 20 min at 4000 rpm using an Eppendorf 5810 bench-top centrifuge. This extract was considered to be the 100 % extract. It was diluted with sterile distilled water to obtain 0.1, 1, 5, 10, 25 and 50 % extracts. Sterile distilled water serves as the control. All procedures after the centrifugation of extract were done in a cross-flow laminar flow cabinet to minimise contamination.

Sterilisation and pre-germination of seeds

Seeds of both test species: annual ryegrass (*Lolium rigidum* Gaudin) and wild radish (*Raphanus raphanistrum* L.) were soaked in 2 % NaClO for 2 min and then thoroughly rinsed with sterile distilled water. They were then transferred to petri plates (9 cm dia) lined with Whatman #1 filter paper. The petri plates were then placed in a Precision Model 818 Low Temperature Incubator (20°C for 48 h with 14 h day/10 h night) to germinate. Wild radish plates were wrapped in aluminium foil prior to placement in the incubator.

Bioassays

Ten pre-germinated seeds of each species were sown in 250-mL beakers lined with Whatman #1 filter paper. Prior to sowing, 4 mL of 0, 0.1, 1, 5, 10, 25, 50 or 100 % extract solution were added to the beaker. Sterile distilled water was used in the control. The beakers were covered with parafilm before being placed in the incubator. All procedures were done in a cross-flow laminar flow cabinet to minimise contamination.

Three replicates were arranged in a randomised complete block design under the experimental conditions mentioned above. After 3 days, root and shoot growth was measured.

A similar experiment was conducted using seeds which were not pre-germinated. As the seeds in the pre-germination bioassay are given 2-days pre-germination time, then 3 days growth in beakers, the seeds in this experiment were sown immediately into the beakers and allowed to grow for 5 days before measurement. As wild radish germinates better in the dark, the beakers were kept in the dark for the first 2 days. Bioassays with germinated and non-germinated seeds were conducted twice.

Stability Study

The shepherd's purse extract's phytotoxicity was tested on annual ryegrass over a period of four months, then again at 256 days. Three 400 mL aliquots of extract were prepared as described above and stored in incubators (4°C dark, 25°C dark and 25°C light). At 0, 1, 2, 4, 8, 16, 32, 64, 128 and 256 days, 40 mL of extract was used in bioassay. After the dilution of original extract (100 %) to 0 (control), 0.1, 1, 5, 10, 25 and 50 % concentrations, both EC and pH were measured for each concentration. The pH was determined with a MODEL pH meter and the electric conductivity (EC) with an Activon Pocket Conductivity Meter. The bioassay in this study was identical to the bioassay using pre-germinated ryegrass as described above, however, the experiment was not repeated over time.

Spray Trial

Two separate spray trials were conducted, one as a pre-emergence spray and one as a post-emergence spray. To determine the influence of soil on the observed phytotoxic effects, soil trials were also conducted. Nursery trays (8 x 9 grid, each square 3 cm x 3 cm) were filled with 50: 50 (sand: potting mix) and placed onto a large shallow tub filled with 1 cm water. Ten sterilised ARG seeds were planted per square and covered with a fine layer of soil mixture, then sprayed with tap water to moisten the soil. In the pre-emergence trial, 5 mL of 100, 200 or 400 % extract solutions were sprayed on each square after two days. The 200 and 400 % solutions were prepared by rotary evaporating the stock 100 % solution using a Buchi rotary evaporator. In the control, 5 mL tap water was sprayed. In the post-emergence trial, 5 mL extract was sprayed 2 weeks after sowing. When required, water was added to the reservoir rather than spraying on the soil surface. Four replicates were arranged in a complete randomised block and the tray was placed in an incubator [15°C (14 h light/10 h dark)]. In the pre-emergence trial, the seedlings were removed after 10 days and the shoots length was measured using a ruler. In the post-emergence trial, shoot length and shoot weight were recorded.

