Cover crop-based ecological weed management: exploration and optimization

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Cover crop-based ecological weed management: exploration and optimization

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Abstract

Cover crop-based ecological weed management: exploration and optimization. In organic farming systems, weed control is recognized as one of the main production-related bottlenecks. System-oriented approaches for ecological weed management are needed and cover crops may form an important component of such an approach. Inclusion of cover crops in crop rotations introduces two important mechanisms through which the development of weed populations may be hampered. In late summer and autumn the successful introduction of cover crops can prevent growth and seed production of weeds through competition. In springtime, cover crop residues incorporated in the upper layer of the soil may suppress or retard weed development and growth due to, among others, allelopathic effects. The main focus of research was put on the weed suppressive effect of cover crop residue material in spring and particularly on identifying management options to maximize this effect. To better appreciate the potential of cover crop residue material the investigations were focused on three aspects, namely allelochemicals in the cover crop, the residence time of the residue-mediated inhibitory potential in the soil and the variability in inhibitory effects on receptor plants. The study was conducted with representatives of three plant families: *Secale cereale* L. (winter rye), *Brassica napus* L. (winter oilseed rape) and *Medicago sativa* L. (lucerne). Mechanical injuring of field grown cover crops enhanced the allelopathic activity per unit biomass. However, this increase was often just sufficient to compensate for loss of plant material resulting from damaging, implying the limited practical significance of damaging. Different options for pre-treatment and incorporation of cover crop residue material were compared and these were found to influence the size and persistence of an inhibitory effect on seedling emergence. Results were found to be cover crop specific. With regard to species' sensitivity our results suggest that for inhibition of a receptor plant not just seed size is important. Only if the time course of sensitivity of the receptor plant matches with the time course of residue-mediated inhibitory potential, significant reductions in seedling establishment can be expected. In light of this, it was postulated that variation in synchronicity of receptor species' sensitivity and potential residue effects may well explain the large degree of variation often noted in field studies of allelopathy.

Keywords: organic farming, ecologically-based weed management, cover crops, green manure, allelopathy, *Secale cereale, Brassica napus, Medicago sativa*

Preface

Interestingly, my involvement in the PhD-trajectory that led to this thesis has its origin in Africa. Thanks to Hawa Coulibaly, who very kindly sent me a present from Mali, I was invited to the office of Aad van Ast who had brought the package with him to Wageningen. As at that time I was close to finishing my studies and interested in becoming a PhD-student, I asked Aad whether there were possibilities for PhD projects in the Crop and Weed Ecology Group. In this way I learned about the project I have worked on for these past few years.

This PhD-project was based on a collaboration between de Crop and Weed Ecology Group, Plant Research International and the PE & RC Graduate School, all part of Wageningen UR. Lammert Bastiaans was my daily supervisor at the Crop and Weed Ecology Group. Without his unconditional support it would not have been possible to complete all the work that is presented in this book. Lammert, you were like a “rots in de branding” for me. I really appreciate I could just knock your door every moment I had a question. Whenever there was a problem or I was worried about something I felt reassured after talking to you. Thank you for all the stimulating discussions, your patience and your advice. At PRI, I would like to thank Bert Lotz for providing funding for the execution of the experiments and Piet Scheepens for his effort in helping to initiate this project. I want to thank Martin Kropff, my promotor, for all the stimulating discussions and the time he allocated to our meetings, especially in the last period when the frequency of our encounters increased significantly.

De experimenten die zijn uitgevoerd in het kader van dit project waren erg arbeidsintensief en zouden onmogelijk zijn geweest zonder alle hulp van de medewerkers van Unifarm. Het grootste deel van de experimenten is uitgevoerd op het biologische proef- en leerbedrijf “Droevendaal”. Ik moet toegeven dat het, onervaren als ik was, in het begin toch wel wennen was aan het werk op de “boerderij”. Het was op z’n minst een uitdaging om jullie Unifarmers te vertellen over de praktische uitvoering van de experimenten terwijl ik nog nooit een zaaimachine van dichtbij had gezien. Gerrit Huisman, Ralph Post, Henk Vleeming, Herman Meurs, Wim Lieftink, Wim van der Slikke, Henk van Roekel, Johan van Woggelum, Johan Scheele, Taede Stoker, Teus van de Pol, Eddy de Boer, John van der Lippe, Gerard Derks, Herman Masselink en alle anderen; ik heb veel van jullie geleerd en ben blij dat ik kon bouwen op jullie jarenlange ervaring. Ik wil jullie allemaal erg bedanken voor jullie inzet, ook bij de wat minder leuke klussen. Ook wil ik de bedrijfsleider van Droevendaal, Andries Siepel, heel erg bedanken voor het meedenken over de experimenten en de alle hulp bij de coördinatie en de uitvoering hiervan. Andries, ik bewonder je inzet en

je harde werken om het proef- en leerbedrijf tot een succes te maken. Echter, niet alle experimenten zijn uitgevoerd in het veld. Het laatste experiment vond plaats in de klimaatkamer. Ik wil André Maassen en de andere kasmedewerkers van Unifarm dan ook bedanken voor de hulp bij de realisatie van dit experiment. Voor sommige experimenten moest speciaal gereedschap worden gemaakt. Ton Blokzijl en Peter de Ruygt, bedankt voor jullie creatieve bijdrage aan dit project. De hoeveelheid werk die moest worden verzet om tot de beschreven resultaten te komen hebben menigmaal geleid tot mijn verblijf in het ruwlab buiten de kantooruren. René Alles, bedankt voor je flexibele houding tijdens de vele keren dat je niet op tijd kon afsluiten omdat ik nog aan het werk was.

I would also like to thank my colleagues Ans Hofman and Aad van Ast from the Crop and Weed Ecology Group for their valuable help with the execution of several experiments. Ans, thank you very much for counting all the seedlings in our ring experiments, even in the weekends or during Easter. Aad, thank you for your help with the experiments of Chapter 6. I also want to thank you for your kind help in revising the reading version of this thesis for spelling and editorial errors. In this last respect, the help of Gon van Laar has been indispensable. Gon, thank you very much for all your effort to make the manuscript of this thesis ready for printing.

I would also like to thank my colleagues from Plant Research International for their help. Roel Groeneveld helped me to learn about the identification of the different weed species encountered in the field and Pieter Pikaar helped me initiating the laboratory bioassays. I would also like to thank Wolter van der Zweerde, Jacques Davies and Bert van Alfen for their help in the execution of the experiments. André Uffing, thank you for lending me the “rainfall machine”. I am very grateful to Geert Stoopen, Patricia van der Zouwen, Yvonne Birnbaum and Steven Groot for providing me with laboratory space and other research facilities. I also appreciate that John de Koning was there to help whenever there was a technical problem.

During the course of this project several collaborations with researchers from other groups and institutes were established. Nicole van Dam from the Netherlands Institute of Ecology (NIOO-KNAW) was involved in the experiments of Chapter 3. Nicole, thank you very much for your contribution to this work, for the interesting discussions about glucosinolates and for allowing me to carry out the glucosinolate analyses in your laboratory. Ciska Raaijmakers, thank you for explaining me how to carry out the glucosinolate extraction and for your help with the analyses.

Eric Gallandt and Erin Haramoto from the University of Maine (USA) made a great contribution to Chapter 5. Thank you very much for providing me your datasets, which allowed for the testing of a new hypothesis. Your collaboration on this matter greatly reinforced our new insights on the importance of synchronicity of receptor

species' sensitivity and potential residue effects, one of the key findings of this project.

The expertise regarding soilborne pathogens of Aad Termorshuizen of the Biological Farming Systems Group has been indispensable for the experiments of Chapter 6. Aad, thank you very much for the pleasant collaboration. I would also like to thank Dine Volker and Wim Blok from the same research group for their help and advice with these experiments. I would like to thank Arjen van de Peppel from the Horticultural Production Chains Group for his help with the CO_2 measurements and for allowing me to use his equipment. Hennie Halm really helped me out by carrying out the nitrogen analyses; thanks a lot!!!

Data analysis would have been complicated without the support of Christian Ritz and Jacques Withagen. I followed a short course on the statistical assessment of dose-response curves taught by Christian during the EWRS congress in Italy. Although this course gave me a pretty good idea about the possibilities of the analysis of dose-response curves, many questions came up when I started to work with the software. Christian did an amazing job in answering all these questions almost instantly after they were asked, I really appreciate this a lot. Thank you!!! However, apart from dose-response curves and I also needed to apply other statistical methods. I think one of the main things I learned from the statistics courses was how to communicate with a statistician, and I practiced these statistical communication skills on a frequent basis with Jacques Withagen. Jacques, thank you very much for all your help, it was very pleasant to work with you.

Other researchers that were involved, especially in the beginning of the research, were Hans Hoek from PPO, and Geertjan Molema and Ben Verwijs from, at that time, the IMAG. Thank you all for your collaboration!

My PhD-experience wouldn't have been the same without all the students that collaborated to this research. Jenneke Vonk, Marios Simos, Ramon Torra Bernat and Kristoffer Jarlov Jensen, thank you very much for your dedication and contribution to this project. I really enjoyed working with you and I have learned a lot from this.

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It is amazing how many people helped me during the course of this project. As I am writing these acknowledgements, more and more names and memories pop up. Ton Elzenbroek helped me with the determination from the development stages of rye, Johan van Nieuwburg en Bart Timmermans helped me to get started with Winrhizo, Dave Polhemus (of Dutch ancestry) sent me winter oilseed rape seeds from the US, the people of Tupea helped with all kinds of computer problems, Gijsbertje Berkhout helped with the finances, the people of the secretariat helped with arranging all kinds of things….Thanks to all!!!

Although 98% of the work of my PhD was carried out in Wageningen, the last bits and pieces were put together far from “home” in Riverside, California. Sonia Zarate, thanks a lot for your support and for giving me a few final pushes to finish. Laura Delgado, I am really happy you helped me to put together the cover of this thesis!

Wageningen is a special place to me, and it is the only place in the world I would really call home. The reason for this good feeling is the many great friends that I made in this small, but cosmopolitan city. The Latin-Dutch crowd, “het meidenclubje”, and all my other friends; I thank you all for the many delicious dinners, the conversations, the parties; simply for sharing such an important part of my life!!! Rixt and Roxi, thanks for being my paranymphs during the defence! Iemke and Rink, thanks for coming out on Sunday to help me with my experiments! What makes Wageningen most special is that this is the place where Edward and I fell in love, and where we got married last summer. Edward, I don’t know how to thank you for all your support! You’ve not only put up with my temper in times of PhD-stress, but also helped me many times with the experimental work. Unfortunately, that wasn’t always good for you, as during the time you got stung by all those angry bees... Gracias por todo tu amor y por compartir tu vida conmigo!

Ik wil ook mijn ouders, Henk en Nelly, bedanken voor hun steun en vooral voor het feit dat ze me altijd de kans en vrijheid gaven om mijn eigen keuzes te maken, om de wereld verder te verkennen en om me te ontwikkelen tot wie ik nu ben.

Contents

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CHAPTER 1

General introduction

Weed management in organic farming systems

In organic farming systems, where the use of pesticides is excluded, 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). Weeds thrive in crops which (initially) have a relatively open canopy structure and the largest weed problems are encountered on sandy soil, particularly with *Stellaria media* and *Chenopodium album* (Wijnands et al., 1999). Simply replacing herbicides by other direct control measures is inadequate. A heavy reliance on mechanical cultivation is undesirable because of damage to soil structure, increased risk of erosion and frost damage to crops, and a strong dependency on weather conditions. Hand weeding is often used as a last resort, and this requires the availability of sufficient labour and is costly. Consequently, the weed problem cannot just be solved by curative tactics; instead weed management should be seen as a component of integrated cropping systems design. Rather than focusing on the detrimental effects of weeds in current crops, the time horizon of interest should be extended and main emphasis should be given to the management of weed populations (Barberi, 2002; Riemens et al., 2007). Therefore, system-oriented approaches to weed management that make better use of alternative weed management tactics need to be developed (Liebman and Davis, 2000; Barberi, 2002).

Cover crops as a companion crop for weed suppression

Cover crops have potential to form an important, pro-active component in such a system-oriented approach. Use of cover crops as a companion crop for weed suppression has been investigated and various strategies have been proposed (Brandsaeter and Netland, 1999). Sowing and growing cover crops together with the main crop resulted in substantial weed suppression (Ilnicki and Enache, 1992; Teasdale and Daughtry, 1993; Brandsaeter, 1996). However, a serious problem of these living mulch cropping systems is yield depression because of competition with the main crop. Attempts to reduce this competition include chemical and mechanical suppression of cover crop growth (e.g. Vrabel, 1983; Grubinger and Minotti, 1990; Brandsaeter et al., 1998) and screening for less competitive cover crops (e.g.

Nicholson and Wien, 1983; Den Hollander et al., 2007). Improved timing of establishment of the cover crop relative to that of the main crop is another option to reduce competition. Müller-Schärer and Potter (1991) proposed delayed sowing of cover plants in field-planted leek to give the crop a competitive advantage. De Haan et al. (1994) tried the opposite and studied a spring seeded smother plant that began senescence 5 weeks after emergence. In line with this, Ilnicke and Enache (1992) suggested to use winter annual legumes sown in late summer. The main crop should then be transplanted into the senescing mulch early next summer. Baumann et al. (2000) proposed the introduction of a competitive second cash crop to minimize the financial consequences of yield reduction in the main crop.

Traditional role of cover crops in Dutch agriculture

In Dutch agriculture, cover crops are not commonly used as a companion crop, but are rather included in the crop-free period in between two main crops. Cover crops have always played a modest role in Dutch agriculture, but the motives for using them have changed over time. Originally, these crops were mainly used as green manure or fodder crops, and this is how they still can be found in the Recommended List of Varieties of Field Crops (Anonymous, 2004). On arable farms, cover crops were mainly used after the main crop, for increasing the organic matter content of the soil. More strict regulations on emission of nutrients have given cover crops an additional role as catch crop, meant to avoid leaching in the crop-free winter period. In some cases, cover crops can contribute to the control of soil borne diseases, such as nematodes. Fodder radish (*Raphanus sativus* L.), for example, is being used as a hatch or trap crop for *Heterodera* spp. – cyst-nematodes that are pathogenic to sugar beet (Anonymous, 2004). For this purpose, the best results are obtained if the crop is sown in spring. In organic farming systems leguminous cover crops are used to supply nitrogen to the soil (e.g. Liebman and Davis, 2000). Cover crops taken up in the main crop-free period of the crop rotation may offer a good prospective for weed management. However, this aspect has not yet received much attention.

Exploring the weed suppressive effects of rotational cover cropping

Inclusion of cover crops in crop rotations in between two main crops provides two important opportunities for interference in the life cycle of weeds. In late summer and autumn the successful introduction of cover crops can reduce growth and, most importantly, seed production of weeds through competition. Indeed, cover crops fill gaps in cropping systems that would otherwise be occupied by weeds (Liebman and

Staver, 2001). This type of niche pre-emption is illustrated in data from McLenaghen et al. (1996) who sowed five winter cover crops or let ground lie fallow after fall-ploughing the sod. The ground cover of weeds was inversely proportional to that produced by the cover crops.

In springtime, cover crop residues incorporated in the upper layer of the soil may suppress or retard weed development and growth due to, among others, allelopathic effects (e.g. Al Khatib, 1997; Eberlein et al., 1998; Gardiner et al., 1999; Ohno et al., 2000). Other factors that can be altered through addition of cover crop residues and that can exert a direct influence on weed development and growth include soil nitrogen dynamics, soil physical characteristics and soilborne pathogens. 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 this (Stevenson, 1986, in Liebman and Mohler, 2001). Crop residues can also affect the physical properties of the soil. Residue-amended soil can for instance better conserve moisture (Liebl et al., 1992; Teasdale and Mohler, 1993). Decomposition of cover crop residues can, however, also result in an increased osmotic pressure of the soil solution, in this way reducing the availability of water to plants. Residues left on the soil surface can lead to decreased soil temperature fluctuations and reduced light penetration, which can both have an inhibitory effect on weed germination (Teasdale and Mohler, 1993; Liebman and Mohler, 2001). Iin some cases, soil organisms, including pathogens (Dabney et al., 1996; Conklin et al., 2002; Manici et al., 2004) and pests (e.g. Hammond, 1990), are stimulated after soil amendment with fresh residue material. Figure 1.1 summarizes the mechanisms through which cover crops, grown in between two main crops, can affect weed establishment, growth and seed production.

Allelopathy

As was mentioned before, allelopathy may form an important mechanism of cover crop residue-mediated inhibition of weeds. The term allelopathy was introduced by Molisch (1937) to designate the process by which one plant negatively affects another by chemicals means, and is derived from the Greek words ‘allelon’ meaning mutual and ‘pathos’ meaning harm or affection. Rice (1984) considered not only negative but also positive effects on the target organisms to be allelopathic, and in addition included microorganisms (bacteria, fungi and micro-algae) in the definition of allelopathy. In 1996, the International Allelopathy Society (IAS) has defined allelopathy as follows: ‘allelopathy refers to any process involving secondary metabolites produced by plants, microorganisms and viruses that influence the growth and development of agricultural

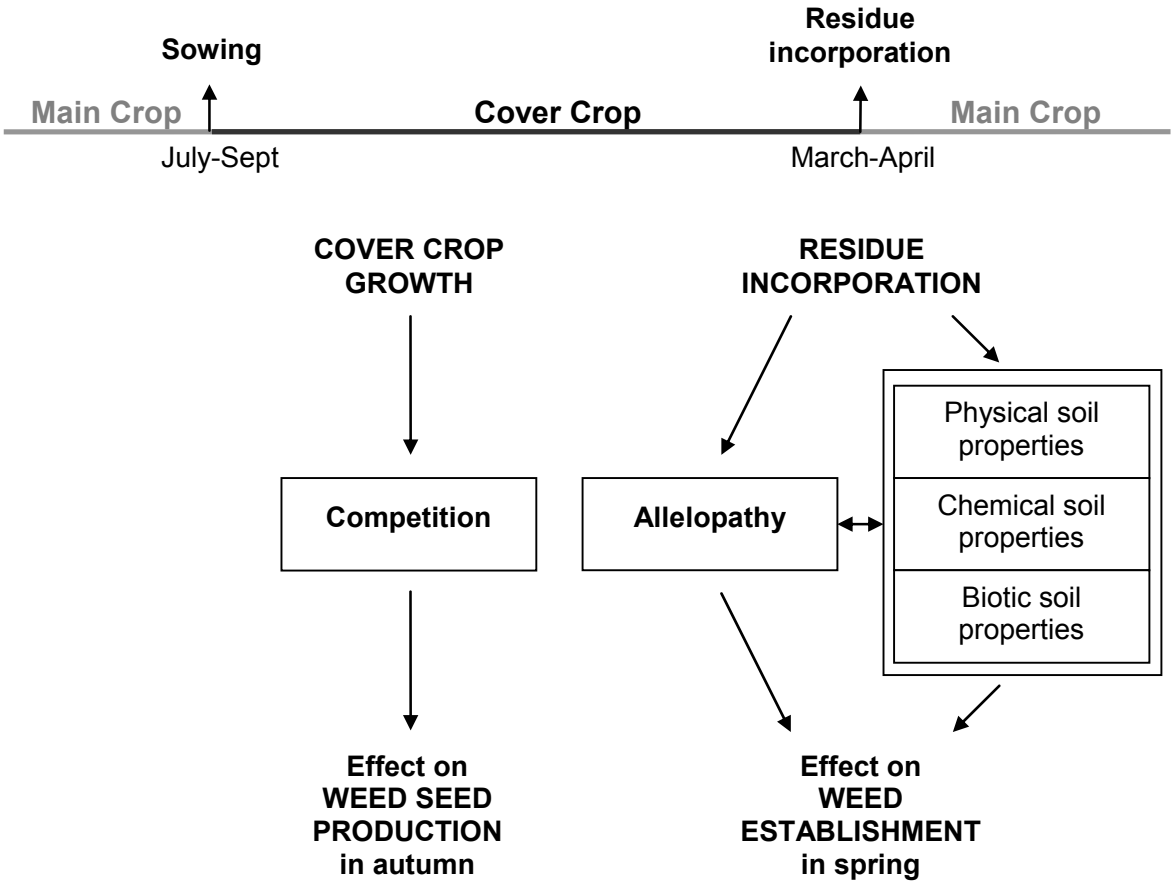

Figure 1.1. Framework showing the mechanisms through which cover crops may contribute to weed management in a crop rotation.

and biological systems'.

We observe here a clear development from a rather limited definition, only involving plant-plant interactions, to a more inclusive definition, depicting the allelopathic phenomenon as a general defence mechanism in plants. This inclusive definition recognizes that similar compounds are involved in the defence against multiple biological threats, including competition by other plants, herbivory and disease. This is a logical view from an evolutionary perspective, as a defence metabolite is cheaper in terms of resource investment if it can serve more than one purpose (Macias et al., 2007).

In our system, there are two possible sources of allelochemicals; allelochemicals can be released directly from the cover crop residues or they can be produced by microorganisms that use the cover crop residues as a substrate. Many different compounds exist that exert a certain level of allelopathic activity and more compounds are still being discovered. Most plant species contain several of these compounds, although in different concentrations. Rice (1984) divided allelopathic compounds into

14 classes and one miscellaneous group according to their biosynthetic origin, and this classification is still valid (Macias et al., 2007). The classes identified include (a) simple water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, and ketones, (b) simple unsaturated lactones, (c) long-chain fatty acids and polyacetylenes, (d) naphthoquinones, anthraquinones, and complex quinones, (e) simple phenols, benzoic acid, and derivatives, (f) cinnamic acid and derivatives, (g) coumarins, (h) flavonoids, (i) tannins, (j) terpenoids and steroids, (k) amino acids and polypeptides, (l) alkaloids and cyanohydrins, (m) sulfides and mustard oil glycosides, and (n) purines and nucleosides.

Conceptual model of allelopathic effects of cover crop residues on weeds

The allelopathic interference of cover crop residues on weed plants is a complicated matter. A large number of component studies, each dealing with a particular aspect of allelopathic interference, has been published. We integrated the knowledge generated by these component studies into a conceptual model. In this conceptual model a series of consecutive phases are distinguished, particularly accumulation of (a) allelochemicals in the cover crop, (b) allelochemicals in the soil, (c) allelochemicals in the receptor plant, and (d) the effect of allelochemicals on the receptor plant. In Figure 1.2, these stages are presented together with the major processes and factors involved.

Allelochemicals in the cover crop

In our system the strongest allelopathic effect is expected after cover crop residues are incorporated in the soil. The amount of allelochemicals that can be incorporated in the soil is a combination of cover crop biomass and the concentration of allelochemicals in the cover crop at the moment of cover crop residue incorporation. Between and within crop species there is a large genetic variation in the allelochemical content of the tissue (e.g. Xuan and Tsuzuki, 2002; Bertholdsson, 2004). At the same time, various studies showed that concentrations of allelochemicals in plants are not stable, but change in response to abiotic and biotic environmental factors, and in relation to age or growth stage (Figure 1.2a).

Nutrient deficiency, for instance, has often been observed to cause an increase in the allelochemical content of plants (e.g. Armstrong et al., 1970; Lehman and Rice, 1972; Mason Sedun and Jessop, 1989; Mwaja et al., 1995). Chaves and Escudero (1999) pointed at various studies that discovered a relationship between flavonoid synthesis and weather conditions, including ultraviolet light, precipitation and temperature.

Mason Sedun and Jessop (1989) found high temperatures (30 °C versus 15 °C day

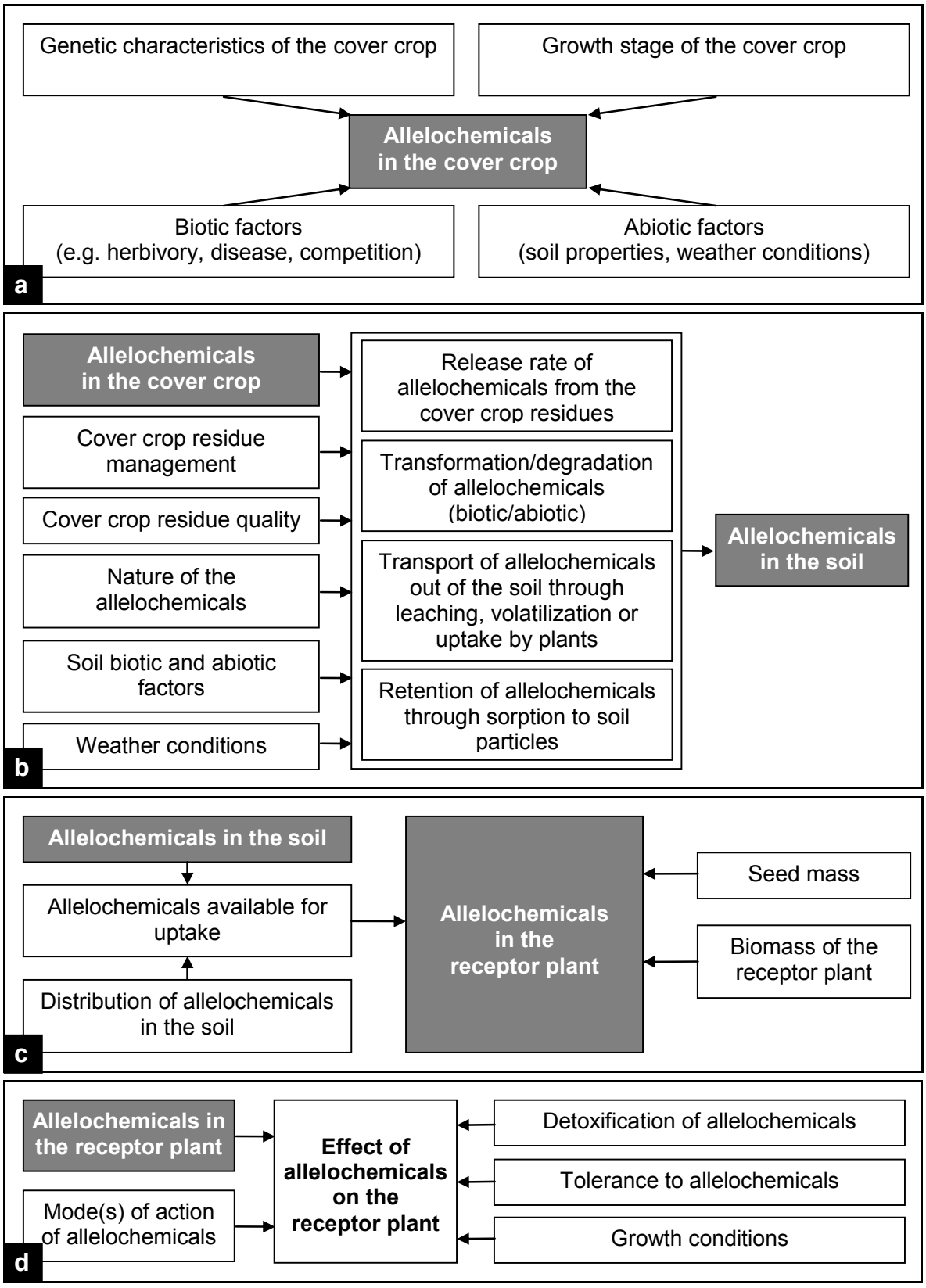

Figure 1.2. Conceptual framework of the allelopathic effects of cover crops, divided in four phases; (a) allelochemicals in the cover crop, (b) allelochemicals in the soil, (c) allelochemicals in the receptor plant and (d) the effect of allelochemicals on the receptor plant.

temperature) and short days (8 hours light versus 16 hours light) during the growth of *Brassica napus* and *Brassica campestris* to increase the allelopathic potential of the residue. There are some indications that (mild) water stress can induce allelochemical synthesis (e.g. Richardson and Bacon, 1993; Estiarte et al., 1994; Liu, 2000).

