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REGULAR ARTICLE

Cover crop residue management for optimizing weed control

H. Marjolein Kruidhof · Lammert Bastiaans · Martin J. Kropff

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Abstract Although residue management seems a key factor in residue-mediated weed suppression, very few studies have systematically compared the influence of different residue management strategies on the establishment of crop and weed species. We evaluated the effect of several methods of pre-treatment and placement of winter rye (*Secale cereale* L.) and winter oilseed rape (*Brassica napus* L.) residue on seedling emergence under field conditions. For both species two cultivars, differing in allelochemical content, were used. Residues incorporated in the upper soil layer exerted a large inhibitory effect on the establishment of the relatively early emerging lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea* L.) seedlings, whereas the inhibitory effect on the slightly later emerging *Stellaria media* L. seedlings was variable, and often a stimulatory effect on the very late emerging *Chenopodium album* L. seedlings was observed. Differences between cover crop cultivars were minor. For winter oilseed rape residue, pre-treatment strongly affected the time-course of residue-mediated effects. Finely ground residues were only inhibitory to seedling establishment during the first two to three weeks, whereas cut residues became inhibitory after this period. For winter rye, residue placement was most important. Residue incorporation gave variable results, whereas placement of winter rye residue on top of the soil inhibited the emergence of all receptor species. In conclusion, the optimal residue management strategy for weed suppression depends both on the cover crop species used and the target weed species.

Keywords Allelopathy · Cover crops · Crop residues · Mulch · Organic farming · Weed control

Introduction

In organic farming systems weed control is recognized as the foremost production-related problem and a major reason for conventional farmers not to convert to organic production (e.g. Kloen and Daniels 2000). Also in conventional farming sytems the use of herbidices is becoming more and more limited, due to changes in the regulatory environment. Simply replacing herbicides by other direct control measures is inadequate. Instead, weed management should be seen as a component of integrated crop management

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H. M. Kruidhof (✉) · L. Bastiaans · M. J. Kropff
Crop and Weed Ecology Group,
Department of Plant Sciences, Wageningen University,
P.O. Box 430, 6700 AK, Wageningen, Netherlands
e-mail: marjolein.kruidhof@gmail.com

H. M. Kruidhof
Plant Research International,
Wageningen University and Research Centre,
P.O. Box 16, 6700 AA, Wageningen, Netherlands

(e.g. Liebman and Davis 2000). Cover crops fit very well in such an integrated approach, as they provide many additional services to the agro-ecosystem, including improved soil quality, increased nutrient cycling and, in some cases, a contribution to pest management (Sarrantonio and Gallandt 2003). With respect to weeds, cover crop residues have been reported to negatively affect germination and establishment of weed seeds (Weston 1996; Ohno et al. 2000; e.g. Kruidhof et al. 2008b). Cover crops that contain a high level of allelochemicals seem well-suited for residue-mediated weed suppression. Of the cover crops that are suitable for temperate climates, both winter rye and winter oilseed rape contain allelochemicals, though of a completely different nature. Following enzymatic action of glucosidases upon tissue damage, winter rye forms hydroxamic acids from its glucoside precursor (4-hydroxy-1,4-benzoxazin-3-ones) (Barnes et al. 1987; Niemeyer 1988), while winter oilseed rape releases glucosinolate breakdown products, including isothiocyanates, oxazolidinethiones, ionic thiocyanate (SCN^-) and organic cyanides (Brown and Morra 1996; Haramoto and Gallandt 2004). Most of the breakdown products of glucosinolates are volatile, whereas hydroxamic acids are water-soluble. In the soil, hydroxamic acids can be transformed into more toxic compounds (Gagliardo and Chilton 1992; Fomsgaard et al. 2004).

In addition to allelopathic effects, crop residues can exert an effect on weed germination and establishment through other mechanisms. Release of nutrients from the residues can stimulate weed germination (e.g. Teasdale and Pillai 2005), whereas temporary immobilization of nutrients from the soil upon decomposition of high C/N residues can inhibit it (Stevenson 1986, in Liebman and Mohler 2001). Crop residues may also affect the physical properties of the soil. Residue-amended soil may for instance better conserve moisture (Liebl et al. 1992; Teasdale and Mohler 1993). Residues left on the soil surface can lead to decreased soil temperature fluctuations and reduced light penetration, which both have been shown to inhibit weed germination (Teasdale and Mohler 1993; Liebman and Mohler 2001). Furthermore, in some cases soil microbial populations, including soil pathogens, are either stimulated (Dabney et al. 1996; Conklin et al. 2002; Manici et al. 2004) or suppressed (Matthiessen and Kirkegaard 2006) after soil amendment with fresh residue material.

The level and the time course of allelochemical release and of other residue-mediated alterations in the soil are largely dependent on the amount and decomposability of the residue, on soil biological, chemical and physical characteristics and on environmental conditions (e.g. Cheng 1992; Liebman and Mohler 2001), but can also be influenced by residue management. Prior to incorporation into the soil, the residues can be pre-treated in several ways, establishing pieces of different sizes and resulting in different levels of cell disruption. These changes may influence the release rate of allelochemicals directly (Morra and Kirkegaard 2002), or indirectly through an alteration in the decomposition rate (e.g. Ambus and Jensen 1997; Angers and Recous 1997). After pre-treatment, several methods can be implemented to incorporate the residue material into the soil, a practice that is referred to as green manuring. Alternatively, the residue material can be retained on the soil surface as mulch through no-tillage or zone tillage techniques. Crop residues retained on the soil surface decompose more slowly than residues incorporated in the soil (e.g. Dou et al. 1995), which may result in a slower release rate of allelochemicals.

Although residue management is a key factor in residue-mediated weed suppression, very few studies have systematically compared the influence of different residue management methods on germination and establishment of crop and weed species. The main objective of this research was therefore to investigate the relation between cover crop residue management, particularly pre-treatment and placement of residue material, and weed suppression for two contrasting cover crop species. In this context we also (1) compared the emergence of four receptor species in relation to residue management, (2) investigated the time course of residue-mediated effects on seedling establishment for different residue treatments and (3) determined whether the effect of residue management was different for cultivars with a high and cultivars with a low allelochemical content. In relation to this, we hypothesized that (1) the effect of residue management strategy on seedling emergence is independent of receptor species, (2) residue-mediated inhibition of seedling emergence takes place earlier with increased level of tissue disruption, and (3) the allelochemical content of the cover crop becomes more important when residues are incorporated in the soil. The study was conducted under field conditions, to be able to measure the actual effect

of residue management, rather than the potential effect which could be measured in controlled environment conditions.

