CHAPTER 3

Influence of *Avena sativa*, *Secale cereale* and three cultivars of *Lolium multiflorum* on *Zea mays* and *Cyperus esculentus* growth under controlled conditions

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1. INTRODUCTION

Winter cover crops play an important role in sustainable agriculture through their ability to improve soil conditions, reduce soil erosion, and suppress weed growth. Weed suppression by cover crops is achieved through modification of environmental factors and the release of allelochemicals by allelopathic plants (Teasdale *et al*., 2007). Selection of a particular cover crop species depends on the purpose for which it will be used. Different cultivars of the same cover crop differ, not only in terms of their general weed suppression abilities but also through the reduction of growth of specific weed species as well (Bordelon & Weller 1997; Vasilakoglou *et al.,* 2006).

Stooling rye (*Secale cereale*) had been considered for weed suppression because of its biomass production and apparent allelopathic potential. Stooling rye reduced weed emergence by 43–100%, depending on the weed species (Shilling *et al*., 1995). Different allelochemicals have been identified in stooling rye, including phenolic acids (Wójcik-Wojtkowiak *et al*., 1990) and benzoxazolinones (Nair *et al*., 1990). Chon & Kim (2004) reported that weed suppression by oats (*Avena sativa*) is a possibility, identifying phenolics and benzoxazolinones as inhibitors of growth. Although annual ryegrass is acknowledged as a good cover crop with regards to weed suppression (Weston 1990), limited information is available on its possible allelopathic effect.

Limitations on a standardized methodology for allelopathy research and inconclusive reports impede research efforts and information on the phenomenon. Various studies that were done in laboratories and greenhouses reported on the alleged allelopathic effect of plants without considering the influence of the growth medium and abiotic and biotic stress factors (Foy & Inderjit 2001). These influences may be lost or modified in a controlled environment. The studies therefore only indicated the possibility of the phenomenon existing but did not prove that allelopathy is operational (Inderjit & Weston 2000). However, laboratory and greenhouse studies can generate meaningful data to understand plant behaviour that may be the result of allelopathic interactions (Inderjit & Weston 2000).

Anecdotal evidence of poor crop establishment in different annual ryegrass cultivar residues and weed suppression by oats (*Avena sativa* L.) emanating from the local farming community, plus previous research done on the suppression abilities of different cover crop species (Norsworthy *et al.,* 2007) and cultivars (Reberg-Horton *et al.,* 2009) prompted the inclusion of oats and two additional annual ryegrass cultivars to address the ability of different cover crop species and cultivars to suppress *Cyperus esculentus* (yellow nutsedge) growth and influence early maize (*Zea mays*) growth in a tunnel experiment.

2. MATERIALS AND METHODS

2.1 Experimental site

A pot experiment was carried out in 2009 in a temperature controlled plastic tunnel at the Cedara Research Station of the KwaZulu-Natal Department of Agriculture and Environmental Affairs, South Africa (latitude 29°32'S; longitude 30°16'E; altitude 1051 m). The temperature during the day (06:00–18:00) was set not to exceed 25°C, while no adjustments were made to the night-time temperature. Plastic pots (195 mm diameter, 200 mm in height) were filled with four kilograms of Umgeni sand consisting of 4.95% clay (<0.002 mm), 3.29% silt (0.002–0.05 mm) and 91.76% sand (0.05–2.00 mm). Before planting the cover crops, the sand was washed with tap water until clean water drained out of the base. Nitrogen (350 kg ha^{-1}), phosphorus (95 kg ha^{-1}) and potassium (250 kg ha^{-1}) were applied as solid NPK 2:3:4 (30%) fertilizer with 0.5% added zinc according to recommendations for annual ryegrass establishment. The balance of nitrogen (286 kg ha^{-1}) was applied at planting as limestone ammonium nitrate (LAN) (28%) and potassium (123 kg ha^{-1}) as potassium chloride (KCl) (50%). During the growth period, water and plant samples were collected for nutrient analysis. Water draining out of the cover crop pots (= leachate) was collected in pots lined with a clear plastic bag which was then used to water the cover crops again. The nutrient solution was therefore recirculated in order to minimize nutrient variation and putative allelochemicals exuded through the roots.

2.2 Treatments

Three cover species, stooling rye cultivar 'Agri Blue', oats cultivar 'Heros' and annual ryegrass cultivars 'Agriton', 'Midmar' and 'Sophia', were planted on 11 May 2009 at 100, 70 and 30 kg ha^{-1} respectively in the pots and covered with a thin layer of sand. No treatments were applied to the cover crops during their growth period. Cover crop leaf growth was cut 100 mm above the soil surface at seven and 15 weeks after emergence (WAE). Twenty one weeks after emergence, the cover crops were killed by spraying glyphosate-isopropylamine (Roundup Turbo SL, 450 g a.i. L^{-1}, Monsanto) at a rate of 2160 g a.i. ha^{-1}, using a flat fan nozzle (Teejet XR 8002VS) at 2 kPa.

Four cover crop treatments were included in the experimental design and instituted two weeks after the cover cops were sprayed with glyphosate-isopropylamine. Treatment one (= leaf+root) consisted of dead cover crop material being left intact in the pots while in the second treatment (= roots) the cover crop leaf material was cut at the soil surface and removed, leaving the roots intact. The leaf material was weighed to obtain samples that equated to dry matter yields equivalent to 5 t ha^{-1} for stooling rye and annual ryegrass, and 4 t ha^{-1} for oats. Pots filled with previously unused sand, treated in the same manner as described for establishing the cover crops, were used in treatment three and four. For treatment three (= leaf material) the weighed leaf material was placed on top of the sand in pots while for treatment four (= soaked leaf material) the leaf material was soaked overnight (24 hours) in tap water. It was then rinsed twice with tap water before being placed on top of the sand. The control was treated in the same manner as for planting cover crops except they were not established (Figure 1).

FIGURE 7 Different cover crop residue treatments into which maize and *C. esculentus* were planted, where (A) represents the control, (B) the soaked or unsoaked leaf material and (C) the root material

Ten maize seeds (Pioneer Seeds PHB 32D99) and ten *C. esculentus* tubers were planted separately into the four treatments to a depth of 50 mm on 29 and 30 October 2009, respectively. In treatments one and two, the soil was loosened before planting the seeds and tubers by wriggling a solid plastic tube that was inserted to the required depth into the soil. Afterwards, all the pots received 500 ml tap water. This was done only at planting. Water draining out of the pots was collected in pots lined with a clear plastic bag which was then used to water the maize and *C. esculentus* plants again once a week. The nutrient solution was therefore recirculated.

Soil temperature during the emergence phase for the maize and *C. esculentus* was measured with a type T thermocouple (copper and constantan) inserted into treatments one, two, three and the control. Treatments three and four were relatively similar as the material in treatment three became soaked after the pots were watered. Therefore, only treatment three was included. Data was recorded for six days with a Campbell Scientific Inc. CRX10® datalogger.

2.3 Data collection

Maize and *C. esculentus* were considered to have emerged when the seedlings protruded 20 mm above the soil surface. The date of final emergence was the last day emergence was measured and expressed as the percentage of seeds planted. Maize height and stem diameter measurements were taken 21 DAE (days after emergence). Height was taken from the soil surface to the ligule of the last fully expanded leaf, and stem diameter just above the soil surface. At the same time, the number of leaves was recorded by counting only fully expanded leaves where the ligule was visible. The foliage (stem and leaves) of maize and *C. esculentus* plants was sampled and oven-dried at 70°C for 48 hours to determine the dry weight. The watering solution and leaves were analysed for nutrient content after harvesting.

2.4 Statistical analysis

The pots were placed on movable trolleys which once a week were pushed to a different location in the tunnel (Figure 2). Treatments were replicated 10 times in a randomized block design, with each trolley representing a block. Data for emergence, maize height, stem diameter and dry weight were analysed using the analysis of variance (ANOVA) procedure in the statistical package Genstat (Payne *et al.*, 2007). Treatment means were compared using Fisher's Least Significant Difference procedure P=0.05.

FIGURE 8 Oats, annual ryegrass and stooling rye at six weeks after being planted into pots and placed on movable trolleys inside a plastic tunnel

2.5 Chemical analysis

Chemical analysis of the leachate collected from the root treatment of the three annual ryegrass cultivars and oats was carried out by an independent laboratory Biochemical and Scientific Consultants cc[2]. A decision was made that in the light of unforeseen financial restrictions, chemical analysis would be done only on the leachate collected from the root treatment (Treatment 2) of the three annual ryegrass cultivars and oats. Analyses on three phenolic acids, vanillic, ferulic and hydroxybenzoic acids as well as the benzoic acid benzoxazolin-2(3*H*)-one (BOA) were performed. The leachate from the root treatments were collected in the dark, 10 days after planting maize and *C. esculentus* and kept in the dark at 3°C until analysis was done.