Solvent Extraction and Fractionation

To obtain enough extract for extraction and fractionation, the values of the aforementioned extract were increased 10-folds. The solvent extraction and fractionation method used here was slightly modified from Weston *et al.* (28). One L of shepherd's purse extract (100 %) was added to a 5 L volumetric flask with 2.5 L acetone (to precipitate the proteinaceous material). The flask was placed in an incubator set at 2°C and the solution was slowly stirred for 48 h. The solution was then filtered through two layers

of Whatman #1 and Whatman #42 filter paper and the acetone was evaporated using a Buchi rotary evaporator. The remaining aqueous extract was then subjected to a series of solvent extractions. The extract was partitioned three times with 300 mL aliquots of each solvent: hexane, dichloromethane, ethyl acetate and *n*-butanol. In the *n*-butanol extraction, an emulsion layer formed, that was then centrifuged for 10 min at 2000 rpm to separate the solvent from the aqueous solution. Pooled solvent fractions were rotary evaporated under vacuum at 40°C and dried with nitrogen. Each dry solvent fraction was stored at 4°C prior to bioassay.

Bioassay of solvent fractions

A stock concentration of 4000 ppm was made from the dried crude extract, the solvent fractions and the dried final aqueous extract by dissolving the appropriate amount of dried material in analytical grade methanol. The stock was serially diluted to obtain 2000, 1000 and 200 ppm. One mL of each solution was added to 250 mL beakers lined with Whatman #1 filter paper and allowed to evaporate to dryness, then 4 mL sterile distilled water was added. Thus, the resulting concentrations used in the bioassay were 1000, 500, 200, 50 ppm. In the control, 1 mL pure methanol was added and allowed to evaporate. From this point, the bioassay was identical to the crude plant extract bioassay described earlier.

Chemical Analysis

Instrumentation: Chemical analysis was conducted on a Varian 3400CX gas chromatograph fitted with a J & W Scientific DB-5 fused silica capillary column (30 m length, 0.25 mm internal diameter and 0.25 um film) coupled with a Varian Saturn 2000 ion trap mass spectrometer. The injector temperature was 280°C. The initial column temperature was a constant 50°C for one minute, increased to 160°C at a rate of 10°C/min, then ramped to 235°C at 5°C/min. The temperature was then brought to 310°C at a rate of 20°C/min and held until the end of the 38 minute run. Helium was the carrier gas with linear velocity 34 cm/s. The mass scan range was set at 50 - 650 m/z in the 70 eV electron impact ionisation (EI) mode. A 1.7 minute filament delay was employed for samples made up in methanol and a 6 minute filament delay was employed for samples derivatised with BSTFA. Data were analysed using the Varian (Walnut Creek, California) Saturn Chromatography Work Station software (Version 1.3).

Identification of compounds

Originally, 1000 ppm solutions of each fraction made in methanol were injected (split-less) straight in to the GC/MS in 1 µL aliquots. However, the ethyl acetate fraction, which was the most phytotoxic in bioassay, appeared to have relatively few compounds, all of which had very low concentrations. Therefore, the contents of all solvent fractions were converted to volatile trimethylsilyl derivatives with BSTFA. One mL of each solvent fraction was added to a 2 mL reactivial and dried with nitrogen. One mL of BSTFA was added to each reactivial which was then capped and heated in a 60°C oven for 20 min prior to injection to insure that the sample was fully derivatised. One µL aliquots of these derivatised samples were also injected (split-less) into the GC/MS using the same temperature programme described above. To identify the compounds present in the

fractions, mass spectra from the samples were compared with mass spectra entries in the NIST 2005 database. Tentative positive identifications were made, if the peak in the fraction sample had a spectral fit value of at least 850 (perfect fit is 1000) in the silylated fractions and 750 in the non-silylated fractions.

RESULTS AND DISCUSSION

Natural plant compounds are an under-utilised source of novel, potentially phytotoxic compounds, offering new sites of herbicide action and activities different from existing synthetic herbicides (8). In addition to herbicidal potential, much work has been done on natural plant products (*e.g.* essential oils) as insecticides (4,15,16,26). Isman (15) lists several plant derived compounds currently being used as insecticides (pyrethrum, neem and rotenone). However, botanically derived herbicides are less prominent, despite the substantial research into natural herbicides.

Haig *et al.* (12) screened more than 160 plant tissues and species for their phytotoxicity towards annual ryegrass in a large-scale search for plants with such herbicidal potential. Three of these plants were selected for extensive analysis. In this study, one of these plants, shepherd's purse, was examined for phytotoxic potential.