Materials and methods

General experimental set-up

Ring experiments were carried out in 2004–2005 (experiment 1 and 2) and 2005–2006 (experiment 3) at the 'Haarweg' of Wageningen University, the Netherlands. Rings with a diameter of 30 cm and a depth of 25 cm were buried in the soil, allowing the upper brim to protrude 1 cm above soil level. The rings were interspaced at 0.5 m distance and the upper 20 cm of the rings were filled with soil that originated from the organic experimental farm "Droevendaal" in Wageningen. The texture of the soil was highly sandy, consisting of 1% clay, 2% silt and 97% sand and had an organic matter content of 4.3% (2004–2005) or 3.2 % (2005–2006) and a pH-KCl of 5.2 (2004–2005) or 4.9 (2005–2006). The soil in the upper 10 cm of the rings had been sterilized with gamma rays (10 kGray) in order to kill all weed seeds two weeks before sowing of the cover crops in September. We assumed that the activity of soil organisms killed by the gamma rays was restored during the cover crop growth period (McNamara et al. 2007). To be able to distinguish between sterilised and non-sterilised soil in the rings, a 1 cm thick layer of silver sand was placed at a depth of 10 cm.

In all three experiments, seeds of winter rye (*Secale cereale* L.) (36 seeds ring^{-1}=127 seeds m^{-2}) and winter oilseed rape (*Brassica napus* L.) (12 seeds ring^{-1}= 42 seeds m^{-2}) were homogeneously distributed and sown at 2 cm depth on September 7. On average 82% of the winter oilseed rape seeds and 83% of the winter rye seeds were recovered as full grown plants the next spring. Fertilization in experiment 1 and 2 was applied in granules (NPK 5-12-24) at a rate of 560 kg ha^{-1} on October 14, 2004. In experiment 3, fertilizer granules with NPK 5-6-13 were applied at a rate of 400 kg ha^{-1} on February 22, 2006.

In spring, the above-ground material of the cover crops was cut at ground level and soil and roots were removed to a depth of 10 cm. Per block, the above-ground plant material was combined per cover crop cultivar, thoroughly mixed, and weighed to determine the average fresh above-ground biomass per ring. The same was done for the soil including the roots. The roots were removed from the soil by hand and were cut into pieces of approximately 1 cm length for experiment 1 and 2. For experiment 3 the roots were discarded. The soil, roots and the above-ground fresh material were divided into equal portions, the same number as there were rings, and for each portion the soil and roots were mixed again. The above-ground cover crop material was added to the rings according to treatment. Immediately after refilling the rings, seeds of two weed and two crop species were sown and newly emerged seedlings were regularly counted.

Experiment 1: effect of residue pre-treatment

In experiment 1, winter rye cv. "Protector" and winter oilseed rape cv. "Emerald" were grown. Plants were harvested, and the residue material pre-treated and incorporated in the soil on March 14 (block 1), March 15 (block 2), March 16 (block 3) and March 18 (block 4), 2006. The main focus of the experiment was on the effect of residue pre-treatment (Table 1). The above-ground cover crop material was cut into pieces of 3 cm length, and thereafter either left untouched, crushed or finely ground. Subsequently, the material was mixed into the upper 10 cm of the soil. A control treatment was included in which no cover crop above-ground material was added to the soil. The experimental design was a completely randomized split-plot with eight replications (2 replications per block). The main plots were assigned to individual rings and consisted of a combination of cover crop species and residue pre-treatment. Each ring was subdivided into four sub-plots of equal size, in each of which a different receptor species was sown. The amount of above-ground fresh biomass that was added to each ring depended on the amount of biomass that was harvested per block and on average amounted to 186 (± 16) g (= 26.3 metric tonnes ha^{-1}) for winter oilseed rape and 171 (± 17) g (= 24.2 metric tonnes ha^{-1}) for winter rye. For the crop species lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea* L.) thirty-three seeds were used, whereas for the weed species *Chenopodium album* L. and *Stellaria media* L. 200 seeds were sown. The seeds were sown immediately after residue incorporation at a depth of 1.5 cm (spinach) or 1 cm (all other species). Weed seeds were purchased from the

Table 1 Fraction emergence of indicator species, sown directly after residue incorporation of winter oilseed rape (WO) or winter rye (WR) in experiment 1 and 2

Exp	Pre-treatment	Placement	Lettuce		Spinach		S. media		C. album	
WO										
1	-	-	0.84 (0.077)	b	0.70 (0.055)	b	0.30 (0.017)	b	0.25 (0.021)	a
	grinding	mixed	0.61 (0.064)	a	0.42 (0.102)	a	0.23 (0.029)	a	0.37 (0.026)	c
	crushing		0.72 (0.058)	ab	0.55 (0.042)	ab	0.26 (0.017)	ab	0.32 (0.031)	bc
	Cutting		0.74 (0.032)	ab	0.66 (0.040)	b	0.26 (0.012)	ab	0.27 (0.024)	ab
2	-	-	0.86 (0.063)	b	0.74 (0.035)	b	0.37 (0.012)	b	0.26 (0.026)	bc
	cutting	mixed	0.68 (0.079)	a	0.61 (0.039)	a	0.27 (0.031)	a	0.23 (0.034)	ab
		mulch	0.67 (0.078)	a	0.49 (0.049)	a	0.26 (0.029)	a	0.19 (0.043)	a
		layer	0.86 (0.040)	b	0.80 (0.028)	b	0.39 (0.017)	b	0.30 (0.028)	c
WR										
1	-	-	0.84 (0.061)	b	0.77 (0.043)	b	0.25 (0.016)	a	0.28 (0.027)	a
	grinding	mixed	0.78 (0.033)	ab	0.46 (0.038)	a	0.23 (0.019)	a	0.31 (0.037)	a
	crushing		0.70 (0.031)	a	0.44 (0.024)	a	0.23 (0.025)	a	0.32 (0.035)	a
	cutting		0.63 (0.037)	a	0.50 (0.043)	a	0.24 (0.026)	a	0.33 (0.019)	a
2	-	-	0.86 (0.054)	c	0.68 (0.025)	b	0.24 (0.024)	bc	0.23 (0.035)	b
	cutting	mixed	0.57 (0.080)	b	0.33 (0.035)	a	0.17 (0.021)	ab	0.30 (0.015)	c
		mulch	0.38 (0.069)	a	0.24 (0.031)	a	0.15 (0.027)	a	0.14 (0.037)	a
		layer	0.88 (0.024)	c	0.68 (0.053)	b	0.26 (0.018)	c	0.23 (0.031)	b

Treatments differed in residue pre-treatment and placement. Different letters (a-c) within a column indicate significant differences at the 0.05 level within an experiment; values in parentheses denote SE.

company Herbiseed (UK), where they were harvested in the year 2000 (*C. album*) or 2002 (*S. media*) and stored at 5°C and RH 10–15% until use. Prior to sowing, half of the *C. album* seeds were pre-treated to break dormancy by burying the seeds, mixed with soil in nylon bags, at a depth of 10 cm in the soil from December 2004 until their use in March 2005.