Additionally, research on plant defence against herbivores and pathogens has shown that many plants can enhance their chemical defence level in response to biotic stress (Karban and Baldwin, 1997). There are also some indications that plant-plant chemical interactions can influence the level of allelochemicals in the plant. Kong et al. (2004) found higher concentrations of a flavone and a cyclohexenone compound, both possessing allelopathic activity, in rice exudates when rice was grown in the presence of *Echinochloa crus-galli*. Dayan (2006) showed that sorgoleone levels in *Sorghum bicolor* increased in plants treated with a crude extract of velvetleaf (*Abutilon theophrasti*) root. We are not aware of any studies addressing the influence of intraspecific competition on the allelochemical content. However, MacLeod and Nussbaum (1977), who investigated the effects of different horticultural practices on the chemical flavour composition of cabbage, found that higher *Brassica oleracea* densities caused an increase in the production of isothiocyanates.

Plant age has been associated with a decrease of hydroxamic acids in both rye (*Secale cereale* L.) (Reberg Horton et al., 2005; Rice et al., 2005) and maize (*Zea mays* L.) (Morse et al., 1991). Clossais-Besnard and Lahrer (1991) observed an accumulation in the glucosinolate content of rape (*Brassica napus* L.) during the vegetative growth and seed maturation stages and a decline during the flowering, germination and early seedling growth stages. That the ability of plants to induce synthesis of allelochemicals in response to environmental conditions is dependent on the growth stage has been shown by Ohnmeiss and Baldwin (2000), who found that whole-plant nicotine contents in tobacco were increased by leaf damage applied in the rosette-stage, but not in the elongation- or flowering-stage.

Allelochemicals in the soil

Following cover crop residue incorporation, allelochemicals are released into the soil upon tissue disruption, by means of leaching or volatilization (Figure 1.2b). Tissue disruption can be established mechanically, by pre-treating the residue prior to incorporation, or via residue decomposition. The decomposition rate of residue material is dependent on residue quality, residue loading rate, soil abiotic and biotic properties, and weather conditions, such as temperature and moisture (e.g. Parr and Papendick, 1978). However, residue decomposition may also be influenced by residue pre-treatment, as the decomposition rate is dependent on particle size (e.g. Ambus and Jensen, 1997; Angers and Recous, 1997), and by residue placement, as residues

retained on the soil surface decompose more slowly than residues incorporated in the soil (e.g. Dou et al., 1995).

Once allelochemicals enter the soil, a number of interacting processes take place, which include *transformation, transport* and *retention* of allelochemicals (Figure 1.2b). Retention of allelochemicals takes place through, for example, sorption to soil particles (Cheng, 1992). Transport of allelochemicals out of the soil can occur by means of leaching, volatilization and/or uptake by plants. Degradation of allelochemicals is mainly accomplished by microorganisms, but some plant species are also able to degrade allelochemicals in their surroundings by exudation of certain enzymes (e.g. Gramss and Rudeschko, 1998). On the other hand, microorganisms can transform allelochemicals into more toxic compounds. For example BOA, an allelochemical mainly occurring in rye, can be transformed by microbes into the more toxic allelochemical 2-amino-phenoxazin-3-one (APO) (Chase et al., 1991; Gagliardo and Chilton, 1992; Understrup et al., 2005). Apart from biochemical transformation, allelochemicals can also be transformed by strictly chemical processes, such as oxidation, reduction, hydrolysis, substitution, complexation and polymerization (Cheng, 1992).

The rates of transformation, transport and retention of allelochemicals can be influenced by numerous factors, including the nature of the allelochemicals, soil abiotic and biotic properties and weather conditions. With respect to the nature of the allelochemicals: their solubility affects their mobility in soil water, their vapour pressure affects their volatility in the air, and their structure affects their affinity to soil particles and their degradability by microorganisms (Cheng, 1992). Allelochemicals can lower their transformation rates by expressing antimicrobial activity, which is for instance the case for the alkaloid caffeine released from coffee litter (Einhellig, 1986). Soil properties, such as the soil mineral and organic matter content, particle size distribution, pH and ion exchange characteristics, and oxidation state, play a prominent role in influencing the behaviour of a chemical in the soil (Cheng, 1992). Cecchi et al. (2004) found soil organic matter, free metal oxides and clay content to be positively correlated to sorption of certain phenolics acids. Soil pH can affect the ionization state of the allelochemicals and, in turn, their mobility (Cheng, 1992). Some factors, such as soil moisture and temperature, indirectly affect the transformation rate of allelochemicals through their influence on microbial activity. At the one side, water-soluble allelochemicals persist for a longer period in a dry environment (Einhellig, 1999). At the other extreme, increased persistence of organic and phenolic acids has been observed in water-logged soils (Wang et al., 1968, 1971; Shindo and Kawatsuka, 1977, c.f. Einhellig, 1986). Soil moisture content can also have an influence on the transport rates of volatile allelochemicals, such as isothiocyanates. Borek et al. (1995)

discovered that allyl isothiocyanate disappeared more rapidly from the soil with reduced soil moisture.

Allelochemicals in the receptor plant

Allelochemicals in the soil can be taken up by receptor plants, which in our system are represented by weed plants (target) and crop plants (non-target; Figure 1.2c). Only allelochemicals that are close enough to the seed or root can be taken up by the receptor plant. The amount of allelochemicals available for uptake does therefore not only depend on the total amount of allelochemicals in the soil, but also on their distribution. The distribution of allelochemicals is in turn determined by the distribution of cover crop residues, which depends on residue management, and by all factors affecting the mobility of the allelochemicals in the soil.

Apart from the allelochemicals available for uptake, both seed mass and total biomass of the receptor plant may affect the concentration of allelochemicals ending up in the plant tissue. Although the amount of allelochemicals taken up by a plant will be higher with increasing total root length, the concentration of allelochemicals in the plant tissue is expected to decline with increasing plant biomass. Uptake of allelochemicals by small-seeded species is thought to generally result in a higher concentration of allelochemicals in the plant tissue compared to large-seeded species. This because small-seeded species usually have a larger root length per unit of root mass (Leishman et al., 2000), which corresponds to a larger absorptive surface area through which allelochemicals might enter (Liebman and Sundberg, 2006).

Furthermore, uptake rates of allelochemicals may depend on root membrane permeability. Although we could not find any studies addressing this issue, there is some indirect proof coming from a study from Shafer and Schönherr (1985) that species differ in membrane permeability for allelochemicals. They found differences as large as one to two orders of magnitude in membrane permeability for phenolic compounds between cuticles of mature tomato (*Lycopersicon*) and green pepper (*Capsicum*) fruits and the adaxial surface of rubber (*Ficus*) leaves, and suggest membrane permeability for phenolics is related to the lipid composition of the membrane.

The effect of allelochemicals on the receptor plant

Allelochemicals can exert a direct effect on the receptor plant, or an indirect effect through inhibition of symbiotic bacteria and fungi (e.g. Dawson and Seymour, 1983; Rose et al., 1983). We will focus here on the direct effects. The effect that allelochemicals in the tissue of the receptor plant will have on the growth or development of the receptor plant depends on the mode(s) of action of the

allelochemicals, the ability of the receptor plant to detoxify the allelochemicals, the tolerance of the receptor plant to allelochemicals and the growth conditions of the receptor plant (Figure 1.2d). The extent of the inhibitory action of allelochemicals is concentration dependent, with very low concentrations often even exerting a stimulatory effect on plant growth (e.g. Lovett et al., 1989). Individual allelochemicals are seldom present in sufficient concentration to negatively affect the receptor plant. However, cover crop residues often contain different kinds of allelochemicals, which can inhibit plant growth through additive or synergistic action (Einhellig, 2004).

To create a better overview of the mode(s) of action of allelochemicals, we divide the effects on receptor plants into three levels: (1) the molecular mechanism of action, (2) the biochemical and physiological responses induced and (3) the visual injury of affected plants. Most allelopathic research focuses on the third level: the allelopathic potential of allelochemicals, plant extracts or plant residues is usually assessed by monitoring the rate of germination, root elongation or overall seedling growth. Inhibition of seed germination often requires higher concentrations of allelochemicals than inhibition of seedling growth (Einhellig, 1999). Rice (1984) went one step deeper and described the effects allelochemicals can have on plant biochemistry and physiology. He included a long list of processes, ranging from the division, elongation and ultra-structure of cells to hormone-induced growth, membrane permeability, mineral uptake, stomatal opening and photosynthesis, respiration, protein synthesis, lipid and organic acid metabolism, enzyme activity and plant-water relationships. However, at that time – and still – it was difficult to separate secondary effects from primary causes. This is nicely illustrated by a review of Einhellig (2004) on the modes of action of phenolic acids, which are among the most widely distributed allelochemicals. The initial actions of phenolic acids are on cell membranes, resulting in non-specific permeability changes that alter ion fluxes and hydraulic conductivity of roots. These membrane perturbations are, however, followed by a cascade of physiological effects that include alterations in ion balance, plant-water relationships, stomatal function, and rates of photosynthesis and respiration. Knowledge on the first level – the molecular mechanisms of action – is still scarce, but has received increasing attention since the last 10–15 years (e.g. Wink et al., 1998; Duke and Dayan, 2006). Whereas herbicides target only a few molecular sites – the approximately 270 herbicides currently on the market have only 17 modes of action, with almost half of them acting on three sites – allelochemicals presumably have a much larger spectrum of target sites (Macias et al., 2007). Apart from gaining more fundamental insight in the modes of action of allelochemicals, an important driving factor for research at this level has been the identification of new molecular targets for herbicide design.

Detoxification of allelochemicals has rarely been studied at the molecular level, but studies on the detoxification of herbicides are plentiful. Because mechanisms that are involved in detoxification of herbicides have certainly developed during evolution to reduce or compensate the reactivity of natural compounds, information on herbicide detoxification is likely to be also valid for allelochemicals. Schulz and Friebe (1999) reviewed the detoxification of allelochemicals by plants, which they describe as a three-step mechanism, which had been first introduced by Cole (1994). The first step in this mechanism deals with increasing the polarity of the incorporated allelochemicals through hydroxylation, dealkylation or oxidation. This is followed by conjugation of the modified allelochemicals with plant constituents such as sugars, malonic acid, or amino acids, in this way masking the reactivity of functional groups that are responsible for the phytotoxic effect. Finally, the conjugated allelochemicals are stored within vacuoles or excreted into the environment. Enzymes such as oxidases, peroxigenases and transferases are necessary for detoxification, and can be either constitutive or induced by allelochemicals. Another strategy is polymerization of absorbed compounds and subsequent deposition within the cell walls. Plant species differ in the rate of allelochemical detoxification and in the mode of enzyme induction (Schulz and Friebe, 1999). It has been hypothesized that large-seeded species may be better to detoxify allelochemicals than small-seeded species, because of the greater seed reserves (Liebman and Sundberg, 2006).

Plant species may also differ in their tolerance to allelochemical stress. Larger-seeded species with greater seed reserves may, for instance, better support seedling respiration during periods of stress-induced carbon deficit (Westoby et al., 2002). Finally, the growth conditions of the receptor plant are of major importance in determining the effect of allelochemical stress. The idea that allelopathic effects are more pronounced when receptor plants are also affected by environmental stresses is widely accepted. Interactions between environmental and allelochemical stress have been reviewed by Einhellig (1999) and Pedrol et al. (2006). In our context it is useful to make a distinction between environmental stress factors that are induced by the presence of cover crop residues and environmental stress factors that act independently of cover crop residues. This first category includes, depending on the specific circumstances, increased activity of soilborne pathogens, pests, water stress, nitrogen deficiency, and reduced light quantity (see also Figure 1.1).

Population increases of *Pythium* (Conklin et al., 2002; Manici et al., 2004), *Rhizoctonia* (Dabney et al., 1996) and *Fusarium* (Lynch, 1987) spp. following crop residue incorporation have been observed. Instead of a merely additive effect, in some cases pathogens and allelochemicals may interact synergistically. Phenolic compounds, for instance, are known to interfere with cell membrane permeability

(Einhellig, 2004), resulting in higher exudation of organic molecules into the spermo- or rhizosphere. As propagule germination and germtube elongation of soilborne pathogens, like *Pythium* spp. (Martin and Loper, 1999) as well as direction of growth (*R. solani*) or movement (*Pythium*), is enhanced by the presence of plant exudates, increased exudation resulting from phenolic action may have caused higher seed(ling) infection rates. Not only pathogens, but also pests residing in the soil can be stimulated in the presence of crop residues. Hammond (1990) observed an increase in the population of the seedcorn maggot *Delia platura* following cover crop residue incorporation in the soil, with largest increases following the incorporation of lucerne (*Medicago sativa* L.), followed by rye (*Secale cereale* L.), soyabean (*Glycine max* L.) and maize (*Zea mays* L.) residues. Instead of cover crop residues causing an increase in pathogen and pest development, allelochemicals from cover crop residues may exert an inhibitory effect on pathogens and pests. A good example to illustrate this is the biofumigation potential of *Brassica* spp., for which volatile breakdown products of glucosinolates are responsible (e.g. Brown and Morra, 1997; Matthiessen and Kirkegaard, 2006).

With regard to abiotic stress factors, decomposition of cover crop residues results in an increased osmotic pressure of the soil solution, and in this way reduces the

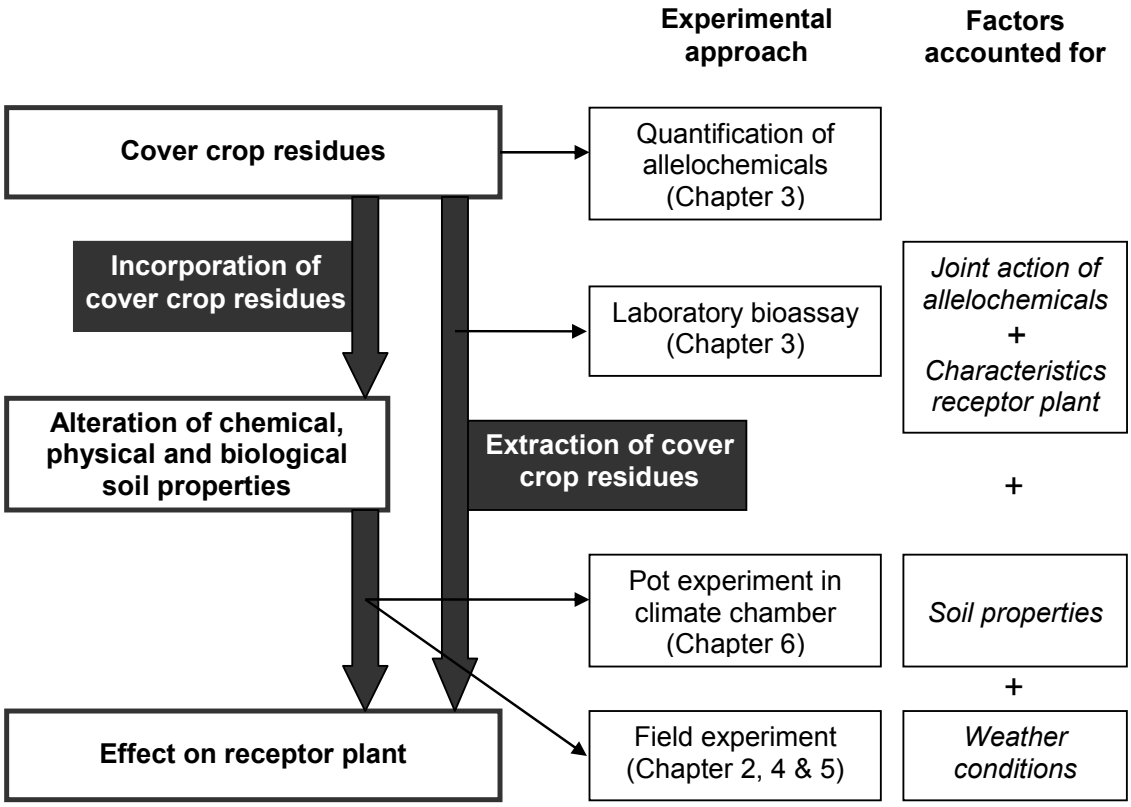

Figure 1.3. The experimental approach used in each chapter and the associated level of complexity.

availability of water to plants. At the same time, however, cover crop residues improve the retention of water in the soil. Decomposition of high C:N residues can cause temporary immobilization of nutrients (Stevenson, 1986, in Liebman and Mohler 2001) and cover crop residues retained on top of the soil can reduce the amount of light reaching the seedling (Teasdale and Mohler, 1993).

Objectives and experimental approach

The central objective of this study was to explore and optimize the contribution of rotational cover cropping to ecological weed management in organic farming systems. We initiated our study on the field scale with a broad exploration of the potential of a series cover crop species, sown at different densities, to suppress weed biomass accumulation in autumn and inhibit weed and crop establishment in spring (Chapter 2). After this broad exploration, several management options to optimize the inhibitory effects of cover crop residues in spring were identified, each of which was linked to one of the consecutive phases of the conceptual model on allelopathic effects of cover crop residues described earlier. Figure 1.3 shows the experimental approach that was used for each experiment and the associated level of complexity.

All experiments were carried out on sandy soil, because of the usually higher weed pressure on these soils and the fact that soil tillage is carried out in spring and not in autumn, as is the case for clay soils. We selected six cover crop species belonging to three different plant families (Table 1.1), which contain different groups of allelochemicals. In the *Poaceae*, hydroxamic acids are the main group of allelochemicals

Table 1.1. Six cover crop species selected for experimentation and their recommended sowing time.

Plant family		Cover crop		Sowing recommended until*
Brassicaceae	winter hardy	Winter oilseed rape	*Brassica napus* cv. Emerald	Mid September
	frost sensitive	Fodder radish	*Raphanus sativus* cv. Brutus	End of August
Poaceae	winter hardy	Winter rye	*Secale cereale* cv. Protector	Beginning of October
	frost sensitive	Italian ryegrass	*Lolium multiflorum* cv. Fabio	End of August
Fabaceae	winter hardy	Lucerne	*Medicago sativa* cv. Mercedes	End of July
	frost sensitive	White lupine	*Lupinus albus* cv. Weibit	Mid August

*Source: Recommended List of Varieties of Field Crops (Anonymous, 2004)

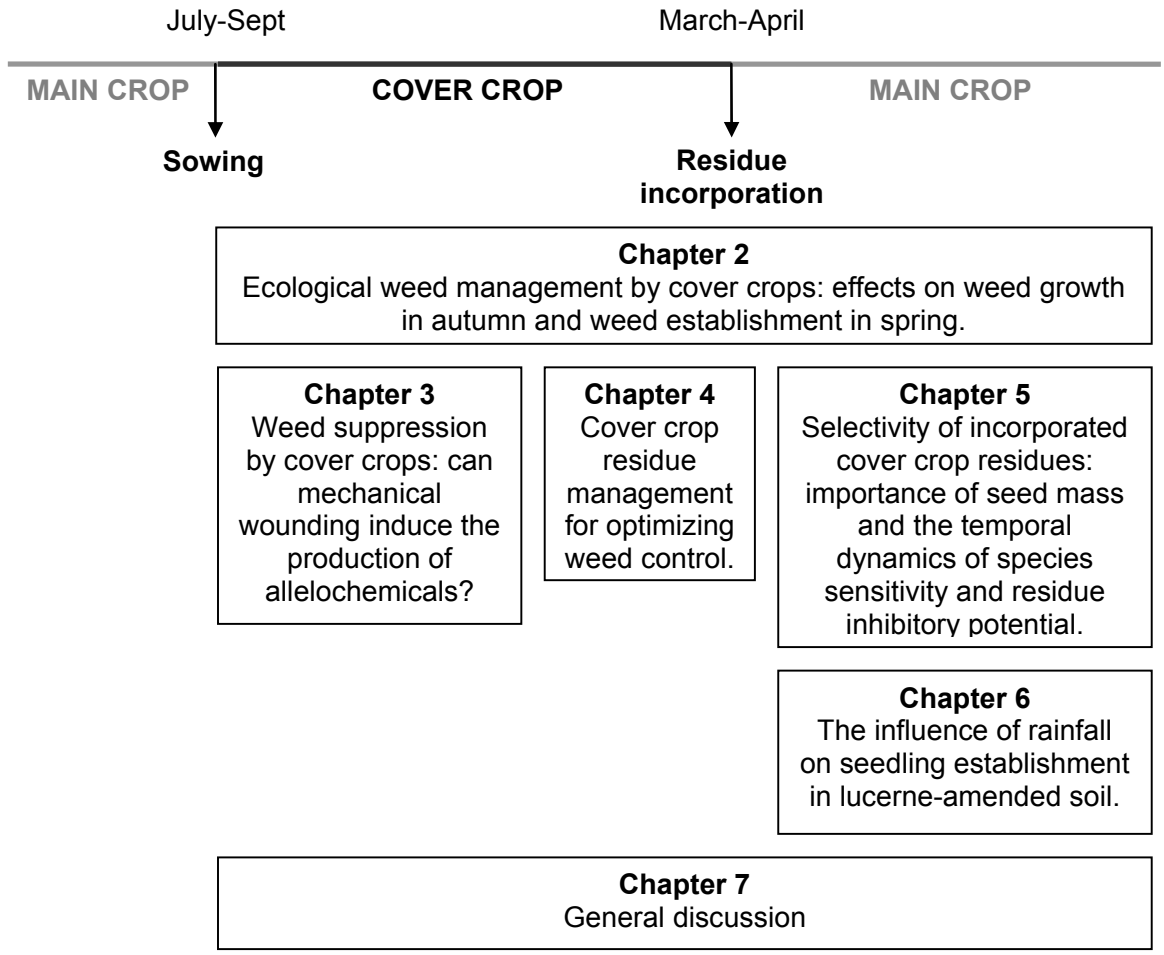

Figure 1.4. Structure of the thesis.

(Niemeyer, 1988). Allelopathy in *Brassica* species has been primarily attributed to the hydrolysis products of glucosinolates, most of which are volatile (Teasdale and Taylorson, 1986; Oleszek, 1987; Bialy et al., 1990; Brown and Morra, 1996; Petersen et al., 2001; Siemens et al., 2002; Haramoto and Gallandt, 2004). Lucerne contains several groups of allelochemicals, including saponins, flavonoids and phenolic acids (Dornbos et al., 1990; Oleszek, 1993; Xuan et al., 2003). White lupine contains quinolizidine alkaloids that act as a herbivore deterrent (e.g. Vilarino et al., 2005), but have also been suggested to influence plant-plant interactions (Wink, 1983). Especially bitter cultivars contain a high level of these alkaloids.

Figure 1.4 summarizes the structure of the thesis. In Chapter 3, mechanically damaging plants as a possibility to increase the allelochemical content of the cover crop just prior to residue incorporation was studied. Mechanical damage was used to mimic herbivore damage, which is known to induce the synthesis of allelochemicals, and therefore has potential to increase the allelochemical content of the cover crop.

In Chapter 4, cover crop residue management options to increase the inhibitory effect of cover crop residues on weeds were explored. The possible influences on cover crop residue pre-treatment and placement on the release rate of allelochemicals was described in the conceptual model earlier in this chapter. Manipulating the release rate of allelochemicals from the residues, and thereby influencing the time course of allelochemicals in the soil, may change the effect on the receptor plant. Apart from an influence of the release rate, cover crop residue management also influences the distribution of allelochemicals in the soil, and therefore the amount of allelochemicals available for uptake by the receptor plant.

In Chapter 5, we wanted to find out whether differences in seed mass could be used to target small-seeded weed species and avoid negative effects on large-seeded crop species. A higher seed mass may result in a lower concentration of allelochemicals in the seedling, and at the same time increase the tolerance to allelochemicals and the ability to detoxify allelochemicals. The results of Chapter 5 showed a sudden and large increase in the inhibitory effects of soil-incorporated lucerne residues, which was likely to be related to a rainfall peak. The influence of a rainfall peak on the strength of lucerne residue-mediated effects was therefore further investigated in Chapter 6.

In a final chapter (Chapter 7) the contribution of the research presented in this thesis to the understanding of cover crop effects on weeds, as well as the possibilities for optimization of these effects through management were discussed. Subsequently, the findings of this thesis were placed in a broader systems perspective. Here, attention was given to the possibilities of combining various cover crop services and the potential of including cover crops as one of the components in an all encompassing ecological weed management strategy. Finally, future research needs regarding cover crop-based ecological weed management were discussed.

CHAPTER 2

Ecological weed management by cover cropping: effects on weed growth in autumn and weed establishment in spring

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Summary
Cover crops grown in the period between two main crops have potential to develop into an important component of a system-oriented ecological weed management strategy. In late summer and autumn the cover crop can suppress growth and seed production of weeds, whereas the incorporation of cover crop residues in spring may reduce or retard weed emergence. Based on these two criteria, six cover crop species were evaluated for their weed suppressive potential in two years of experimentation. Fodder radish, winter oilseed rape and winter rye had the strongest competitive ability in autumn; the competitive strength of Italian ryegrass was intermediate and white lupine and lucerne were poor competitors. Competitiveness was strongly correlated to early light interception. Surprisingly, doubling the recommended sowing density did not increase weed suppressive ability. Although a poor competitor in the fall, after incorporation in spring lucerne had the strongest inhibitory effect on seedling establishment, followed by winter oilseed rape and white lupine. Winter rye and fodder radish did not affect seedling establishment, whereas Italian ryegrass was not evaluated because of re-growth after incorporation. Establishment of the indicator species lettuce and sugar beet reflected the response of *Chenopodium album* in residue-amended soil, suggesting the validity of using crop seeds as indicator species in comparative studies. Competition in autumn and subsequent residue-mediated suppression of weed establishment in spring varied among the cover crop species evaluated, with winter oilseed rape offering relatively strong effects during both periods.

Keywords: cover crops, ecologically-based weed management, competition, green manure, allelopathy, organic farming

Introduction

In organic farming systems, where the use of pesticides is excluded, 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). Simply replacing herbicides by other direct control measures is inadequate. A heavy reliance on mechanical cultivation is undesirable because of damage to soil structure, increased risk of erosion and frost damage to crops and a strong dependency on weather conditions. Hand weeding is therefore often used, and this requires the availability of sufficient labor and is costly. Consequently, the weed problem cannot just be solved by curative tactics; instead weed management should be seen as a component of integrated cropping systems design. Rather than focusing on the detrimental effects of weeds in current crops, the time horizon of interest should be extended and main emphasis should be given to the management of weed populations (Barberi, 2002; Riemens et al., 2007a). Consequently, systems-oriented approaches to weed management that make better use of alternative weed management tactics need to be developed (Liebman and Davis, 2000; Barberi, 2002).

Cover crops have potential to form an important, pro-active component in such a system-oriented approach. Cover crops are grown for various reasons, like prevention of nitrogen leaching, improvement of soil structure, soil enrichment by nitrogen fixation and control of soil borne diseases, such as nematodes (Sarrantonio and Gallandt, 2003). A promising strategy is to grow cover crops during the period that the main crop is absent. Inclusion of cover crops in crop rotations introduces two important mechanisms through which the development of weed populations may be hampered. In late summer and autumn, the successful introduction of cover crops can prevent growth, development and, most importantly, seed production of weeds through competition. Indeed, cover crops fill gaps in cropping systems that would otherwise be occupied by weeds (Liebman and Staver, 2001). In springtime, cover crop residues incorporated in the upper layer of the soil may suppress or retard weed emergence and growth due to allelopathic effects (e.g. Al Khatib, 1997; Ohno et al., 2000), to stimulation of soilborne pathogens (Conklin et al., 2002) or to an interaction of these two factors.