[2] Biochemical and Scientific Consultants cc, P.O. Box 469, Hilton, 3245, South Africa, drsandybye@mweb.co.za. Tel: 033 343 1414, Fax: 033 343 1478

The qualitative analysis of the phenolic acid content was performed by means of a Waters Module 1 HPLC with UV/Vis detector, detection wavelength 220 nm, autosampler and Clarity software. Vanillic acid, supplied by Fluka Chemicals, ferulic acid and 4-hydroxybenzoic acid, both supplied by Sigma-Aldrich Chemicals, were used as standards. 11.4 mg vanillic acid, 17.6 mg ferulic acid and 16.4 mg hydoxybenzoic acid were accurately weighed out into a 50 ml volumetric flasks and each dissolved in 10ml mobile phase by ultrasonicating for five minutes. These were then made up to volume with the mobile phase which consisted of 800 ml ultra pure water, 200 ml acetonitrile and 0.25 ml trifluoroacetic acid. The standard solutions were filtered through 0.45 µm filter paper and 5 µl of each standard solution was injected separately and in duplicate. The annual ryegrass cultivar 'Midmar' solution was filtered and 20 µl was injected in duplicate with 50 µl of filtered solutions of the annual ryegrass cultivars 'Agriton' and 'Sophia' and oats. Comparisons were made by a Novelab C_{18} column (4.6 x 150 mm, 5 µm) at 0.5 ml min^{-1} flow.

The BOA analysis was performed according to the method of (Chiapusio *et al.*, 2004). Qualitative analysis was carried out by a Waters Module 1 HPLC with UV/Vis detector, detection wavelength 270 nm, autosampler and Millenium software. A 4.6 x 150 mm, 5 µm column filled with Novelab C_{18}, with 0.5 ml min^{-1} flow rate was used for the procedure; 12.5 mg BOA standard (Sigma-Aldrich Chemicals) was accurately weighed into a 100 ml volumetric flask and dissolved in 10 ml mobile phase by ultrasonicating for 5 minutes. This was then made up to volume with the mobile phase which consisted 800 ml ultra pure water, 195 ml acetonitrile and 5 ml glacial acetic acid. The mobile phase was filtered through a glass filter and ultrasonicated for 20 minutes to de-gas. The standard solution was filtered through 0.45 µm filter paper and 5 µl was injected in duplicate. Each of the annual ryegrass cultivars test solutions was filtered and 200 µl injected in duplicate while 150 µl was used for the oats solution.

The differences in injection volumes between the test samples in both chemical analyses were due to the fact that there were large peaks that were eluted before the peaks of interest in these particular samples. These large peaks were interfering with the peaks of interest at the higher injection volumes, but the chromatogram showed better resolution at the lower injection volumes displaying more accurate results.

3. RESULTS

3.1 Nutrient analysis

3.1.1 Cover crop growth period

Nutrient analyses of the cover crop leaf material and leachate collected during the 21 week growth period are given in Tables 1 and 2. Due to the low sulphur content measured at seven WAE (weeks after emergence), an adjustment was made by the addition of ammonium sulphate. At 15 WAE, the nitrogen, phosphorus and potassium content were lower than at seven WAE but the analysis indicated that the nutrient content was still adequate for cover crop and subsequent maize growth.

TABLE 14 Nutrient content of oats, stooling rye and three cultivars of annual ryegrass leaf material collected at seven and 15 weeks after emergence

	Nutrient content										
Treatment	**Ca**	**Mg**	**N**	**P**	**K**	**S**	**Na**	**Zn**	**Cu**	**Fe**	**Al**
			(%)						(mg kg^{-1})		
						7 WAE[1]					
Oats	0.36	0.25	4.66	0.47	3.84	0.19	1005.0	46	20.6	224	131
Stooling rye	0.58	0.28	4.93	0.54	4.71	0.21	422.3	68	28.9	310	205
Annual ryegrass											
cv. 'Agriton'	0.49	0.31	5.48	0.51	5.25	0.19	644.8	36	13.4	139	71
cv. 'Midmar'	0.45	0.32	5.31	0.53	5.79	0.21	884.9	26	11.0	360	58
cv. 'Sophia'	0.49	0.33	4.91	0.45	5.16	0.19	645.0	38	8.9	242	48
						15 WAE					
Oats	0.74	0.40	3.15	0.27	2.56	1.10	241.2	20	3.2	78	34
Stooling rye	0.40	0.41	3.33	0.24	2.54	1.40	885.1	20	3.2	93	32
Annual ryegrass											
cv. 'Agriton'	1.08	0.59	3.42	0.28	3.46	2.21	986.5	32	4.6	123	44
cv. 'Midmar'	1.03	0.57	3.86	0.26	3.96	2.08	1214.0	30	4.2	121	45
cv. 'Sophia'	0.86	0.47	3.60	0.28	3.41	1.88	790.4	30	4.7	116	32

[1] WAE denotes weeks after emergence

Leachate analysis at seven WAE indicated high chloride content possibly due to the build-up of chloride in the pots (Table 2). More water was given to the cover crops from seven WAE because of increased growth which contributed to leaching of chloride from the system. Although nutrient content was lower at 15 WAE, it was still sufficient for maize growth (Thibaud, personal communication).[3]

[3] G. R. Thibaud, DAEARD, Private Bag X9059, PMB, 3200

TABLE 15 Nutrient content of the water solution (=leachate) collected from pots in which oats, stooling rye and annual ryegrass grew at seven and 15 weeks after emergence

	Cations				Anions		EC	pH	SAR
	Na	Ca	Mg	K	Alkalinity	Cl			
Treatment		(me L^{-1})			(me L^{-1})		(mS m^{-1})	(KCl)	
					7 WAE[1]				
Oats	1.17	5.05	3.07	0.13	0.96	9.5	111.1	6.42	0.58
Stooling rye	1.27	11.39	7.91	0.13	1.75	33.8	363.4	6.30	0.41
Annual ryegrass	5.09	15.25	8.23	0.7	1.02	20.8	234	6.39	1.49
					15 WAE				
Oats	0.60	2.04	0.45	0.02	0.74	0.9	31.05	6.15	0.05
Stooling rye	0.18	2.47	0.79	0.04	0.64	1.5	42.6	6.13	0.14
Annual ryegrass	0.06	1.98	0.67	0.02	0.46	0.8	33.37	5.72	0.05

[1] WAE denotes weeks after emergence

3.1.2 Maize and C. esculentus growth period

Leaf and water analyses were carried out 21 days after maize and *C. esculentus* emergence and the results appear in Tables 3 and 4. Leaf analysis of the maize seedlings growing in the different cover crop residue treatments indicated that no nutrient deficiencies occurred that could have a negative impact on maize growth (Buys 1991; James 2009). Leaf analysis was not done on *C. esculentus* as no benchmark is available for comparison. Analysis of the leachate showed that adequate nutrients were available for growth (Thibaud, personal communication)[4] despite the generally low values in the root treatment.

[4] G.R. Thibaud, DAEARD, Private Bag X9059, PMB, 3200

TABLE 16 Leaf nutrient content of maize seedlings growing in different cover crop residue treatments 21 days after emergence

	Nutrient content										
Treatment	**Ca**	**Mg**	**N**	**P**	**K**	**S**	**Na**	**Zn**	**Cu**	**Fe**	**Mn**
	(%)						**(mg kg^{-1})**				
Control	0.44	0.27	3.84	0.41	5.84	0.27	1002.6	53	5.6	432	110
Leaf+root											
Oats	0.71	0.46	3.44	0.37	3.83	0.45	606.7	33	4.1	235	272
Stooling rye	0.68	0.52	3.97	0.48	4.89	0.91	716.0	36	3.8	174	329
Annual ryegrass											
cv. 'Agriton'	0.46	0.36	3.32	0.34	4.45	0.53	795.9	28	2.1	109	211
cv. 'Midmar'	0.60	0.47	3.91	0.49	4.76	0.61	594.1	36	3.5	201	339
cv. 'Sophia'	0.49	0.39	3.95	0.39	4.42	0.81	491.7	30	4.1	126	303
Roots											
Oats	1.00	0.61	4.65	0.33	2.21	0.92	480.6	30	5.2	174	284
Stooling rye	0.69	0.49	4.41	0.36	3.28	0.73	676.7	33	5.2	205	356
Annual ryegrass											
cv. 'Agriton'	0.66	0.44	4.15	0.37	3.79	0.62	234.4	32	4.0	153	475
cv. 'Midmar'	0.75	0.51	4.19	0.53	3.99	0.67	380.2	36	4.2	186	483
cv. 'Sophia'	0.68	0.47	4.29	0.30	3.28	0.76	323.4	27	3.5	182	499
Leaf material											
Oats	0.48	0.31	3.68	0.75	5.89	0.31	874.1	31	5.4	112	128
Stooling rye	0.41	0.26	3.85	0.75	6.13	0.30	957.0	42	5.2	109	117
Annual ryegrass											
cv. 'Agriton'	0.50	0.28	4.00	0.61	6.21	0.31	363.3	45	4.1	156	126
cv. 'Midmar'	0.62	0.31	4.28	1.06	6.50	0.38	679.5	55	5.9	151	164
cv. 'Sophia'	0.44	0.29	2.69	0.45	5.29	0.25	466.8	39	3.6	113	117
Soaked leaf material											
Oats	0.43	0.31	3.12	0.47	5.25	0.24	632.0	33	3.7	102	107
Stooling rye	0.40	0.29	3.02	0.40	4.97	0.24	657.0	23	4.1	110	105
Annual ryegrass											
cv. 'Agriton'	0.39	0.26	3.19	0.40	5.74	0.24	274.4	38	3.8	139	101
cv. 'Midmar'	0.46	0.30	3.61	0.58	5.85	0.28	664.6	33	6.0	177	122
cv. 'Sophia'	0.36	0.28	2.60	0.38	4.89	0.21	613.8	28	3.3	82	89