Experiment 2: effect of residue placement

In experiment 2, three different methods of residue placement of winter rye cv. "Protector" or winter oilseed rape cv. "Emerald" and a control treatment were compared (Table 1). The receptor species and the experimental set-up were the same as experiment 1, only now with 1 replication per block for a total of 8 blocks. Residues were harvested, cut and placed in three different ways in the rings on March 21 (block 1–4) and March 23 (block 5–8), 2005. Residues were mixed through the upper 10 cm of the soil, left on top of the soil as a surface mulch or placed in a layer at 10 cm below soil surface. Again, a control treatment was included in which no cover crop above-ground material was added to the soil. The quantity of above-ground fresh biomass that was added to each ring depended on the amount of biomass that was harvested per block and on average amounted to 619 (± 141) g (= 88 metric tons ha^{-1}) of fresh winter oilseed rape material and 422 (± 152) g (= 60 metric tons ha^{-1}) of fresh winter rye material.

Experiment 3: temporal effect of combinations of residue pre-treatment and placement as affected by cultivar

For experiment 3, two cultivars were selected for each cover crop species. The winter oilseed rape cultivar "Athena" was chosen for its relatively low glucosinolate content (Brown et al. 2005) and the cultivar "Dwarf Essex" for its relatively high glucosinolate content (Gardiner et al. 1999; Dandurand et al. 2000). The winter rye cultivar "Wheeler" has often been used in allelopathy studies (e.g. Barnes and Putnam 1986; Mwaja et al. 1995; Reberg Horton et al. 2005) and was compared to the Dutch cultivar "Protector". In this third experiment a selection of treatments from experiment 1 and 2 were combined, resulting in a total of four treatments; (1) control treatment (no residues), (2) grinding and mixing through the upper soil layer, (3) cutting and mixing through the upper soil layer and

(4) cutting and placing residues on top of the soil as surface mulch. The amount of fresh biomass that was used for each ring was kept at 300 g (= 42.4 metric tons ha^{-1}) for all rings. When the average amount of fresh biomass per ring was lower than 300 g, cover crop material was added from the same species grown on the same soil in field plots at the organic experimental farm "Droevendaal". Fifty lettuce seeds were sown at 0 days, 3 days, 6 days, 9 days, 15 days, 21 days and 29 days after residue incorporation (DAI). The experimental design was a completely randomised split-plot with four replications and the experiment was carried out twice (experiment 3a and 3b). The main plots consisted of a combination of cover crop cultivar and residue treatment and the sub-plots contained seeds of the different lettuce sowing times. The rings were prepared on March 28 (block 1&2, experiment 3a), March 29 (block 3&4, experiment 3a), April 4 (block 1&2, experiment 3b) and April 5 (block 3&4, experiment 3b), 2006.

Soil measurements For every two blocks of the main experiment, one extra block was installed for additional measurements. In these extra blocks, electrical conductivity (EC), pH, soil water content (percentage by weight), and available NO_3^- and NH_4^+ were determined at different times after residue amendment. Soil cores to a depth of 10 cm were taken on March 29 (only experiment 3a), April 5 (only experiment 3b), April 15, April 21, April 27, May 4 and May 15, 2006 and dried at 37 °C during 5 days. Soil samples were stored in plastic jars in the dark at room temperature until analysis. For EC and pH measurements, 8.00±0.03 g of loose, dry soil was dissolved in 40.0 ml demineralised water (room temperature) and placed on a rotary shaker for 30 min. The EC was measured with a TetraCon 32, WTW EC-meter directly after shaking. The pH was measured 2 h after shaking with an inoLab, WTW, pH-meter. NO_3-N and NH_4^+-N concentrations in the soil were analysed with a Continuous Flow Analyzer (Technicon AutoAnalyzer II) after extraction with 0.01 M $CaCl_2$ (Houba et al. 2000).

Cultivar differences Just prior to residue incorporation, above-ground plant material of the cover crops was collected for determination of the allelopathic potential. In each block of experiment 3a and 3b, two samples per cultivar were analysed, resulting in a total of 32 samples per cover crop species. Immediately after harvesting, the plant material was frozen in liquid nitrogen and stored in a freezer at minus 30°C until the samples were placed in a freeze-dryer for 7 days. The dried material was ground to pass a 1.5 mm sieve and stored in plastic jars in the dark and at room temperature until use. For winter oilseed rape the glucosinolate content was measured, whereas for winter rye the allelopathic potential of the plant material was assessed by means of a bioassay.

Glucosinolate analysis was performed by using high performance liquid chromatography (HPLC) as described by van Dam et al. (2004). Glucosinolate detection was performed with a PDA detector (200–350 nm) with 229 nm as the integration wavelength. Sinigrin (sinigrin monohydrate, ACROS, New Jersey, USA) was used as an external standard. Correction factors at 229 nm from Buchner (1987) and the EC (1990) were used to calculate the concentrations of the glucosinolates. Desulfoglucosinolate peaks were identified by comparison of HPLC retention times and UV spectra with standards kindly provided by M. Reichelt, MPI Chemical Ecology, Jena (Germany) and a certified rapeseed standard (Community Bureau of Reference, Brussels, code BCR-367R).

For the winter rye bioassay, a concentration series of 8%, 4%, 2%, 1%, 0.5% and 0% wt/wt extracts were prepared by mixing ground, freeze-dried, winter rye material in de-ionised water. Lettuce seeds were used as an indicator species in Petri dish bioassays. After 4 days the total root length per Petri dish was determined. Further details about extract preparation, bioassay and root length measurement have been described by Kruidhof et al. (2008a).

Data analysis

Analysis of seedling emergence data The total fraction of emerged seedlings and the time at which 50% of the seedlings had emerged (T_{50} emergence) were analysed separately for winter oilseed rape and winter rye. The time at which 50% of the seedlings had emerged was determined by fitting a three-parameter logistic curve through the emergence data of each treatment, assuming a binomial distribution. A General Linear Mixed Model (GLMM) with an Iterative Reweighted Residual Maximum Likelihood (IRREML) procedure in the Genstat 9 statistical package (Payne et al. 2006) was implemented for data analysis, with a

binomial distribution for the total fraction of emerged seedlings. This analysis was followed by a Wald test to test for significance of main and interaction effects. To test for significant differences between treatments, pairwise t tests were used.

The fraction of seedlings that emerged within successive one-week periods was analysed separately for each receptor species with the GLIM (Generalized Linear Model) procedure for binomial data (Payne et al. 2006). In this binomial analysis we used the number of non-emerged seeds at the start of each new period as the total number of entries. Pairwise t-tests were used to test for significance between treatments.

Analysis of glucosinolate data The GLIM procedure was also used to compare treatment means of the total glucosinolate content, the content of three distinct classes of glucosinolates, as well as the content of individual glucosinolates.