In order to evaluate the potential of this strategy for ecological weed management, we compared the potential of six cover crop species for their competitive ability in autumn and for the inhibitory effect of their incorporated residues on weed seedling establishment in spring. We also assessed whether these aspects were cover crop density dependent. In relation to this the following hypotheses were formulated: (1) autumn weed suppression is positively correlated to early light interception by the

cover crop, (2) weed suppression by cover crops can be further enhanced by increasing sowing density and (3) suppression of weed establishment in spring is greatest for overwintering cover crop species and least or absent for winter-killed species. In order to include a broad and balanced range of cover crop species, we selected both a winter hardy and a frost-sensitive species from each of the families *Brassicaceae, Poaceae* and *Fabaceae*.

Materials and methods

Experimental set-up

Field experiments were carried out in 2003–2004 and 2004–2005 at the biological experimental farm "Droevendaal" of Wageningen UR, Wageningen, the Netherlands. The experimental fields were located on sandy soil with an organic matter content of 3.6–4.5% and a pH-KCl of 4.7–5.7. On all experimental fields the preceding crop was triticale (*Triticum aestivum* L. × *Secale cereale* L.), which was harvested on July 11, 2003 and July 20, 2004.

In the summer of 2003, two field experiments (A and B) were established using a randomized complete block design with four replicates. The treatments consisted of 6 different cover crops (Table 2.1) and one control treatment (no cover crop). Each plot was divided into four sub-areas, three of which were used for data collection in autumn (A1–A3) and one that was used for experimentation in spring (A4). Total plot size was 54 m^2 (6 × 9 m). Fertilization was applied on July 14 by injecting 15,000 kg ha^{-1} of cow manure (NPK 5.6, 4.2, 9.7) into the soil followed by soil tillage with a disc harrow the next day.

The seedbed in experiment A was prepared on July 24 with a reciprocating harrow. Cover crops were broadcast sown with a harrow behind the sowing machine on July 24 (white lupine and Italian ryegrass) and July 25 (fodder radish, winter oilseed rape, winter rye and lucerne). After sowing, the soil was superficially tilled with a reciprocating harrow to place the seeds at an approximate depth of 2 cm after which a Cambridge roller was used to slightly compact the soil. Seedbed preparation in experiment B was carried out on August 19 with a reciprocating harrow. The next day a rotary cultivator was used to create a more compact seedbed. Cover crops were sown at a row spacing of 12.5 cm and at a depth of 3–4 cm on August 22 (white lupine) or at a depth of 2–3 cm on August 21 (all other cover crops). Sowing density of each cover crop species was based on the recommended seeding rate (kg ha^{-1}) mentioned in the Recommended List of Varieties of Field Crops (Anonymous, 2004).

In both experiments seeds of white lupine and lucerne were inoculated with *Rhizobium lupini* and *Rhizobium meliloti*, respectively. Because of the extreme hot and

dry weather conditions in July and August 2003, experiment A was sprinkle irrigated on August 7 (5 mm), August 8 (10 mm) and August 14 (10 mm) and both experiments were sprinkle irrigated on August 23 (10 mm).

For experiment C, which started in the summer of 2004, the three winter-hardy cover crop species of experiment A and B were sown at three different densities (Table 2.1). The lowest density of each cover crop species was set to the recommended seeding rate; additional densities were 1.5 and 2.0 times the recommended rate. The experiment was arranged as a randomized complete block design with 4 replications. Plot size was 90 m^2 (10.5 × 8.5 m) for lucerne treatments and 77 m^2 (9 × 8.5 m) for winter rye and winter oilseed rape treatments. Each plot was subdivided into two areas used for data collection in autumn (A1 and A2) and one area for experimentation in spring (A4). Area 4 received the same treatments as areas 1 and 2, but was divided into two parts to which a different amount of fertilizer was applied.

The experimental field was ploughed to a depth of 30 cm on July 29 and limed with 5000 kg ha^{-1} Dolokal (80% $CaCO_3$, 19% $MgCO_3$) on August 1. One day before sowing, plots were fertilized with 1600 (A1–2, A4a) or 800 (A4b) kg ha^{-1} NPK (5-6-13) ecological fertilizer granules (Ecostyle). Lucerne, inoculated with *Rhizobium meliloti*, was sown on August 2, and winter rye and winter oilseed rape were sown on September 2. Due to the different sowing dates, two control plots were included in the experiment. All nine cover crop × sowing density combinations were sown at a depth of 2 cm and a row spacing of 12.5 cm. No irrigation was applied.

In area A2 natural weeds were allowed to grow, whereas all other areas were kept weed-free. In area A3 (experiment A and B), *Vicia sativa* was introduced as a model weed, and manually sown between the cover crop plants at 2 cm depth and with an interplant distance of 15 cm. In experiment A, two times 77 seeds per plot were sown on August 1 (7–8 days after sowing (DAS); block 1 and 2) and August 4 (10–11 DAS; block 3 and 4). In experiment B, two times 32 *V. sativa* seeds per plot were sown on September 2 (11–12 DAS).

Observations in autumn

The number of emerged cover crop seedlings was recorded. Height and light interception (experiment A and B) or soil cover (experiment C) fraction were monitored on a weekly basis in area A1. Light interception was measured on 10 positions within each plot, using a SunScan Canopy Analysis System (Delta T Devices Ltd., Cambridge, UK). Soil cover was visually assessed by using a 0.5×0.5 m frame containing a grid of 100 squares of 5×5 cm. At 6 positions within each plot the fraction of green surface was estimated within the 10 squares forming a diagonal within the frame. Above-ground dry weight (DW) of the cover crops was measured at 101 DAS

(experiment A), 76 DAS (experiment B), 73 DAS (lucerne, experiment C) and 67 DAS (winter oilseed rape and winter rye, experiment C). Dry weight per unit area was determined by clipping at ground level in two quadrats of 0.25 m^2 and subsequent drying at 70 °C for at least 3 days.

Above-ground DW of natural weeds was determined by clipping weed plants from a randomly placed 1×1 m quadrat at ground level. Weeds were harvested on September 11 and October 16 (experiment A), on October 16 and November 10 (experiment B), and on September 20 (only lucerne) and November 8 (all cover crop species; experiment C). For weed species present in high abundance DW was separately determined.

In experiment A, half of the *V. sativa* plants were harvested on September 11 and the other half on November 11 by cutting plants at ground level. In experiment B, harvesting of the *V. sativa* plants was carried out on October 16 and November 10.

Analysis of autumn data

Daily values for light interception or soil cover fraction were obtained for each plot by fitting a three-parameter logistic curve through the values measured at each sampling date, assuming a binomial distribution:

$$f\left(t\left(b,c,T_{50}\right)\right)=\frac{c}{1+\exp\left\{-b\left(\left(t\right)-\left(T_{50}\right)\right)\right\}} \quad (1)$$

In equation 1, parameter c represents the upper limit, which was fixed to the maximum measured value of each plot. Parameter T_{50} is the time (expressed in DAS) at which 50% of the maximum value was reached and parameter b is the maximum relative rate of increase. Time, expressed in DAS, is denoted by t.

The generalized linear model (GLIM) procedure was used to test for differences in parameter values and for differences in DW of natural weeds (g m^{-2}) and of *V. sativa* (mg $plant^{-1}$). For maximum values of light interception and soil cover, the GLIM procedure was applied with a binomial distribution. Pairwise t-tests were used to test for significance between treatments.

For experiment A and B the area under the light interception curve (AULIC) was calculated for each treatment:

$$AULIC=\sum_{i=0}^{n}\left[\left(t_i-t_{i-1}\right)\cdot\left(y_i+y_{i+1}\right)\cdot\tfrac{1}{2}\right] \quad (2)$$

In equation 2, t_i represents time (DAS), y_i is the light interception fraction on the i^{th} day after sowing and n is the day of harvest of natural weeds or *V. sativa*. Correlation coefficients (r) between AULIC and the DW of natural weeds and *Vicia sativa* were

calculated. The AULIC was also obtained for shorter time periods starting from sowing of the crop, to find out whether early light capture provided a better indication for weed suppressive ability of a cover crop. All statistical procedures were carried out using the Genstat 9 statistical package (Payne et al., 2006).

Observations in spring

Above-ground DW of cover crops was determined on March 22, 2004 in experiment A and B and on April 4, 2005 in experiment C. On March 31, 2004, cover crops growing in area A4 of experiment A were cut in pieces with a flail mower and mixed through the upper 10 cm of the soil with a rotary cultivator. From April 5–7, 2005, the same procedure was followed in experiment C, except that after flailing and before incorporation the cover crops were cut at 1 cm below ground level. This was done in order to avoid re-growth problems with Italian ryegrass and winter rye that were encountered in the previous year. Only plots with the highest and the lowest sowing density were incorporated. In experiment B, cover crop material was not incorporated due to time constraints and high weed pressure.

After residue incorporation, 100 seeds of lettuce (*Lactuca sativa* L.) and sugar beet (*Beta vulgaris* L.) were sown in each plot at 2 cm depth, either in rows (experiment A) or in quadrats (experiment C), and with a mutual distance of 5 cm. Seedling emergence of both species was determined. Additionally, in experiment A the number of emerged natural weeds growing within a 1×1 m square were counted on May 3, 10, 17 and 24.

Analysis of spring data

All data of experiment A and B were analysed using the GLIM (Generalized Linear Model) procedure. For the total fraction of emerged lettuce and sugar beet seedlings, a binomial distribution was used. Pairwise t-tests were used to test for significance between treatments. The emergence fractions of lettuce and sugar beet were correlated to the number of emerged *C. album* seedlings.

In experiment C, a GLMM analysis (General Linear Mixed Model) using the IRREML (Iterative Reweighted Residual Maximum Likelihood) procedure was used to test whether cover crop species, sowing density and fertilization rate had an effect on lettuce and sugar beet emergence. This analysis was followed by a Wald-test to test for significance of main and interaction effects. To test for significant differences between treatments, t-tests were used. The effect of cover crop density was tested for all data by using the GLIM procedure. Again, all statistical procedures were carried out using the Genstat 9 statistical package (Payne et al., 2006).

Table 2.1. Cover crop species, frost resistance (WH = winter hardy, FS = frost-sensitive), recommended sowing time, sowing density (kg ha^{-1} and # seeds m^{-2}) and % establishment of the cover crop for experiment A, B and C. Values in parentheses denote the SE.

Exp		Cover crop		Abbr	Sowing recommended until*	Sowing density (kg ha^{-1})	Sowing density (# seeds m^{-2})	Establishment (%)
A	WH	Lucerne	*Medicago sativa* cv. Mercedes	LU	End of July	25	1282	13 (1.0)
	FS	White lupine	*Lupinus albus* cv. Weibit	WL	Mid August	160	47	57 (5.0)
	WH	Winter oilseed rape	*Brassica napus* cv. Emerald	WO	Mid September	7	139	60 (4.5)
	FS	Fodder radish	*Raphanus sativus* cv. Brutus	FR	End of August	15	82	55 (5.7)
	WH	Winter rye	*Secale cereale* cv. Protector	WR	Beginning October	150	473	37 (2.7)
	FS	Italian ryegrass	*Lolium multiflorum* cv. Fabio	IR	End of August	30	682	34 (3.3)
B	WH	Lucerne		LU		25	1282	43 (6.9)
	FS	White lupine		WL		160	47	69 (9.3)
	WH	Winter oilseed rape		WO		7	139	63 (6.8)
	FS	Fodder radish		FR		15	82	68 (8.2)
	WH	Winter rye		WR		150	473	20 (2.1)
	FS	Italian ryegrass		IR		30	682	58 (6.9)
C	WH	Lucerne		LU_1		25	1282	98 (1.1)
	WH			LU_2		38	1923	95 (2.4)
	WH			LU_3		50	2564	86 (7.4)
	WH	Winter oilseed rape		WO_1		7	139	79 (1.2)
	WH			WO_2		11	208	74 (1.7)
	WH			WO_3		14	277	73 (0.5)
	WH	Winter rye		WR_1		150	473	89 (3.8)
	WH			WR_2		225	710	82 (4.4)
	WH			WR_3		300	946	80 (3.0)

*Source: Recommended List of Varieties of Field Crops (Anonymous, 2004)

Results

Establishment of the cover crop species

Establishment of cover crop plants in experiment A and B (on average 49%) was rather poor when compared to experiment C (on average 84%; Table 2.1). In experiment A, seedling establishment was probably reduced due to dry conditions. For lucerne, only 13% of its seeds turned into viable seedlings. Establishment of Italian ryegrass and winter rye was intermediate (34–37%), whereas the other species reached 55–60% establishment. In experiment B, lucerne was discarded as its seedlings were outcompeted by weeds due to a high weed pressure combined with a late sowing date. Establishment of winter rye seedlings was severely reduced due to bird predation. Establishment of the other cover crop species was slightly better than that in experiment A (58–69%). In experiment C, establishment slightly decreased with increasing sowing density for all cover crop species. Consequently, the realized plant density averaged over all three cover crops, was 1: 1.42: 1.81 instead of 1: 1.5: 2.

Cover crop growth characteristics

Maximum light interception fraction (LI) was lowest for white lupine (0.86 and 0.68 in experiment A and B, respectively) and lucerne (0.87 in experiment A). All other cover crops reached a maximum LI above 0.93 (Figure 2.1, Table 2.2). Differences between cover crop species in the time needed to reach 50% of the maximum LI (T_{50}) were more pronounced in experiment A than in experiment B. In both experiments fodder radish had the shortest T_{50}, followed by winter oilseed rape. Whereas in experiment A the T_{50} value of winter rye was only slightly longer than that of fodder radish and winter oilseed rape, winter rye had the longest T_{50} in experiment B. This probably resulted from the relatively low percentage of established winter rye seedlings in experiment B.

All cover crop species in experiment C reached a maximum soil cover fraction above 0.96 (Table 2.2), but differed in T_{50}. In spite of the earlier sowing date, lucerne took nearly twice as long to reach 50% soil cover (48 days) compared to winter oilseed rape and winter rye (25 and 23 days, respectively). T_{50} also differed between sowing densities. In winter rye, the highest sowing density reached 50% soil cover about 3 days earlier than the lowest sowing density. For the other two species this difference was 5-6 days.

Height differences were largest in experiment A, where fodder radish and white lupine were about three times taller as the three shortest growing species winter rye, Italian ryegrass and lucerne. In experiment B and C, height differences among the tested species were present, but these differences were less pronounced (Table 2.2).

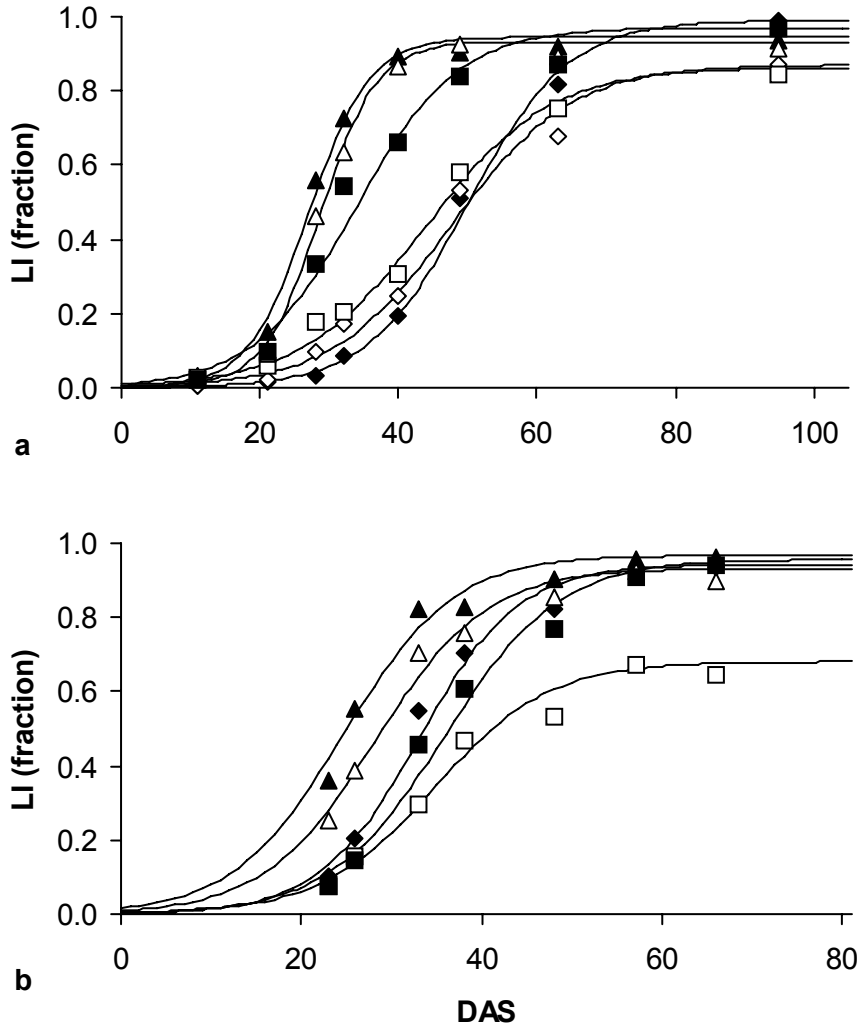

Figure 2.1. Light interception (fraction) over time (DAS) of different cover crop species for experiment A (a) and experiment B (b). Markers indicate observed values and lines represent the fitted light interception curves (Equation 1). FR (closed triangles), WO (open triangles), WR (closed squares), WL (open squares), LU (open diamonds), IR (closed diamonds). See Table 2.1 for full names of cover crops.

In experiment A, the highest above-ground biomass in autumn was observed for fodder radish (723 g m^{-2}), followed by Italian ryegrass, white lupine and winter oilseed rape (419–454 g m^{-2}; Figure 2.2). Biomass in autumn of winter rye was slightly lower (295 g m^{-2}) and lucerne accumulated by far the lowest biomass (194 g m^{-2}). In experiment B, the accumulated biomass of all cover crops was lower than in experiment A, with fodder radish and white lupine showing the largest difference. In experiment C, all cover crop species obtained a very similar maximum amount of biomass (about 400 g m^{-2}). The biomass of the frost-sensitive species fodder radish and white lupine was severely reduced when measured in spring. The biomass of the other species remained unchanged, except for winter oilseed rape in experiment C,

Table 2.2. Estimated parameter values obtained by fitting a logistic function (Equation 1) to the observed values of light interception (LI; experiment A and B) or soil cover (SC; experiment C). Maximum LI, SC and height are the maximum values observed in the field. See Table 2.1 for full names of cover crops.

Exp	Cover crop	Max LI/SC (fraction)		T_{50} LI /SC (DAS)		*b* LI /SC (day^{-1})		Max height (cm)	
A	FR	0.95 (0.013)	c	27.1 (0.69)	a	0.23 (0.014)	b	88.7 (8.75)	c
	IR	0.99 (0.000)	d	50.0 (2.33)	c	0.14 (0.000)	a	29.1 (0.73)	ab
	LU	0.87 (0.038)	a	47.6 (1.74)	c	0.12 (0.008)	a	30.0 (0.88)	ab
	WL	0.86 (0.067)	ab	43.8 (2.19)	c	0.11 (0.014)	a	83.2 (2.75)	c
	WO	0.93 (0.014)	bc	28.7 (0.66)	a	0.25 (0.008)	b	41.8 (3.28)	b
	WR	0.97 (0.007)	cd	33.8 (1.80)	b	0.14 (0.009)	a	27.5 (0.96)	a
B	FR	0.97 (0.002)	b	24.6 (2.58)	a	0.16 (0.008)	a	43.8 (2.02)	b
	IR	0.94 (0.038)	b	32.9 (1.11)	b	0.18 (0.012)	a	26.0 (1.09)	a
	WL	0.68 (0.047)	a	34.7 (1.62)	b	0.16 (0.010)	a	41.2 (1.48)	b
	WO	0.93 (0.029)	b	28.2 (1.03)	a	0.16 (0.016)	a	40.7 (3.04)	b
	WR	0.96 (0.010)	b	35.8 (1.18)	b	0.16 (0.006)	a	23.1 (0.67)	a
C	LU_1	0.97 (0.008)	a	50.4 (0.88)	f	0.08 (0.003)	a	30.0 (0.35)	b
	LU_2	0.98 (0.006)	a	48.1 (0.67)	e	0.07 (0.001)	a	24.0 (0.68)	a
	LU_3	0.99 (0.003)	a	44.7 (0.64)	d	0.07 (0.003)	a	25.1 (0.83)	a
	WO_1	0.96 (0.010)	a	27.5 (1.23)	c	0.14 (0.009)	b	46.0 (1.93)	e
	WO_2	0.98 (0.004)	a	24.9 (0.92)	b	0.16 (0.014)	bc	48.0 (2.20)	e
	WO_3	0.98 (0.006)	a	21.7 (0.88)	a	0.14 (0.011)	b	48.0 (1.47)	e
	WR_1	0.98 (0.008)	a	24.7 (1.01)	b	0.17 (0.013)	bc	35.0 (0.91)	c
	WR_2	0.98 (0.004)	a	22.6 (0.66)	a	0.20 (0.021)	d	38.0 (0.71)	d
	WR_3	0.97 (0.015)	a	21.4 (0.66)	a	0.18 (0.016)	cd	34.0 (0.91)	c
	LU	0.98 (0.004)	a	47.7 (0.80)	b	0.07 (0.002)	a	26.4 (0.86)	a
	WO	0.97 (0.004)	a	24.7 (0.89)	a	0.15 (0.007)	b	47.3 (1.03)	c
	WR	0.98 (0.006)	a	22.9 (0.59)	a	0.18 (0.010)	c	35.7 (0.68)	b

b: relative rate of increase (day^{-1}); T_{50}: moment at which 50% of the maximum was reached (DAS). Different letters (a–g) within a column indicate significant differences at 0.05 level within an experiment; values in parentheses denote SE.

which increased in biomass. Italian ryegrass, which was selected as a frost-sensitive species, did not loose biomass during winter. However, it should be noted that Italian ryegrass, especially when autumn-sown, is only moderately frost-sensitive during the first winter. This, in combination with the relatively mild winter of 2003–2004, explains the overwintering of Italian ryegrass. No effect of fertilization rate or sowing density on cover crop biomass of lucerne and winter oilseed rape was observed. For winter rye, biomass in the treatment that combined the highest sowing density with the

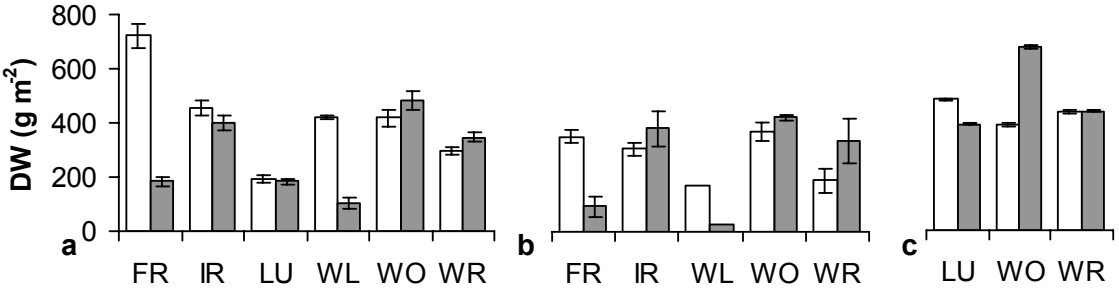

Figure 2.2. Above-ground dry weight (g m^{-2}) of various cover crops in autumn (white) and spring (grey), in experiment A (a) , B (b) and C (c). Autumn values represent maximum DW and spring values represent DW determined on March 22 (experiment A and B) or April 4 (experiment C). Vertical bars represent mean values ± SE. See Table 2.1 for full names of cover crops.

highest fertilization rate was higher compared to all other winter rye treatments (471 g m^{-2} vs. on average 384 g m^{-2}).

Weed growth in autumn

Both at 48 and 83 DAS, the biomass of natural weeds in experiment A, which consisted for 98% of *Chenopodium album*, was severely reduced by fodder radish, winter oilseed rape and winter rye (> 70% reduction; Table 2.3). White lupine caused a lower decrease in weed biomass (about 40%). Lucerne only reduced weed pressure at 48 DAS (39%), and Italian ryegrass did not affect weed biomass. The biomass of natural weeds in experiment B, which mainly consisted of *Poa annua* (29%) and *Stellaria media* (47%), was decreased by all cover crop species, except for lucerne, which was completely overgrown by weeds and therefore discarded from the experiment. Of the remaining species, white lupine gave a poorer weed suppression than the other cover crops. A large difference in biomass of natural weeds in the two control treatments of experiment C was observed. The additional tillage operation in the control treatment of winter rye and winter oilseed rape, which was conducted one month after tilling the whole field, resulted in a five times lower weed biomass compared to that of the lucerne control treatment, which was only tilled once (Table 2.3). Also, the species composition differed, with *P. annua* and *S. media* being dominant in the winter rye and winter oilseed treatments and a more diverse weed flora being present in the control plots of lucerne (e.g. *C. album*, *Polygonum spp*. and *Echinochloa crus-galli*). All cover crop species caused a reduction in weed biomass relative to their control treatment, varying from 79% (lucerne) to 92% (winter oilseed rape) and 98% (winter rye). Weed suppression was similar across sowing densities for all cover crop species. In general, *V. sativa* and natural weeds responded similarly to the different cover crop species (Table 2.3). However, at the first harvest *V. sativa* was

Table 2.3. Dry weight (DW) of natural weeds (g m^{-2}) and *Vicia sativa* (mg $plant^{-1}$) at the first (H1) and second (H2) harvest time in experiment A, B and C. See Table 2.1 for full names of cover crops.

Exp	Abbr	DW natural weeds (g m^{-2})		DW natural weeds (g m^{-2})		DW *Vicia sativa* (mg $plant^{-1}$)		DW *Vicia sativa* (mg $plant^{-1}$)	
A		H1: 48 DAS		H2: 83 DAS		H1: 48 DAS		H2: 109 DAS	
	control	147 (27.3)	d	327 (22.8)	c	264 (7.7)	b	6013 (184.0)	d
	FR	39 (7.6)	a	47 (3.9)	a	74 (9.9)	a	269 (104.2)	a
	IR	124 (7.3)	cd	281 (3.7)	bc	242 (63.2)	b	2617 (433.5)	b
	LU	90 (17.5)	bc	241 (41.9)	bc	342 (1.7)	bc	4121 (516.4)	c
	WL	92 (12.5)	bc	188 (83.1)	b	356 (16.3)	c	2412 (299.4)	b
	WO	40 (12.3)	a	29 (7.4)	a	88 (15.3)	a	222 (34.9)	a
	WR	44 (5.0)	ab	33 (13.8)	a	112 (16.6)	a	795 (194.9)	a
B		H1: 56 DAS		H2: 81 DAS		H1: 56 DAS		H2: 81 DAS	
	control	211 (9.5)	c	273 (14.5)	c	298 (48.6)	cd	636 (63.0)	c
	FR	42 (7.6)	a	74 (27.9)	a	101 (23.7)	a	137 (25.7)	a
	IR	59 (7.0)	a	52 (3.5)	a	218 (9.0)	bc	331 (21.7)	b
	WL	120 (24.8)	b	149 (36.6)	b	340 (24.6)	d	520 (44.5)	c
	WO	43 (13.6)	a	49 (13.0)	a	121 (6.6)	ab	175 (16.5)	a
	WR	77 (16.7)	a	86 (26.7)	ab	245 (47.8)	cd	352 (56.0)	b
C		H1: 49 DAS		H2: 98 DAS					
	control	155 (21.4)	b	494 (55.1)	b				
	LU_1	59 (11.9)	a	111 (17.7)	a				
	LU_2	55 (21.9)	a	165 (9.7)	a				
	LU_3	45 (11.7)	a	139 (24.4)	a				
				H: 67 DAS					
	control			90.4 (13.7)	b				
	WO_1			9.9 (1.18)	a				
	WO_2			5.9 (1.31)	a				
	WO_3			6.3 (1.94)	a				
	WR_1			2.2 (0.66)	a				
	WR_2			2.6 (0.96)	a				
	WR_3			1.4 (0.79)	a				
	WO			7.4 (0.96)	b				
	WR			2.1 (0.43)	a				

Different letters (a–e) within a column indicate significant differences at 0.05 level within an experiment; values in parentheses denote SE.

less suppressed than the naturally occurring weeds, and was even stimulated in the presence of white lupine and lucerne.