Bioassay

In bioassay, the shepherd's purse extract significantly inhibited the growth of both test species (Fig. 1). Results for the non-germinated and pre-germinated bioassays are not significantly different, so only the pre-germinated results were presented. ARG was significantly more affected by the shepherd's purse extract than wild radish ($LSD._{weed} = 4.3$, $p < 0.05$). The root growth of both test species was significantly reduced at concentrations of 5 % and above ($LSD_{concentration} = 8.6$, $p < 0.001$). Extract concentrations required for 50 % inhibition (ED_{50}) of annual ryegrass and wild radish root growth were 8.3 and 11.3 %, respectively.

Herbicide resistant annual ryegrass (HR-ARG) was also inhibited by the extract similar to non-HR-ARG. Therefore, the shepherd's purse extract and the chemicals found therein could potentially offer an alternative weed control option for HR-ARG.

Stability Study

pH and EC: The extracts pH changed over time under both sets of 25°C storage conditions, ranging from 6.4 at Day 0 to 5.5 at Day 128, regardless of the presence of light. The only observable difference between the light and dark treatments was that the extract stored in the light was murky as compared to a relatively translucent extract, when stored in dark. The pH of extract stored at 4°C had a smaller range, from 6.4 at Day 0 to 5.9 at Day 128. Although these pH values changed over time, they are still within the general accepted levels for plant growth.

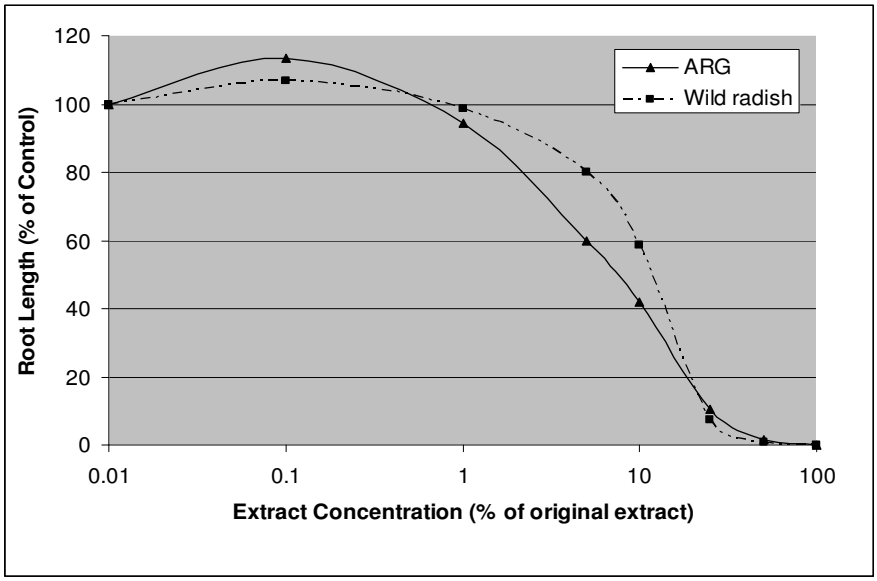

Figure 1. Effects of extract concentration on root growth of two test species ($L.S.D._{interactions}$ = 12.2., $p < 0.001$)

Only the EC of 100 % extract changed over time, with the greatest changes observed in those extracts stored at 25°C. EC values of light and dark solutions went up from 10.96 dS/m to a value of 11.72 and 11.75 dS/m, respectively. The solution stored at 4°C, however, only increased to 11.35 dS/m. The EC values remained at 0.02, 0.19, 0.85, 1.50, 3.53 and 6.36 for 0.1, 1, 5, 10, 25 and 50 % extract, respectively, during the experiment.

Phytotoxicity: The storage conditions did not significantly affect the phytotoxicity of the shepherd's purse extract. After storage for 256 days there were no differences in phytotoxicity, which suggests that this extract has potential as a novel herbicide. The compounds would not break down before the farmer had a chance to spray. This observed phytotoxic effect on root growth is more likely due to the presence of extract rather than pH or electrical conductivity (EC) effects. The pH of shepherd's purse extract ranged from 5.4 - 6.4, which is well within the reported limits suitable for plant growth. The EC ranged from 0.03 dS/m in the 0.1 % extract to 6.6 dS/m in the 50 % extract. The EC of the 100 % extract ranged from 10.96-11.75 dS/m. Fujii *et al.* (10) reported that an EC below 1 mS/cm (1 dS/m) was not harmful to lettuce germination, whereas, a value greater than 10mS/cm (10 dS/m) strongly inhibited the lettuce germination and growth. It is important to remember that lettuce is a very sensitive test species, so it is possible that ARG may be able to withstand a greater range of EC values. In this study, the only concentration with an EC value over 10 dS/m was the 100 % extract. However, even at 50 % which was within the EC limits for suitable plant growth, there was more than 95 % inhibition in root

growth, which suggests that it is the compounds in the extract rather than properties such as EC or pH which are responsible for the root growth inhibition.