TABLE 17 Nutrient content of leachate collected from different cover crop residue treatments in which maize seedlings were growing in 21 days after emergence

Treatment	Na	Ca	Mg	K	Alkalinity	Cl	EC	pH
	Cations				Anions		EC (mS m^{-1})	pH (KCl)
				(me L^{-1})				
Control	2.8	5.7	4.2	2.8	0.6	11.8	219.7	6.7
Leaf+root								
Oats	1.1	8.7	3.7	0.3	0.2	5.5	181.7	4.1
Stooling rye	1.4	7.3	3.4	0.8	0.3	3.1	139.6	5.6
Annual ryegrass								
cv. 'Agriton'	1.7	5.3	3.1	0.7	0.5	6.9	118.1	6.1
cv. 'Midmar'	2.3	9.6	5.5	0.8	0.4	6.5	221.4	5.2
cv. 'Sophia'	1.5	6.8	3.0	0.5	0.3	3.0	161.6	4.0
Roots								
Oats	1.1	6.4	2.6	0.1	0.2	1.0	133.4	3.6
Stooling rye	1.1	2.2	1.4	0.3	0.4	1.3	71.30	6.2
Annual ryegrass								
cv. 'Agriton'	1.6	2.6	1.5	0.3	0.5	1.0	75.9	5.8
cv. 'Midmar'	1.1	2.4	1.5	0.2	0.3	1.3	59.30	5.7
cv. 'Sophia'	1.2	2.8	1.7	0.2	0.2	1.1	84.60	3.9
Leaf material								
Oats	2.6	3.1	2.8	1.7	0.5	6.4	119.7	5.7
Stooling rye	2.4	4.7	3.8	1.0	0.4	7.8	163.9	6.2
Annual ryegrass								
cv. 'Agriton'	1.5	4.3	3.5	0.8	0.5	7.1	152.0	6.3
cv. 'Midmar'	2.6	6.9	6.5	1.5	0.4	11.5	253.3	5.8
cv. 'Sophia'	2.3	6.7	5.1	3.8	0.4	9.2	198.9	5.1
Soaked leaf material								
Oats	1.9	1.9	1.2	0.2	0.5	3.6	66.6	5.7
Stooling rye	1.1	3.4	2.9	1.8	1.0	8.4	139.4	6.4
Annual ryegrass								
cv. 'Agriton'	2.7	2.3	2.1	1.7	0.7	6.8	115.0	6.3
cv. 'Midmar'	1.9	2.9	2.5	2.0	0.7	5.4	109.2	6.3

cv. 'Sophia'	2.3	1.8	1.3	0.6	0.61	4.1	67.7	5.6

3.2 Soil temperature

Soil temperatures were measured for six days after maize and *C. esculentus* emergence and are shown in Table 5. Similar temperatures were measured in the leaf+root and leaf material treatments with residues placed on top of the soil surface. The higher ambient temperatures measured in the tunnels on 8 and 9 November 2009 could possibly be responsible for the higher soil temperatures recorded on 9 and 10 November 2009.

TABLE 18 Soil temperatures measured with a type T thermocouple during maize and *C. esculentus* emergence in the different cover crop residue treatments

	Soil temperature (°C)						
Treatment	**6 Nov**	**7 Nov**	**8 Nov**	**9 Nov**	**10 Nov**	**11 Nov**	**Mean**
Control	8.40	8.83	8.84	12.64	12.20	9.50	**10.07**
Leaf+root	8.49	9.32	8.32	13.40	12.49	9.94	**10.32**
Roots	7.88	8.44	8.09	12.43	11.68	9.30	**9.64**
Leaf material	8.56	9.35	8.49	13.07	12.58	10.16	**10.37**

3.3 Final emergence

The interaction between the cover crop residue type and species did not influence maize and *C. esculentus* emergence significantly, only the main effect of cover crop residue type (Appendix B, Tables 1 and 2). Maize seedling emergence in the leaf+root treatment was significantly inhibited compared to the control but not compared to the other residue treatments (Table 6). Final emergence percentages amongst the roots and two leaf material treatments were not significantly different. *C. esculentus* emergence was significantly inhibited in the leaf+root and roots treatments compared to the control and two leaf material treatments. No significant differences were measured amongst the two leaf material and control treatments.

TABLE 19 Influence of different cover crop residues on the final number of maize and *C. esculentus* seedlings that emerged five and seven days after planting (statistical analysis in Appendix B Tables 1 and 2)

	Maize	***Cyperus esculentus***
Treatment	**Final emergence (%)**	
Control	96.00 a	50.00 a
Leaf+root	88.45 b	9.60 c
Roots	94.41 ab	20.60 b
Leaf material	93.65 ab	39.00 a
Soaked leaf material	95.05 ab	47.40 a

	Maize	***C. esculentus***
	Treatment	
SED	2.92	5.46
LSD	7.45	10.76
CV. (%)	9.1	52.2

[1] Means followed by the same letter are not significantly different at $P \leq 0.05$

[2] Means are compared for test species separately

3.4 Growth parameters

3.4.1 Maize height growth

The interaction between the different cover crops species and the residue treatments was significant 21 DAE (Appendix B Table 3). Maize seedlings growing in the control treatment were significantly taller compared to the other treatments, except the soaked leaf material treatment (Table 7 and Figures 3–7). Height growth was less reduced in the leaf material treatment compared to the leaf+root and roots treatments. Maize seedlings growing in the root material of the different cover crops were the shortest while those in the soaked leaf material the tallest.

No significant maize height growth inhibition between the different annual ryegrass cultivars was observed in the leaf+root treatment. Height growth was significantly reduced by the root material of the ryegrass cultivar 'Midmar' compared to the other cover crop species in the same treatment. No significant height growth difference amongst the different residue treatments were measured in the soaked leaf material.

Maize seedlings growing in the leaf+root and roots treatments of oats and stooling rye had relatively similar heights but was significantly shorter compared to the two leaf material treatments of the same cover crop species. The height growth was relatively similar in the leaf+root and leaf material treatments of the annual ryegrass cultivars 'Agriton' and 'Midmar'. Both was significantly taller compared to maize in the roots treatment of the two same cover crops. The maize growing in the soaked leaf material was significantly taller compared to the other residue treatments of the same cover crop species.

TABLE 20 Influence of three cover crop species residues on maize height growth 21 days after emergence (statistical analysis in Appendix B, Table 3)

	Cover crop species				
	Oats	**Stooling rye**	**Annual ryegrass**		
			'Agriton'	**'Midmar'**	**'Sophia'**
Treatment	**Height growth (mm)**				
Control			103.64 a		
Leaf+root	40.34 fg	42.34 efg	53.09 de	44.73 ef	48.39 def
Roots	43.85 efg	41.23 fg	33.57 g	28.05 h	46.80 def
Leaf material	73.86 b	65.26 bc	46.74 ef	41.79 fg	57.82 cd
Soaked leaf material	99.13 a	101.25 a	97.58 a	93.73 a	100.86 a

	Species*Treatment
SED	5.8
LSD	11.01
CV. (%)	20.1

[1] Means followed by the same letter are not significantly different at $P \leq 0.05$

3.4.2 Maize diameter growth

The interaction amongst the different cover crops species and the residue treatments was significant 21 DAE (Appendix B Table 4). The influence of the residue treatments on height growth did not always translate into the same effect on diameter growth (Table 8). The maize plants growing in the soaked leaf and leaf material had relatively similar diameters compared to the control while significantly smaller diameters were measured in the leaf+root and roots treatments.

The stem diameter of maize seedlings was the widest in leaf+root and roots treatments of the annual ryegrass 'Midmar' compared to the other cover crops in the same treatments. Variation in diameter growth was observed in the leaf material treatment with significant smaller diameters in the annual ryegrass cultivars 'Agriton' and 'Midmar' compared to the other cover crop residues. In the soaked leaf material treatment, maize seedlings growing through the oats and annual ryegrass cultivar 'Sophia' residues had significantly wider diameters compared to the other cover crop species as well as the control. Diameters were similar in the soaked leaf and leaf material treatments of oats and stooling rye. In all the annual ryegrass cultivars maize seedlings in the soaked leaf material had significantly wider diameters compared to the leaf material treatment.