Correlation between cover crop light interception and weed suppressive ability
Biomass of natural weeds and *V. sativa* was negatively correlated with the area under the light interception curve (AULIC), with p-values ranging from <0.001 to 0.064. For *V. sativa* and the second harvest of natural weeds in experiment A, it was observed that the correlation between AULIC and biomass could be further improved when the light interception fraction was accumulated over a shorter period of time. The optimized correlations between AULIC and the biomass of natural weeds and *V. sativa* are shown in Figure 2.3, for the two harvest times of experiment A and B.

Weed suppression by cover crop residues in spring
The effect of residue incorporation on the emergence of the indicator species lettuce and sugar beet differed per cover crop species (Figure 2.4). Lettuce and sugar beet responded similarly to the different cover crop residues, but the emergence of lettuce was more reduced than that of sugar beet. In both experiments, lucerne residue had the strongest negative impact on seedling establishment, followed by white lupine in experiment A. Winter oilseed rape reduced emergence of both lettuce and sugar beet in experiment C, but only reduced the emergence of lettuce in experiment A. Residues of winter rye and fodder radish did not affect the emergence of the indicator species and Italian ryegrass was omitted from the analysis, because of severe problems with re-growth following residue incorporation. In experiment C, fertilization rate did not affect lettuce and sugar beet emergence, neither did sowing density of winter rye and winter oilseed rape. However, the highest sowing density of lucerne reduced the emergence of lettuce with 35% more than the lowest sowing density (p=0.003, data not shown).

The emergence of natural weeds in experiment A was characterized by a large variation and no differences in emergence were detected between the different cover crop residues and the control treatment. More than 50% of the emerged weed plants were *C. album* plants and, in contrast to the other weed species, this species was present in all plots. Even though the overall variance in *C. album* emergence was considerably lower than that of the total weed population, differences in the emergence between treatments were not significant (p=0.102). Correlation analysis, however, revealed a correlation between the emergence of *C. album* and the total emergence of both lettuce (r = 0.82–0.76; p = 0.044–0.081) and sugar beet (r=0.92–0.76; p=0.009–0.077) on all different *C. album* counting dates (Figure 2.5).

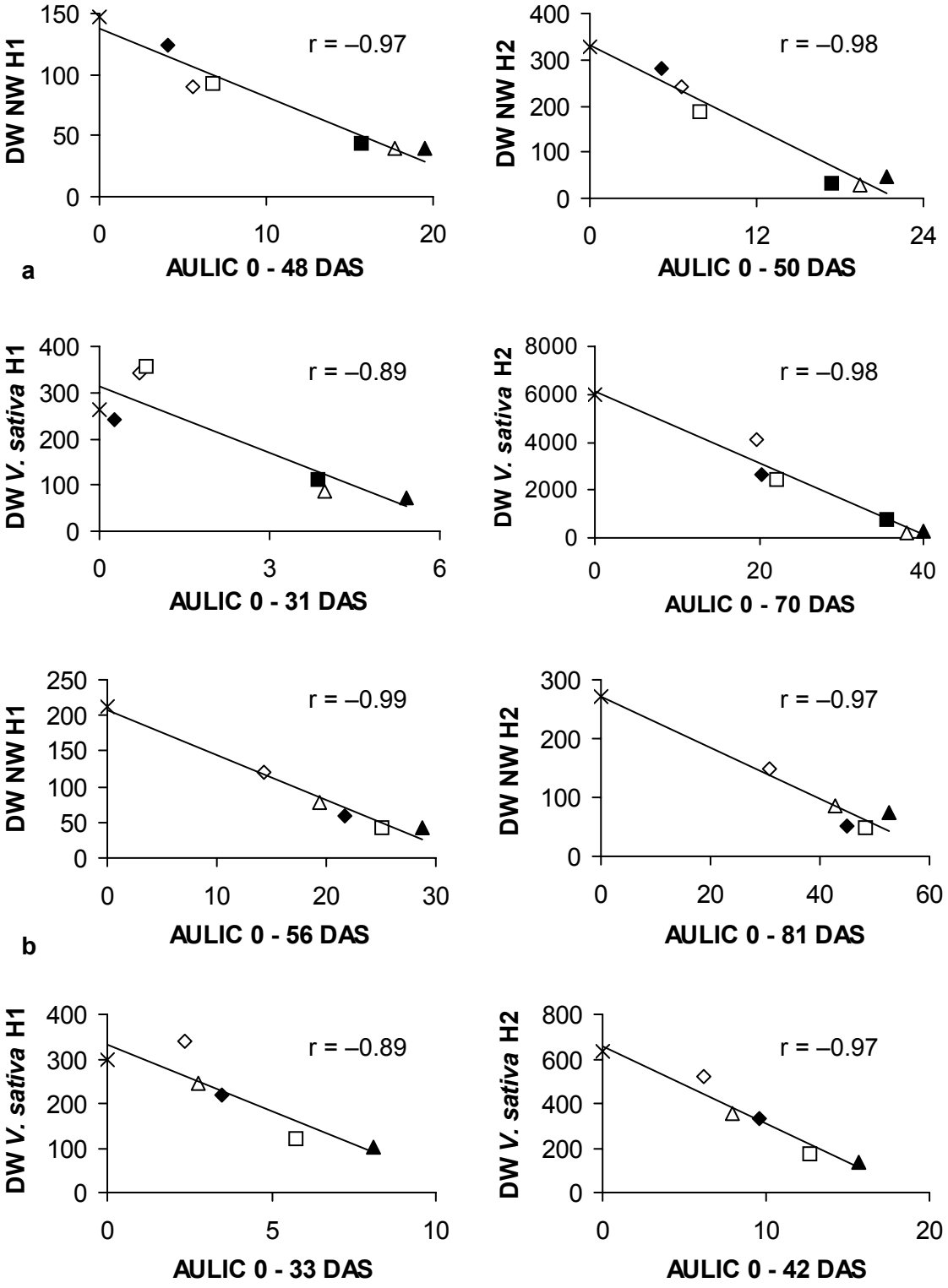

Figure 2.3. Correlations (r) between the area under the light interception curve (AULIC; Equation 2) and the biomass of natural weeds (NW, g m^{-2}) or the biomass of *Vicia sativa* (g $plant^{-1}$) for the first (H1) and second (H2) harvest time of experiment A (a) and experiment B (b). Light interception was accumulated from sowing till the moment an optimum correlation was obtained. Control (crosses), FR (closed triangles), IR (closed diamonds), LU (open diamonds), WL (open squares), WO (open triangles), WR (closed squares). See Table 2.1 for full names of cover crops.

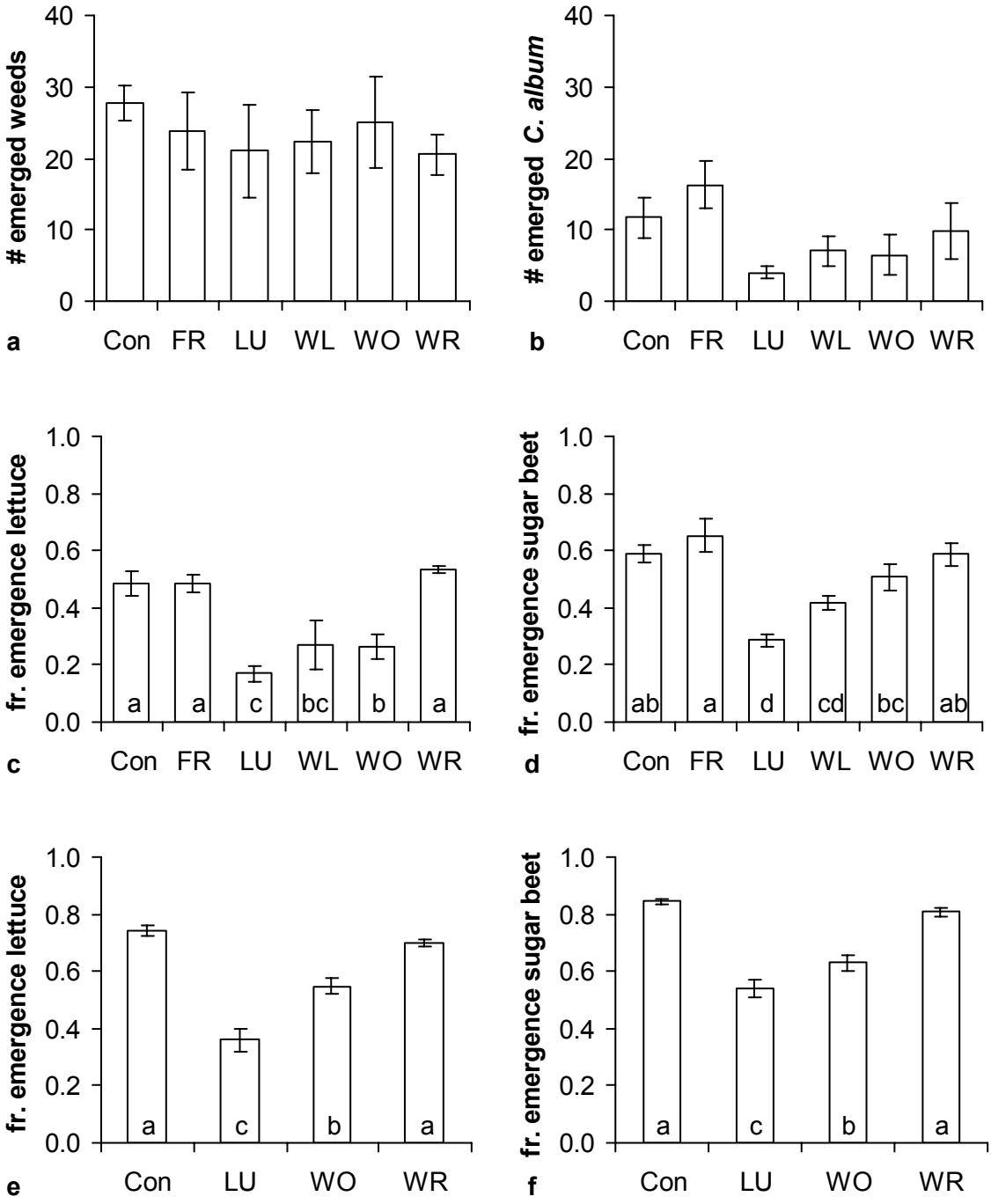

Figure 2.4. Total emergence (# seedlings m^{-2}) of naturally occurring weeds (a) and *C. album* separately (b) in experiment A and fraction emergence of lettuce and sugar beet in experiment A (c, d) and C (e, f). See Table 2.1 for full names of cover crops. Vertical bars represent mean values ± SE. Different letters within one figure indicate significant differences at the 0.05 level.

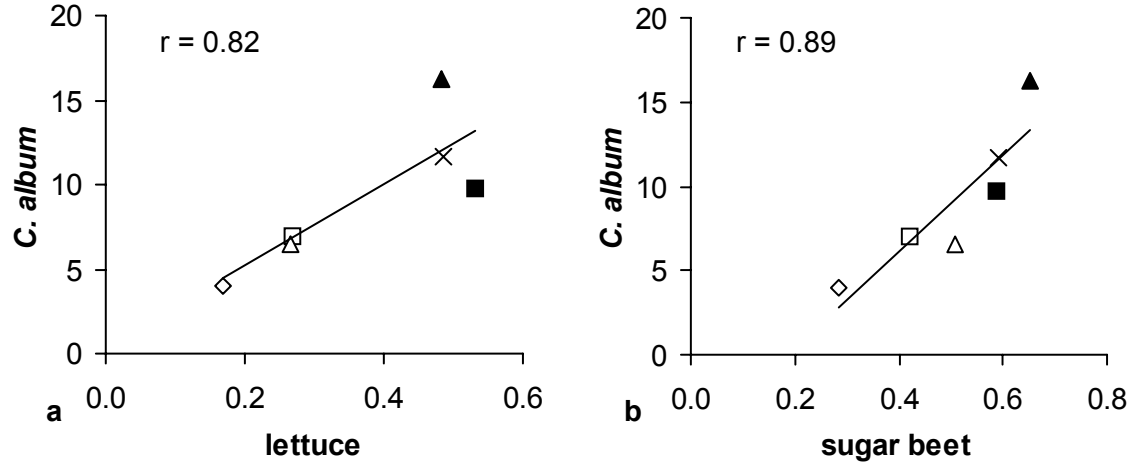

Figure 2.5. Correlation (r) between the fraction emergence of lettuce (a) or sugar beet (b) and the number of emerged *C. album* seedlings counted on May 10. Control (crosses), FR (closed triangles), LU (open diamonds), WL (open squares), WO (open triangles), WR (closed squares). See Table 2.1 for full names of cover crops.

Discussion

Cover crop competitiveness in autumn

Our first hypothesis, which stated that autumn weed suppression is positively correlated to early light interception by the cover crop, is sustained by the strong negative correlation we found between cumulative light interception (AULIC) and weed biomass. The fact that in most cases this correlation could be further improved when the light interception fraction was accumulated over a shorter period of time than the whole season, indicates that early light interception is relatively more important for competition than late light interception. However, the relative importance of earliness of light interception seemed to be dependent on the height increase of the weed species present. In experiment B the short-statured species *S. media* and *P. annua* were the dominant weed species. Due to their limited height, these species were not able to outgrow even the slowest growing cover crops and consequently the best correlation between weed biomass and AULIC was obtained when light interception was accumulated until harvest of the weeds. In this case early light interception was not more important than late light interception. In experiment A, the tall growing *C. album* was the dominant weed species. Biomass of *C. album* in the slow growing Italian ryegrass and lucerne was considerably higher than expected based on the AULIC accumulated over the whole season (data not shown), indicating that the relative advantage of the weed early on was well exploited. Consequently, accumulation of the light interception fraction over a shorter period of time (until 50 DAS) gave a much better fit (Figure 2.3).

The finding that early light interception is important for the competitive ability of a species is in line with research on the development of more weed-competitive crop cultivars (Bertholdsson, 2005; Zhao et al., 2006a, b). Typically, characteristics commonly identified to make crops more competitive to weeds, such as rapid germination, early above-ground growth and vigour, rapid leaf area and canopy establishment, large leaf area development and duration, and greater plant height (Pester et al., 1999), are all related to early light interception.

In general, *Vicia sativa* and natural weeds responded similarly to the different cover crop species. Interestingly, we observed that, at the time of first harvest, the biomass of *V. sativa* in the control was lower than that of *V. sativa* growing in lucerne (experiment A) and white lupine (experiment A and B; Table 2.3). In competition, the plants of *V. sativa* showed a clear shade response characterized by thin leaves and longer but thinner stems. Additionally, they used the cover crop plants for support. The results suggest that in these particular cover crops this adaptive strategy of the model weed initially led to a higher biomass production.

Cover crop density effects in autumn

Because of the expected earlier canopy closure at higher sowing densities we expected to reach higher weed suppression by increasing the cover crop sowing density. This positive relationship between sowing density and weed suppression has been found in most literature (e.g. Olsen et al., 2005). We indeed observed the time needed to reach 50% soil cover (T_{50}) for all cover crop species to be reduced when doubling the seeding rate. However, this did not translate into a lower weed pressure. For winter rye and winter oilseed rape the lack of effect of sowing density on weeds may be explained by the dominance of the short-statured species *P. annua* and *S. media*. In experiment B it was shown that for these weed species, which due to their limited height were not able to outgrow any of the cover crops, late light interception was as important as early light interception. Therefore, the earlier canopy closure in these cover crops as established by doubling the seeding rate might have exerted a lower effect than it would have in the presence of more competitive weeds. However, a good explanation for the situation in the lucerne plots, where the weed species composition was more diverse, is still lacking.

Inhibitory effects of cover crop residues on weed establishment in spring

Our hypothesis stating that suppression of weed establishment in spring is greatest for overwintering cover crop species and least or absent for winter-killed species has to be rejected as the winter-hardy winter rye did not affect establishment of the indicator species, whereas the frost-sensitive white lupine did inhibit seedling emergence.

For winter rye it is surprising that, in spite of the numerous publications on winter rye allelopathy (e.g. Barnes et al., 1986; Chase et al., 1991; Burgos and Talbert, 2000; Rice et al., 2005), we did not observe any inhibitory effect on seedling emergence. This may be due to differences in cultivar, and/or to the development stage of the winter rye plants at the moment of residue incorporation. Reberg Horton et al. (2005) found that the allelopathic potential of winter rye declines with development, and consequently it cannot be excluded that a better result would have been obtained with residue incorporation at an earlier stage.

For white lupine it is equally surprising that, despite the fact that its residues were almost completely decomposed, it caused a severe reduction in the establishment of the indicator species. White lupine contains quinolizidine alkaloids that act as a herbivore deterrent (e.g. Vilarino et al., 2005), but have also been suggested to influence plant-plant interactions (Wink, 1983). The white lupine cultivar "Weibit" that was used in our experiment was a bitter cultivar with a high alkaloid content. As alkaloids in *Lupinus* are produced in green leaves and shoots (Waller and Nowacki, 1978; in Williams and Harrison, 1983) it seems most likely that the inhibitory effect in the white lupine plots is caused by alkaloids that leached from the shoot and were still present in the soil in spring. However, data on the fate of alkaloids in the soil are scarce, and results are difficult to compare since the persistence of allelochemicals is largely influenced by soil type and weather conditions (Levitt et al., 1984; Starr et al., 1996).

Lucerne residues exerted the strongest inhibitory effect on seedling establishment of all indicator species in both experiment A and C, followed by winter oilseed rape. However, the average reduction of 54% was clearly higher than that of winter oilseed rape (31%). For lucerne an effect of plant density on seedling emergence in spring was observed, with 35% lower emergence of lettuce in the plots with a high sowing density. Although above-ground dry weight was slightly higher for the high plant density (376 g m^{-2}) compared to the low plant density (346 g m^{-2}), this small difference is unlikely to have caused the large difference in emergence.

Both for lucerne and winter oilseed rape there is a vast amount of literature on the allelopathic properties of these species. Lucerne contains several groups of allelochemicals, including saponins, flavonoids and phenolic acids (Dornbos et al., 1990; Oleszek, 1993; Xuan et al., 2003). In a pot experiment in a growth chamber, Xuan et al. (2005) found a strong inhibitory effect of lucerne residues on weeds. Barnyardgrass and *Monochoria vaginalis* growth was reduced by 80–100% for up to 10 days after incorporation and by 50% after 20–25 days. Allelopathy in *Brassica* species has been mainly attributed to the hydrolysis products of glucosinolates (e.g. Brown and Morra, 1996; Haramoto and Gallandt, 2005). Haramoto (2005) and Al

Khatib (1997) found reductions in the emergence of bioassay species following *Brassica* cover crop residue incorporation in the field of 23–34% and 30%, respectively, which were very similar to our results. Boydston and Hang (1995), however, found much higher reductions of weed density (73–85%) following oilseed rape incorporation in spring in a loamy sand soil.

Fodder radish residues did not exert any effect on seedling establishment. This was not unexpected as there was very little fresh above-ground material that remained after winter. Furthermore, there is virtually no literature that reports on allelopathic properties of fodder radish. Due to severe problems with the re-growth of Italian ryegrass following residue incorporation, the effects on seedling establishment could not be evaluated.

C. album establishment in the different cover crop residues was clearly correlated to that of lettuce and sugar beet, despite the slower emergence of *C. album* relative to lettuce and sugar beet. In the control treatment, T_{50} of emergence of lettuce and sugar beet was reached on April 15 and April 19, 2004, respectively, whereas emergence of *C. album* started at the end of April. Our results suggest that, in comparative studies, lettuce and sugar beet can be used as indicators of the inhibitory potential of cover crop residues on weeds. Use of crop seeds has several advantages over using weeds. Firstly, crop seedling emergence is more homogeneous due to the lack of dormancy and, secondly, there is no interference from a background population of the same species already present in the field.

Methodological aspects

The light interception curve is characterized by three parameters, i.e. T_{50}, the maximum light interception (parameter c) and the maximum relative rate of increase (parameter b). In other studies these parameters have been used to correlate to weed biomass (e.g. Den Hollander et al., 2007), and also in this case a preliminary analysis indicated that T_{50} gave a good correlation with weed biomass. However, the advantage of taking the area under the light interception curve is that the weed biomass in the control plots can also be included in the analysis.

Practical considerations

Ideally, autumn cover crops would both prevent weed seed production and reduce weed establishment in the subsequent cash crop. Based on the current results, winter oilseed rape, which was found to have a strong competitive ability during autumn and an intermediate inhibitory effect on seedling emergence in spring, seems the best choice for weed management. Fodder radish and winter rye were not effective in spring, but severely reduced weed biomass in autumn. They offer a good prospect for

reducing weed seed production in autumn, as often times weed biomass is positively correlated to weed seed production (e.g. Lutman, 2002). In contrast, lucerne and white lupine offer a good prospect for reducing weed establishment in spring, but are very weak competitors in autumn. Italian ryegrass was an intermediate competitor, but the inhibitory effects of its residues on seedling establishment could not be evaluated due to re-growth following incorporation.

Although lucerne would be a less obvious choice for the proposed system because of its weak competitive ability in autumn, the strong inhibitory effect of lucerne residues could be exploited in other settings. Lucerne grown as a fodder crop during a two-year period is an important component of many crop rotations. If farmers are aware of the weed-suppressive potential of lucerne residues, lucerne residue management can be adapted in order to optimize weed control in the next crop. Furthermore, regular mowing also allows for a better regulation of taller weed species in the crop.

This research was focused on direct weed suppression by the cover crop. However, experiment C showed that delayed sowing in combination with a stale seedbed can, regardless of cover crop species, severely reduce weed pressure and weed species composition. After tillage of the whole field, the winter rye and winter oilseed rape plots were tilled again one month later, just prior to sowing. This resulted in a more than five times lower weed biomass in these control plots as compared to the control plots of lucerne, which were only treated once. For reducing weed seed production in autumn, it may therefore be beneficial to combine a cover crop that can be sown relatively late with a stale seedbed preparation. On the other hand, a later sowing date also implies a reduced amount of residue that can be incorporated in spring.

In general, our results provide a good prospect for the use of autumn-sown cover crops for weed management and form a basis for further research on the optimization of this system.

CHAPTER 3

Weed suppression by cover crops: can mechanical wounding induce the production of allelochemicals?

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Summary

The effect of mechanical wounding of the field-grown cover crops winter rye, winter oilseed rape and lucerne on the allelopathic activity of plant residue was studied per unit biomass and per unit area. We investigated how the allelopathic activity of residues of intact and damaged cover crops changed over time. Cover crops were sown in late summer and damage was applied in spring. For lucerne and winter rye, lettuce seedling bioassays were used to determine allelopathic activity, whereas for winter oilseed rape the glucosinolate content was quantified by HPLC. The experiment clearly demonstrated that mechanical wounding enhanced the allelopathic activity per unit biomass of all three cover crop species, but the species differed in the onset and the duration of the response to mechanical wounding. The temporal pattern of allelopathic potential of intact cover crop plant material in spring was characterized by a linear decline for winter rye and a steep decline at the onset of the flowering stage for winter oilseed rape, whereas for lucerne no specific pattern was observed. For all three species, the allelopathic activity per unit area (biomass × allelopathic activity per unit biomass) was highest at the end of the sampling period, mainly as a result of an increased biomass. Comparing the increase in allelopathic activity per unit biomass after damaging to the change in this parameter over time, it became clear that the impact of damaging is minor and often just sufficient to compensate for the loss in plant biomass resulting from mechanical damaging. Therefore, it can be concluded that the increase in allelopathic activity due to damaging is of little significance for farmers.

Keywords: *Brassica napus*, *Secale cereale*, *Medicago sativa*, allelopathy, weed management, weeds, induced resistance, glucosinolates, phenology

Introduction

In organic farming systems, where the use of pesticides is excluded, 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). Cover crops, grown during the crop-free period in between two main crops, have potential to form an important, pro-active component of weed management in organic farming systems. They can combine a reduced weed seed production in autumn through competition with a reduced and/or delayed weed growth in spring through an allelopathic effect of incorporated crop residues (e.g. Weston, 1996; Haramoto and Gallandt, 2004).

Rice (1984) gave the following definition of allelopathy: "any direct or indirect harmful or beneficial effect by one plant (including micro-organisms) on another through the production of chemical compounds that escape into the environment". In our system, the strongest allelopathic effect of cover crops on weeds that grow in the main crop in spring is expected when the cover crop residues are incorporated shortly before the main crop is sown/planted. To enhance the allelopathic effect, it is important to use cover crops that contain a high level of allelochemicals at the moment of residue incorporation. Between and within crop species there is a large genetic variation in the allelochemical content of the tissue (e.g. Xuan and Tsuzuki, 2002; Bertholdsson, 2004). At the same time, various studies showed that concentrations of allelochemicals in plants are not stable. The level of allelochemicals in the plant is influenced by abiotic and biotic stresses in combination with age or growth stage (Mwaja et al., 1995; Einhellig, 1999; Reberg Horton et al., 2005).

Research on plant defense against herbivores and pathogens has shown that many plants can enhance their chemical defense level in response to biotic stress, a process called induction (Karban and Baldwin, 1997). Responses that take place within hours or days after injury are categorized as "rapidly-induced responses". Their occurrence may be either local and restricted to the injured tissue or systemic throughout the plant (Baldwin, 1994). Many groups of chemicals that protect the plant against herbivores can also act as allelochemicals. For example, hydrolysis products of glucosinolates, which predominantly occur in the *Brassicaceae* family, act as a defense against generalist insect herbivores (Chew, 1988; Mithen, 2001) and also negatively affect germination and growth of a number of plant species (Oleszek and Jurzysta, 1987; Vaughn and Boydston, 1997). Likewise, hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), secondary metabolites present in the *Poaceae*, are widely recognized as allelopathic agents (Barnes et al., 1987; Burgos and Talbert, 2000) and can be induced by herbivores (e.g. Gianoli and Niemeyer, 1996; Collantes et al., 1999).

To our knowledge, the first article which describes an experiment that brings herbivore-induced defense and allelopathy together was published by Siemens et al. (2002). They investigated the cost of defense against herbivores in the context of plant competition. This study, conducted with *Brassica rapa,* showed that in the absence of competitors defense costs were significant, whereas in the presence of *Lolium perenne* defense costs were balanced by an increased competitiveness. The link between herbivore-induced defense and allelopathy was further strengthened by the observation that a simultaneous increase in glucosinolate and myrosinase concentration negatively affected *L. perenne* seedling growth. This indicates that in some plants resource-mediated competition can shift towards chemical-mediated competition when these plants are induced by herbivores to synthesize defensive chemicals.

The evolutionary history of allelochemicals is not clear. Whittaker and Feeney (1971) hypothesized that, because of their chemical nature, allelopathic substances may only be secondarily functional in plants, having arisen initially in response to herbivore pressures. This theory is contrasted by studies from Kong et al. (2004) and Dayan (2006), which suggest that sorghum and rice respond to the presence of other plant species by releasing a higher amount of allelochemicals.

Several studies have shown that artificial damage causes effects that are similar to those resulting from herbivore damage (Bodnaryk, 1992; Mithofer et al., 2005). This could create possibilities to enhance the concentration of allelochemicals in cover crops by mechanically damaging the crop before residue incorporation. As in most experiments mechanical damage has been applied to young plants grown under laboratory or greenhouse circumstances (Bodnaryk, 1992; Mithofer et al., 2005) it is not known whether mechanical damage can induce the production of defense chemicals in older, field-grown plants. Additionally, it is essential to know how the concentration of allelochemicals changes with time after wounding to establish the optimal time to incorporate the cover crop.