Spray Trials

Although these effects were observed under sterile conditions, it was difficult to predict the effects of these phytochemicals in soil due to the myriad influences of soil properties and microflora on the persistence of phytochemicals. Therefore, the shepherd's purse extract was also applied to trays of 50: 50 sand/peat moss, a substrate commonly used in herbicide resistance trials (2) in both pre- and post-emergence trials. The pre-and post-emergence trials had similar results, hence, only the post-emergent results were presented (Fig. 2). All three extract concentrations (100, 200 and 400% extract) significantly inhibited the shoot growth of ARG (LSD. $_{length}$ = 8.5, LSD $_{weight}$ = 14.3, $p < 0.001$). The 400 % extract inhibited the shoot length and weight by >70 % compared to the control. In the 200 and 400 % extract treatments, the majority of plants were in various stages of necrosis when sampled.

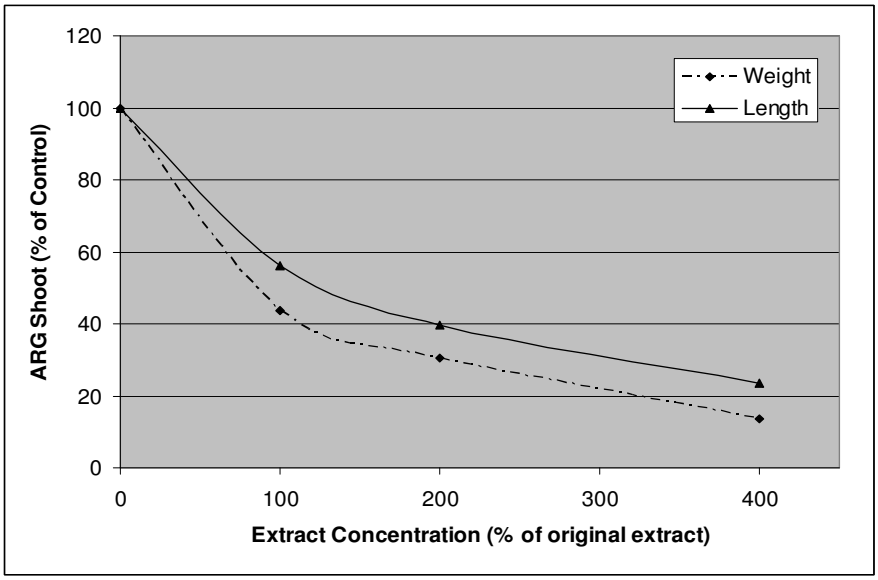

Figure 2. Effects of shepherd's purse extract on shoot growth of ryegrass when sprayed 2 weeks post-emergence (L.S.D.$_{length}$ = 8.5, L.S.D.$_{weight}$ = 14.3, $p < 0.001$).

In bioassay, the 100 % extract almost completely inhibited the root and shoot growth, while in both pre and post-emergence spray trials, only shoot growth was inhibited by >40 %. During establishment and early growth, small difference in plant growth rates greatly affects the plants' success (11). Thus, inhibiting the growth of ARG would confer a competitive advantage to the growing wheat crop with which ARG competes in Australian winter wheat crops.

Bioassay of Solvent Fractions

In addition to examining the agronomic factors of shepherd's purse phytotoxicity, we also studied the chemicals aspects underlying the phenomenon. All fractions were phytotoxic to annual ryegrass. There were significant differences between the fractions (LSD = 3.2, $p < 0.001$) in their ability to inhibit ARG root growth (Fig. 3) and followed the order: *n*-butanol > hexane > dichloromethane > ethyl acetate, from least to most phytotoxic. All concentrations significantly inhibited the ARG root growth (LSD = 3.6, $p < 0.001$). At 1000 ppm, only *n*-butanol was significantly less phytotoxic than other fractions.