TABLE 21 Influence of three cover crop species residues on maize diameter growth 21 day after emergence (statistical analysis in Appendix B, Table 4)

	Cover crop species				
	Oats	Stooling rye	Annual ryegrass		
			'Agriton'	'Midmar'	'Sophia'
Treatment	Diameter growth (mm)				
Control			0.68 c		
Leaf+root	0.24 g	0.25 g	0.27 fg	0.34 ef	0.25 g
Roots	0.29 efg	0.26 g	0.26 g	0.36 e	0.31 efg
Leaf material	0.78 a	0.64 c	0.52 d	0.49 d	0.66 c
Soaked leaf material	0.77 a	0.67 c	0.69 c	0.64 c	0.76 ab

	Species*Treatment
SED	0.0386
LSD	0.761
CV. (%)	17.9

[1] Means followed by the same letter are not significantly different at $P \leq 0.05$

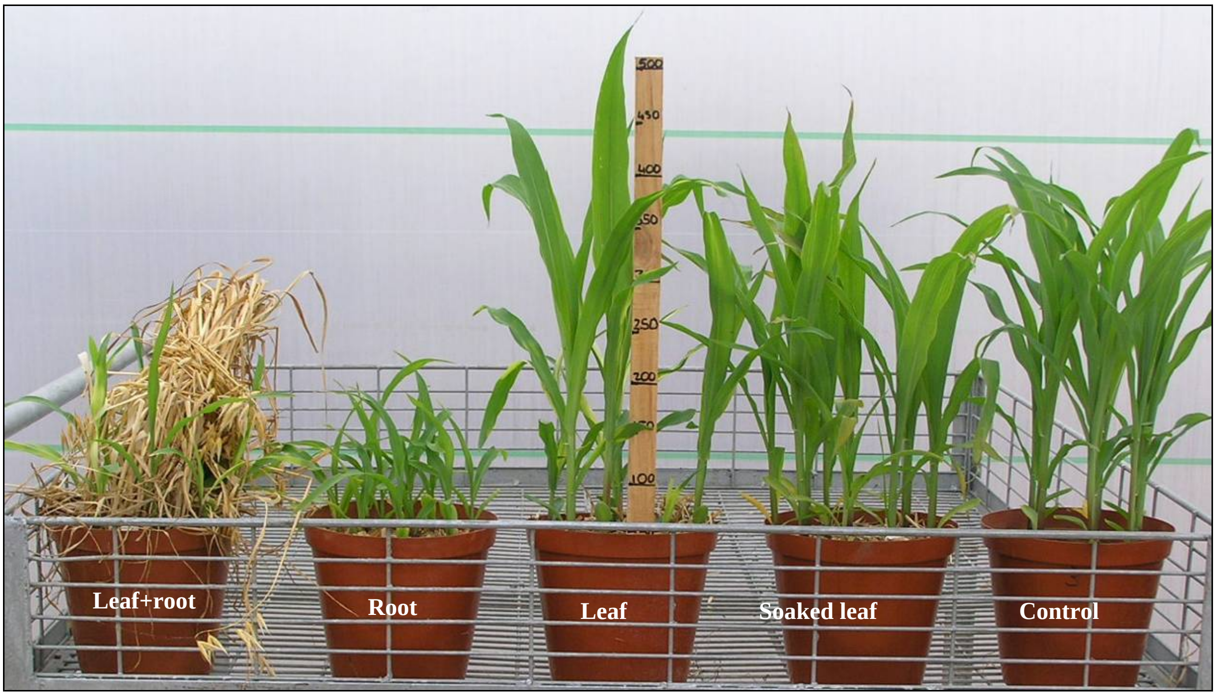

FIGURE 9 Influence of different oats residue treatments on maize growth 21 days after emergence

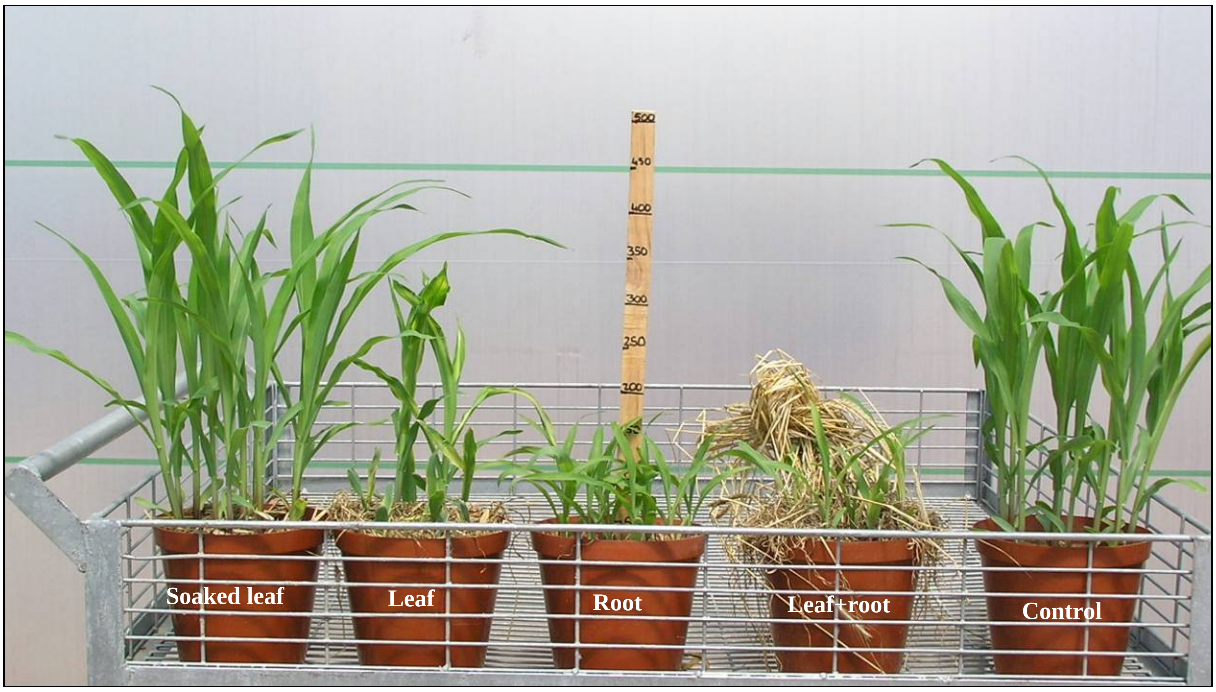

FIGURE 10 Influence of different stooling rye residue treatments on maize growth 21 days after emergence

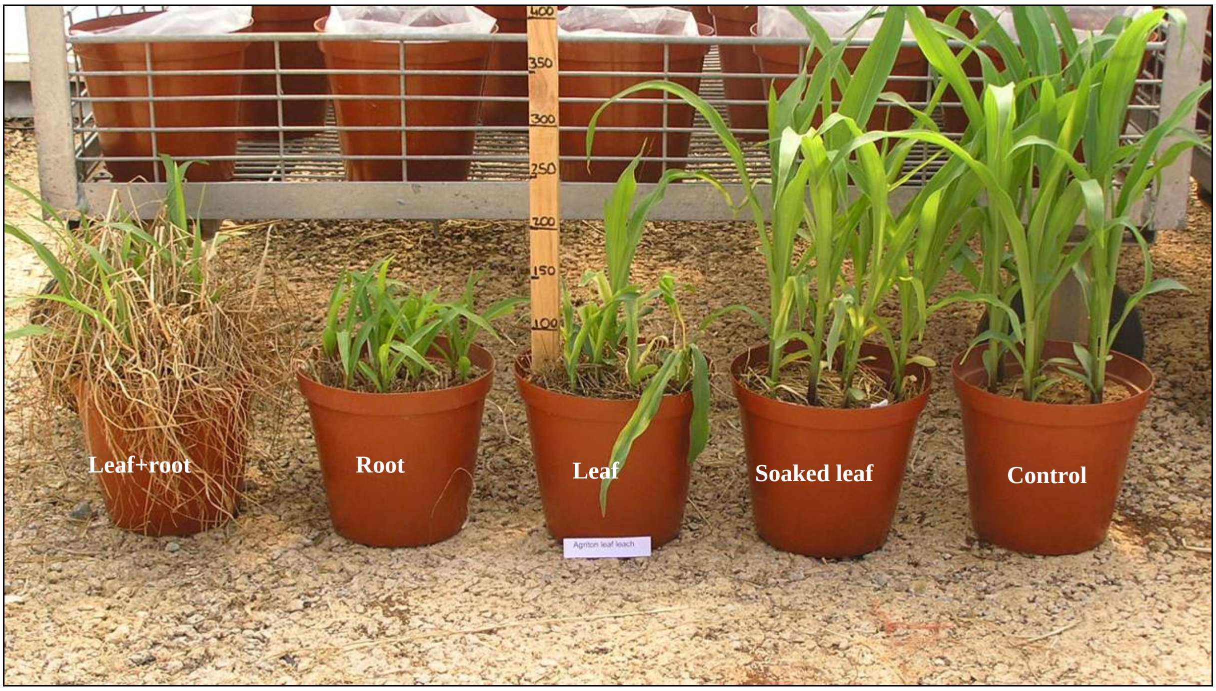

FIGURE 11 Influence of different annual ryegrass cv. 'Agriton' residue treatments on maize growth 21 days after emergence