The above-mentioned considerations resulted in the following research questions: (1) Can mechanical damaging of cover crops applied in spring induce the production of allelochemicals? (2) How does the allelopathic activity per unit cover crop biomass (concentration) and per unit area (concentration × biomass) in intact and mechanically damaged cover crop plants change over time? (3) Are the responses to mechanical damage similar for species from different plant families?

Materials and methods

General

Three cover crop species from three plant families were selected; winter rye (*Secale*

cereale L.) from the *Poaceae* family, winter oilseed rape (*Brassica napus* L.) from the *Brassicacae* family, and lucerne (*Medicago sativa* L.) from the *Fabaceae* family. Each species contains different groups of allelochemicals. The group of hydroxamic acids is the main group of allelochemicals in the *Poaceae* (Niemeyer, 1988). Allelopathy in *Brassica* species has been mainly attributed to the hydrolysis products of glucosinolates (Haramoto and Gallandt, 2004). Lucerne contains several groups of allelochemicals, including saponins, flavonoids and phenolic acids (Dornbos et al., 1990; Oleszek, 1993; Xuan et al., 2003). All species were field-grown and whole plants were assayed. The general allelopathic potential of lucerne and winter rye was determined using plant extracts in seedling bioassays with lettuce as a test plant. For winter oilseed rape, extract preparation with conservation of all allelopathic compounds is difficult as during extraction of winter oilseed rape material glucosinolates can be hydrolyzed into volatile, allelochemical compounds. These hydrolysis products are likely to escape from the extract before put into contact with lettuce seeds. For that reason, the allelopathic potential of winter oilseed rape was approximated by chemical quantification of glucosinolates by High Performance Liquid Chromatography (HPLC).

Experimental field set-up

Winter rye cv. Protector, winter oilseed rape cv. Emerald and lucerne cv. Mercedes were grown at the biological experimental farm "Droevendaal", the Netherlands. The experimental field was located on a sandy soil with an organic matter content of 4.3%. On July 29, 2004, the field was limed with 5000 kg ha^{-1} Dolokal to increase the pH from 4.7 to 5.5. One day before sowing of each crop, the plots were fertilized with 1600 kg NPK (5-6-13) ecological fertilizer granules (Ecostyle). Lucerne, inoculated with *Rhizobium meliloti*, was sown on August 2, 2004 at a sowing density of 37.5 kg seeds ha^{-1}. Winter rye and winter oilseed rape were sown on September 2, 2004 at densities of 225 kg and 10.5 kg seeds ha^{-1}, respectively. No irrigation was applied. Seedling emergence was 95%, 88% and 84% for lucerne, winter rye and winter oilseed rape, respectively. The experimental design was a split-split-plot with four replications, with cover crop species as main plots, intact or damaged plants as sub-plots and harvest times as sub-sub-plots.

The above-ground tissue of winter rye was damaged on March 22, 2005 (stem elongation stage) by driving twice through the plot with a rotary harrow (400 rpm, driving speed 2.5 km h^{-1}). Lucerne was damaged on April 18, 2005 (vegetative stage) by a street sweeper machine (AP, type VHG2500). Its brush, containing 50% steel and 50% high quality synthetic material, "swept" through the upper half of the 42 cm tall lucerne plants. The first method resulted in crushing of winter rye leaves and the

second method in the scratching of lucerne leaves. Both methods aimed at a minimal loss of plant material. The effect of artificial damage on winter oilseed rape was investigated at two different moments in time; on March 22 (stem elongation stage) and on April 18 (flower bud development/ flowering stage), 2005. As it was not possible to mechanically damage winter oilseed rape with a machine without damaging the stem, damage was applied manually by cutting each leaf twice with a scissors. The cuts were made at each side of – and parallel to – the main nerve, covering approximately two-third of the length of the leaf. At April 18, mechanical damage included the removal of the inflorescence by cutting the stem at 45 cm from the top.

Collection of plant material

Plant material was collected for the determination of the concentration of allelochemicals and biomass. Intact plants were harvested one time in autumn 2004 and seven times in spring 2005. At two, seven and fourteen days after damaging, both intact and damaged plants were sampled (Table 3.1). Plant material was collected in the morning by harvesting whole plants from the field and rinsing the roots carefully with water. As some of the plants of winter oilseed rape suffered from infection with *Phoma lingam*, the causal agent of blackleg disease, care was taken to only sample those plants without symptoms. For the determination of the concentration of

Table 3.1. Sampling dates, dates on which damaging was applied (italic) and development stages of winter rye, winter oilseed rape and lucerne. Samples of intact plants were collected on all dates and "×" indicates dates on which samples from damaged plants were collected.

Sampling date	Winter rye		Winter oilseed rape		Lucerne	
12-10-2004	tillering		leaf production		vegetative	
22-03-2005	DAMAGING		DAMAGING 1			
24-03-2005	stem elongation	x	stem extension	x	vegetative	
29-03-2005	stem elongation	x	stem extension	x	vegetative	
04-04-2005	stem elongation	x	flower bud development	x	vegetative	
12-04-2005	booting		flower bud development		vegetative	
18-04-2005			DAMAGING 2		DAMAGING	
20-04-2005	booting		flower bud development/ flowering	x	vegetative	x
25-04-2005	head emergence		flowering	x	vegetative	x
02-05-2005	head emergence		flowering/ pod extension	x	vegetative	x

allelochemicals, two (lucerne and winter rye) or three (winter oilseed rape) small aluminum boxes per plot were filled with whole plants to an approximate fresh weight of 100 grams. The boxes with plant material were frozen in liquid nitrogen and stored in the freezer at minus 30 ºC until they were placed in a freeze-dryer for 7 days. The dried material was ground to pass a 1.5 mm screen and stored in plastic jars until use. For biomass determination, intact plants (winter oilseed rape) or intact and damaged plants (winter rye and lucerne) were harvested from a 0.5×0.5 m^2 quadrat from each plot. Plants were dissected in root and shoot material in order to monitor major changes in root: shoot ratio over time or through damaging. The plant material was dried in a stove at 70 ºC for at least 48 hours. For intact winter oilseed rape plants harvested at 2, 7 and 14 days after the second damaging, the concentration of allelochemicals and the biomass were determined separately for the inflorescence and the remaining plant parts, to enable a better comparison with damaged plants. At each harvest date, the development stage of winter rye and winter oilseed rape was determined. Lucerne, unlike the other two cover crop species, did not develop from the vegetative to the generative stage. Different vegetative stages are not clearly distinguished in lucerne, and are therefore not mentioned.

Bioassays with winter rye and lucerne extracts

8% wt/wt extracts were prepared by dissolving 3.2 grams of ground plant material in 40 ml of de-ionized water. The mixture was shaken during thirty minutes on a platform shaker at 250 rpm and subsequently centrifuged for fifteen minutes at 12000 rpm. After this, the supernatant was filtered through a thick filter paper (T300) and through a Celtron® 0.45 µm filter. For each plot two 8% extracts were prepared, resulting in a total of 88 extracts (11 treatments × 4 field blocks × 2 plant samples). The extracts of the first sample were immediately used in a bioassay, whereas the extracts of the second sample were stored at 4 ºC in the dark until the next morning.

The 8% extracts were diluted with de-ionized water to obtain 4%, 2%, 1% en 0.5% extracts. A control of de-ionized water completed the concentration series. Of each extract, five ml was added to a Petri dish (9 cm diameter), containing a thick (T300) filter paper on the bottom and a thinner filter paper (Whatman nr. 1) on top. Lettuce (*Lactuca sativa*) was used as an indicator species, because of its rapid germination and its sensitivity to allelochemicals (Macias et al., 2000). Lettuce seeds, from a non light-sensitive variety, were obtained from Rijk Zwaan®. The seeds were surface-sterilized by shaking them for 5 min in a 0.5% NaOCl solution obtained from commercial bleach, followed by rinsing for 2 min with de-ionized water. Fifteen lettuce seeds were added to each Petri dish and the Petri dishes were incubated in a completely randomized design in a germination chamber at 20 ºC in the dark.

After four days, the roots of the germinated seedlings were cut and coloured red in a safranine O solution. WinRhizo LA 1600 (Regent Instruments, Canada) was used to determine the root length after scanning the roots with the settings positive film and grayscale at 200 dpi.

Chemical analysis of glucosinolates

The glucosinolate content of the plant material of winter oilseed rape was determined by using High Performance Liquid Chromatography (HPLC) as described by Van Dam et al. (2004). This was done for 3 samples per plot, resulting in a total of 168 samples (14 treatments × 4 field blocks × 3 plant samples). The inflorescences of the intact plants, harvested at the three dates following mechanical damage on April 18, were pooled per plot, resulting in 12 extra samples (3 treatments × 4 field blocks). 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 oilseed rape standard (Community Bureau of Reference, Brussels, code BCR-367R).

Statistical analysis of bioassays

The data from the bioassays of winter rye and lucerne 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 log-logistic curve (Equation 1).

$$f\left(x\left(b_i, d_i, e_i\right)\right) = \frac{d_i}{1+\exp\left\{b_i\left(\log\left(x\right)-\log\left(e_i\right)\right)\right\}} \qquad (1)$$

In this formula, d is the upper limit, which in this case represents the total root length (cm) per Petri dish of the control. Parameter e (often referred to as ED_{50}) is the dose (% w/w) producing a response halfway the upper limit and parameter b is proportional to the slope around e. The extract dose is denoted by x and the index letter i refers to the treatment. Prior to curve fitting a Box-Cox transformation was carried out (Oberg and Davidian, 2000).

To adjust for inter-assay random effects, a nonlinear mixed-model approach within

drc was implemented (Nielsen et al., 2004). In this nonlinear mixed-model, a variance parameter, representing a random effect, was attached to each of the three fixed parameters *b*, *d* and *e*. The full model, including all fixed and variance parameters, was gradually reduced based on a likelihood ratio test (Pinheiro and Bates, 2000). Model reduction on variance parameters was followed by model reduction on the fixed parameters. An approximate t-test conducted on the fixed parameters that were contained in the final model was used to test for significance of difference between treatments.

For each cover crop species, four different datasets were separately analysed. The first dataset contained data from intact plants for all different sampling dates. The other three datasets contained data of damaged and intact plants at two days, one week and two weeks after damaging. Additionally, a regression analysis of the ED_{50} estimates of intact plants over time was conducted, based on mean treatment values (Genstat Release 9, Payne et al. (2006)).

Statistical analysis of glucosinolates

For winter oilseed rape, the content of different glucosinolates present in the plant samples was quantified. Analysis of variance (ANOVA) was used to compare treatment means of the total glucosinolate content as well as the content of three distinct classes of glucosinolates (Genstat Release 9, Payne et al. (2006)). The glucosinolate content of intact plants at the different sampling times was compared. Additionally, differences in glucosinolate levels between damaged and intact plants were analysed at two days, one week and two weeks after damaging. This was done independently for the two damaging moments. The effect of damage applied on April 18 was assessed by comparing the glucosinolate levels in the lower part of the plants, as on this second batch of damaged plants removal of the inflorescence was part of the applied damage. A Box-Cox transformation was performed before ANOVA to obtain homogeneity of variance. Differences between treatment means were compared using an LSD-test. A regression analysis of the total glucosinolate content of intact plants over time was conducted based on mean treatment values.

Statistical analysis of biomass

For winter oilseed rape, the amount of glucosinolates per unit area was calculated by multiplying the total dry weight of winter oilseed rape per m^2 by total glucosinolate content. For lucerne and winter rye, the allelopathic activity per unit area was calculated by dividing total dry weight per m^2 of each cover crop by the ED_{50} value. For each species, an analysis of variance (ANOVA) of total dry weight, root fraction and glucosinolate content or allelopathic activity per unit area was carried out. Just as

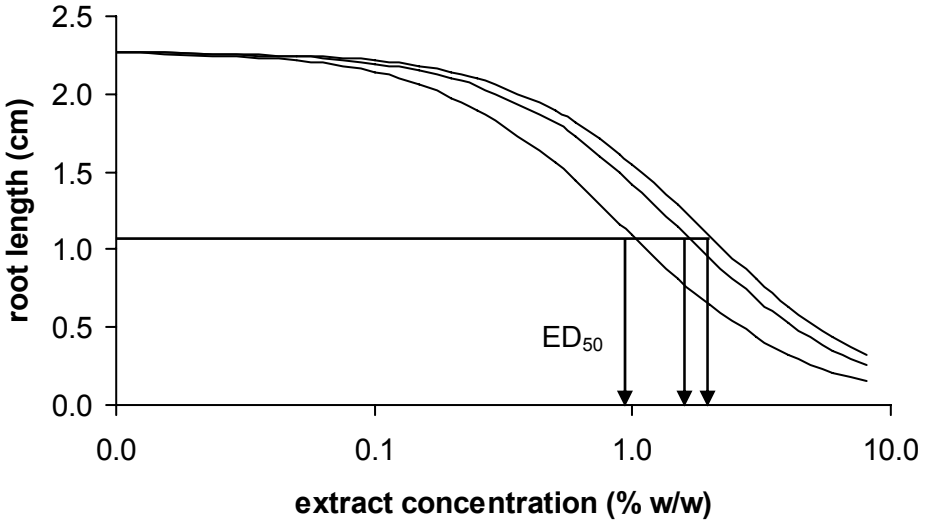

Figure 3.1. Examples of three-parameter logistic dose-response curves (Equation 1) fitted to observed data. Differences in relative allelopathic potency of the different samples were expressed in shifts in ED_{50} (parameter e).

with the results of the bioassays and the glucosinolate content, this analysis provided the basis for a comparison between sampling dates as well as a comparison of the effect of damaging at two days, one week and two weeks after damaging. Regression analysis of various characteristics against time was always conducted on the basis of mean treatment values.

Results

A lack-of-fit test (Ritz et al., 2005) on the full model showed that the three-parameter logistic curve provided an adequate description of the relation between winter rye and lucerne extract dose and lettuce root length. Model reduction allowed the removal of the fixed parameters *b* and *d* in all datasets. Average estimates of parameters *b* and *d* were 1.33 and 1.82 cm for winter rye and 1.18 and 1.52 cm for lucerne, respectively. The allelopathic effect of the different treatments was consequently compared on the basis of parameter *e* (ED_{50}) (Figure 3.1).

Winter rye

On March 24, 2005, the ED_{50} value was 3.36% (w/w), which was 81% higher than on October 12, 2004. The ED_{50} value continued to increase linearly with approximately 0.0342% (w/w) day^{-1} (R^2=0.94), until the last measurement on May 2, 2005 (Figure 3.2a, Table 3.2). Please keep in mind that a low ED_{50} value reflects a high allelopathic

Table 3.2. ED_{50} value (% w/w), root fraction, total dry weight (DW) (g m^{-2}) and the allelopathic activity per unit area (DW/ED_{50}) of winter rye. Data are represented as means with SE within parentheses. Different letters indicate significant differences at the 0.05 level between sampling dates. * indicates a significant difference at the 0.05 level, + indicates a significant difference at the 0.10 level and ns = "not significant".

Harvest date		ED_{50} (% w/w)		Root fraction		DW (g m^{-2})		DW/ED_{50}	
INTACT PLANTS									
12-10-2004		1.86 (0.344)	a	0.10 (0.010)	a	314 (5.1)	a	169 (2.8)	b
24-03-2005		3.36 (0.409)	b	0.16 (0.006)	bc	300 (6.4)	a	89 (1.9)	a
29-03-2005		3.24 (0.402)	b	0.20 (0.016)	de	340 (28.7)	a	105 (8.9)	a
04-04-2005		3.51 (0.417)	bc	0.17 (0.016)	bcd	350 (37.9)	a	100 (10.8)	a
12-04-2005		3.86 (0.459)	bcd	0.21 (0.014)	e	422 (80.3)	ab	109 (20.8)	a
20-04-2005		4.27 (0.467)	cd	0.19 (0.007)	cde	522 (78.2)	b	122 (18.3)	a
25-04-2005		4.32 (0.470)	d						
02-05-2005		4.49 (0.483)	d	0.15 (0.014)	b	740 (73.3)	c	165 (16.3)	b
DAMAGE 22-03-2005									
24-03-2005	damaged	3.53 (0.364)	ns	0.22 (0.020)	+	215 (17.3)	*	61 (4.9)	*
	intact_ref	3.18 (0.348)		0.16 (0.006)		300 (6.4)		94 (2.0)	
29-03-2005	damaged	3.67 (0.440)	ns	0.21 (0.023)	ns	257 (10.0)	+	70 (2.7)	*
	intact_ref	3.19 (0.404)		0.20 (0.016)		340 (28.7)		106 (9.0)	
04-04-2005	damaged	3.04 (0.288)	*	0.19 (0.011)	ns	292 (6.5)	ns	96 (2.1)	ns
	intact_ref	3.72 (0.336)		0.17 (0.016)		350 (37.9)		94 (10.2)	

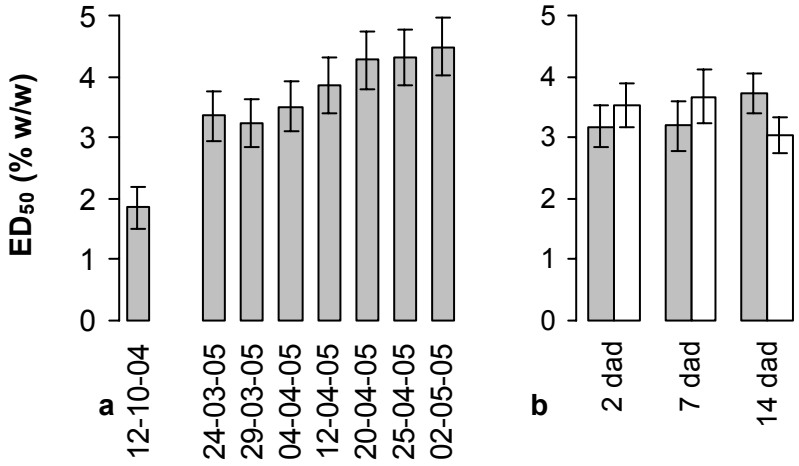

Figure 3.2. ED_{50} estimates of intact winter rye plants at different sampling times (a) and ED_{50} estimates of damaged (white) and intact (grey) winter rye plants at 2, 7 and 14 days after damaging (dad) at 22-03-2005 (b). Vertical bars represent treatment means ± SE.

activity of the cover crop residue material. The total dry weight of winter rye was similar on October 12, 2004 and March 24, 2005 (± 300 g m^{-2}). From March 24 onwards, winter rye growth was nearly linear with an average daily increase of 10.9 g m^{-2} day^{-1} (R^2=0.91) (Table 3.2). The root fraction in spring fluctuated between 0.15 and 0.21 and was significantly higher than the root fraction in autumn (Table 3.2). Despite a reduced allelopathic activity per unit plant material, the allelopathic activity per unit area (DW/ED_{50}) increased linearly with 1.71 g (ED_{50}-equivalent extract solution) m^{-2} day^{-1}, measured from March 24 until May 2, 2005 (R^2=0.83) (Table 3.2). The difference between the ED_{50} values of damaged and intact plants was not significant at two days and one week after damaging. However, two weeks after damaging the injured winter rye plants had a significantly lower ED_{50} value, indicating a higher allelopathic effectiveness, compared to the intact winter rye plants (p=0.040) (Figure 3.2b, Table 3.2). The biomass of the damaged crop was reduced with 28% at two days (p=0.005) and 24% at one week (p=0.055) after damaging, whereas after two weeks the difference in biomass between the damaged and intact crop was no longer significant (p=0.240) (Table 3.2). The increase in root fraction in the damaged plants at two days after damaging was marginally significant (p=0.062) (Table 3.2). The overall effect of mechanical damaging on the allelopathic effectiveness of winter rye residue material per unit area at two days and one week after damaging was negative. Two weeks after damaging the effect became neutral (Table 3.2).

Table 3.3. ED_{50} value (% w/w), root fraction, total dry weight (DW) (g m^{-2}) and the allelopathic activity per unit area (DW/ED_{50}) of lucerne. Data are represented as means with SE within parentheses. Different letters indicate significant differences at the 0.05 level between sampling dates. * indicates a significant difference at the 0.05 level, + indicates a significant difference at the 0.10 level and ns = "not significant".

Harvest date		ED_{50} (% w/w)		Root fraction		DW (g m^{-2})		DW/ED_{50}	
INTACT PLANTS									
12-10-2004		1.40 (0.138)	bc	0.22 (0.001)	a	518 (5.7)	c	370 (4.1)	b
24-03-2005		1.22 (0.125)	b	0.51 (0.004)	d	312 (27.2)	a	255 (22.3)	a
29-03-2005		0.97 (0.107)	a	0.30 (0.013)	c	372 (21.9)	ab	384 (22.5)	b
04-04-2005		1.31 (0.131)	bc	0.23 (0.013)	ab	500 (25.8)	c	382 (19.7)	b
12-04-2005		1.53 (0.149)	cd	0.27 (0.027)	bc	458 (59.2)	bc	299 (38.7)	ab
20-04-2005		1.30 (0.131)	bc	0.21 (0.013)	a	673 (30.4)	d	518 (23.4)	c
25-04-2005		1.39 (0.138)	bc	0.23 (0.007)	a	722 (71.0)	d	519 (51.1)	c
02-05-2005		1.87 (0.178)	d	0.20 (0.006)	a	1047 (48.9)	e	560 (26.2)	c
DAMAGE 18-04-2005									
20-04-2005	damaged	1.07 (0.129)	ns	0.22 (0.008)	ns	541 (56.5)	ns	506 (52.8)	ns
	intact_ref	1.16 (0.137)		0.21 (0.013)		673 (30.4)		580 (26.2)	
25-04-2005	damaged	0.92 (0.148)	+	0.26 (0.008)	ns	650 (8.0)	ns	706 (10.6)	ns
	intact_ref	1.15 (0.175)		0.23 (0.007)		722 (71.0)		628 (61.7)	
02-05-2005	damaged	1.63 (0.218)	+	0.23 (0.011)	*	940 (54.4)	ns	576 (33.4)	ns
	intact_ref	2.03 (0.265)		0.20 (0.006)		1047 (48.9)		516 (24.1)	

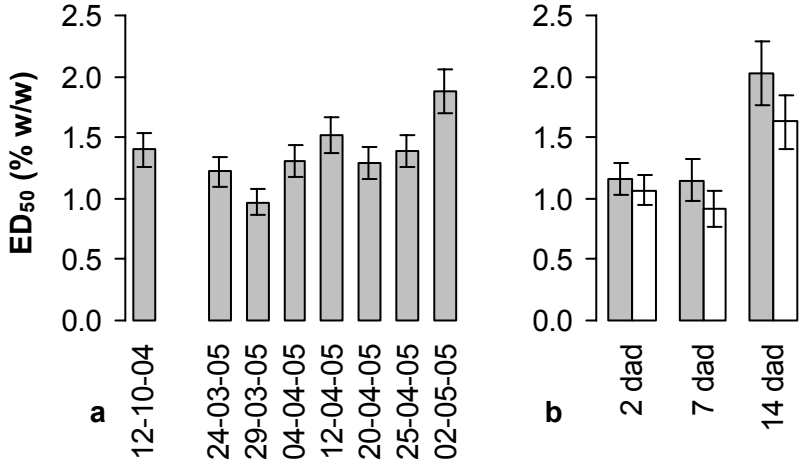

Figure 3.3. ED_{50} estimates of intact lucerne plants at different sampling times (a) and ED_{50} estimates of damaged (white) and intact (grey) lucerne plants at 2, 7 and 14 days after damaging (DAD) at 18-04-2005 (b). Vertical bars represent treatment means ± SE.

Lucerne

The ED_{50} value of lucerne showed no consistent trend with time and ranged from 0.97 on March 29, 2005 to 1.87 on May 2, 2005 (Figure 3.3a, Table 3.3). The total biomass of lucerne had decreased with approximately 200 g m^{-2} between October 12, 2004 and March 24, 2005. In the same period, the root fraction in the lucerne plants increased with 132% (Table 3.3). This decrease in biomass and increase of the root fraction was due to the dying off of the above-ground biomass in winter. In spring, the total dry weight of the intact crop steadily increased with, on average, 16.4 g m^{-2} day^{-1} (R^2=0.86). At the same time, the root fraction started to decrease rapidly until it stabilized in April. The allelopathic activity of lucerne residue material per unit area (DW/ED_{50}) increased linearly with 7.03 g (ED_{50}-equivalent extract solution) m^{-2} day^{-1}, measured from March 24 until May 2, 2005 (R^2=0.69), except for a drop in allelopathic activity per unit area at April 12, 2005 (Table 3.3).

At two days after damaging, no significant difference between damaged and intact lucerne plants was observed. However, the ED_{50} value of the damaged plants was 20% lower than the ED_{50} value of the intact plants at one week (p=0.057) and two weeks (p=0.073) after damaging (Figure 3.3b, Table 3.3). The biomass reduction resulting from damaging lucerne plants was not significant at any harvest time. The root fraction, however, was 15% higher (p=0.033) in the damaged plant at two weeks after damaging (Table 3.3). Despite the increase in allelopathic activity per unit biomass, no significant difference in effectiveness per unit area was observed between damaged and intact lucerne plants at any of the sampling dates (Table 3.3).

Table 3.4. Trivial names and side chain (R) structures of glucosinolates detected in winter oilseed rape.

Trivial name	R side chain	Group
Progoitrin	(2R)-2-hydroxy-3-butenyl	aliphatic
Epiprogoitrin	(2S)-2-hydroxy-3-butenyl	aliphatic
Gluconapoleiferin	2-hydroxy-4-pentenyl	aliphatic
Gluconapin	3-butenyl	aliphatic
Glucobrassicanapin	4-pentenyl	aliphatic
Glucoraphanin	4-methylsulfinylbutyl	aliphatic
Glucoalyssin	5-methylsulfinylpentyl	aliphatic
Glucoerucin	4-methylthiobutyl	aliphatic
4-Hydroxyglucobrassicin	4-hydroxy-3-indolylmethyl	indole
Glucobrassicin	3-indolylmethyl	indole
4-Methoxyglucobrassicin	4-methoxy-3-indolylmethyl	indole
Neo-glucobrassicin	1-methoxy-3-indolylmethyl	indole
Gluconasturtiin	2-phenylethyl	aromatic

Winter oilseed rape

Thirteen different glucosinolates were detected in the winter oilseed rape extracts, eight of which belong to the group of aliphatic glucosinolates, four belong to the group of indole glucosinolates and one belongs to the group of aromatic glucosinolates (Table 3.4). On average, the aliphatic, aromatic and indole glucosinolates constituted 74%, 20% and 6% of the total glucosinolate content, respectively.

The total glucosinolate concentration showed a strong peak on the first sampling date in spring. This was due to a large increase of the aliphatic and aromatic glucosinolates, which were approximately 4.5 and two times higher, respectively, than the levels observed on October 12, 2004. On March 29, 2005, the glucosinolate concentration was reduced again. The level of aliphatic glucosinolates was only about 2.7 times higher than the level in autumn, whereas the level of aromatic glucosinolates had nearly returned to its initial autumn value. From March 29 until the last measurement on May 2, 2005, there was no clear trend in the concentration of aliphatic glucosinolates, whereas the concentration of aromatic glucosinolates showed a linear decline of about 0.0754 nmol g^{-1} day^{-1} (R^2=0.96). In contrast to the other glucosinolate groups, the concentration of indole glucosinolates in spring was approximately three times lower than in autumn. From March 24 to May 2, 2005, the decline continued linearly with on average 0.0172 nmol g^{-1} day^{-1} (R^2=0.90) (Figure 3.4a). In the period between October 12, 2004 and March 24, 2005, winter oilseed rape

continued to grow and the total dry weight increased from 130 to 487 g m^{-2}. From March 24 onwards, the total dry weight steadily increased with approximately 19.2 g m^{-2} day^{-1} (R^2= 0.89) (Table 3.5). The root fraction of the intact plants did not significantly change between harvest times and fluctuated between 0.09 and 0.12 (Table 3.5). The total glucosinolate content of winter oilseed rape per unit area on October 12, 2004 was only 1.43 mmol m^{-2} and had increased almost 10-fold, to 13.87 mmol m^{-2}, on March 24, 2005. On March 29, the total glucosinolate content of the winter oilseed rape crop had dropped again to 8.11 mmol m^{-2}, after which it gradually increased to 15.24 mmol m^{-2} on May 2, 2005 (Table 3.5).