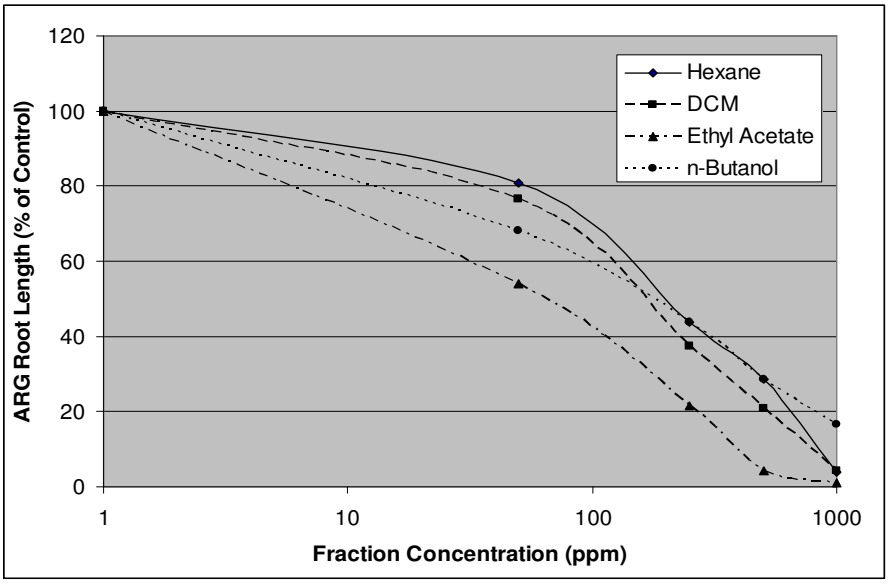

Figure 3. Phytotoxicity of solvent fractions from shepherd's purse on ryegrass root growth ($L.S.D._{interactions} = 7.1$, $p < 0.001$)

Estimated ED_{50} values showed the varying phytotoxicities of individual fractions. All fractions and extracts were 2-5 times more phytotoxic against root growth than shoot growth (Table 1). The most phytotoxic fraction against root growth was ethyl acetate (ED_{50} value of 74 ppm). The next most phytotoxic fraction (dichloromethane) had an ED_{50} which was 100 ppm higher than ethyl acetate. Hexane and *n*-butanol were the least phytotoxic fractions, with ED_{50} values of 217 and 200 ppm, respectively. ED_{50} values for shoot growth ranged from 460 ppm in ethyl acetate to 1000+ ppm in *n*-butanol. *n*-Butanol, the crude extract and the final remaining extract all had ED_{50} values > 1000 ppm and only slightly suppressed the ARG shoot growth by 8, 14 and 1 %, respectively, at the highest concentration used.

Table 1. Mass of fraction obtained via extraction in solvent series and the phytotoxicity of individual fractions in bioassay against annual ryegrass

		ED_{50} (ppm)	
Fraction	Mass of Fraction (mg)	Roots	Shoots
Crude extract[+]	1346.7	343	1000+*
Hexane	22.8	217	490
Dichloromethane	106.9	186	636
Ethyl acetate	154.1	74	461
n-butanol	1120.8	200	1000+*
Final remaining extract[+]	1809.5	367	1000+*

[+] from a 50 mL sample; *Concentration is reported as 1000+ if 50% inhibition did not occur at any tested concentrations

The ethyl acetate solvent fraction was significantly more phytotoxic than the other solvent fractions. However, the dose-response curves of all fractions had similar shape, which suggested that similar compounds may be present in many fractions. In fact when the fractions were analysed via GC/MS, carryover of certain compounds between the fractions was observed (Table 2). Thus, the bioassay data was reflecting what was found in chemical analysis data.

Chemical Analysis

All fractions were subjected to GC/MS analysis using NIST 2005 for tentative identification of compounds. In some cases, compounds overlapped between the fractions, mostly between the dichloromethane (DCM) and ethyl acetate (EA) fractions. In bioassay, all fractions inhibited the ARG root growth by 95 %, except for *n*-butanol which caused 83 % inhibition at 1000 ppm, the concentration used for injection. It is not surprising, then, that there would be similar compounds in the fractions. The observed phytotoxic effect could also be quantitative rather than qualitative. The least inhibitory fraction, *n*-butanol, had fewer high matches with compounds in the NIST 2005 database, even though 6 compounds carried over from the ethyl acetate fraction. One compound was carried over from the hexane fraction (HEX) into the DCM fraction and 3 compounds were carried over from the EA fraction to the *n*-butanol (*n*-BUT) fraction. Eight compounds overlapped between the DCM and EA fractions and two of these were carried over to the final solvent in the series, *n*-butanol, in very small amounts. The compounds tentatively identified from the solvent fractions using NIST 2005 are listed in Table 2.