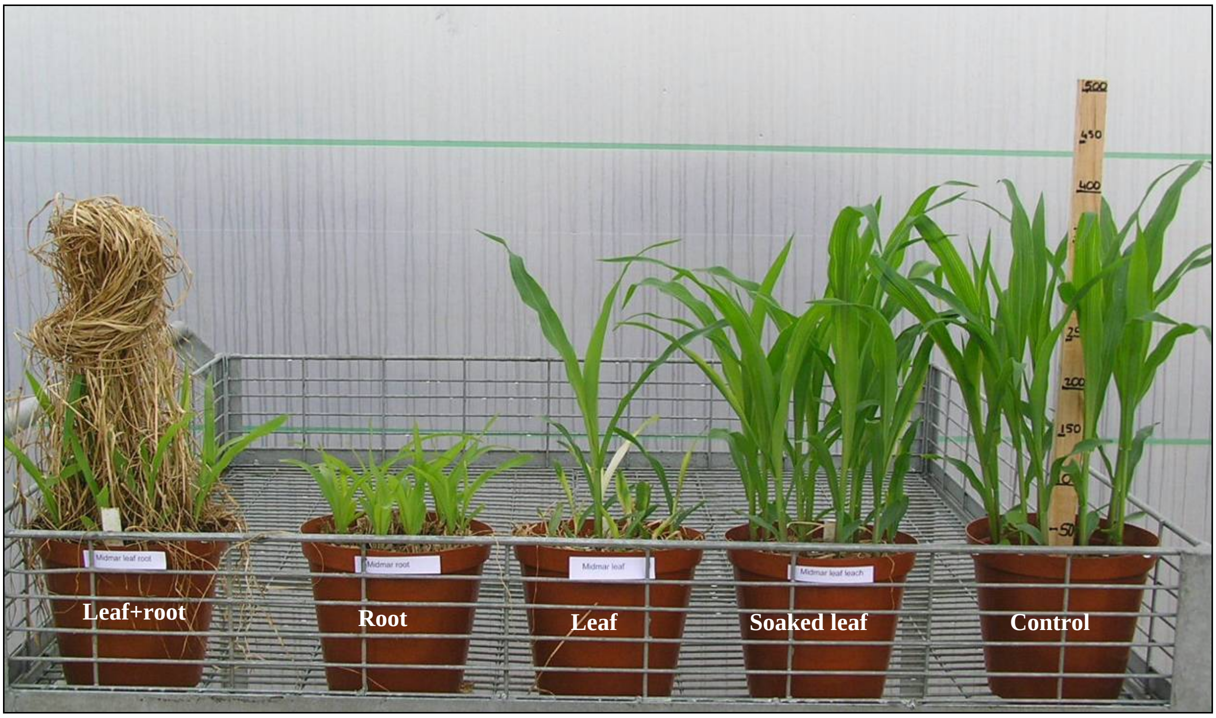

FIGURE 12 Influence of different annual ryegrass cv. 'Midmar' residue treatments on maize growth 21 days after emergence

FIGURE 13 Influence of different annual ryegrass cv. 'Sophia' residue treatments on maize growth 21 days after emergence

3.4.3 Number of fully expanded maize leaves

The interaction amongst the different cover crops species and the residue treatments was significant 21 DAE (Appendix B Table 5). Seedlings in the soaked leaf material and roots treatments had a relatively similar number of expanded leaves as the control (Table 9). Fewer leaves were counted in the leaf+root and leaf material treatments compared to the control.

Small variations in the number of leaves amongst the different cover crop species in each residue treatment occurred with the most variation amongst cover crop species in the leaf material treatment. Maize seedlings growing in the leaf+root residues of the annual ryegrass cultivar 'Agriton' had significantly more expanded leaves compared to the other cover crop species in the same treatment, except the annual ryegrass cultivar 'Sophia'. The root and leaf material of the ryegrass cultivar 'Midmar' suppressed maize leaf growth significantly more than the other cover crop species in the same two treatments. No significant differences occurred amongst the cover crop species in the soaked leaf material treatment.

The seedlings in the oats residue treatments had a similar number of expanded leaves, except in the leaf+root treatment where fewer leaves were counted. In both the stooling rye and annual ryegrass cultivar 'Sophia' the roots and soaked leaf material treatments had a relatively similar number of leaves and significantly more than the other two treatments. With regards to the annual ryegrass cultivars 'Agriton' and 'Midmar', significantly more leaves were counted in the soaked leaf material treatment compared to the roots treatment with both having significantly more expanded leaves than the leaf+root and leaf material treatments.

TABLE 22 Influence of three cover crop species residues on the number of fully expanded maize leaves 21 days after emergence (statistical analysis in Appendix B, Table 5)

	Cover crop species				
	Oats	Stooling rye	Annual ryegrass		
			'Agriton'	'Midmar'	'Sophia'
Treatment	Number of leaves				
Control			2.99 ab		
Leaf+root	1.70 hi	1.54 i	2.08 g	1.63 hi	1.89 gh
Roots	2.87 abc	2.79 abc	2.63 cde	2.14 fg	2.89 abc
Leaf material	2.70 bcd	2.45 de	2.06 g	1.73 hi	2.39 ef
Soaked leaf material	2.99 ab	3.01 a	3.01 a	3.00 a	3.06 a

	Species*Treatment
SED	0.145
LSD	0.286
CV. (%)	13.2

[1] Means followed by the same letter are not significantly different at $P \leq 0.05$

3.4.4 Dry weight of maize seedlings

The interaction between the cover crops species and the different residue treatments was significant 21 DAE (Appendix B Table 6). Growth, as measured by the dry weight of the seedlings, was severely inhibited by the leaf+root and roots treatments of all the cover crop species (Table 10). Growth in the soaked leaf material was generally significantly better compared to control and leaf material treatment. Although the leaf material suppressed growth compared to the soaked leaf material, it was relatively similar to the control.

No significant differences in growth were measured amongst the different cover crop species in the leaf+root and roots treatments. The leaf material of the annual ryegrass cultivars 'Agriton' and 'Midmar' were more suppressive towards maize growth than the other cover crop species in the same treatment. Growth was suppressed by the soaked leaf material of the ryegrass cultivar 'Midmar' when compared to the other cover crop species in the same treatment, but it was not significantly suppressive compared to the control.

Overall, seedlings grew better in the soaked leaf material of the cover crops followed by the leaf material and then the root and leaf+root material.

TABLE 23 Influence of three cover crop species residues on maize dry weight 21 days after emergence (statistical analysis in Appendix B, Table 6)

	Cover crop species				
	Oats	Stooling rye	Annual ryegrass		
			'Agriton'	'Midmar'	'Sophia'
Treatment	Dry weight per plant (g)				
Control			0.60 c		
Leaf+root	0.07 f	0.09 f	0.10 f	0.08 f	0.08 f
Roots	0.11 ef	0.13 ef	0.09 f	0.09 f	0.13 ef
Leaf material	0.62 c	0.53 c	0.28 d	0.22 de	0.56 c
Soaked leaf material	0.81 b	0.84 ab	0.86 ab	0.60 c	0.94 a

	Species*Treatment
SED	0.058
LSD	0.114
CV. (%)	34.6

[1] Means followed by the same letter are not significantly different at $P \leq 0.05$

3.4.5 Dry weight of C. esculentus seedlings

The type of cover crop species and residual treatment significantly influenced the dry weight of *C. esculentus* seedlings 21 DAE (Appendix B, Table 7). The dry weight of *C. esculentus* seedlings in the soaked leaf material and control treatments was relatively similar (Table 11 and Figures 8–12). *C. esculentus* growth was severely inhibited in the leaf+root and roots treatments while the dry weight in the leaf material treatment was less than the control.

No significant differences in dry weight were observed amongst the different cover crop species in the leaf+root and roots treatments. In the leaf material treatment, the three annual ryegrass cultivars significantly inhibited growth compared to the oats and stooling rye. Regardless of the overnight soaking of the leaf material of the ryegrass cultivar 'Midmar', *C. esculentus* growth was still significantly inhibited compared to the soaked leaf material of the other cover crops.

As was the case with maize, *C. esculentus* grew better in the soaked leaf material of the cover crops followed by the leaf material and then the root and leaf+root material.