The increase in total glucosinolate concentration as the result of mechanical damaging on March 22, 2005 was only significant at one week after damaging (p=0.027). This was due to the induction of aliphatic glucosinolates, whose concentration in the damaged plants was increased with 25% (p=0.010) at one week after damaging. At two days after damaging, the increase of indole glucosinolates in the damaged plants was marginally significant (p=0.090), but this increase was no longer present after one and two weeks (Figure 3.4b).

The glucosinolate level in plants damaged on April 18, 2005, from which in addition to leaf cutting the inflorescence was removed, was compared to the glucosinolate level in intact, reference plants from which the inflorescence was removed just before freezing the plant material. Damaging in this case resulted in a marginally significant increased total glucosinolate level in the damaged plants at one (p=0.052) and two weeks (p=0.055) after damaging. At one week after damaging this increase in total glucosinolate content was due to a significant (p=0.016) increase of 56% in the concentration of aliphatic glucosinolates in the damaged plants. After two weeks, when the rise in aliphatic glucosinolates was no longer significant, the level of aromatic glucosinolates in the damaged plants was significantly increased (p=0.004) with 35%. The indole glucosinolates, which comprised only a small part of the total glucosinolate content, were present at a marginally significant higher concentration in the damaged plants at two weeks after damaging (p=0.063) (Figure 3.4c).

Mechanical damaging on March 22, 2005, resulted in a 23% higher total glucosinolate content per unit area at one week after damaging (p=0.003) (Table 3.5). As cutting in leaves did not cause a direct change in the biomass of the damaged plants, this gain in total glucosinolate content per unit area directly resulted from the increased glucosinolate content per unit plant material. The effect of damaging on April 18, 2005 on the total glucosinolate content per unit area was assessed by comparing the lower part of damaged plants with the intact, whole plants. The contribution of the inflorescence that was cut from the damaged plants to the total glucosinolate content per unit area, therefore, was ignored. The loss of the

Table 3.5. Winter oilseed rape total glucosinolate (GLS) concentration (nmol g^{-1}), root fraction, total dry weight (DW) (g m^{-2}) and the total glucosinolate content per m^2 (mmol m^{-2}). Data are represented as means with SE within parentheses. Different letters indicate significant differences at the 0.05 level between sampling dates. * indicates a significant difference at the 0.05 level, + indicates a significant difference at the 0.10 level and ns = "not significant".

Harvest date		Tot GLS (nmol g^{-1})		Root fraction		DW (g m^{-2})		Tot GLS (mmol m^{-2})	
INTACT PLANTS									
12-10-2004		10.93 (1.772)	c	0.10 (0.010)	a	130 (3.3)	a	1.43 (0.246)	a
24-03-2005		28.58 (1.912)	a	0.11 (0.010)	a	487 (18.3)	bc	13.87 (0.931)	d
29-03-2005		17.61 (1.713)	b	0.12 (0.007)	a	452 (39.1)	b	8.11 (1.393)	b
04-04-2005		16.26 (1.734)	b	0.12 (0.014)	a	584 (97.5)	c	9.29 (1.426)	b
12-04-2005		16.69 (1.025)	b	0.10 (0.005)	a	937 (104.2)	d	15.79 (2.270)	d
20-04-2005		13.49 (0.661)	b	0.11 (0.003)	a	976 (23.3)	d	13.12 (0.355)	cd
25-04-2005		10.32 (0.429)	c	0.10 (0.006)	a	939 (63.2)	d	9.95 (0.381)	bc
02-05-2005		12.66 (0.507)	bc	0.09 (0.005)	a	1212 (151.9)	d	15.24 (1.629)	d
DAMAGE 22-03-2005									
24-03-2005	damaged	34.39 (5.024)	ns					16.96 (3.090)	ns
	intact_ref	28.58 (1.912)						13.87 (0.931)	
29-03-2005	damaged	21.53 (2.562)	*					9.99 (1.945)	*
	intact_ref	17.61 (1.713)						8.11 (1.393)	
04-04-2005	damaged	15.40 (1.548)	ns					9.30 (2.353)	ns
	intact_ref	16.26 (1.734)						9.29 (1.426)	
DAMAGE 18-04-2005 (without inflorescence)									
20-04-2005	damaged	11.56 (1.366)	ns	0.13 (0.004)		850 (12.6)		9.85 (1.275)	ns
	intact_ref	9.49 (0.945)						13.12 (0.355)	
25-04-2005	damaged	10.15 (0.516)	+	0.11 (0.007)		817 (58.4)		8.07 (0.133)	*
	intact_ref	7.10 (0.746)						9.95 (0.381)	
02-05-2005	damaged	11.05 (1.215)	+	0.11 (0.006)		1040 (133.8)		11.32 (1.475)	+
	intact_ref	8.99 (0.567)						15.24 (1.629)	

inflorescence, which contained an approximately 3.5 times higher glucosinolate concentration than the lower plant part (data not shown), was not compensated for by the induction of glucosinolates in the lower part of the damaged plants. Consequently, damaging had a negative effect on the total glucosinolate content per unit area, which was significant at one week after damaging ($p=0.017$) and marginally significant at two weeks after damaging ($p=0.052$) (Table 3.5).

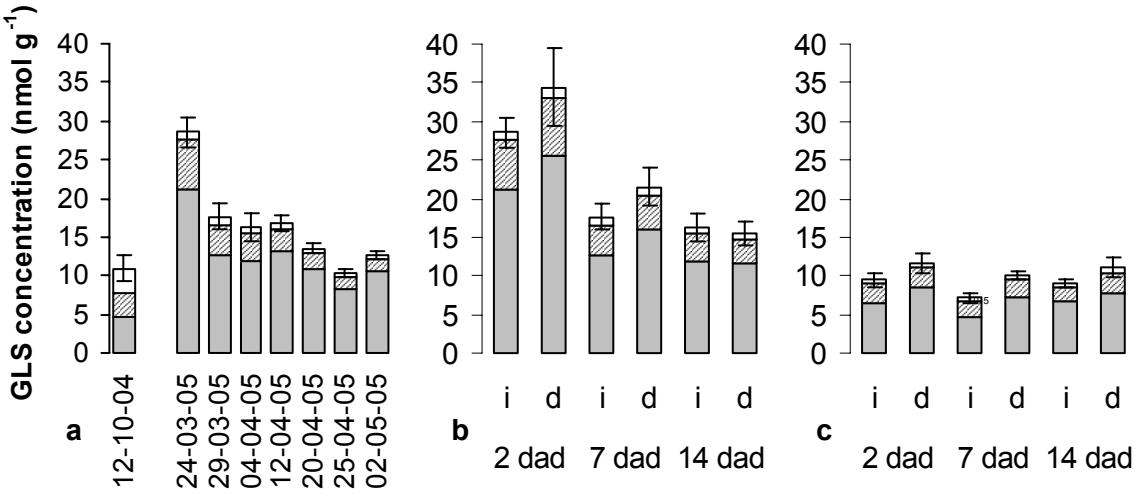

Figure 3.4. Content of aliphatic (grey), aromatic (stripes) and indole (white) glucosinolates of intact winter oilseed rape plants at different sampling times (a); intact (i) and damaged (d) plants at 2, 7 and 14 days after damaging (dad) at 22-03-2005 (b); the lower part of intact (i) and damaged (d) plants at 2, 7 and 14 days after damaging (dad) at 18-04-2005 (c). Vertical bars represent treatment means of total glucosinolate content ± SE.

Discussion

Induction of allelochemicals by mechanical damaging

The results of our study show that mechanical damage can enhance the allelopathic effect of lucerne and winter rye residue material and can cause an increase in the concentration of glucosinolates in winter oilseed rape. To our knowledge this is the first time that induction of allelochemicals by mechanical damaging is demonstrated in older, field-grown crops on the basis of whole-plant assays.

It is remarkable that induction was detected in older, field-grown plants of all three cover crop species at the whole-plant level. Firstly because plants, before mechanical damage was applied, may already have been induced to a certain extent during the months they were growing on the field, leading to a lower impact of additional artificial damaging. Secondly because responses to wounding are usually stronger in younger plants (Ohnmeiss and Baldwin, 2000; Van Dam et al., 2001; Agrell et al., 2003a). Winter rye and winter oilseed rape were in the stem elongation stage when damaged on March 22, 2005 and winter oilseed rape had reached the flowering stage when damaged on April 18, 2005. Lucerne, a biennial crop, although still in its vegetative stage, had also already grown for several months. Boege and Marquis (2005) suggest that young plants, in general, are more inducible than mature plants, as the induction of defense mechanisms only occurs in tissues that are actively growing and the relative proportion of actively growing versus differentiated tissue is greater in

younger plants. Empirical data sustaining this hypothesis were provided by Ohnmeiss et al. (2000), who found that whole-plant nicotine contents in tobacco were increased by leaf damage applied in the rosette-stage, but not in the elongation- or flowering-stage. Agrell et al. (2003a) also could not detect a response to leaf herbivory of lucerne in the flowering stage, whereas a significant response was observed in the vegetative stage. Baldwin and Ohnmeiss (1993) observed that in later growth stages inflorescence removal was more effective in causing an increase in nicotine in *Nicotiana attenuata* foliage than leaf damage. Removal of the inflorescence in winter oilseed rape indeed led to an increased glucosinolate content in the lower plant part. The induction of glucosinolates in the lower plant part following inflorescence removal may be explained by a partial reversion of the plant to the vegetative growth stage.

Whole-plant assaying eliminates the chance of finding an increased content of allelochemicals in certain tissues as a consequence of re-allocation of allelochemicals already present. The increased allelopathic effectiveness of damaged winter rye and lucerne plant material and the enhanced glucosinolate content of damaged winter oilseed rape therefore suggest a *de novo* biosynthesis of the involved chemicals. However, the possibility remains that the increased root fraction observed in damaged winter rye and lucerne plants at 2 days and 2 weeks after damaging, respectively, contributed to the rise in overall allelopathic strength of the damaged plant material. Both qualitative and quantitative differences in allelochemical content of roots and shoot have been reported. In lucerne, for instance, the saponin compounds that showed highest inhibitory effects on wheat seedling growth (Oleszek, 1993) were only detected in roots and not in the shoot (Nowacka and Oleszek, 1994). In winter rye it seems less likely that an enhanced root fraction contributed to the rise in overall allelopathic strength as aqueous crude extract of shoot tissue contained a several times higher hydroxamic acid content and was more toxic to lettuce and tomato root length than extract of root tissue (Rice et al., 2005).

Temporal patterns of induced allelochemicals

Temporal changes in allelochemical content of damaged plants have mainly been studied following herbivore damage. Agrell et al. (2003b) found that herbivore damage of non-flowering 5 to 7-weeks old lucerne plants induced the synthesis of defense compounds. They found a peak in the concentration of defense chemicals in damaged plants at one week after damaging, but in contrast to our findings, could no longer detect any effect at fourteen days after damaging. Induction of hydroxamic acids by Asian corn borer feeding on maize (Huang et al., 2006) or by aphid infestation of wild wheat (*Triticum uniaristatum*) (Gianoli and Niemeyer, 1997) for a period of 48 hours only yielded a localized effect detected immediately after herbivore

removal, but not later. Damage of a relatively large magnitude, such as applied in the winter rye defoliation experiment of Collantes et al. (1997), did lead to a longer lasting induction of hydroxamic acids in winter rye seedling shoots. The effect was present from 5 days to at least up to 11 days after defoliation. In our study, an increased allelopathic effect of damaged plants compared to intact plants was detected only at two weeks after damaging. Studies that show the temporal patterns of induction in winter oilseed rape are scarce. A study of Bodnaryk (1992), in which the cotyledons of *Brassica napus* seedlings were punctured with a needle, showed the levels of 3-indolylmethyl glucosinolate in the cotyledons to increase starting at 4 hours after wounding until their senescence after one week. Increased levels were also detected in the first true leaves at one and two weeks after wounding, but not in the second true leaves measured at two weeks after wounding. In contrast to our study, in which the concentration of aliphatic glucosinolates was most affected by damaging, several studies report increased levels of indole glucosinolates linked with an unchanged or decreased concentration of aliphatic and aromatic glucosinolates following wounding of above-ground tissues of *Brassica* spp (Koritsas et al., 1991; Bodnaryk, 1992; Van Dam et al., 2004). However, these studies have all been carried out with young, laboratory-grown plants. In another experiment by Koritsas (1991), which investigated the induction of field-grown winter oilseed rape plants in spring following infestation with cabbage stem flea beetle (*Psylliodes chrysocephala*), infestation only led to increased levels of indole glucosinolates in the directly attacked plant parts (the petioles), whereas in the laminae adjacent to the infested petioles and in the roots the levels of aromatic and most aliphatic glucosinolate compounds increased as well.

Temporal patterns of constitutive allelochemicals

Congruent with our results, Reberg Horton et al. (2005) found the allelopathic activity of rye aqueous extracts, originating from rye plants harvested on March 1, April 5 and April 26, to decline with time. In contrast, Rice et al. (2005), who sampled rye from March 24 (stem elongation) to June 17 (flowering), did not find a temporal pattern in the allelopathic activity of rye aqueous extracts. Both authors found the hydroxamic acid DIBOA to decline with time. While we observed a substantial increase in the total glucosinolate content between the leaf production and stem elongation stage of winter oilseed rape, Clossais-Besnard and Larher (1991) observed a decrease in total glucosinolate content in greenhouse-grown *Brassica napus* cv. Drakkar and a slight increase in total glucosinolate content in *B. napus* cv. Chine 32 in approximately the same period. It may be that, apart from cultivar differences, the exposure of our field-grown plants to low temperatures has caused this difference. In spring, the temporal pattern of total glucosinolate concentration of *B. napus* cv. Drakkar in the experiment

of Clossais-Besnard and Larher (1991) was very similar to that of our intact winter oilseed rape plants. Unlike for the other two cover crop species, we did not observe a clear pattern of phytotoxicity in time for lucerne, probably because lucerne did not change from the vegetative to the reproductive phase in spring 2005. A study in which sampling of lucerne plants was spread over a longer period showed that the immature lucerne residues contained more allelochemicals than older residues (Guenzi et al., 1964). Furthermore, Wyman et al. (1991) observed that saponins with allelopathic activity in lucerne roots were most abundant during bud appearance and lowest at one month after full bloom.

Practical considerations

For practical application of mechanical wounding of cover crops for weed control, the main focus should be on the allelopathic activity per unit area rather than on the allelopathic activity per unit plant biomass. This is the combination of biomass and allelopathic activity per unit plant material. For all three cover crops we observed that the highest allelopathic activity per unit area in spring was measured at the last sampling date, mainly as a result of an increased biomass. This suggests that, later in spring, biomass becomes the main determinant of allelopathic activity per unit area, and it would therefore be best to wait as long as possible with cover crop residue incorporation.

Induction of allelochemicals in older, field-grown plants is the most striking observation in our experiment. However, when comparing the increases in concentration of allelochemicals or in allelopathic activity per unit plant material after damaging with the changes in these parameters over time, we have to conclude that the impact of damaging is not very large. Furthermore, looking at the change in allelopathic activity per unit area following damaging, it becomes clear that the slight increase in the allelopathic activity per unit biomass was accompanied by a loss of plant material due to damaging. Conservative estimates of the overall effect of damaging were made as it was assumed that any plant material removed as a result of damaging did no longer contain allelochemicals at the time of residue incorporation. For winter rye and lucerne the allelopathic activity per unit plant material following damaging was just sufficient to compensate for the loss of plant material as a result of damaging. Removing the inflorescence of winter oilseed rape even resulted in a clear negative impact on the total glucosinolate content per unit area as inflorescence removal implied the loss of plant material with a relatively high glucosinolate content. Only in the case of winter oilseed rape damage in the stem elongation stage at one week after damaging, a significant increase of glucosinolates per unit area was observed. This can partly be explained by the fact that for this crop large scale

application of mechanical damage was not feasible and manually applied leaf cutting did not involve biomass loss. The minor impact of damaging on the allelopathic activity per unit biomass combined with the loss of biomass resulting from damaging leads to the conclusion that mechanical damaging of cover crops is of little significance for farming practice.

CHAPTER 4

Cover crop residue management for optimizing weed control

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Summary

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 ways 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. 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. Whereas residue incorporation gave variable results, placement of winter rye residue on top of the soil inhibited the emergence of all indicator 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. Simply replacing herbicides by other direct control measures is inadequate. Instead, weed management should be seen as a component of integrated crop management (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 (Chapter 2, Weston, 1996; Ohno et al., 2000). Especially cover crops that contain a high level of allelochemicals seem well-suited for residue-mediated weed suppression. Of the cover crops that fit to 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, oxazolidine-thiones, 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).

Apart from 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 this (Stevenson, 1986, in Liebman and Mohler 2001). Crop residues can also affect the physical properties of the soil. Residue-amended soil can 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 can both have an inhibitory effect on weed germination (Teasdale and Mohler, 1993; Liebman and Mohler, 2001). Furthermore, in some cases soil microbial populations, including soilborne pathogens, are stimulated after soil amendment with fresh residue material (Dabney et al., 1996; Conklin et al., 2002; Manici et al., 2004).

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 quality 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 different levels of cell disruption. These changes in particle size and cell disruption of residue material 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 till 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 a 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 lower release rate of allelochemicals.

Although residue management seems 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, for two contrasting cover crop species, the relationship between weed suppression and cover crop residue management strategy, particularly pre-treatment and placement of residue material on or in the soil. In this context, we also (1) compared the emergence of four indicator 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 strategy 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 indicator species, (2) residue-mediated inhibition of seedling emergence takes place earlier with increased levels 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 an effect based on all relevant residue-mediated mechanisms and their interactions.

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 location “the Haarweg” of the Wageningen University in

Wageningen, the Netherlands. Rings with a diameter of 30 cm and a depth of 25 cm were buried in the soil, allowing 1 cm to protrude 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. To be able to distinguish between sterilized and non-sterilized 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) and winter oilseed rape (*Brassica napus* L.) (12 seeds/ring) 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 5-12-4) 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. For each 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 in pieces of approximately 1 cm^2 for experiment 1 and 2. For experiment 3 the roots were discarded. The soil, roots and the above-ground fresh material were divided in equal portions, similar to the number of rings, and for each portion the soil and roots were mixed again. The above-ground material was cut into pieces of 3 cm, perpendicular to the main plant axis, and thereafter either left untouched, crushed or ground. Subsequently, the rings were refilled with the soil, and shoot residues were added in three different ways: mixed through the upper 10 cm of the soil, left on top of the soil or placed in a layer at a depth of 10 cm in the soil. Directly after refilling the rings, seeds of two weed and two crop species were sown and newly emerged seedlings were regularly counted.

Experiment 1

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 4.1). The experimental design was a completely randomized split-plot with eight replications. 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 in four sub-plots of equal size, in each of which a different indicator 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 168 g (= 23.7 tons ha^{-1}) for winter oilseed rape and 171 g (= 24.2 tons 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 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* 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

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 4.1). The indicator species and the experimental set-up were equal to experiment 1. Residues were harvested, cut and placed in the rings on March 21 (block 1–4) and March 23 (block 5–8), 2005. 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 g (= 88 tons ha^{-1}) of fresh winter oilseed rape material and 422 g (= 60 tons ha^{-1}) of fresh winter rye material.

Experiment 3

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 a mulch. The amount of fresh biomass that was used for each ring was kept at 300 g (= 42.4 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, 3, 6, 9, 15, 21 and 29 days after residue incorporation (DAI). The experimental design was a completely randomized 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 and 2, experiment 3a), March 29 (block 3 and 4, experiment 3a), April 4 (block 1 and 2, experiment 3b) and April 5 (block 3 and 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, the electrical conductivity (EC), the pH, the soil water content (percentage by weight), and the 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 the EC and pH measurements, 8.00 ± 0.03 g of loose, dry soil was dissolved in 40.0 ml de-ionized 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 hours 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 $CaCl_2$.

Cultivar differences. Just prior to residue incorporation, above-ground plant material of the cover crops was collected for determination of the allelopathic potential. Of 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 screen and stored in plastic jars in the dark and at room temperature until use. For winter rapeseed 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-ionized 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 in Chapter 3.

Data analysis

Analysis of seedling emergence data. Both 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 T_{50} emergence was determined by fitting a three-parameter logistic curve through the emergence data of each treatment, assuming a binomial distribution and while keeping the upper limit fixed to the total number of emerged seedlings. A General Linear Mixed Model (GLMM) with an Iterative Reweighted Residual Maximum Likelihood (IRREML) procedure (Genstat Release 9, 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 indicator species with the GLIM (Generalized Linear Model) procedure for binomial data (Genstat Release 9, 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 in Chapter 3.

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 4.1). An interaction between residue pre-treatment and indicator 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 indicator species. For lettuce, spinach and *S. media* only grinding resulted in a reduction of seedling emergence, with 28%, 44% and 27%, respectively. In contrast, for *C. album* grinding as well as crushing of residue material resulted in an increase in the number of emerged seedlings. When *C. album* was excluded from the dataset, also cutting and crushing were found to significantly lower emergence 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. *S. media* and *C. album* emergence, in contrast, was not affected by any of the winter rye residue treatments.

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

Table 4.1. Fraction emergence of indicator species, sown directly after residue incorporation of winter oilseed rape (WO) or winter rye (WR) in experiment 1 and 2. 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.

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

Figure 4.1 shows seedling emergence of each indicator species in successive one-week periods as affected by amendment of differently pre-treated winter oilseed rape residues. Within the first 3 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 ground winter oilseed rape residue between 4 and 8 WAI. Cut winter oilseed rape residue initially still reduced *C. album* emergence, but with time gradually started to stimulate *C. album* emergence. Also for winter rye, early emerging indicator species (lettuce and spinach) were inhibited by the residues, whereas the establishment of the later emerging indicator species (*S. media* and *C. album*) was not affected. However, unlike for winter oilseed rape, the time course of *C. album* emergence was not affected by winter rye residue pre-treatment (data not shown).

Experiment 2 – effect of residue placement

In experiment 2, all residues were cut in 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 4.1). For winter oilseed rape, residue placement had a similar effect on all indicator 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, seedling emergence of none of the indicator species was affected. For winter rye, an interaction between residue placement and indicator species was found ($p<0.001$). This was mainly due to a difference in response of the indicator 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 indicator species, mulching caused the strongest inhibition in emergence, with 56%, 65%, 38% and 39% reduction for lettuce, spinach, *S. media* and *C. album*, respectively. Also for 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 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 and 34 days for *S. media* and *C. album* seedlings, respectively. For all four indicator species and both cover crop species, the T_{50} emergence in the mulch treatment was increased with on average 2–3 days.

Similar to experiment 1, mixing of residue through the upper soil layer initially

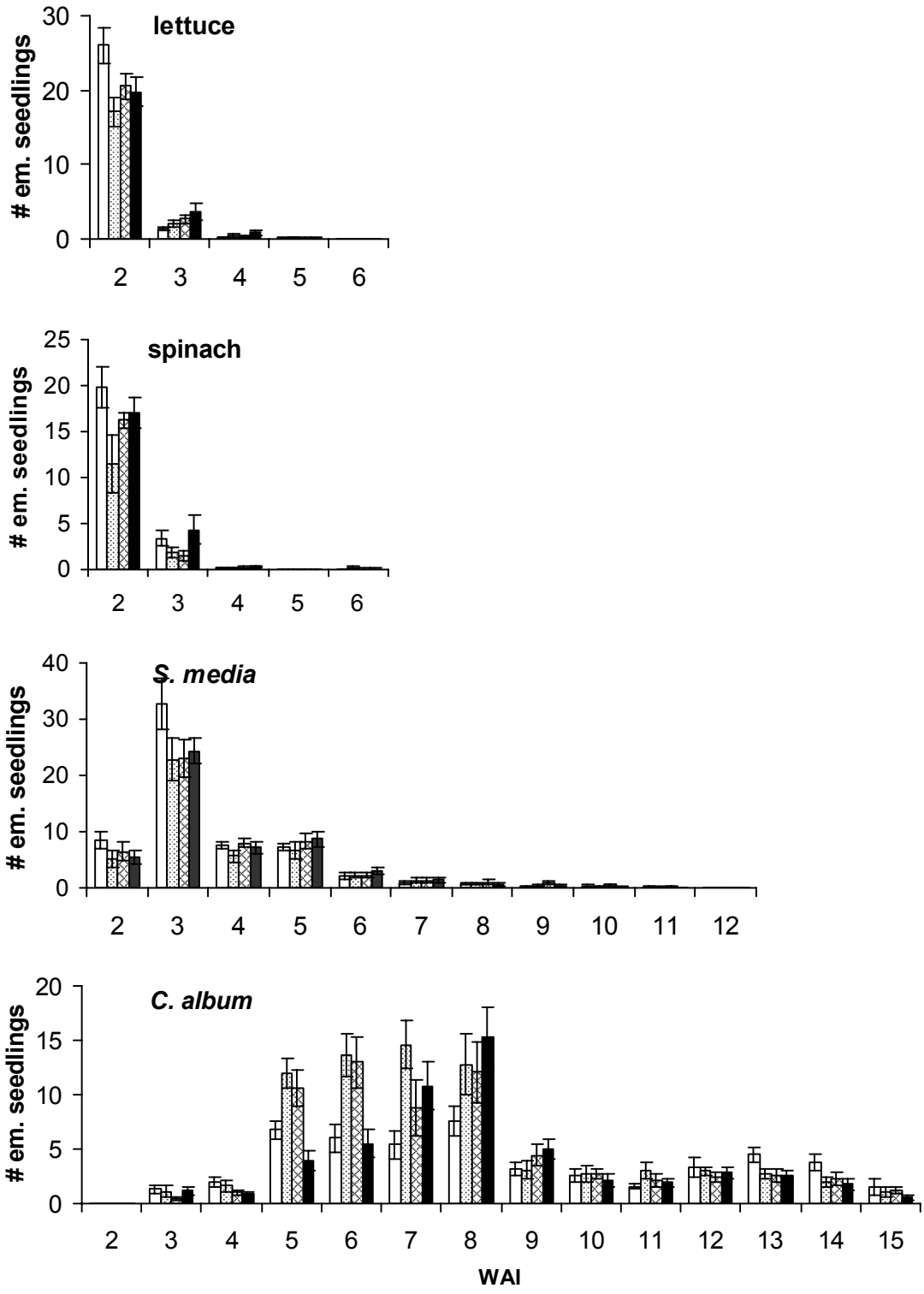

Figure 4.1. Weekly seedling emergence data of four indicator species as affected by pre-treatment of winter oilseed rape residue in experiment 1: control (white), ground (stippled), crushed (cross-hatching) or cut residues (black). WAI = "weeks after incorporation". Vertical bars represent means ± SE.

reduced the emergence of *C. album* seedlings, whereas this inhibitory effect disappeared with time (winter oilseed rape) or changed into a stimulatory effect (winter rye).

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

Frequent sowing of lettuce seeds at different moments 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 ground and cut winter oilseed rape residues (Figure 4.2). Grinding reduced lettuce emergence earlier than cutting. The inhibitory effects of the 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 winter oilseed rape mulch treatment could not be established, because of the interference of snails 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 in either experiment 3a or 3b (data not shown). In experiment 3a, the emergence of lettuce seedlings, averaged over all sowing times, was inhibited by ground cv. Wheeler residues (21%) and by cut and mixed cv. Wheeler residues (13%), whereas the same treatments with residues of cv. Protector did not affect lettuce emergence. When used as a mulch, both winter rye cultivars exerted a 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} emergence 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} emergence of lettuce seedlings was only 4.5 days as opposed to 18 days in the control treatment.