The crude chemical analysis of shepherd's purse extract did not aim to identify the specific compound(s) that are responsible for the observed phytotoxic affects but aimed to compare the compounds present in ethyl acetate fraction with other fractions as leads for future work. All identifications were tentative and were made using the best matches from the latest NIST 2005 database, with a match of at least 850 (out of a possible 1000) in the derivatised samples and purity values greater than 500. Many classes of compounds cannot be adequately analysed via GC/MS, thus the chemical work presented here is by no means exhaustive.

Table 2. Fractions containing compounds with high spectral matches using the NIST 2005 database

Compound	Fractions with high mass spectral matches			
	HEX	DCM	EA	*n*-BUT
2 or 3 or 4-hydroxymandelic acid		√	√	√
2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a,trimethyl-,	√*			
2,3-benzopyrrole (indole)	√**			
2-hydroxybenzoic acid (salicylic acid)			√	√
2-hydroxypropanoic acid (L-lactic acid)		√	√	√
2-methoxy-4-vinylphenol	√*	√*	√*	
2-methoxyphenol (*o*-guaiacol)	√*			
2-methylbenzaldehyde (o-Tolualdehyde)	√*	√*		
2-methylphenylisocyanate			√*	
3,4 dihydroxybenzoic acid (protocatechuic acid)			√	
3,4 dihydroxyhydrocinnamic acid			√	√
3-hydroxybenzoic acid			√	
3-methoxy-4-hydroxybenzenepropanoic acid		√	√	
3-methoxy-4-hydroxybenzoic acid (vanillic acid)		√	√	
4,5-dihydro-3-hydroxy-4,4-dimethyl-2-furanone (pantoyl lactone)		√		
4-hydroxybenzeneacetic acid			√	
4-hydroxybenzoic acid			√	
4-hydroxyhydrocinnamic acid		√	√**	
4-hydroxyphenylethanol (tyrosol)		√	√	
α-hydroxybenzeneacetic acid		√	√	
α-hydroxybenzenepropanoic acid		√	√	√
benzeneacetic acid		√	√	
benzoic acid	√	√		
ß-hydroxyethylbenzene (phenylethylalcohol)	√*	√*		
butanedioic acid (succinic acid)		√	√	√
coumarin (2H-1-benzopyran-2-one)	√*			
coumarin, 3,4-dihydro-4,4,7-trimethyl	√*			
hexadecanoic acid (palmitic acid)	√			
hydroferulic acid			√*	
methylhydroquinone (toluhydroquinone)		√*		
octadecanoic acid (stearic acid)	√			
octanoic acid (caprylic acid)	√	√		
vanillylpropanoic acid		√	√	

*detected in non-silylated fractions only; ** detected in both non-silylated and silylated fractions

Of the compounds tentatively detected in the fractions, several have previously been reported as having pesticidal effects. Many phenolic acids suppress or inhibit germination and plant growth in bioassay, although their role in field situations remains a controversial issue. However, one compound detected in the first three solvents, a break-down product of a common phenolic acid, was highly bioactive compared to its precursor. Bioactive vinylphenols such as 2-methoxy-4-vinylphenol can be produced by the decarboxylation of cinnamic acids and these may contribute to the overall phytotoxicity of plant extract.