TABLE 24 Influence of three cover crop species residue on *C. esculentus* dry weight 21 days after emergence (statistical analysis in Appendix B, Table 7)

	Cover crop species				
	Oats	**Stooling rye**	**Annual ryegrass**		
			'Agriton'	**'Midmar'**	**'Sophia'**
Treatment	**Dry weight per plant (g)**				
Control			0.22 cd		
Leaf+root	0.02 g	0.02 g	0.03 fg	0.01 g	0.02 g
Roots	0.03 fg	0.03 fg	0.02 g	0.02 g	0.03 fg
Leaf material	0.16 e	0.14 e	0.08 f	0.08 f	0.06 fg
Soaked leaf material	0.31 ab	0.26 bc	0.25 c	0.18 de	0.33 a

	Species*Treatment
SED	0.0286
LSD	0.0564
CV. (%)	58.3

[1] Means followed by the same letter are not significantly different at $P \leq 0.05$

FIGURE 14 Influence of different oats residue treatments on *C. esculentus* growth 21 days after emergence

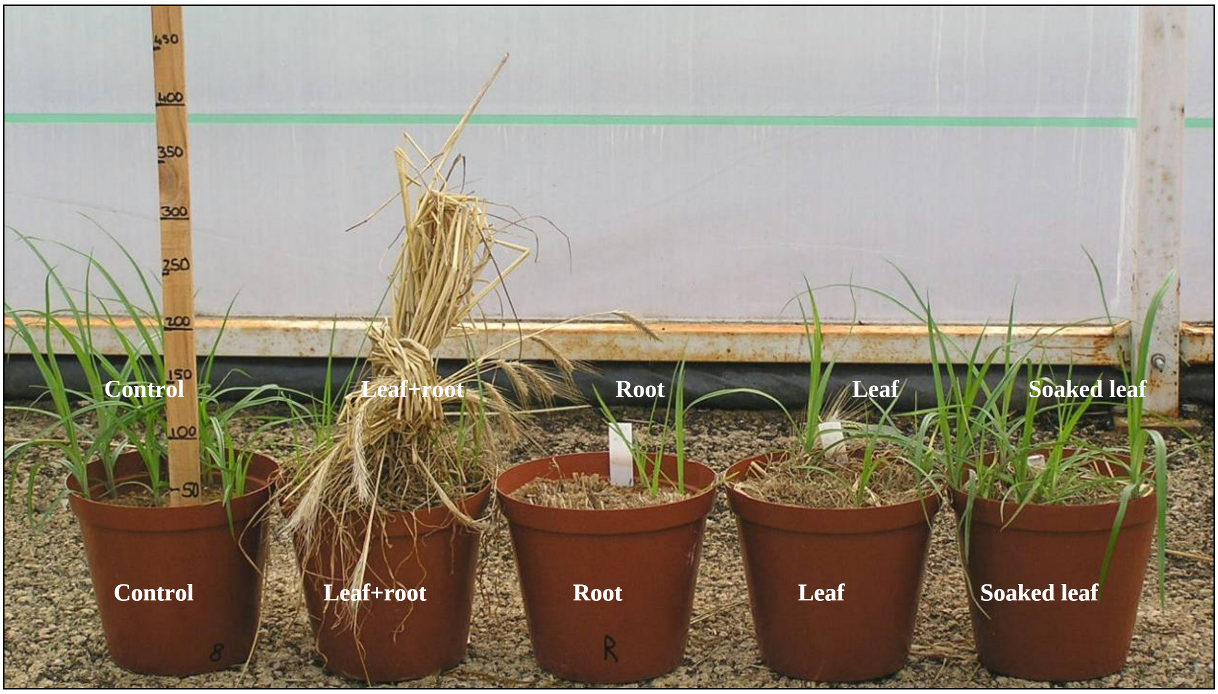

FIGURE 15 Influence of different stooling rye residue treatments on *C. esculentus* growth 21 days after emergence

FIGURE 16 Influence of different annual ryegrass cv. 'Agriton' residue treatments on *C. esculentus* growth 21 days after emergence

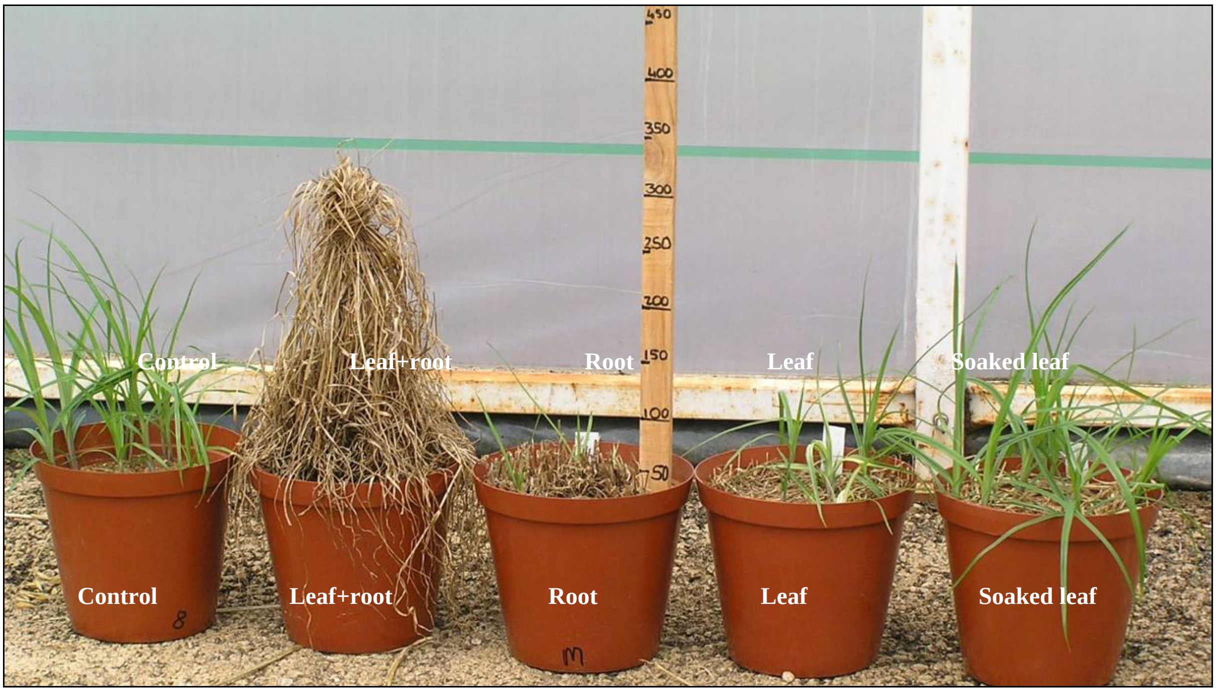

FIGURE 17 Influence of different annual ryegrass cv. 'Midmar' residue treatments on *C. esculentus* growth 21 days after emergence

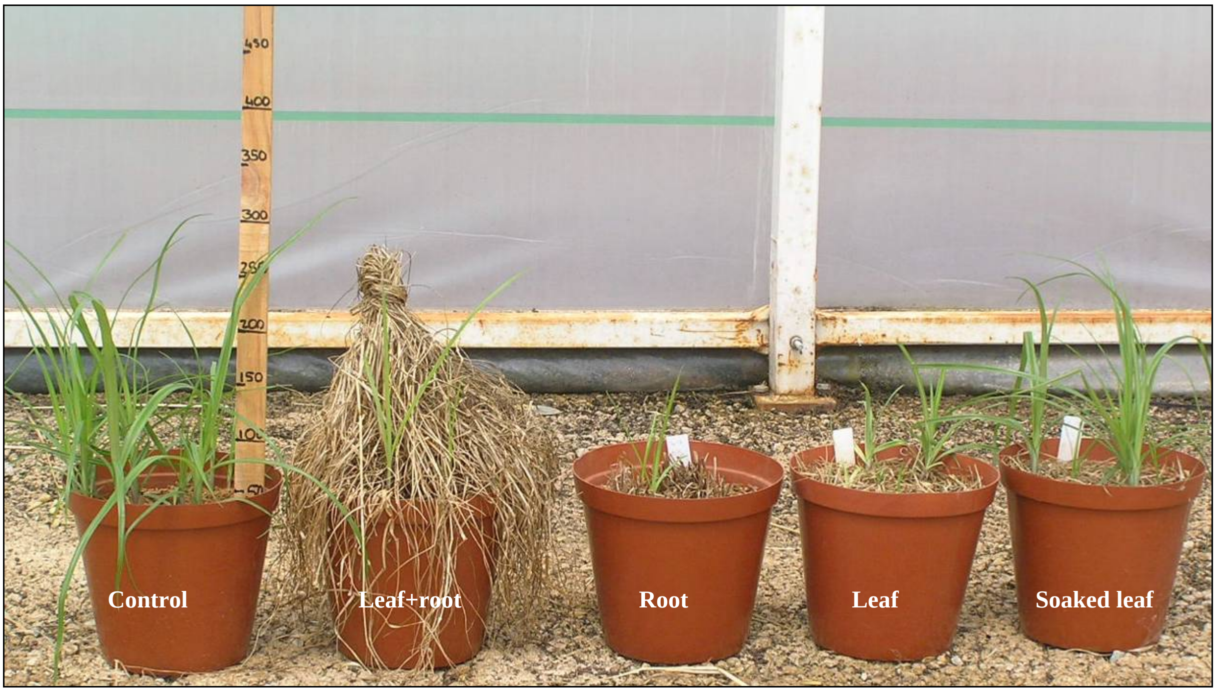

FIGURE 18 Influence of different annual ryegrass cv. 'Sophia' residue treatments on *C. esculentus* growth 21 days after emergence

3.5 Chemical analysis

Laboratory analysis indicated the presence of different concentrations of certain phenolic acids and benzoxazolin-2(3*H*)-one (BOA) in the leachate collected in the root treatment of the three annual ryegrass cultivars and oats (Table 12). The ryegrass cultivar 'Midmar' tested positive for three out of the four allelochemicals, followed by oats and the ryegrass 'Sophia' with two out of four. 'Midmar' had the highest concentrations of BOA and hydoxybenzoic acid compared to the other root treatments, and slightly less ferulic acid than oats. All three annual ryegrass cultivars exuded the allelochemical BOA through their roots with 'Midmar' and 'Sophia' containing hydroxybenzoic acid as well. The root leachate of oats contained ferulic acid and BOA. Vanillic acid was not detected in any of the root leachate of the cover crops tested.