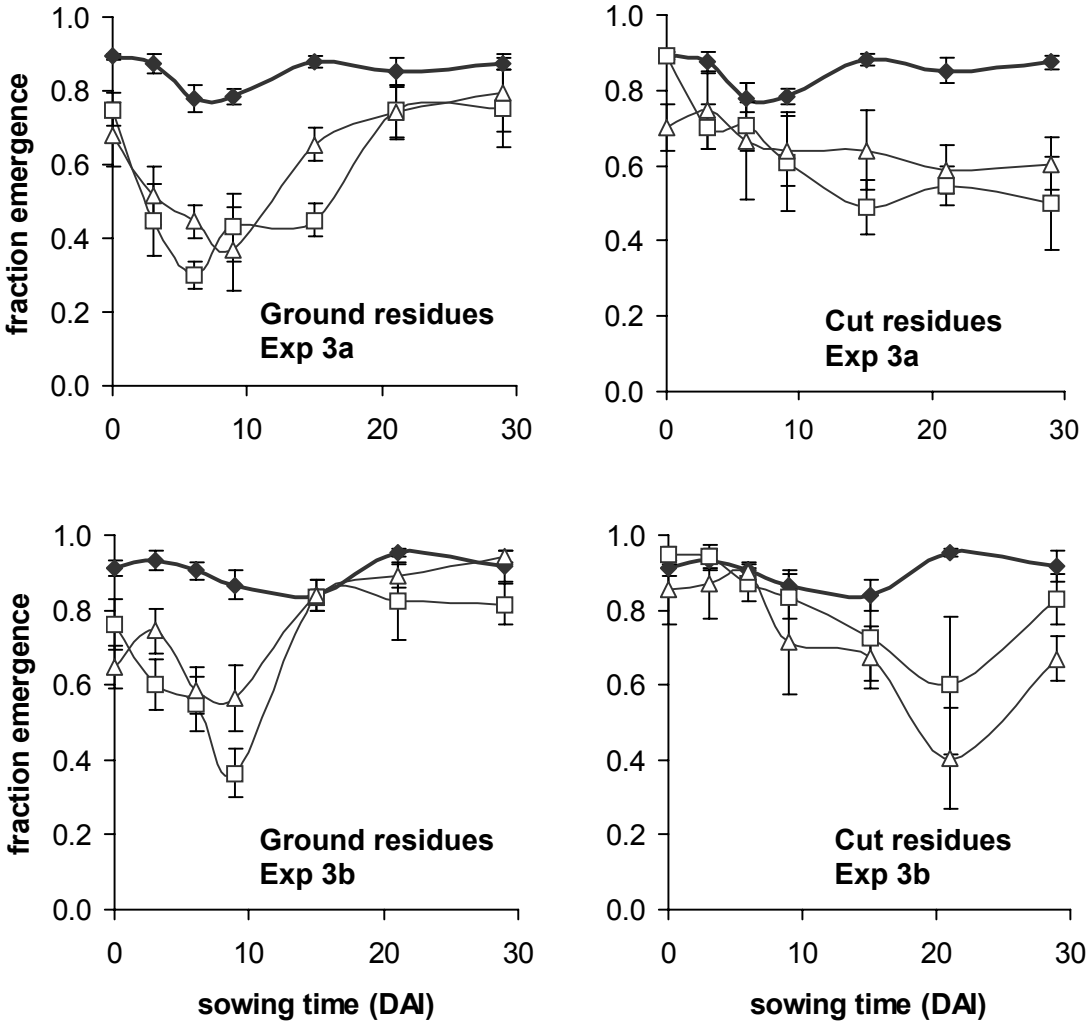

Figure 4.2. Fraction emergence of lettuce sown at different days after winter oilseed rape residue incorporation (DAI) in experiment 3a and 3b. Thick lines = control treatment (no residues), open triangles = winter oilseed rape cv. Dwarf Essex, open squares = winter oilseed rape cv. Athena. Vertical bars represent mean values ± SE.

The influence of residue amendment on soil characteristics

The EC in the control treatment was rather stable (Figure 4.3). 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 through which ground residues were mixed, and was similar to the EC in soil containing cut residues at 29 DAI and later. For winter rye, in contrast, the EC in the soil with 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 through which the ground residues were mixed. Cover crop species and cultivar did

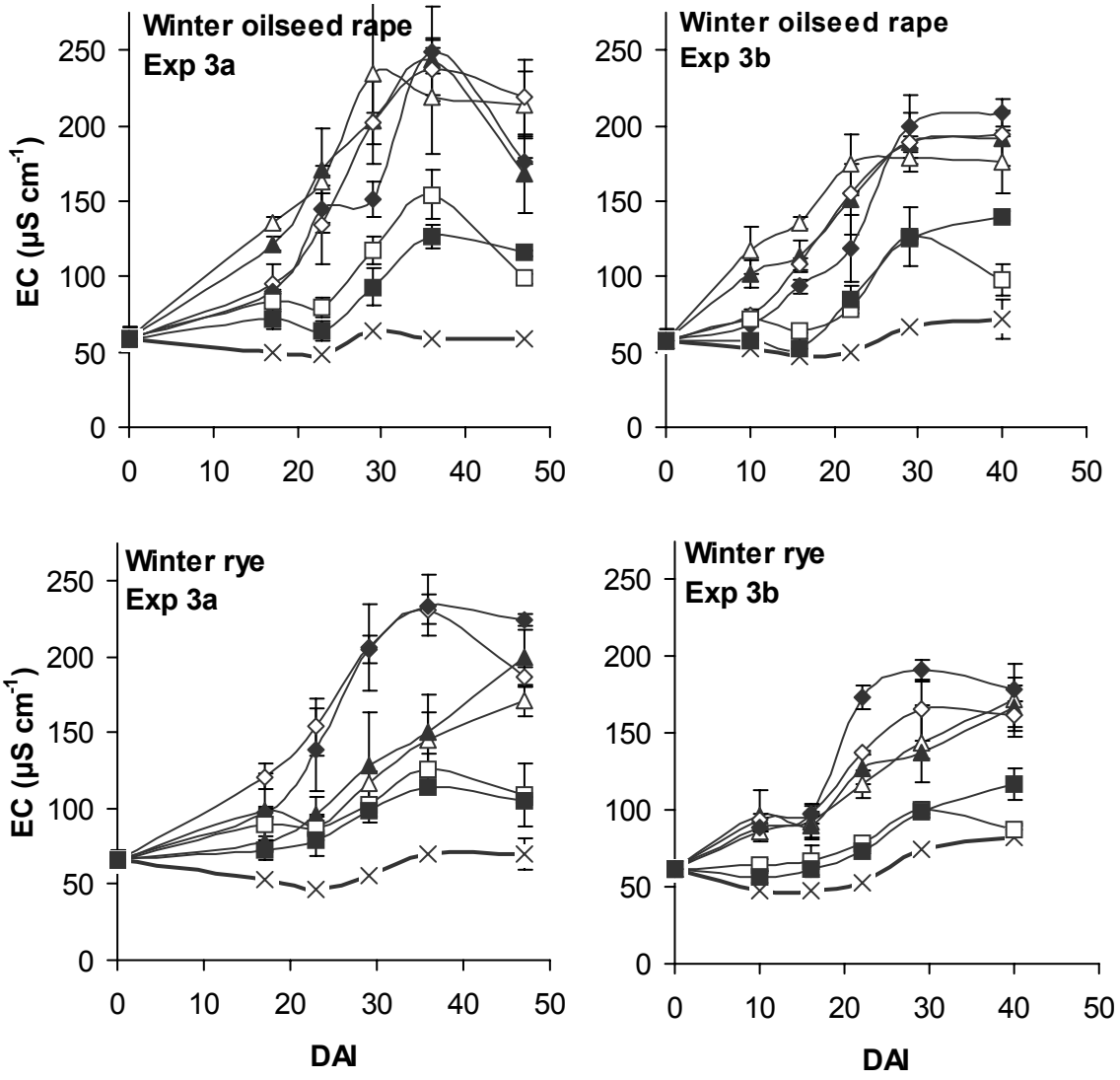

Figure 4.3. Electrical conductivity (EC, µS cm^{-1} at 25 °C) measured in a mixture of 20% dry soil and 80% de-ionized water. Soil samples were taken at different days after residue incorporation (DAI) experiment 3a and 3b. Closed markers = cultivars Wheeler and Dwarf Essex, open markers = cultivars Protector and Athena. Crosses = control treatment (no residues), triangles = ground and mixed, diamonds = cut and mixed, squares = cut and mulch. Vertical bars represent mean values ± SE.

not influence the strength 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.

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

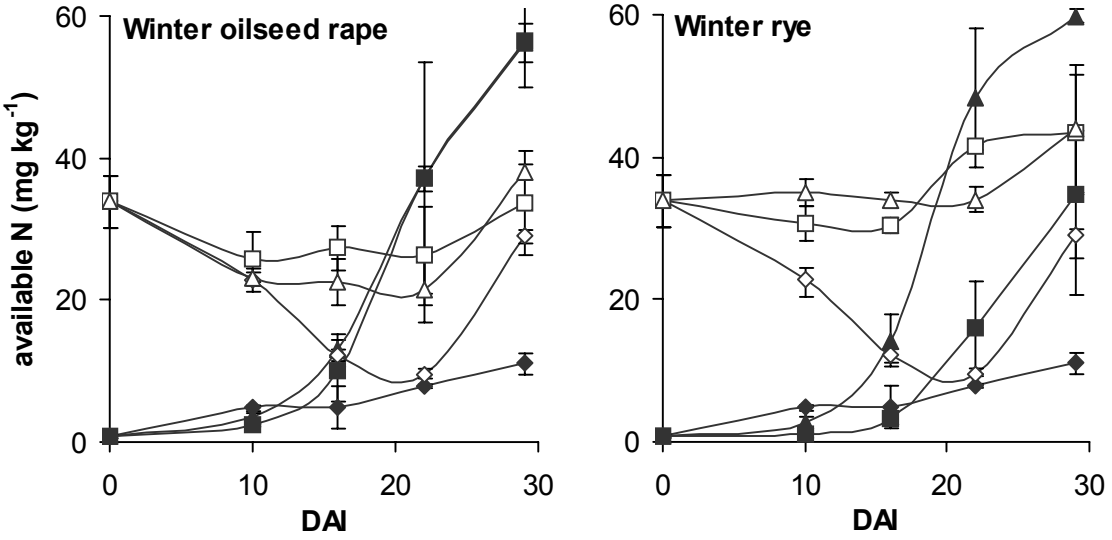

Figure 4.4. Available $N\text{-}NO_3$ (closed markers) and $N\text{-}NH_4$ (open markers) (mg kg^{-1}) measured in soil samples taken at different days after residue incorporation (DAI) in experiment 3b. Diamonds = control treatment (no residues), squares = ground and mixed, triangles = cut and mixed. Vertical bars represent mean values ± SE.

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; Figure 4.4). 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 4.2). Dwarf Essex contained 120 μmoles of glucosinolates per g dry residue, whereas this was only 71 μmoles 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).

Table 4.2. 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 conc Dwarf Essex	GLS conc Athena	
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*	***

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. Congruent with our hypothesis, we showed that inhibition of seedling emergence by incorporated winter oilseed rape residues takes place earlier with increased level of tissue disruption. Ground winter oilseed rape residues inhibited lettuce seedling emergence only during the first two to three weeks following residue incorporation, whereas inhibition of lettuce seedling emergence by cut residues started after this period (experiment 3; Figure 4.2).

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 likely explains why ground residues were inhibitory to seedling emergence earlier in time than cut residues. With tissue disruption not only allelochemicals, but also many other compounds, including salts, were released into the soil. We measured the release of salts from the residues into the soil over time by measuring the electrical conductivity (EC) in extracts of dried soil samples. In correspondence with the difference in temporal patterns observed for inhibition of seedling emergence, the EC in the soil containing the ground residues increased earlier in comparison to the soil with the cut residues (Figure 4.3).

The effect of *Brassica* residue pre-treatment on subsequent isothiocyanate release (Morra and Kirkegaard, 2002), as well as the isothiocyanate release pattern over time in *Brassica* amended soil (Gardiner et al., 1999) have been investigated before. However, as far as we know only our study 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 in the upper soil layer. Peak concentrations of isothiocyanates were reached much faster than the peak inhibitory effects on lettuce emergence of either the ground or cut residues in our experiment. Isothiocyanate (ITC) concentrations reached a peak at 30 hours 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 come to the idea that freezing and thawing may not have simply increased the overall ITC release efficiency, but instead mainly 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 even 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. We monitored the electrical conductivity (EC), which is directly related to the osmotic pressure (Xuan et al., 2005), over time in the residue-amended soil. After approximately 30 DAI (exp. 3a) or 23 DAI (exp. 3b), the EC in the soil water of the residue-amended soil reached a peak through a combination of a high concentration of salts (Figure 4.3) and a low soil water content, whereas at the same time lettuce emergence was no longer reduced by the ground residues.

Winter rye. For winter rye, no clear patterns in lettuce seedling inhibition over time were observed for either ground or cut residues. Furthermore, the emergence of the indicator species was not influenced by residue pre-treatment. This is surprising, because like for glucosinolate breakdown products, the release of the hydroxamic acid DIBOA 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, c.f. Fomsgaard et al. 2004). BOA, 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.

Unlike for winter oilseed rape, the EC increased faster in soil containing cut winter rye residues than in soil containing ground winter rye residues. This is surprising, but may be explained by leaching of salts released from ground residues to 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 ground residue was largest, and 7.9 mm in experiment 3b.

Effects of residue placement on seedling emergence

Winter rye was most effective in reducing seedling emergence when used as a 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 almost 1.5 times lower amount of winter rye residue applied.

However, under dry circumstances 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 indicator species

Contrary to our hypothesis and our findings in Chapter 2, we found large differences in the response of the indicator species to the cover crop residues. These differences seem clearly related to the emergence rate of the indicator 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 will 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. Similar to our hypothesis about residue-mediated inhibitory effects, for winter oilseed rape the time of this transition was influenced by the level of tissue disruption. 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; Figure 4.1).

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 reasons. 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 a mulch, inhibitory effects were similar. This is congruent with our hypothesis, which states that the allelochemical 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 bioassays, 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 the results in Chapter 3 and with several publications that state that the hydroxamic acid concentration in rye decreases with age (Reberg Horton et al., 2005; Rice et al., 2005). 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.

Practical considerations

For winter rye, using residue as a mulch was the most effective weed management strategy. For winter oilseed rape, mulch was less effective and included the risk of attracting snails. However, soil-incorporated winter oilseed rape residue greatly reduced weed establishment during the first weeks following incorporation. The exact timing of the inhibitory effect could be influenced by residue pre-treatment, as residue cutting delayed the inhibitory peak compared to residue grinding.

CHAPTER 5

Selectivity of incorporated cover crop residues: importance of seed mass and the temporal dynamics of species' sensitivity and residue inhibitory potential

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Summary

In laboratory bioassays, it has often been observed that cover crop residues inhibit germination and early growth of small-seeded species while, in comparison, large-seeded species better tolerate residue-mediated stress. As most weed seeds are one to three orders of magnitude smaller than most crop seeds, there is potential for selective weed suppression by cover crop residues in large-seeded crops. The aim of our study was to assess to what extent seed mass determines species sensitivity to cover crop residues under field conditions. Two experiments were conducted in which crop and weed species differing in seed mass were sown in control soil (no residues) and soil with recently-incorporated lucerne, winter oilseed rape or winter rye residues. Seedling emergence was monitored over time. In the first experiment, sowing of lettuce at different times after residue incorporation revealed a continuous decline of residue-mediated effects of lucerne and winter oilseed rape over time. In the second experiment a sudden and large increase in the residue-mediated inhibitory potential of lucerne residues was detected, which was likely due to the occurrence of a large rainfall event 7 to 9 days after residue incorporation. Winter rye residues had no effect on seedling establishment. In most cases we found a positive relationship between seed mass and relative emergence, indicating that small-seeded species are indeed more sensitive to residue-mediated effects. However, re-analysis of datasets of two earlier-published experiments (Haramoto

and Gallandt, 2005) revealed that seed mass only becomes important in determining the strength of the residue-mediated effect *after* accounting for the time of emergence of the receptor plant. Our findings suggest that residue-mediated inhibition of the receptor plant only occurs when there is an overlap of the time course of the sensitivity of the receptor plant and the time course of the residue-mediated inhibitory potential, and that seed mass is of secondary importance. Variation commonly reported in research on residues or allelopathy may be explained by environmental or management effects on the synchronicity of these processes.

Keywords: organic farming, ecologically-based weed management, cover crops, green manure, seed size, allelopathy

Introduction

Incorporated cover crops commonly inhibit emergence and growth of weed and crop species (Chapter 2, Weston, 1996; Ohno et al., 2000). To take advantage of cover crop residues for weed suppression, residue-mediated effects must selectively target weed species, whereas negative effects on the crop should be avoided. It has often been observed that, compared to large-seeded species, germination and early growth of small-seeded species is more inhibited by cover crop residues (Burgos and Talbert, 2000; Petersen et al., 2001; Liebman and Sundberg, 2006). Several mechanisms to explain this phenomenon have been summarized by Liebman and Sundberg (2006). Firstly, small-seeded species usually have a larger root length per unit of root mass (Leishman et al., 2000), which confers a larger absorptive surface area through which allelochemicals might enter. Secondly, seed size is positively related to seed reserves, which allows larger-seed species to better support seedling respiration during periods of stress-induced carbon deficit (Westoby et al., 2002). Analogously to this, it may be that species with greater seed reserves are better able to detoxify allelochemicals, though this still remains to be tested. As the majority of weed seeds are one to three orders of magnitude smaller than most crop seeds (Mohler, 1996), there is potential for selective weed suppression by cover crop residues in large-seeded crops.

Most experiments that established a relationship between seed mass and sensitivity to allelochemicals were carried out in the laboratory, using Petri dishes lined with filter paper as a medium (Burgos and Talbert, 2000; Liebman and Sundberg, 2006). Burgos and Talbert (2000) studied the differential activities of BOA and DIBOA – two major allelopathic compounds in *Secale cereale* – and crude water extract of *S. cereale* in culture dish bioassays using several vegetable and weed species. They found that inhibition of germination by BOA and DIBOA occurred only in small- to medium-seeded species, whereas large-seeded species were tolerant to the allelochemicals from *S. cereale*. Liebman and Sundberg (2006) tested the effect of a 2% and a 1% aqueous extract of red clover shoots on 18 weed and 44 crop species whose 100-seed masses ranged from 20 to 26,250 mg. For both extract concentrations radicle inhibition was inversely proportional to seed mass.

It is questionable, however, whether the results from these laboratory bioassays can simply be extrapolated to field conditions. Weidenhamer et al. (1987) demonstrated that the inhibitory effect of allelochemicals was dependent on the amount of allelochemicals available per seed rather than on the concentration. Consequently, the choice of keeping the number of seeds per Petri dish the same for all species, irrespective of seed mass, may well have created an artifact in the

experiment of Liebman and Sundberg (2006). Besides this, the proportional area of the seed that is in contact with the extract in a Petri dish becomes smaller with increasing seed size, because moistening occurs only from one side. This as opposed to seeds buried in the soil, which are moistened from all sides. Furthermore, under field conditions factors other than seed mass may well have a considerable influence on the fate of weed and crop seeds in cover crop residue-amended soil.

The aim of our study was to assess to what extent seed mass determines species sensitivity to cover crop residues under field conditions. Consequently, seedling emergence in residue-amended soil was determined for a range of crop and weed species differing in seed mass.

Materials and methods

Cover crops

We selected three cover crop species belonging to three different plant families: winter rye (*Secale cereale* L. cv. 'Protector') from the *Poaceae* family; winter oilseed rape (*Brassica napus* L. cv. 'Emerald') from the *Brassicaceae* family; and lucerne (*Medicago sativa* L. cv. 'Mercedes') from the *Fabaceae* family. These three families contain different groups of allelochemicals. In the *Poaceae*, hydroxamic acids are the main group of allelochemicals (Niemeyer, 1988). Allelopathy in *Brassica* species has been primarily attributed to the hydrolysis products of glucosinolates, most of which are volatile (Teasdale and Taylorson, 1986; Oleszek, 1987; Bialy et al., 1990; Brown and Morra, 1996; Petersen et al., 2001; Siemens et al., 2002; Haramoto and Gallandt, 2004). Lucerne contains several groups of allelochemicals, including saponins, flavonoids and phenolic acids (Dornbos et al., 1990; Oleszek, 1993; Xuan et al., 2003).

Experiment 1

This experiment was conducted in the year 2004–2005 at the biological experimental farm "Droevendaal", the Netherlands. The experimental field was located on a sandy soil, which consisted of 1% clay, 2% silt and 97% sand and had an organic matter content of 4.3%. On July 29, 2004, the field was limed with 5000 kg/ha Dolokal to increase the pH from 4.7 to 5.5. One day before sowing of each crop, either 800 or 1600 kg ha^{-1} NPK (5-6-13) ecological fertilizer granules (Ecostyle) were applied. Sowing was performed at 2 cm depth and with a row spacing of 12.5 cm. For all three cover crops two sowing densities were used. Lucerne, inoculated with *Rhizobium meliloti*, was sown at 25 and 50 kg seeds ha^{-1} on August 2, 2004. On September 2, 2004, winter rye and winter oilseed rape were

sown at 150 and 300 kg ha^{-1} or at 7 and 14 kg seeds ha^{-1}, respectively. The emergence rate was on average 92%, 76% and 84% for lucerne, winter oilseed rape and winter rye, respectively. The experimental design was a completely randomized split-split-plot with four blocks. Cover crop species by sowing densities were main plots, fertilization split-plots and receptor plant species split-split plots. Each block included two control main plots (no cover crop), in which soil tillage and fertilization were either carried out at the same time as in the lucerne plots or at the same time as in the winter rye and winter oilseed rape plots. Main plot size was 3 m × 9 m.

On April 5 (block 1), 6 (block 2 and 3) and 7 (block 4), the cover crop above-ground biomass was cut into pieces with a flail mower and mixed through the upper 10 cm of the soil with a rotary cultivator. Between flail mowing and incorporation of the cover crop material, the roots were cut at 1 cm below ground level in order to avoid problems with re-growth. At the time of residue incorporation, winter oilseed rape had reached the flower bud development stage, whereas winter rye was at the end of the stem elongation stage and lucerne was still in the vegetative stage. The dry weight of the above-ground residues incorporated into the soil was 361 g m^{-2} for lucerne, 626 g m^{-2} for winter oilseed rape and 406 g m^{-2} for winter rye.

Eight different crop species ranging in 100-seed mass from 100 to 35,000 mg (Table 5.1) were used as bioassay indicator or "receptor" plant species. In each plot, 100 seeds per receptor plant species were manually sown directly after cover crop residue incorporation in a uniform pattern with a mutual distance of 5 cm. Lettuce was also sown at 6 and 13 days after incorporation (DAI). The relative sowing depth of each species was chosen on the basis of its 100-seed mass (Table 5.1). The total number of emerged seedlings was determined by regular counting and removing of emerged seedlings until no more seedlings emerged.

Experiment 2

This experiment was carried out with cover crop residue material originating from experiment 1. Approximately one week prior to experiment 1 residues were incorporated at location "the Haarweg" of Wageningen University in Wageningen, the Netherlands. Rings with a diameter of 30 cm and a depth of 25 cm were buried in the soil, allowing 1 cm to protrude above soil level. The rings were spaced at 0.5 m intervals in the field. The upper 20 cm of the rings were filled with soil that originated from the same site as experiment 1. The soil in the upper 10 cm of the rings had been sterilized with gamma rays (10 kGray) to kill all weed seeds. By eliminating the background population of weed seeds in the soil, ubiquitous weed species could be included in the experiment. To stimulate the recovery of microbial

Table 5.1. Receptor species used in experiment 1 and 2, as characterized by 100-seed mass (mg) and the time at which 50% of the seedlings had emerged in the ring experiment (T_{50} emergence, days after sowing (DAS)). Sowing depth (cm) is given for both experiments as well as the surface area that was used for sowing in experiment 2.

Indicator species		100-seed mass (mg)	T_{50} emergence (DAS)	sowing depth exp 1 (cm)	sowing depth exp 2 (cm)	surface area exp 2
common chickweed	*Stellaria media*	46	14.4		0.0-1.0	¼ ring
common lambsquarters	*Chenopodium album*	69	16.5		0.0-1.0	¼ ring
lettuce	*Lactuca sativa*	100	10.6	1.5	1.0	¼ ring
carrot	*Daucus carota*	120	16.3	1.5	1.0	¼ ring
onion	*Allium cepa*	350	19.6		1.0	¼ ring
spinach	*Spinacia oleracea*	1000	8.9	2.0	1.5	½ ring
sugar beet	*Beta vulgaris*	1200	10.5	2.0	1.5	½ ring
common vetch	*Vicia sativa*	3300	9.0	3.0	2.0	¾ ring
wheat	*Triticum sativum*	3700	8.1	3.0	2.0	¾ ring
yellow pea	*Pisum sativum*	29000	8.4	4.0	3.0	1 ring
maize	*Zea mays*	35000	17.9	4.0	3.0	1 ring

activity, sterile soil was mixed with 1% non-sterilized soil, and stored while kept moist at 25 ºC for a period of 6 weeks prior to the experiment.

On March 30 (block 1), March 31 (block 2 and 3) and April 1 (block 4), cover crop plants were harvested from the field of experiment 1 and on the same day incorporated in the upper 5 cm of the soil in each ring. Prior to incorporation, the roots were rinsed carefully and subsequently cut in pieces of 1 cm, whereas the shoot material was cut into pieces of 3 cm, perpendicular to the main plant axis. The amount of residue incorporated per unit area in the rings was 1.5 times the dry weight per unit area incorporated in experiment 1.

Immediately after residue incorporation, 9 crop species and 5 weed species, ranging in 100-seed mass from 46 to 35,000 mg, were sown at a rate of 200 (weeds) or 50 (crops) seeds per replicate. Relative sowing depth and surface area used for sowing were based on the 100-seed mass of the species (Table 5.1). Weed seeds were purchased from Herbiseed (UK), where they were harvested in the year 2000 (*Chenopodium album* L.), 2001 (*Persicari maculosa* L.) or 2002 (*Stellaria media* L., *Polygonum aviculare* L. and *Poa annua* L.) and stored at 5 °C and RH 10–15% until use. To break dormancy, one-half of the *C. album, P. maculosa* and *P. aviculare* seeds were pre-treated 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. Because of the very low overall emergence of *P. annua*, *P. maculosa* and *P. aviculare*, these three species were discarded from the experiment.

The experimental design was a completely randomized split-plot with four blocks: cover crop species were main plots, and receptor plant species split-plots. Both the total number of emerged seedlings as well as the emergence rate were determined by regular counting and removing of emerged seedlings up to 40–42 days after sowing (DAS).

Data analysis experiment 1 and 2

The relative emergence of each receptor plant species was calculated by dividing the total number of emerged seedlings in each residue treatment by the average total number of emerged seedlings in the control treatment.

As the emergence in the control treatment of experiment 1 was independent of both the fertilization level and the time at which soil tillage and fertilization were implemented, emergence in all control plots was averaged, resulting in a single value per receptor plant species. Subsequent analysis of variance (ANOVA, Genstat release 9, Payne et al., 2006) showed that also the relative emergence of the receptor plant species was independent of cover crop sowing density and fertilization level in all three cover crop species. We, therefore, eliminated these two factors from the

further data analysis and used the resultant means for subsequent analyses.