Other detected compounds with potential phytotoxicity were: indoles, coumarins and 2-methylphenyl isocyanate. Coumarin is more phytotoxic than majority of common phenolic acids (8). Two coumarins (coumarin and 4,4,7-trimethylchroman-2-one/ coumarin, 3,4-dihydro-4,4,7-trimethyl) were detected in the hexane fraction. Lydon and Duke (19) report that coumarin is allelopathic, while Harborne *et al.* (14) list coumarin as antifungal and piscicidal. Phytochemical activities of coumarin include germination

suppression (1), root growth inhibition (5) and suppression of early growth (6). It is possible that the detected coumarins may have contributed to the overall phytotoxicity of shepherd's purse extract. One exciting detected compound was 2-methylphenyl isocyanate, which is related to methyl isocyanate (a compound used to manufacture carbamate pesticides) (9). This compound is also related to isothiocyanates which are pesticidal. Thiocyanic acid and sinigrin (a glucosinolate) are also present in shepherd's purse (7). Sinigrin is phytotoxic to cress and wheat (29) and thiocyanic acid is related to isothiocyanates which have herbicidal properties (3). Two bioactive compounds, garbanzol and luteolin, are present in shepherd's purse (14), but these were not detected in this study.

Other compounds tentatively identified in this study were: 2-pyrrolidinone**,** pantoyl lactone (4,5-dihydro-3-hydroxy-4,4-dimethyl-2-furanone) and 5,6,7,7a-tetrahydro-4,4,7a,trimethyl-2(4H)-benzofuranone, but these have not been previously reported from plants. Compounds similar to the latter, a tetrahydrobenzofuranone, has been isolated from a marine cyanobacterium (*Kyrtuthrix maculans*) (18). It is possible that the shepherd's purse extract contains compounds similar to those listed. Equally plausible, GC/MS analysis may not be suitable for identification of certain compounds in the extract. Thus, although the aforementioned tentatively identified compounds had high matches with entries in the NIST 2005 database, they may not be present in Shepherd's purse extract.

Although application of Shepherd's purse crude extract provided 60 % control of ARG, a large volume was required to obtain the observed effects in field. This makes it too expensive for widespread use as weed control. However, further chemical analysis of bioactive fractions may determine which compounds are specifically responsible for the phytotoxic effect, to discover the novel herbicide chemistries for ARG control.

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REFERENCES

1. Abenavoli, M.R., Cacco, G., Sorgona, A., Marabottini, R., Paolacci, A.R., Ciaffi, M. and Badiani, M. (2006). The inhibitory effects of coumarin on the germination of durum wheat (*Triticum turgidum* Ssp *Durum*, Cv. Simeto) seeds. *Journal of Chemical Ecology* **32**: 489-506.
2. Broster, J.C. and Pratley, J.E. (2006). A decade of monitoring herbicide resistance in *Lolium rigidum* in Australia. *Australian Journal of Experimental Agriculture* **46**: 1151-1160.
3. Brown, P.D. and Morra, M.J. (1995). Glucosinolate-containing plant tissues as bioherbicides. *Journal of Agricultural and Food Chemistry* **43:** 3070-3074.
4. Calmasur, O, Aslan, I. and Sahin, F. (2006). Insecticidal and acaricidal effect of three Lamiaceae essential oils against *Tetranychus urtica* Koch and *Bemisia tabaci* Genn. *Industrial Crops and Products* **23**: 140-146.
5. Chon, S.U. and Kim, Y.M. (2004). Herbicidal potential and quantification of suspected allelochemicals from four grass crop extracts. *Journal of Agronomy and Crop Science* **190**: 145-150
6. Chon, S.U., Kim, Y.M. and Lee, J.C. (2003). Herbicidal potential and quantification of causative allelochemicals from several Compositae weeds. *Weed Research* **43**: 444-450.
7. Duke, J.A. (1992). *Phytochemical Constituents of GRAS Herbs and Other Economic Plants*. CRC Press New York. 654 pp.