TABLE 25 Concentrations of benzoxazolin-2(3*H*)-one (BOA) and three phenolic acids in the leachate collected from oats and annual ryegrass root material

	Phenolic acids			
	Vanillic acid	Ferulic acid	Hydroxybenzoic acid	BOA
		(ppb)		
Oats	0	16	0	7
Annual ryegrass				
cv. 'Agriton'	0	0	0	5
cv. 'Midmar'	0	14	440	20
cv. 'Sophia'	0	0	15	4

4. DISCUSSION

Stooling rye and annual ryegrass residues suppressed maize emergence and *C. esculentus* density in the field experiment. This is in agreement with Burgos and Talbert (1996), Reddy (2001) and Kravchenko and Thelen (2007) who found that residues of wheat (*Triticum aestivum*), stooling rye, oats and clovers (*Trifolium* spp.) suppressed sweet corn, soyabean (*Glycine max*) and maize emergence. The reduction was attributed to the creation of physical barrier, lower soil temperatures and allelopathy. However, results from the present study indicate that none of the above influenced maize emergence as no significant difference in emergence was observed amongst treatments, with and without residues. The conclusion that a physical barrier did not influence emergence in the field experiment, is therefore substantiated. There is a possibility that poor planting practices, such as insufficient seed coverage due to the amount of residue on the soil surface and planting by hand could have reduced maize emergence. Teasdale *et al.* (2008) reported reduced sweet corn emergence in stooling rye and vetch (*Vicia villosa*) residues but suggested that it was due to the planting procedure.

The differences observed in maize emergence in the field experiment were ascribed to possible fluctuations in soil temperature amongst the treatments. Teasdale and Mohler (1993) suggested that a delay in germination could be expected with lower soil temperatures under cover crop residues. Temperatures measured in the present study varied amongst the treatments, however, it had no influence on maize emergence and therefore the conclusion drawn from the field experiment is refuted. Also, the lower temperature in the roots and control treatments is in contrast to findings of Kravchenko and Thelen (2007) who measured higher temperatures in wheat root and no-cover treatments compared to wheat straw and roots placed on top of the soil surface.

Ormeño-Núñez *et al.* (2008) found that a stooling rye mulch of 5 t ha^{-1} inhibited *C. esculentus* emergence and subsequent growth and suggested that it was due to allelopathy and the formation of a physical constraint. However, significantly fewer *C. esculentus* tubers sprouted in treatments containing root residues, implicating that the leaf residue layer did not, as expected, restrict emergence. The possibility that low soil temperatures and tuber size influenced emergence is small as *C. esculentus* tuber sprouting is more dependent on favourable moisture conditions than temperature and tuber size does not influence emergence (Stoller *et al.*, 1972).

Seedling growth can be influenced by variations in soil moisture and temperature, nutrient deficiencies and the presence of putative allelochemicals. Nutrient analysis of leachate and maize leaves collected from the different treatments indicated that nutrients were present in adequate quantities. This finding supports the conclusion that growth suppression measured in the field experiment was not due to N immobilization.

The influence of the leaf material treatment on growth was confounded to some extent by possible glyphosate damage (Figure 13). Maize and *C. esculentus* leaves were injured as the seedlings grew through the cover crop leaf residues containing glyphosate-isopropylamine residues. For confirmation purposes, ten Roundup- Ready and PHB 32D99 maize seeds were planted in plastic pots filled with sand and replicated five times. Unwashed cover crop leaf material was placed on the soil surface and pots were watered with a nutrient solution.

Roundup-Ready maize seedlings growing through the residues had no signs of leaf chlorosis, while especially the younger leaves of the non-Roundup-Ready cultivar, showed signs of leaf chlorosis. Tesfamariam *et al.* (2009) found in a pot experiment that the dry weight of sunflower seedlings (*Helianthus annuus*) was reduced after being planted into rye residues that were sprayed with glyphosate. They attributed the damage to the bio-availability of glyphosate in the stooling rye residues to subsequent cultivated crops.

FIGURE 19 Glyphosate damage symptoms on non-Roundup-Ready maize seedlings (bottom) compared to Roundup-Ready seedlings (top) growing through cover crop residues containing glyphosate-isopropylamine residues

Results from the field experiment indicated that growth inhibition was primarily due to the cover crop species as the annual ryegrass cultivar 'Midmar' inhibited maize and *C. esculentus* growth more than stooling rye, with both cover crops being more suppressive than the weed residues. This observation was confirmed in the pot experiment. Annual ryegrass suppressed maize and *C. esculentus* growth the most followed by stooling rye and oats. Of the three annual ryegrass cultivars, 'Midmar' was the most suppressive. Reddy (2001) found that the total weed biomass was the lowest in ryegrass and the highest in stooling rye residues 10 weeks after soybean planting. Ryegrass also suppressed soyabean growth more than stooling rye. Similar results involving glucosinolate-producing cover crops and cultivars were reported by Norsworthy *et al.* (2007). *Digitaria sanguinalis* (crab finger-grass) growth was more reduced by Indian mustard [*Brassica juncea* (L.) Czer.] four weeks after bell-pepper (*Capsicum annuum*) transplanting than meadowfoam (*Limnanthes alba*), oilseed rape (*B. napus*) and brown mustard (*B. juncea* L.). They also highlighted the importance of cultivar selection as Indian mustard cultivar F-E75 resulted in greater *D. sanguinalis* control than Indian mustard cultivar F-L71.

In comparing the influence of separated cover crop root and leaf material, the root material of the different cover crop species caused more maize and *C. esculentus* growth inhibition than the leaf material. Differences in the extent of growth inhibitions by different plant parts have been reported previously with Barnes and Putnam (1986) indicating that rye shoots were the primary cause of growth inhibition but that root and shoot growth can act together in the field. Stone *et al.* (1998) pointed out that wheat growth was inhibited by both whole ryegrass plants and separated roots in comparison to interference from only leaves and stems and ryegrass interference with wheat primarily takes place below ground (Snaydon & Howe 1986). Breland (1996) concluded that the suppression of grain establishment after the incorporation of fresh annual ryegrass material was due to phytotoxic substances.

Growing the maize and *C. esculentus* in sand exposed the plants to higher concentrations of allelochemicals as soils with high organic matter and clay content generally retain allelochemicals more than sandy soil (Schmidt & Ley 1999). Allelochemicals are released from plant material through leaching, root exudation, decomposition and volatilization (Belz 2004). Chemical analysis of leachate collected from the roots treatment indicated the presence of known allelochemicals. The root material contained higher concentrations of allelochemicals and upon decomposition of the material the allelochemicals were leached from the material. By soaking the leaf material overnight in water, allelochemicals were leached out of the material as indicated by the reduction in growth suppression by the soaked leaf material treatment compared to the others.

It is possible that the lower nutrient content in the roots treatment did not reduce growth *per se* but by inducing stressful conditions, the presence of the allelochemicals could have exacerbated the suppression of growth. Higher levels of BOA and 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4H) (DIBOA) were measured in stooling rye grown under low to moderate fertility than under high fertility (Mwaja *et al.*, 1995). The present chemical analysis also indicated that the annual ryegrass cultivar 'Midmar' contained higher concentrations of allelochemicals compared to the other two cultivars.

Allelochemical content not only differs amongst cultivars, but also over time. The concentration of DIBOA and BOA in eight field-grown cultivars of stooling rye ranged from 137–1469 µg g^{-1} dry tissue (Burgos *et al.*, 1999). Reberg-Horton *et al.* (2009) reported different DIBOA concentrations in stooling rye, depending on the cultivar and harvest date. Lower concentrations were measured later in the season except the late maturing cultivar 'Wheeler', which retained higher DIBOA concentrations later in the seasons than the other cultivars. BOA is released from DIBOA (2,4-dihydroxy-1,4 benzoxazin-3 one) during decomposition of residues or through root exudation (Chiapusio *et al.*, 2004). Because 'Midmar' tested positive for BOA, it had to contain DIBOA as well. The growth inhibition was therefore due to the combined effect both allelochemicals as it is unlikely that growth inhibition is due to BOA alone with DIBOA being more allelopathic than BOA (Burgos & Talbert 2000). However, the influence of the other two known allelochemicals as well as the unknown compounds present, should not be disregarded as the allelopathic effect on plants is often the result of a combination of these compounds (Einhellig 1996; Inderjit & Nayyar 2002).

5. CONCLUSIONS

The results of this study indicate clearly that the different cover crop species, cultivars and residue type affected maize and *C. esculentus* growth differently as both *C. esculentus* emergence and growth were inhibited but only maize seedling growth was suppressed. This effect will have to be taken in consideration when planning a weed control strategy involving cover crops. The presence of allelochemicals was confirmed in the different cover crop species, but the concentration thereof differed amongst cultivars and species. Presumably, the allelochemical content will also differ amongst the different residue types as the degree of suppression was different. Inhibition of maize and *C. esculentus* in the field experiment was therefore primarily caused by the presence of these allelochemicals and the extent of the inhibition was increased in the field experiment as both root decomposition and leaching from the leaf material occurred.