Analysis of winter rye bioassay The data from the bioassay of winter rye were analysed using drc, an add-on package for the language and environment R (R_Development_Core_Team 2005). The drc package is especially developed for the analysis of dose-response curves and allows simultaneous fitting of nonlinear regression models (Ritz et al. 2005). The variable "total root length per Petri dish" was fitted with a three-parameter logistic curve, resulting in estimates for the upper limit, the ED_{50} and the slope around the ED_{50}. The upper limit represents the total root length per Petri dish of the control (0% extract) and the ED_{50} is the dose (% w/w) producing a response halfway the upper limit. Further details about the procedure have been described by Kruidhof et al. (2008a).

Results

Experiment 1 – effect of residue pre-treatment

In experiment 1, the total fraction of emerged crop seedlings in the control treatment was on average 0.84 for lettuce and 0.74 for spinach. For weeds this was lower, with on average 0.28 for *S. media* and 0.27 for *C. album* (Table 1). An interaction between residue pre-treatment and receptor species ($p<0.001$) was found with regard to the influence of both winter oilseed rape and winter rye residues on seedling emergence. For winter oilseed rape, the response of *C. album* to the residue treatments was clearly different from the response of the other three receptor species. For lettuce, spinach and *S. media* only grinding resulted in a reduction of seedling emergence, by 28%, 44% and 27%, respectively. In contrast, for *C. album* both grinding and crushing of residue material resulted in an increase in the number of emerged seedlings. When *C. album* was excluded from the dataset, cutting and crushing were also found to reduce emergence significantly compared to the control treatment, though grinding reduced emergence more than the other two residue treatments. In case of winter rye, all residue treatments caused a reduction in emergence of the crop species, except for grinding, which did not affect lettuce emergence. The emergence of *S. media* and *C. album*, in contrast, was not affected by any of the winter rye residue treatments.

The time needed to reach 50% seedling emergence (T_{50}) was similar for all residue treatments. However, the T_{50} differed between receptor species. In the control treatment, the T_{50} was on average 8.5 daysand 10 days for lettuce and spinach, respectively. The T_{50} of *S. media* was longer, on average 18 days. *C. album* emergence started relatively late and was characterised by different flushes which extended over a long time period. As the cumulative emergence curve did not follow a logistic function, the T_{50} was visually estimated to be 49 days.

Figure 1 shows seedling emergence of each receptor species in successive one-week periods as affected by amendment of differently pre-treated winter oilseed rape residues. Three weeks after incorporation (WAI), when almost all lettuce and spinach seedlings as well as the majority of the *S. media* seedlings emerged, seedling emergence was reduced in the residue-amended soil compared to the control soil. *C. album* emergence started later and was stimulated by finely ground winter oilseed rape residue between 4 and 8 WAI. Initially cut winter oilseed rape residue reduced *C. album* emergence, but with time *C. album* emergence increased. Also for winter rye, early emerging receptor species (lettuce and spinach) were inhibited by the residues, whereas the establishment of the later emerging receptor species (*S. media* and *C. album*) was not affected. However, the time course of *C. album* emergence was

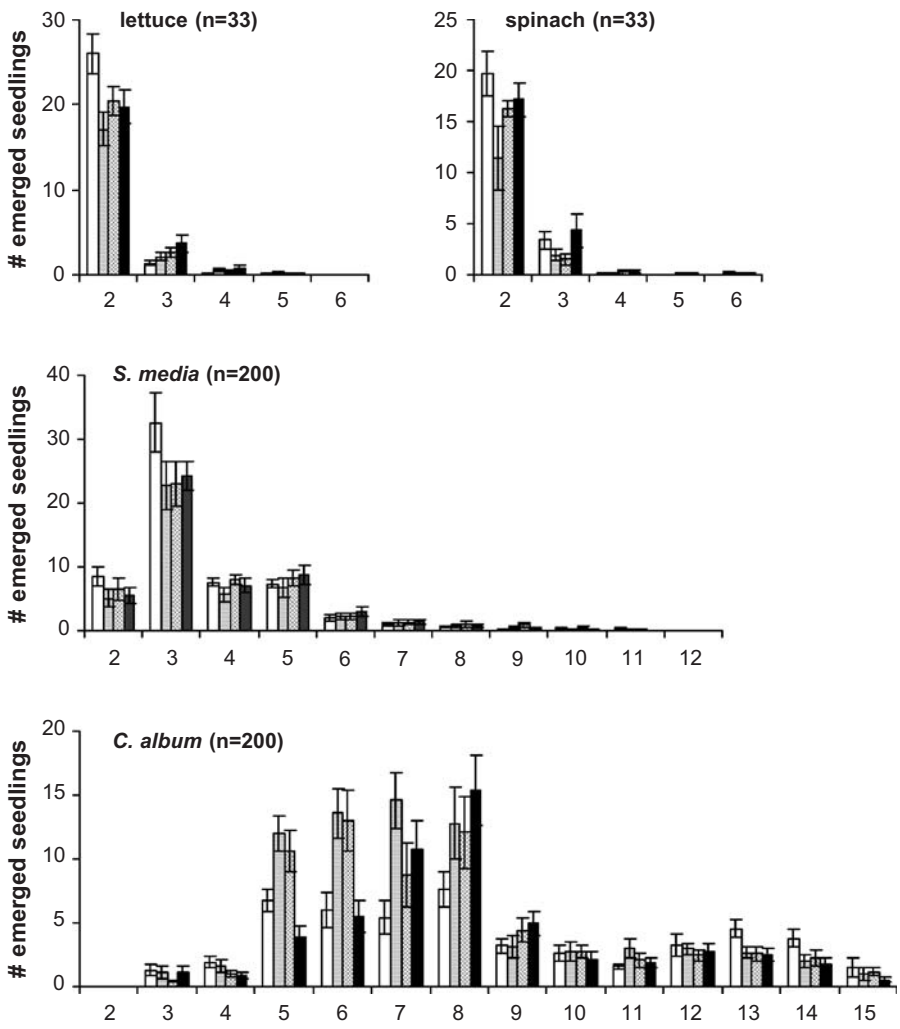

Fig. 1 Weekly seedling emergence data of four indicator species as affected by pre-treatment of winter oilseed rape residue in experiment 1: control (white), ground (stippled), crushed (cross-hatching) or cut residues (black). WAI = "weeks after incorporation". Vertical bars represent means ± SE

not affected by residue pre-treatment (data not shown).

Experiment 2 – effect of residue placement

In experiment 2, all residues were cut into pieces of 3 cm and the focus was on the effect of residue placement. The total fraction of emerged seedlings in the control treatments was comparable to experiment 1 (Table 1). For winter oilseed rape, residue placement had a similar effect on all receptor species. Seedling emergence was reduced when residue was either mixed through the soil (average reduction 20%) or left on top of the soil (average reduction 29%). When residues were placed in a layer at 10 cm depth, emergence of none of the receptor species was affected. For winter rye, there was an interaction between residue placement and receptor species ($p<$ 0.001). This was mainly due to a difference in the response of receptor species to mixing of the residue through the upper soil layer. Whereas the emergence of both crop species in this treatment was reduced (lettuce - 34%; spinach - 51%), the emergence of *S. media* was not affected and the emergence of *C. album* was increased (30%). For all receptor species, mulching caused the strongest inhibition of emergence, with 56%, 65%, 38% and 39% reduction for lettuce, spinach, *S. media* and *C. album*, respectively. With winter rye, residue placed in a layer at 10 cm depth did not affect seedling emergence.