8. Duke, S.O. and Dayan, F.E. (2005). Modes of action of phytotoxins from plants. In: *Allelopathy: A Physiological Process with Ecological Implications*, (Eds., M.J. Reigosa, N. Pedrol and L. González). pp 511-536. Kluwer Academic Publisher, Amsterdam, The Netherlands.
9. Environmental Protection Agency U.S.A. (1986). *Health and Environmental Effects Profile for Methyl Isocyanate*. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, Cincinnati, OH.
10. Fujii, Y., Shibuya, T. and Yasuda, T. (1990). Method for screening allelopathic activities using logistic function (Richards' function) fitted to lettuce seed germination and growth curves. *Weed Research,* Japan **35**: 353-361.
11. Grime, J.P. (1979), *Plant Strategies and Vegetation Processes*. John Wiley and Sons: Chichester, UK. 222 pp.
12. Haig, T., Pratley, J., An, M., Haig, T. and Hildebrand, S. (2005). Using allelopathy to search for new natural herbicides from plants. *Proceedings, 4th World Congress on Allelopathy,* Charles Sturt University, Wagga, Australia. 565-568 pp.
13. Haramoto, E.R. and Gallandt, E.R. (2004). Brassica cover cropping for weed management: A review. *Renewable Agriculture and Food Systems* **19**: 197-198.
14. Harborne, J.B., Baxter, H. and Moss, G.P. (1999). Phytochemical Dictionary: *A Handbook of Bioactive Compounds From Plants*. 2nd edition. Taylor and Francis, London. 976 pp.
15. Isman, M.B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review in Entomology* **51**: 45-66.
16. Laurent, D., Vilaseca, L.A., Chantraine, J.M., Ballivian, C., Saavedra, G. and Ibanez, R. (1997). Insecticidal activity of essential oils on *Triatoma inrestans*. *Phytotherapy Research* **11**: 285-290.
17. Lazzeri, L., Curto, G., Leoni, O. and Dallavalle, E. (2004). Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita* (Kofoid et White) Chitw. *Journal of Agricultural and Food Chemistry* **52**: 6703-6707.
18. Lee, S.C. and Brown, G.D. (1998). Tribenzylbutyrolactones and dibenzyldiphenyl-4,5,6,7-tetrahydrobenzofuranones from *Kyrtuthrix maculans*. *Journal of Natural Products* **61**: 29-33.
19. Lydon, J. and Duke, S.O. (1989). The potential of pesticides from plants In: *Herbs, Spices and Medicinal Plants: Recent Advances in Botany, Horticulture and Pharmacology*, (Eds., L.Craker, and J. Simon). Vol. **4**:1-41. Oryx Press, Phoenix,USA.
20. Mayton, H.S., Olivier, C., Vaughn, S.F. and Loria, R. (1996). Correlation of fungicidal activity of Brassica species with allyl isothiocyanate production in macerated leaf tissue. *Phytopathology* **86**: 267-271.
21. Norsworthy, J.K. and Meehan, J.T. (2005). Herbicidal activity of eight isothiocyanates on Texas panicum (*Panicum texanum*), large crabgrass (*Digitaria sanguinalis*), and sicklepod (*Senna obtusifolia*). *Weed Science* **53**: 515-520.
22. Olivier, C., Vaughn, S.F., Mizubuti, E.S.G. and Loria, R. (1999). Variation in allyl isothiocyanate production within Brassica species and correlation with fungicidal activity. *Journal of Chemical Ecology* **25**: 2687-2701.
23. Petersen, J., Belz, R., Walker, F. and Hurle, K. (2001). Weed suppression by release of isothiocyanates from turnip-rape mulch. *Agronomy Journal* **97**: 37-43.
24. Peterson, C.J., Tsao, R. and Coats, J.R. (1998). Glucosinolate aglucones and analogues: Insecticidal properties and a QSAR. *Pesticide Science* **54**: 35-42.
25. Preston, C., Roush, R.T. and Powles, S.B. (1999). Herbicide resistance in weeds of southern Australia: Why are we the worst in the world? *Proceedings, 12th Australian Weeds Conference*. (Eds., A.C. Bishop, M. Boersma and C.D. Barnes). pp. 454-459. Tasmanian Weed Society, Hobart, Australia.
26. Regnault-Roger, C. (1997). The potential of botanical essential oils for insect pest control. *Integrated Pest Management Reviews* **2**: 25-34.
27. Tawaha, A.M. and Turk, M A. (2003). Allelopathic effects of black mustard (*Brassica nigra*) on germination and growth of wild barley (*Hordeum spontaneum*). *Journal of Agronomy and Crop Science* **189**: 298-303.
28. Weston, L.A., Burke, B.A. and Putnam, A.R. (1987). Isolation, characterization and activity of phytotoxic compounds from quackgrass [*Agropyron repens* (L.) Beauv.]. *Journal of Chemical Ecology* **13**: 403-421.
29. Vaughn, S.F. and Berhow, M.A. (1999). Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). *Journal of Chemical Ecology* **25**: 2495-2504.
30. Zomlefer, W.B. (1994). *Guide to Flowering Plant Families*. Chapel Hill: The University of North Carolina Press. 430 pp.