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APPENDIX B Statistical analysis

TABLE 1 ANOVA for the final emergence percentage of maize in the different cover crop residues

Source of variation	DF	SS	MS	VR	F pr
Block stratum	9	1325.2	147.3	2.07	
Block.*Units* Sratum					
Control	1	92.10	92.10	1.29	0.257
Control.Species	4	611.62	152.91	2.15	0.077
Control. Ryegrass vs oats+rye	1	191.61	191.61	2.69	0.103
Control. 'Sophia' vs 'Agriton'.'Midmar'	1	399.22	399.22	5.60	0.019
Control.Treat	3	1361.30	453.77	6.37	<0.001
Control. leaf+root vs root	1	887.01	887.01	12.45	<0.001
Control.leaf+soaked	1	48.41	48.41	0.68	0.411
Control.Species.Treat	12	1127.82	93.98	1.32	0.211
Control. Ryegrass vs Oats+rye. leaf+root vs root	1	96.93	96.93	1.36	0.245
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+root vs root	1	0.69	0.69	0.01	0.922
Control. Ryegrass vs Oats+rye. leaf+soaked	1	156.47	156.47	2.20	0.140
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+soaked	1	84.11	84.11	1.18	0.279
Residual	175	12470.5			
TOTAL	**204**	**16924.9**			

TABLE 2 ANOVA for the final emergence percentage of *C. esculentus* in the different cover crop residues

Source of variation	DF	SS	MS	VR	F pr
Block stratum	9	4429.0	492.1	1.98	
Block.*Units* Sratum					
Control	1	4140.2	4140.2	16.69	<0.001
Control.Species	4	208.0	52..0	0.21	0.933
Control. Ryegrass vs oats+rye	1	200.1	200.1	0.81	0.370
Control. 'Sophia' vs 'Agriton'.'Midmar'	1	6.7	6.7	0.03	0.870
Control.Treat	3	44269.5	14756.5	59.50	<0.001
Control. leaf+root vs root	1	3025.0	3025.0	12.20	<0.001
Control.leaf+soaked	1	1764.0	1764.0	7.11	0.008
Control.Species.Treat	12	1208.0	100.7	0.41	0.960
Control. Ryegrass vs Oats+rye. leaf+root vs root	1	150.0	150.	0.60	0.438
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+root vs root	1	367.5	367.5	1.48	0.225
Control. Ryegrass vs Oats+rye. leaf+soaked	1	20.2	20.2	0.08	0.776
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+soaked	1	140.8	140.8	0.57	0.452
Residual	180	44641.0			
TOTAL	**209**	**98895.7**			

TABLE 3 ANOVA for the height growth of maize in the different cover crop residues

Source of variation	**DF**	**SS**	**MS**	**VR**	**F pr**
Block stratum	9	2190.2	243.4	1.56	
Block.*Units* Sratum					
Control	1	18117.6	18117.6	116.48	<0.001
Control.Species	4	4189.6	1047.4	6.73	<0.001
Control. Ryegrass vs oats+rye	1	1529.8	1529.8	9.83	0.002
Control. 'Sophia' vs 'Agriton'.'Midmar'	1	1953.4	1953.4	12.56	<0.001
Control.Treat	3	107383.8	35794	230.12	<0.001
Control. leaf+root vs root	1	1252.4	1252.4	8.05	0.005
Control.leaf+soaked	1	42883.1	42883.1	275.70	<0.001
Control.Species.Treat	12	6491.4	541.0	3.48	<0.001
Control. Ryegrass vs Oats+rye. leaf+root vs root	1	1143.0	1143.0	7.35	0.007
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+root vs root	1	907.7	907.7	5.84	0.017
Control. Ryegrass vs Oats+rye. leaf+soaked	1	1939.7	1939.7	12.47	<0.001
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+soaked	1	232.5	232.5	1.49	0.223
Residual	175	27220.3	155.5		
TOTAL	**204**	**162222.5**			

TABLE 4 ANOVA for the diameter growth of maize seedlings in the different cover crop residues

Source of variation	**DF**	**SS**	**MS**	**VR**	**F pr**
Block stratum	9	0.112	0.012	1.67	
Block.*Units* Sratum					
Control	1	0.418	0.418	56.17	<0.001
Control.Species	4	0.176	0.044	5.93	<0.001
Control. Ryegrass vs oats+rye	1	0.031	0.031	4.18	0.043
Control. 'Sophia' vs 'Agriton'.'Midmar'	1	0.058	0.058	7.82	0.006
Control.Treat	3	7.362	2.454	329.75	<0.001
Control. leaf+root vs root	1	0.016	0.016	2.12	0.147
Control.leaf+soaked	1	0.189	0.189	25.39	<0.001
Control.Species.Treat	12	0.613	0.051	6.87	<0.001
Control. Ryegrass vs Oats+rye. leaf+root vs root	1	0.00007	0.00007	0.01	0.924
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+root vs root	1	0.010	0.010	1.32	0.252
Control. Ryegrass vs Oats+rye. leaf+soaked	1	0.104	0.104	13.92	<0.001
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+soaked	1	0.010	0.010	1.33	0.251
Residual	175	1.302	0.007		
TOTAL	**204**	**9.651**			

TABLE 5 ANOVA for the number of fully expanded leaves of maize seedlings in the different cover crop residues

Source of variation	DF	SS	MS	VR	F pr
Block stratum	9	0.87	0.10	0.92	
Block.*Units* Sratum					
Control	1	3.01	3.01	28.66	<0.001
Control.Species	4	5.17	1.29	12.30	<0.001
Control. Ryegrass vs oats+rye	1	0.83	0.83	7.86	0.006
Control. 'Sophia' vs 'Agriton'.'Midmar'	1	1.96	1.96	18.68	<0.001
Control.Treat	3	43.04	14.35	136.56	<0.001
Control. leaf+root vs root	1	20.00	20.00	190.37	<0.001
Control.leaf+soaked	1	14.00	14.00	133.22	<0.001
Control.Species.Treat	12	6.27	0.52	4.97	<0.001
Control. Ryegrass vs Oats+rye. leaf+root vs root	1	1.66	1.66	15.84	<0.001
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+root vs root	1	0.72	0.72	6.87	0.010
Control. Ryegrass vs Oats+rye. leaf+soaked	1	1.73	1.73	16.42	<0.001
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+soaked	1	0.63	0.63	6.02	0.015
Residual	175	18.38	0.11		
TOTAL	**204**	**75.16**			

TABLE 6 ANOVA for the dry weight per plant of maize seedlings in the different cover crop residues

Source of variation	DF	SS	MS	VR	F pr
Block stratum	9	0.13	0.01	0.86	
Block.*Units* Sratum					
Control	1	0.52	0.52	31.38	<0.001
Control.Species	4	0.85	0.21	12.82	<0.001
Control. Ryegrass vs oats+rye	1	0.21	0.21	12.41	<0.001
Control. 'Sophia' vs 'Agriton'.'Midmar'	1	0.50	0.50	29.71	<0.001
Control.Treat	3	17.41	5.80	348.11	<0.001
Control. leaf+root vs root	1	0.01	0.01	0.80	0.373
Control.leaf+soaked	1	3.38	3.38	202.86	<0.001
Control.Species.Treat	12	1.07	0.90	5.38	<0.001
Control. Ryegrass vs Oats+rye. leaf+root vs root	1	0.002	0.002	0.16	0.687
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+root vs root	1	0.006	0.006	0.39	0.534
Control. Ryegrass vs Oats+rye. leaf+soaked	1	0.23	0.23	13.58	<0.001
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+soaked	1	0.03	0.03	2.06	0.453
Residual	175	2.90	0.02		
TOTAL	**204**	**22.26**			

TABLE 7 ANOVA for the dry weight per plant of *C. esculentus* seedlings in the different cover crop residues

Source of variation	**DF**	**SS**	**MS**	**VR**	**F pr**
Block stratum	9	0.06	0.007	1.62	
Block.*Units* Sratum					
Control	1	0.14	0.14	33.59	<0.001
Control.Species	4	0.08	0.02	4.66	0.001
Control. Ryegrass vs oats+rye	1	0.04	0.04	9.80	0.002
Control. 'Sophia' vs 'Agriton'.'Midmar'	1	0.02	0.02	5.17	0.024
Control.Treat	3	1.98	0.66	161.75	<0.001
Control. leaf+root vs root	1	0.001	0.001	0.45	0.503
Control.leaf+soaked	1	0.66	0.66	160.80	<0.001
Control.Species.Treat	12	0.14	0.01	2.87	0.001
Control. Ryegrass vs Oats+rye. leaf+root vs root	1	0.0002	0.0002	0.07	0.796
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+root vs root	1	0.0004	0.0004	0.11	0.744
Control. Ryegrass vs Oats+rye. leaf+soaked	1	0.01	0.01	2.73	0.100
Control. 'Sophia' vs 'Agriton'.'Midmar'. leaf+soaked	1	0.06	0.06	14.19	<0.001
Residual	173	0.71			
TOTAL	**202**	**3.03**			