In the control treatment, the T_{50} values of lettuce and spinach emergence were 8 days and 9.5 days, respectively, and comparable to experiment 1. Mid-emergence times of the weed species were much

shorter than those in experiment 1, with 13 days and 34 days for *S. media* and *C. album* seedlings, respectively. For all four receptor species and both cover crop species, the T_{50} in the mulch treatment was increased with on average 2–3 days.

Similarly to experiment 1, mixing of residue through the upper soil layer initially reduced the emergence of *C. album* seedlings, this inhibitory effect then disappeared with time (winter oilseed rape) or changed into a stimulatory effect (winter rye).

Experiment 3 – temporal effect of residue combinations of pre-treatment and placement as affected by cultivar

Sowing of lettuce seeds at different times after residue incorporation facilitated the monitoring of the time course of residue-mediated suppression of seedling emergence. In both experiment 3a and 3b, a clearly distinct time course of inhibition of lettuce emergence was observed between finely ground and cut winter oilseed rape residues (Fig. 2). Grinding reduced lettuce emergence earlier than cutting. The inhibitory effects of the finely ground residues on emergence were strongest for lettuce sown on 3, 6 and 9 DAI in experiment 3a (on average 49% reduction) and on 6 and 9 DAI in experiment 3b (on average 42% reduction). In contrast, the inhibitory effect of the cut residues was strongest for lettuce sown on 15, 21 and 29 DAI in experiment 3a (on average 36% reduction) and on 21 DAI in experiment 3b (on average 47% reduction). For both winter oilseed rape cultivars similar results were observed. The temporal pattern of inhibition of lettuce emergence in the mulch treatment could not be established, because of the interference of molluscs that were found in several of the rings containing winter oilseed rape residues on the soil surface.

For winter rye, no clear temporal pattern in lettuce emergence was observed for either experiment 3a or 3b (data not shown). In experiment 3a, the emergence of lettuce seedlings, averaged over all sowing times, was inhibited by finely ground Wheeler residues (21%) and by cut and mixed Wheeler residues (13%), whereas the same treatments with residues of cv. Protector did not affect lettuce emergence. When used as mulch, both winter rye cultivars exerted a

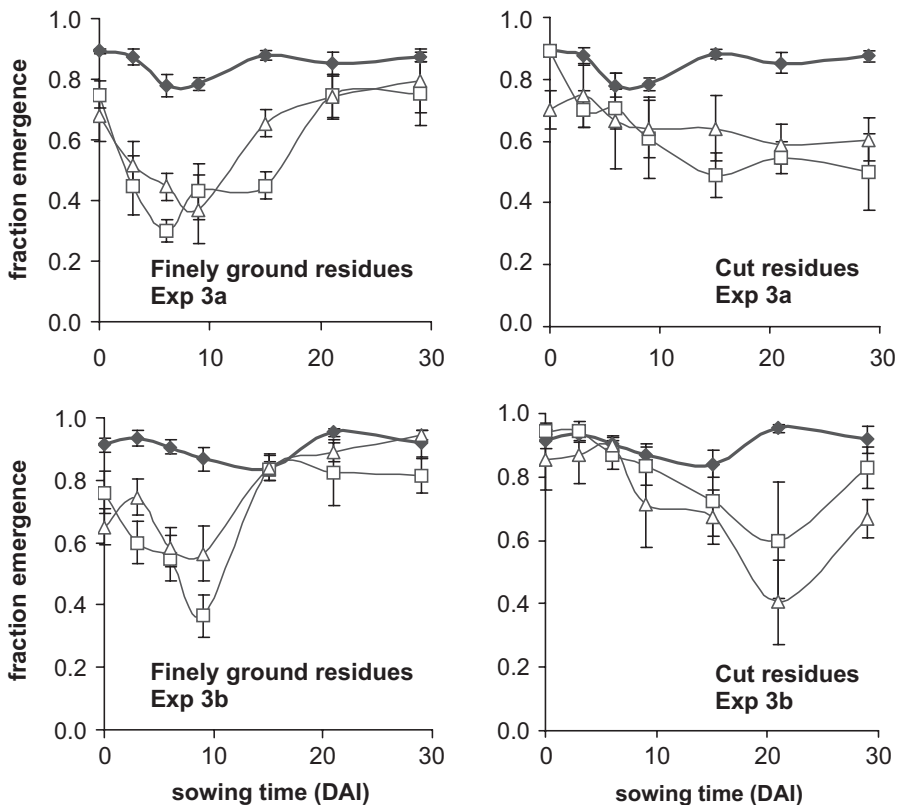

Fig. 2 Fraction emergence of lettuce sown at different days after winter oilseed rape residue incorporation (DAI) in experiment 3a and 3b. Thick lines = control treatment (no residues), open triangles = winter oilseed rape cv. Dwarf Essex , open squares = winter oilseed rape cv. Athena. Vertical bars represent mean values ± SE

similar influence on the emergence of lettuce (14% reduction). In experiment 3b, winter rye residues were less effective and reduction of lettuce emergence never exceeded 10% when averaged over all sowing times. Consequently no differences between cultivars were observed.

For winter oilseed rape, no treatment effects on the T_{50} of lettuce emergence were detected. For winter rye, the presence of mulch initially caused an average increase in the T_{50} of 1.7 days in both experiments. However, for the last three lettuce sowing dates, lettuce emergence in the mulch treatment was earlier than in the control treatment. This was particularly obvious for the last sowing date of experiment 3b where the T_{50} of lettuce seedlings was only 4.5 days as opposed to 18 days in the control treatment.

The influence of residue amendment on soil characteristics

The EC in the control treatment was rather stable (Fig. 3). Addition of residue to the soil led to an increase in the EC, which lasted for at least 40 days. For winter oilseed rape, the EC was initially highest in the soil into which finely ground residues were mixed, and was similar to the EC in soil containing cut residues at 29 DAI and later. In winter rye, in contrast, the EC in the soil with finely ground residues was initially lower than in the soil with cut residues. This was true for both experiment 3a and 3b, but the difference was larger for experiment 3a. For both cover crop species, the EC values in the mulch treatment were intermediate between the control soil and the soil into which the finely ground residues were mixed. Cover crop species and cultivar did not influence the value or pattern of EC over time. pH-H_2O values, measured in the same 20% soil extracts as the EC, ranged from 6.0 to 6.6, but differences were not significant.

The moisture content in the upper 10 cm of the soil in the control treatment decreased from 13% (w/w) on March 31, 2005 to 7.5% (w/w) on April 27, 2005. Soil of the mulch treatment conserved 33% (winter oilseed rape) to 41% (winter rye) more water than the

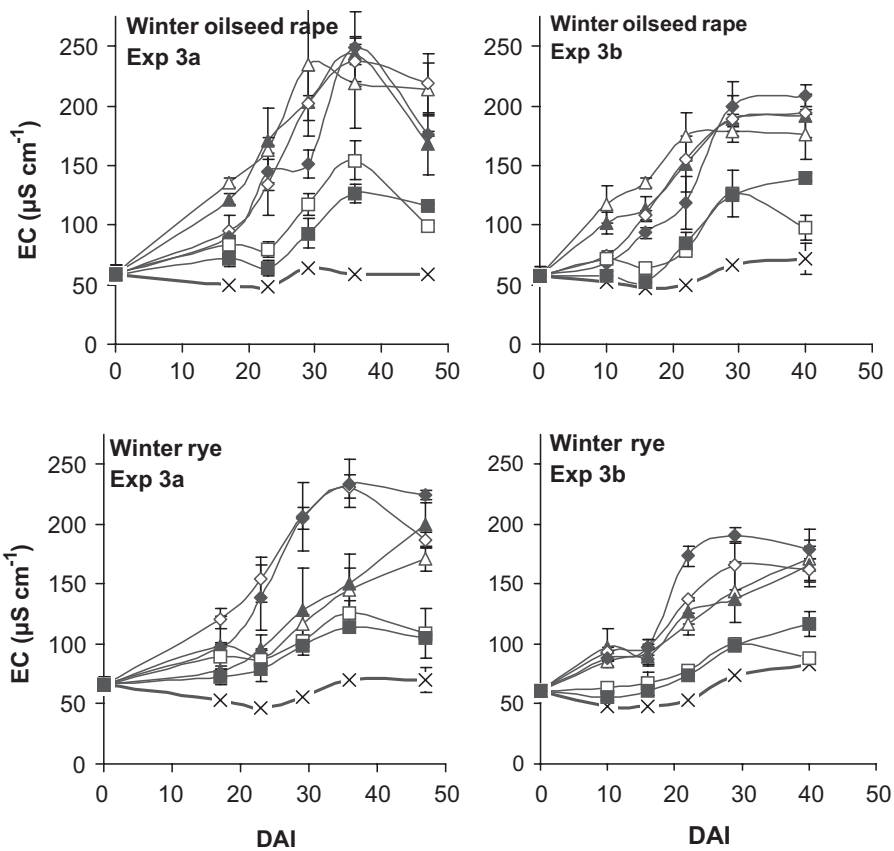

Fig. 3 Electrical conductivity (EC, µS cm^{-1} at 25°C) measured in a mixture of 20% dry soil and 80% deionized water. Soil samples were taken at different days after residue incorporation (DAI) experiment 3a and 3b. Closed markers = cultivars Wheeler and Dwarf Essex, open markers = cultivars Protector and Athena. Crosses = control treatment (no residues), triangles = finely ground and mixed, diamonds = cut and mixed, squares = cut and mulch. Vertical bars represent mean values ± SE

control soil. Soil moisture was also conserved when cut or finely ground residues were mixed through the soil, although to a lesser extent.

The available nitrate content in the control soil of experiment 3b was relatively stable, whereas in the residue-amended soil for both cover crop species a considerable increase in the nitrate content was observed at the last two sampling dates (April 28 and May 4; Fig. 4). In contrast to nitrate, the available ammonium content in the control soil was not stable. During the first three weeks it decreased by approximately three-fold, after which it increased again to its initial value. The available ammonium content in the residue-amended soil remained rather stable for both cover crop species.

Cultivar differences in allelopathic potential

Large differences in total glucosinolate concentration were detected between the two winter oilseed rape cultivars Athena and Dwarf Essex ($p<0.001$; Table 2). Dwarf Essex contained 120 nmoles of glucosinolates per mg dry residue, whereas this was only 71 nmoles for Athena. The concentration of indole glucosinolates was similar for both cultivars, while the concentration of alipathic glucosinolates was almost twice as high and the concentration of the aromatic glucosinolate gluconasturtiin was approximately three times as high in Dwarf Essex compared to Athena. Only the concentration of the alipathic glucosinolate gluconapin and the indole glucosinolates glucobrassicin and 4-methoxyglucobrassicin, which made up respectively 6%, 12% and 2% of the total average glucosinolate concentration, were not higher in Dwarf Essex compared to Athena. No differences were found between residues harvested on March 28–29 (experiment 3a) and residues harvested on April 4–5 (experiment 3b).

For winter rye, we assessed the allelopathic potential of the cultivars Protector and Wheeler by comparing the ED_{50}, the extract dose at which lettuce root length was reduced by 50%. The ED_{50} values did not differ between the two winter rye cultivars Protector and Wheeler for both experiment 3a and 3b. We did, however, find a difference in ED_{50} of the residue extracts between experiment 3a and 3b. The residues that were used in experiment 3a had an average ED_{50} value of 1.98 compared to an average ED_{50} value of 2.68 for the residues in experiment 3b. This indicates that the residues in the last experiment were less effective in reducing lettuce root length.

Discussion

Effects of residue pre-treatment on seedling emergence over time

Winter oilseed rape Inhibition of seedling emergence by incorporated winter oilseed rape residues takes place earlier where there is increased tissue disruption of the residues. Finely ground winter oilseed rape residues inhibited lettuce seedling emergence only for the first two to three weeks following residue incorporation, whereas inhibition of lettuce seedling emergence by cut residues started after this period (experiment 3; Fig. 2).

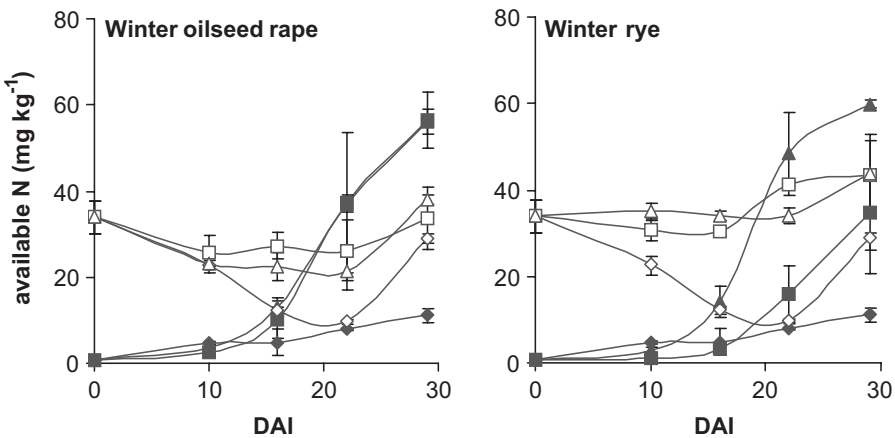

Fig. 4 Available N-NO_3 (closed markers) and N-NH_4 (open markers) (mg kg^{-1}) measured in soil samples taken at different days after residue incorporation (DAI) in experiment 3b. Diamonds = control treatment (no residues), squares = finely ground and mixed, triangles = cut and mixed. Vertical bars represent mean values ± SE

Table 2 Glucosinolates (GLS) concentrations (μmoles g^{-1}) in winter oilseed rape cv. Dwarf Essex and winter oilseed rape cv. Athena averaged over experiment 3a and 3b. *** means a significant difference at the $p<0.001$ level, ns = not significant

Trivial name	R side chain	GLS concDwarf Essex	GLS concAthena	
Alipathic GLS				
Progoitrin	(2R)-2-hydroxy-3-butenyl	33.0	14.4	***
Gluconapoleiferin	2-hydroxy-4-pentenyl	14.3	4.72	***
Gluconapin	3-butenyl	5.64	5.34	ns
Glucobrassicanapin	4-pentenyl	38.7	22.8	***
Glucoalyssin	5-methylsulfinylpentyl	3.65	1.10	***
TOTAL alipathic		95.3	48.4	***
Indole GLS				
Glucobrassicin	3-indolylmethyl	10.1	13.5	***
4-Methoxyglucobrassicin	4-methoxy-3-indolylmethyl	1.78	1.81	ns
Neo-glucobrassicin	1-methoxy-3-indolylmethyl	9.31	5.68	***
TOTAL indole		21.2	21.0	ns
Aromatic GLS				
Gluconasturtiin	2-phenylethyl	3.96	1.36	***
TOTAL GLS		120	70.7	***

As in living winter oilseed rape plants glucosinolates and the corresponding enzyme myrosinase are compartmentalized in different cells (e.g. Rask et al. 2000), tissue disruption is needed to convert glucosinolates into the more toxic volatile breakdown products. In our experiment this occurred mechanically, by grinding or crushing, and/or as a result of decomposition in the soil. This would explain why finely ground residues inhibited seedling emergence earlier than cut residues. During tissue disruption many compounds will be released from plant cells and it was observed that EC in the soil containing finely ground residues increased earlier than in the soil containing cut residues (Fig. 3), showing a temporal pattern similar to that of the inhibition of seedling emergence.

The effect of *Brassica* residue pre-treatment on subsequent isothiocyanate release (Morra and Kirkegaard 2002), as well as the isothiocyanate release pattern over time of *Brassica* amended soil (Gardiner et al. 1999) have been investigated previously. However, as far as we know only our study has combined these two aspects and addressed the effect of *Brassica* residue pre-treatment on seedling emergence over time. Furthermore, rather than focusing on specific allelochemical compounds, we focused on the overall effect of the residues. Gardiner et al. (1999) monitored glucosinolate degradation products released in soil following whole-plant plough-down of *Brassica napus* cv. Dwarf Essex and Humus into the upper soil layer. Peak concentrations of isothiocyanates were reached much faster than the peak inhibitory effects on lettuce emergence of either the finely ground or cut residues in our experiment. Isothiocyanate (ITC) concentrations reached a peak at 30 h after plough-down, after which they declined to approximately one-third of the peak concentration at 2–3 days after incorporation, and had dropped to below detection limit after 3 weeks. Morra and Kirkegaard (2002) concluded that freezing and thawing of *Brassica* tissue resulted in maximum ITC release efficiency. This freezing and thawing of *Brassica* tissue is comparable to grinding in our experiment, as both treatments result in a high level of tissue disruption. However, when comparing their results with the results of our experiment, we conclude that freezing and thawing may not have simply increased the overall ITC release efficiency, but instead speeded up ITC release. Their data sustain this view as the difference in ITC release between the frozen and fresh residues was largest directly after residue incorporation and became rapidly smaller. On the last sampling date, 5 days after incorporation, ITC release was slightly higher in the fresh compared to the frozen residues.

Although glucosinolate breakdown products are usually held responsible for allelopathic effects, other, yet unknown, allelochemicals could have been involved in the observed effects. Bending and Lincoln (1999) suggested that the bio-fumigant properties of crucifer tissues represent the combined effect of low quantities of highly toxic ITC and large quantities of mildly toxic non-glucosinolate derived volatile

S-containing compounds produced during decomposition. Nevertheless, the phytotoxic effect of these volatile S compounds on germination and early growth of weeds remains to be established. Brown and Morra (1996) found water extracts of hydrolyzed shoot and leaf tissue of *Brassica napus* to completely inhibit lettuce germination and concluded that participation of an unknown compound(s) in addition to water-soluble glucosinolate degradation products seems probable. However, as they added only 29 mL water to 5.5 g of plant tissue, and did not include a control treatment to account for osmotic pressure, the observed effects could have possibly been influenced by osmotic stress.

It is unlikely that seedling emergence in our experiments was directly affected by osmotic stress. After approximately 30 DAI (exp. 3a) or 23 DAI (exp. 3b) lettuce emergence was no longer reduced by the finely ground residues. However, at the same time the EC of the soil water of the residue-amended soil reached a peak as a result of a high concentration of salts (Fig. 3) and low soil water content; this indicates a high osmotic pressure (Xuan et al. 2005).

Winter rye For winter rye, no clear patterns in lettuce seedling inhibition over time were observed for either finely ground or cut residues. Furthermore, the emergence of the receptor species was not influenced by residue pre-treatment. This is surprising, because similarly to glucosinolate breakdown products, the release of the hydroxamic acid DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one) is also dependent on enzymatic cleavage of the glucose moiety, and this is likely to be accelerated by mechanical tissue disruption. However, DIBOA is unstable with a half-life of 1 day or less in an aqueous solution (pH 5–7) at room temperature and rapidly hydrolyzes into the less toxic BOA (Bredenberg et al., 1962, in Fomsgaard et al. 2004). BOA (benzoxazolin-2(3H)-one), in turn, can be broken down by microbes into the more toxic allelochemical 2-amino-phenoxazin-3-one (APO) (Chase et al. 1991; Gagliardo and Chilton 1992). Because of the difference in toxicity of the subsequent hydroxamic acid derivatives and the dependence of their half-lives on many factors, including the prevailing temperature and soil moisture, temporal patterns of phytotoxicity will be difficult to predict.

The EC increased faster in soil containing cut winter rye residues than in soil containing finely ground winter rye residues. This is surprising, but may be explained by leaching of salts released from finely ground residues into deeper soil layers shortly after residue incorporation. This seems likely, as within the first 3 days following residue incorporation 18.2 mm rain had fallen in experiment 3a, for which the difference between the EC of soil containing cut and finely ground residue was largest, and only 7.9 mm of rain had fallen over the same period in experiment 3b.

Effects of residue placement on seedling emergence

Winter rye was most effective in reducing seedling emergence when used as mulch. More importantly, the strong reduction in the establishment of the late emerging *C. album* indicates that the inhibitory effect of mulch continued for a long time. This coincides with many publications showing that winter rye mulch provides excellent weed control (e.g. Barnes and Putnam 1983; Liebl et al. 1992; Masiunas et al. 1995). In experiment 2, winter rye mulch was more effective than winter oilseed rape mulch in reducing seedling establishment, despite the 32% lower amount of winter rye residue applied.

However, under dry conditions lettuce emerged more rapidly in the winter rye mulch treatment compared to bare soil, most likely because of the positive effect of the mulch on soil moisture conservation. We clearly observed this in experiment 3b, where lettuce seedlings that were sown up to 9 DAI emerged more rapidly in bare soil compared to mulch-covered soil, whereas this was the other way around for seedlings that were sown at 15 DAI and later. This coincided with the lack of rainfall after 13 DAI.

Differences between receptor species

Contrary to our hypothesis and the findings of Kruidhof et al. (2008b), we found large differences in the response of the receptor species to the cover crop residues. These differences seem clearly related to the emergence rate of the receptor species in the control soil. Residues incorporated in the upper soil layer exerted a large inhibitory effect on the establishment of the relatively early emerging lettuce and spinach seedlings, whereas the strength of the inhibitory effect on the slightly later emerging *S. media* seedlings was variable,

and often a stimulatory effect on the very late emerging *C. album* seedlings was observed. This possible relationship between emergence rate and sensitivity to residue-mediated effects may be an important aspect to take into account when assessing the sensitivity of different crop and weed species to cover crop residues.

Transition from inhibitory to stimulatory effects

A transition from inhibitory to stimulatory effects over time was observed for incorporated residues of both cover crop species. For winter oilseed rape the time of this transition was influenced by the level of tissue disruption. Finely ground winter oilseed rape residues stimulated the establishment of the very late emerging *C. album* from the beginning, whereas cut residues initially reduced *C. album* establishment and started to stimulate *C. album* establishment later in time (experiment 1; Fig. 1).

There are two possible explanations for the fact that *C. album* emergence was stimulated in the residue-amended soil. Firstly, it is widely recognized that low concentrations of allelochemicals can be stimulating to weed germination and early growth (e.g. Lovett et al. 1989). Secondly, the observed stimulation could be a response to increased nitrate levels in the residue-amended soil, because nitrate stimulates weed seed germination (e.g. Henson 1970; Bouwmeester and Karssen 1993). Nitrate release was not monitored in experiment 1, but soil sample analysis of experiment 3b showed a rapid increase in the release of nitrogen from the winter oilseed rape residues, starting at 16 DAI.

Cultivar differences

Despite strong differences in glucosinolate content, the two winter oilseed rape cultivars used in experiment 3a and 3b did not cause any differences in lettuce emergence. This may be due to a number of factors. Firstly, it is important to consider the relative phytotoxicity of the various glucosinolates and their breakdown products present in winter oilseed rape. The indole glucosinolate glucobrassicin (3-indolylmethyl) was the only glucosinolate present in a higher concentration in winter oilseed rape cv. Athena compared to cv. Dwarf Essex. Although isothiocyanates usually have a larger phytotoxic effect than glucosinolates, glucobrassicin was one of the few glucosinolates found to exert a direct inhibitory effect on the root elongation of wheat seedlings (Bialy et al. 1990). The toxicity of various isothiocyanates has been compared in at least three studies (Bialy et al. 1990; Petersen et al. 2001; Norsworthy and Meehan 2005). However, none of these studies included indole ITC's. If indole ITC's would have high phytotoxicity relative to the other ITC's, this could explain part of the lack of cultivar differences on lettuce emergence. Secondly, the importance of high-glucosinolate containing cultivars or species for weed suppression and bio-fumigation is emphasized in many publications (e.g. Eberlein et al. 1998; Kirkegaard and Sarwar 1998; Bellostas et al. 2007). However, both Warton et al. (2001) and Siemens et al. (2002) stress that not only the glucosinolate concentration, but more importantly the combination of the glucosinolate and myrosinase concentration, determines the allelopathic or bio-fumigation potential of *Brassica* species. This notion might explain the lack of differences in effect on lettuce emergence between the two cultivars. Thirdly, winter oilseed rape might contain other non-glucosinolate derived allelochemical compounds, which might have been partly responsible for the observed inhibitory effects on seedling emergence.

In the field, we observed that after residue incorporation the winter rye cultivar Wheeler exerted a stronger inhibitory effect on lettuce emergence than the Dutch winter rye cultivar Protector. However, when residues were used as mulch, inhibitory effects were similar. This is in line with our hypothesis, which states that the allelochemicals content of the cover crop becomes more important when residues are incorporated in the soil. This hypothesis is based on the idea that mulch affects seedling establishment mainly through physical alterations, which are independent of cultivar. However, cultivar differences observed in the field were not sustained by the laboratory bio-assays, where the ED_{50} of the winter rye residue extracts was similar for both cultivars. We can therefore not be certain if cultivar differences observed in the field were due to allelopathic effects.

Laboratory bioassays did, however, show a difference in the ED_{50} between residues used in experiment 3a and residues used in experiment 3b. The ED_{50} was higher in the one-week older residues used in experiment 3b, indicating a lower allelopathic potential of these residues. This coincides with several publications

that state that the hydroxamic acid concentration in rye decreases with age (Reberg Horton et al. 2005; Rice et al. 2005; Kruidhof et al. 2008a). It also coincides with the observations in the field, where lettuce emergence in the treatments with soil-incorporated residues was, on average, less inhibited in experiment 3b than in experiment 3a.

Conclusion

The relation between cover crop residue management strategy, particularly pre-treatment and placement of residue material on or in the soil, and weed suppression was investigated for two contrasting cover crop species; winter rye and winter oilseed rape. At the application rates used, residue applied as surface mulch was clearly the most effective weed management strategy for winter rye. Winter rye surface mulch provided a relatively strong and consistent inhibition of emergence of the receptor species, whereas the inhibitory effect of soil-incorporated winter rye residues was weaker and the time course of inhibition difficult to predict. Residue management of winter oilseed rape, however, showed a completely opposite picture. First, mulching was less effective than residue incorporation and included the risk of attracting molluscs. Second, the time course of the inhibitory effects of soil-incorporated residues could be actively influenced by residue pre-treatment. Residue cutting delayed the inhibitory peak compared to residue grinding, without affecting the strength of the inhibitory effect. This finding offers a useful tool to actively steer the time course of winter oilseed rape residue-mediated inhibitory effects. Furthermore, our results suggest that the response of the receptor species to cover crop residues depends on the emergence time of these receptor species. Further investigation of this phenomenon may provide better insight into differences in sensitivity of receptor plants to residue-mediated effects. Contrary to our expectations, using a cultivar with high allelochemical content did not translate into stronger inhibitory effects under field conditions. This unexpected outcome emphasizes the need of research specifically aimed at uncovering the actual mechanisms underlying observed residue inhibitory effects.

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