In experiment 2, the time needed to reach 50% seedling emergence (T_{50} emergence) was determined for each receptor plant species by fitting a three-parameter logistic curve through the emergence data in the control treatment, assuming a binomial distribution and while keeping the upper limit fixed to the total number of emerged seedlings.

The relation between seed mass and the relative emergence of the receptor species was determined for each cover crop species separately with a linear regression analysis (Genstat Release 9, Payne et al., 2006), in which the natural logarithm of the 100-seed mass (mg) was used as the explanatory variable and the average relative emergence as the response variable.

Analysis of two additional datasets

A close inspection of the data from both experiments raised the idea that the level of residue-mediated inhibition of the receptor plant was influenced by the time of emergence of the receptor plant. As a test of this hypothesis, two additional datasets published earlier by Haramoto and Gallandt (2005) were re-analysed. These data originated from two field experiments that were designed to test the influence of seed mass on seedling emergence in cover crop residue-amended soil in the field. These experiments indicated that seed mass was a poor predictor of a species' establishment; however, this conclusion was based on analyses of seed mass as the sole explanatory variable. Thus, we wanted to test whether, by accounting for the T_{50} emergence, a larger part of the variation in the relative emergence of weed and crop species could be explained.

The field experiments, conducted in the years 2002 and 2003, contained six cover crop species whose residues were incorporated in the upper soil layer. Directly after residue incorporation different weed and crop species were sown, with 100-seed masses ranging from 35 to 30,767 mg (Table 5.2). Each experiment contained four replicates and was based on a split-plot design with cover crop as the main plot and receptor plant species as the subplot. Among the six cover crop species included in the experiments were three *Brassica* species; canola cv. 'Hyola' (*Brassica napus* L.), winter oilseed rape cv. 'Dwarf Essex' (*Brassica napus* L.) and yellow mustard cv. 'Idagold' (*Sinapis alba* L.). The data obtained for these three species were pooled and used for further analysis.

The relative emergence of each receptor plant species, as well as the time needed to reach 50% seedling emergence (T_{50} emergence), were calculated as described above. We tested the influence of time of emergence and seed mass on plant

Table 5.2. Receptor species used in field experiments of Haramoto and Gallandt (2005), as characterized by 100-seed mass (mg) and the time at which 50% of the seedlings had emerged (T_{50} emergence, DAS) in the years 2002 and 2003.

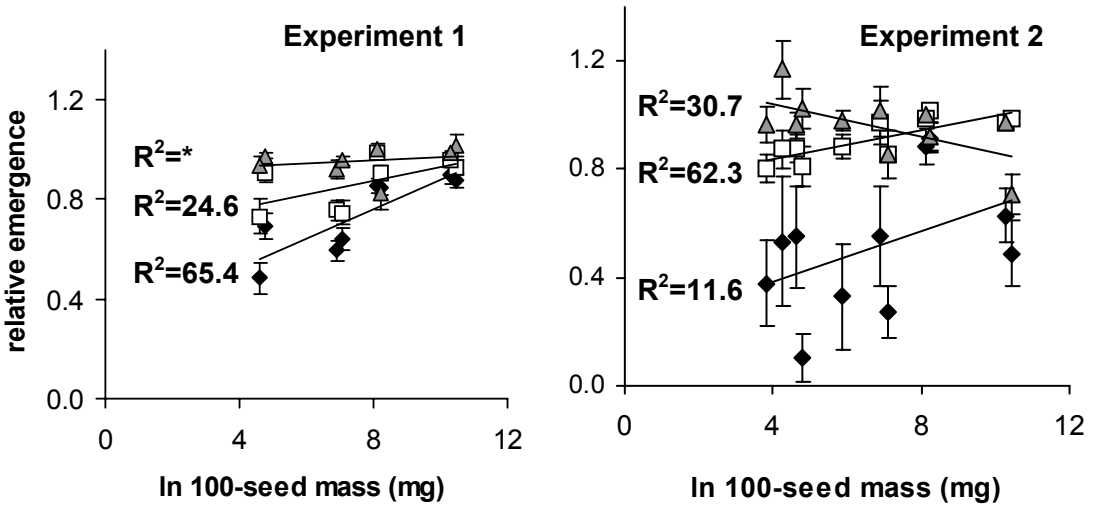
Receptor species		100-seed mass (mg)	T_{50} emergence 2002 (DAS)	T_{50} emergence 2003 (DAS)
redroot pigweed	*Amaranthus retroflexus*	35	11.0	8.3
carrot	*Daucus carota*	127	12.2	8.8
lettuce	*Lactuca sativa*	131	6.2	6.9
yellow foxtail	*Setaria glauca*	165	13.4	8.7
wild mustard	*Brassica kaber*	176	6.8	6.5
tomato	*Lycopersicon esculentum*	279	-	8.5
broccoli	*Brassica oleracea*	422	-	5.3
rapeseed	*Brassica napus*	472	-	5.2
velvetleaf	*Abutilon theophrasti*	739	5.8	5.9
spinach	*Spinacia oleracea*	1035	8.4	-
rye	*Secale cereale*	2681	-	4.8
cucumber	*Cucumis sativus*	2743	6.5	5.8
field pea	*Pisum sativum*	14769	8.1	5.3
green bean	*Phaseolus vulgaris*	30767	-	6.7

sensitivity to residue-mediated effects by linear regression using the average relative emergence of the receptor plant species as the response variable and 100-seed mass (ln(mg)), T_{50} emergence (days) or both as the explanatory variables. The receptor plant species *Chenopodium album* and *Galinsoga ciliata* were discarded from the analyses. *C. album* was discarded because of the large background population present in the field plots of both experiments. The reason to discard *G. ciliata* was the very low emergence in 2002 (on average only 16 out of 160 viable seeds emerged in the control plots) and the very large variation in emergence in the control treatment in 2003 (ranging from 23 to 392 seedlings).

Results and discussion

Experiment 1

In experiment 1, lucerne had the greatest overall inhibitory effect on seedling emergence (26%), followed by winter oilseed rape that inhibited seedling emergence an average of 14%. Winter rye did not inhibit the emergence of any of the receptor plant species, except for that of wheat (18% inhibition). A vast amount of literature on allelopathic properties of these three cover crop species is available. In a pot



Separate intercepts, separate slopes
WR: y = 0.90 + 0.007x (p=0.581)
WOR: y = 0.65 + 0.028x (p=0.120)
LU: y = 0.29 + 0.059x (p=0.009)

Separate intercepts, separate slopes
WR: y = 1.16 – 0.030x (p=0.045)
WOR: y = 0.73 + 0.027x (p=0.002)
LU: y = 0.19 + 0.047x (p=0.163)

Figure 5.1. Relative emergence of receptor species as a function of 100-seed mass (ln(mg)) and cover crop species in experiment 1 and 2, triangles = winter rye (WR); squares = winter oilseed rape (WOR); diamonds = lucerne (LU). Vertical bars represent SE and * indicates that the residual variance exceeded the variance of the response variate.

experiment in a growth chamber, Xuan et al. (2005) found a strong inhibitory effect of lucerne residues on weeds. Barnyardgrass and *Monochoria vaginalis* growth was reduced by 80–100% for up to 10 days after incorporation and by 50% after 20–25 days. Haramoto and Gallandt (2005) and Al Khatib (1997) found reductions in the emergence of bioassay species following *Brassica* cover crop residue incorporation in the field of 23–34% and 30%, respectively, which were very similar to our results. Boydston and Hang (1995), however, found much higher reductions of weed density (73–85%) following rapeseed incorporation in spring in a loamy sand soil. Because of the numerous publications on allelopathy in winter rye (e.g. Barnes et al., 1986; Chase et al., 1991; Mwaja et al., 1995; Burgos and Talbert, 2000; Rice et al., 2005), it was surprising we did not observe any inhibitory effect of this cover crop species on seedling establishment.

We observed a positive relationship between seed mass and relative emergence of the receptor plant species in lucerne-amended soil (p=0.009; Figure 5.1), meaning that heavier seeds were less affected by lucerne residues than lighter seeds. For winter oilseed rape, we observed a similar trend, although not significant (p=0.120). In both winter oilseed rape- and lucerne-amended soil, the relative emergence of carrot was higher than expected on the basis of its seed mass.

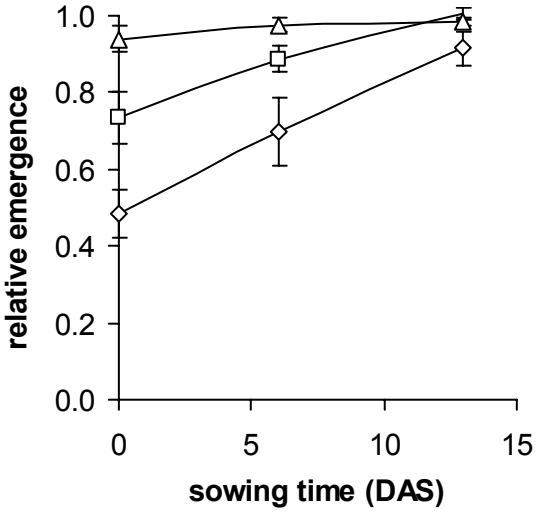


Figure 5.2. Relative emergence of lettuce sown at 0, 6 and 13 days after incorporation of winter rye (triangles), winter oilseed rape (squares) or lucerne (diamonds) residue. Vertical bars indicate mean value ± SE.

The relative emergence of lettuce rapidly increased with later sowing times for both lucerne and winter oilseed rape (Figure 5.2). This indicates that the strength of the residue-mediated effects of these two cover crop species decreased with time after incorporation and had virtually disappeared, at least as evidenced by this receptor species, after approximately two to three weeks. This is congruent with most findings that decomposing plant residues in soil exhibit the greatest inhibition at the early stages of decomposition and that phytotoxicity declines as decomposition proceeds (e.g. Patrick et al., 1963; Ohno et al., 2000; An et al., 2001; Xuan et al., 2005).

When comparing the T_{50} emergence of the receptor plant species, it was clear that carrot and maize emerged much later than the other species used in experiment 1 (Table 5.1). If we combine this observation with the rapidly declining strength of residue-mediated inhibitory effects on lettuce emergence, it seems logical that carrot emerged better than expected on basis of its seed mass. At the time carrot germinated and emerged, the residue-mediated inhibitory potential had already disappeared. Maize was already less affected by the residues because of its large seed mass and its relative emergence could, therefore, not be further increased by a late emerging time.

Experiment 2

Of all cover crop species in experiment 2, lucerne again exerted the largest overall inhibitory effect on seedling emergence, which at 49% was also much higher than in

experiment 1. One explanation for this is that there was 1.5 times more residue per unit area incorporated in experiment 2. Winter oilseed rape had an overall inhibitory effect of 9% and, as noted in experiment 1, winter rye did not inhibit seedling emergence, expect for that of maize (29% inhibition).

Although the overall inhibitory effect of winter oilseed rape was low, we did observe a positive relationship between seed mass and relative emergence of the receptor plant species in winter oilseed rape-amended soil ($p=0.002$; Figure 5.1). The emergence of species with a 100-seed mass of up to 350 mg was inhibited 10 to 20%, whereas the emergence of heavier seeds was not inhibited. In contrast, winter rye residues resulted in a negative relationship between seed mass and relative emergence ($p=0.045$). This was largely the result of a slight stimulation of the emergence of the small-seeded *C. album* in combination with an inhibition of the emergence of the large-seeded maize. Seedling emergence in soil amended with lucerne residue was highly variable and no relationship between seed mass and relative emergence was observed.

If we analyse the large variation in emergence of receptor plant species in the lucerne-amended soil further by looking at each block separately, we observe a remarkable pattern. The emergence of receptor plant species was much lower in block 4 compared to block 1, and was usually intermediate in block 2 and 3 (Figure 5.3). This pattern was repeated in all receptor plant species, but differed in level. For instance, wheat and common vetch were not inhibited by the lucerne residues in block 1, 2 and 3 and only slightly inhibited in block 4. Emergence of lettuce and spinach were not inhibited in block 1, inhibited by approximately 50% in block 2 and 3 and completely inhibited in block 4. Lucerne inhibited sugar beet by approximately 50% in block 1, by 75% in block 2 and 3 and by 100% in block 4.

The large rainfall event of 21.5 mm that occurred on April 8, 2005, is most likely the responsible factor for this remarkable pattern. As the four blocks were incorporated on subsequent days, the rainfall peak occurred at 9 days after residue incorporation (DAI) in block 1, 8 DAI in block 2 and 3, and 7 DAI in block 4. Considering time of seedling emergence in relation to the time of the rainfall peak for the different receptor plant species in the different blocks, a consistent pattern is observed. The lucerne residues did not affect establishment of seedlings that emerged before the rainfall peak, and inhibition of seedling emergence became stronger with increasing time between the date of emergence and the date of the rainfall peak. This was true for all species. Surprisingly, in case of the late-emerging species *S. media*, *C. album* and onion, for which emergence in all blocks occurred after the rainfall peak, establishment of the first-sown seeds was not inhibited. This seems only logical if one assumes that germination was the stage when plants were

most sensitive to residue-mediated effects. Seeds that germinated before the rainfall peak then escaped from the inhibitory effects, whereas seeds that germinated after the rainfall peak were severely inhibited. Plants are likely to be most sensitive to residue-mediated inhibitory effects in the early stages of development. In case of allelopathic effects, the concentration of allelochemicals in plant tissue is expected to decline with increasing plant biomass. In case of soilborne pathogen effects, infection rates are especially high during the germination process, because of the presence of exudates released by seeds imbibing water (Martin and Loper, 1999). In experiment 1, the same rainfall peak did not differentially affect the emergence of the different receptor plant species in the lucerne-amended soil, as the rainfall peak occurred only one to three days after residue incorporation and none of the seedlings had germinated before the rainfall peak.

One explanation for the increased inhibitory residue-mediated effects of lucerne on seedling emergence following the large rainfall peak may be an increase in the activity of soilborne pathogens. Soilborne pathogens can be stimulated after soil amendment with fresh residue material (Dabney et al., 1996; Conklin et al., 2002; Manici et al., 2004). At high soil moisture content, for example, *Pythium* spp. can gain a competitive advantage over other saprophytes. This because *Pythium* spp. are more tolerant of low O_2 concentrations than many other soil microorganisms (Martin and Loper, 1999), and the combination of a high soil moisture with a high microbial activity in residue-amended soil causes a rapid decrease in the concentration of O_2.

Another explanation could be that the rainfall peak stimulated the release of the water-soluble allelochemicals from the lucerne residues into the soil and/or allowed for a better distribution of the allelochemicals in the soil. However, the rainfall peak could also have caused allelochemicals to leach to deeper soil layers.

It was striking that the strength of the residue-mediated inhibitory effect was block-dependent only for lucerne. We offer two explanations why rainfall affected the residue-mediated inhibitory effect of lucerne and not that of winter oilseed rape or winter rye. A first possible explanation is related to the interaction between allelochemicals and pathogens. Allelochemicals in *Brassica* spp., on the one hand, are known for their biofumigation potential (e.g. Brown and Morra, 1997). Volatile glucosinolate break-down products released from winter oilseed rape residues may have, therefore, not only inhibited seedling emergence, but also suppressed soilborne pathogens. In this way allelochemicals released from winter oilseed rape residues could have counteracted the possible stimulating effect of rainfall on the activity of these pathogens. Allelochemicals in lucerne, on the other hand, may have acted additively or even synergistically with soilborne pathogens. Phenolic compounds, one of the groups of allelochemicals present in lucerne residues, are known to

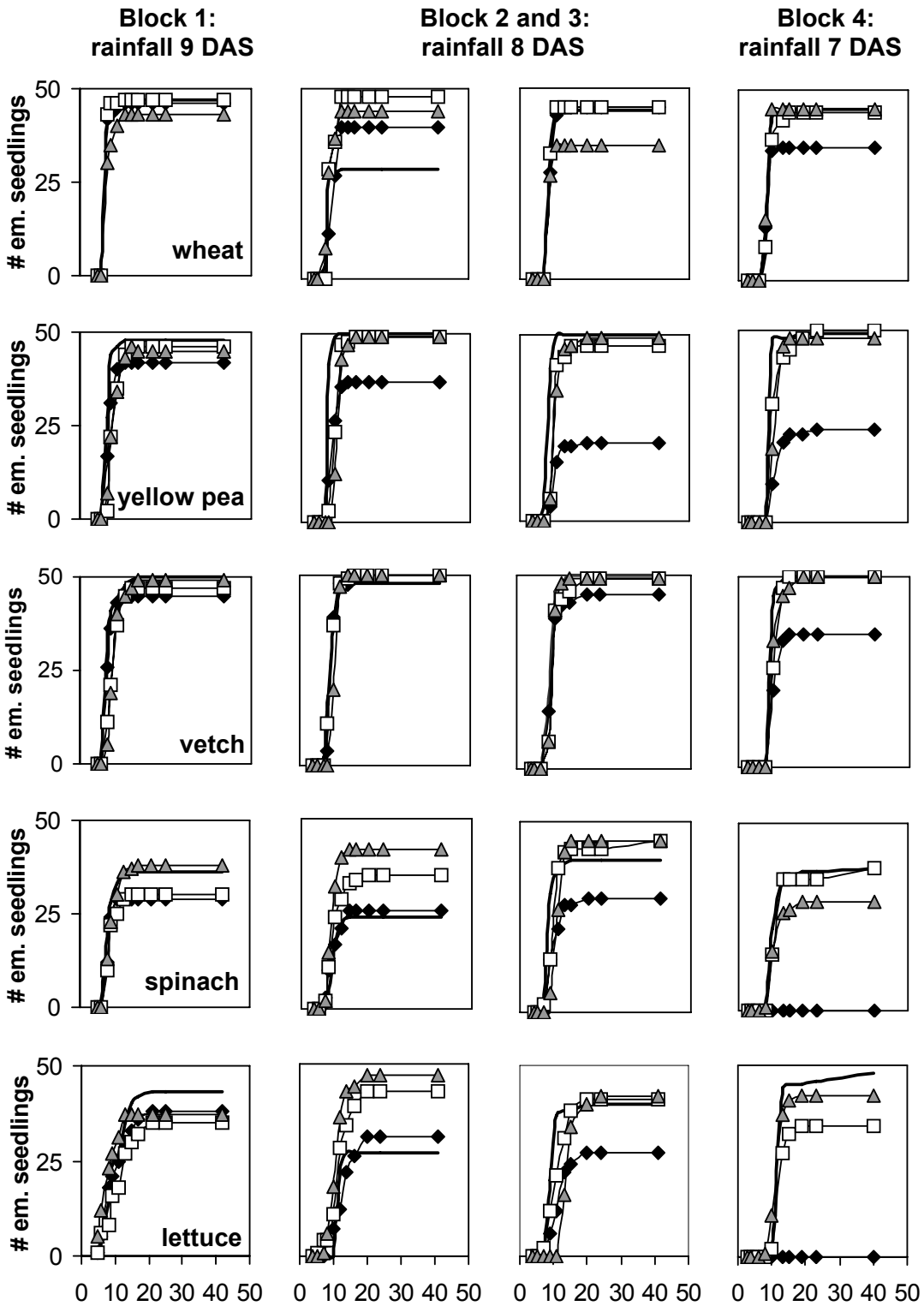
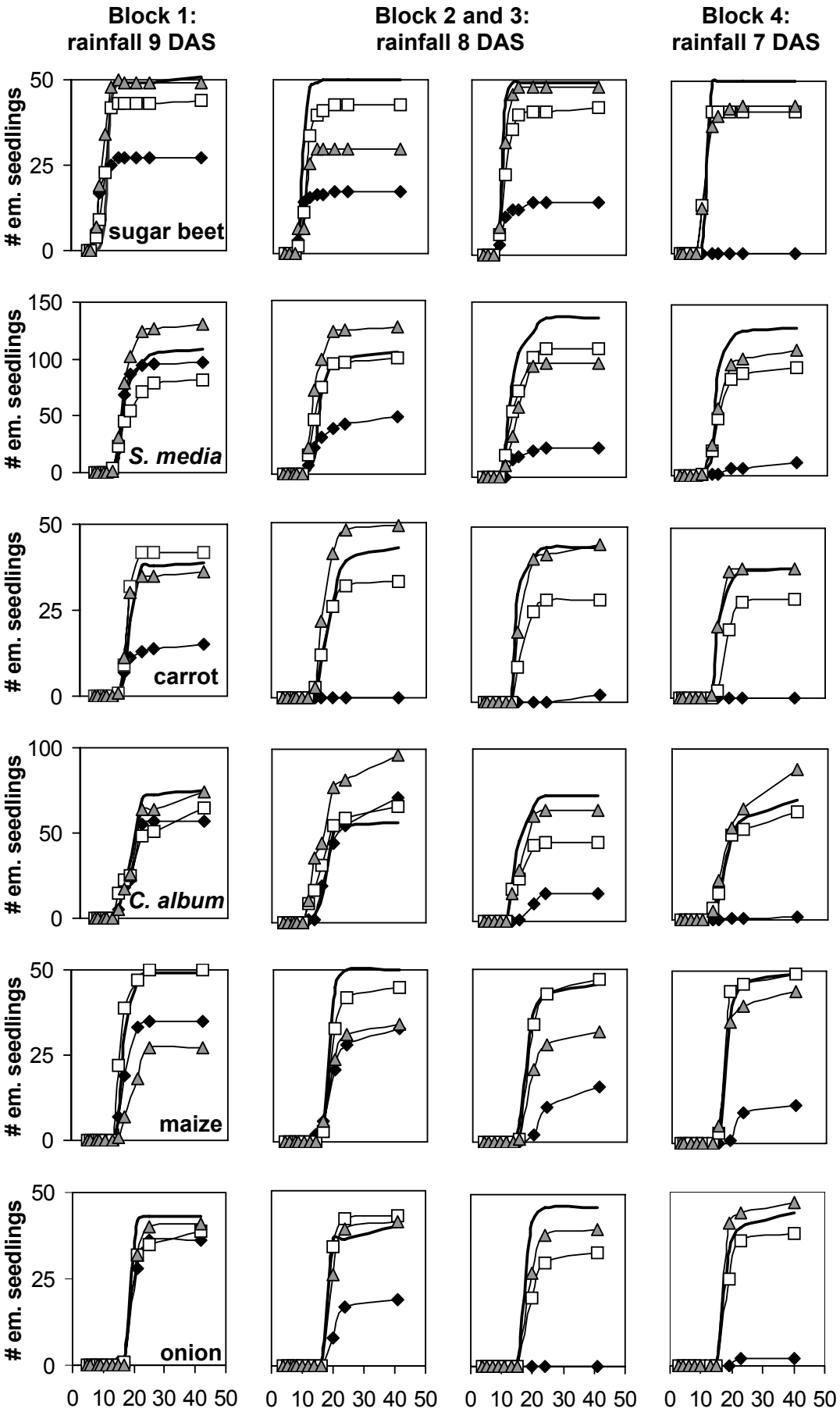


Figure 5.3. Emergence over time (DAS) of indicator species in experiment 2, represented separately for each block. Thick lines represent the emergence in the control treatment, black diamonds = lucerne, white squares = winter oilseed rape and grey triangles = winter rye.

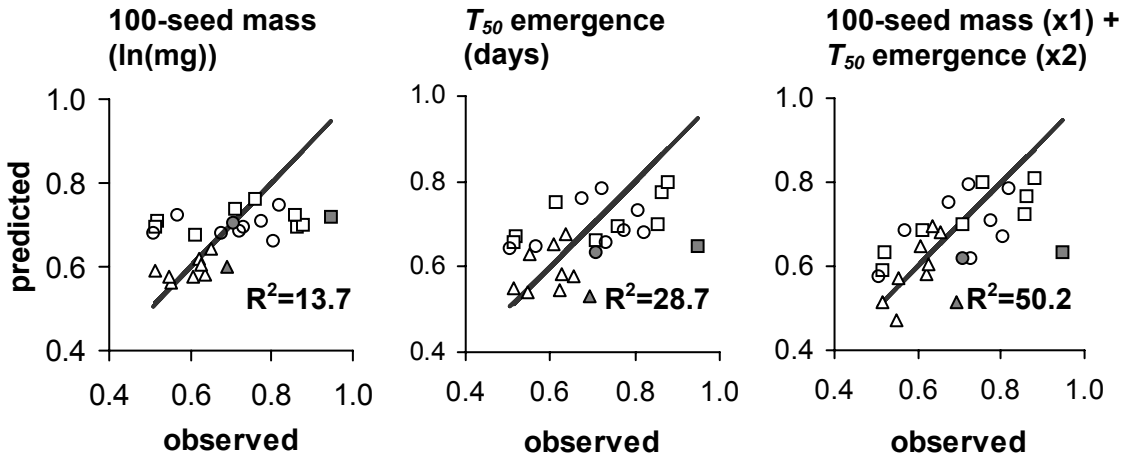

Block 1:
rainfall 9 DAS
Block 2 and 3:
rainfall 8 DAS
Block 4:
rainfall 7 DAS
em. seedlings
sugar beet
S. media
carrot
C. album
maize
onion
0 10 20 30 40 50

interfere with cell membrane permeability (Einhellig, 2004), resulting in leakage of organic molecules into the surrounding soil. As propagule germination and germtube growth of, for instance, *Pythium* spp. is enhanced by the presence of plant exudates (Martin and Loper, 1999), phenolic action may have caused higher seed(ling) infection rates. A second possible explanation is related to the difference in decomposability of residues of lucerne, winter oilseed rape and winter rye. The relatively low C:N ratio of lucerne residues may have allowed for a faster increase in overall microbial, including soilborne pathogen, activity in lucerne-amended soil.
Although rainfall did not cause the residue-mediated effect of winter oilseed rape to increase, it may have prolonged the inhibitory effect on seedling emergence. This because volatile glucosinolate-breakdown products released from winter oilseed rape residues are better retained in soil with a high moisture content (Borek et al., 1995). The observation that the relatively late emerging, but small-seeded species carrot, *S. media*, onion and *C. album* were also inhibited by winter oilseed rape residues substantiates this theory.

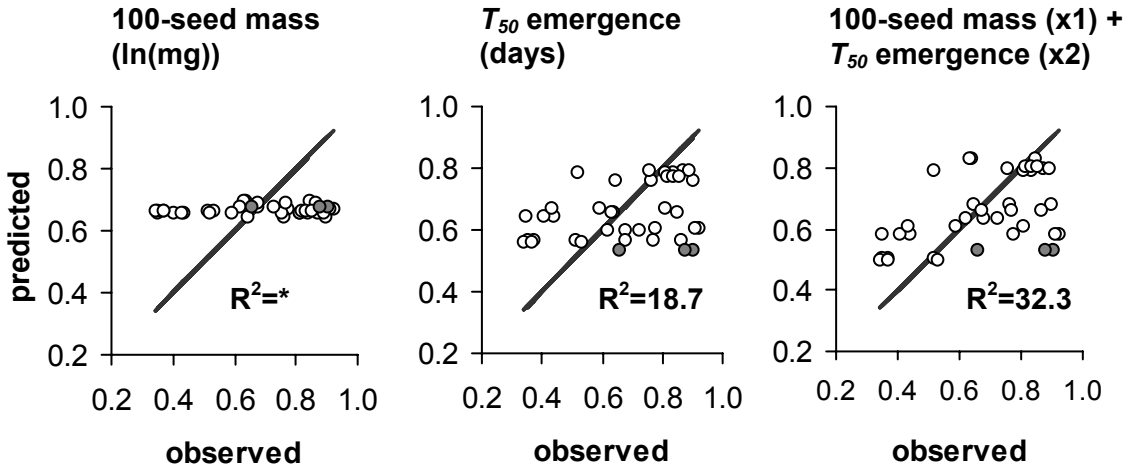
Additional datasets
Results of experiment 1 suggested that carrot emerged better than expected on basis of its seed mass, which could be due to the late emerging time of carrot in combination with the declining strength of residue-mediated inhibitory potential over time. Results of experiment 2 suggested that the time of germination relative to the rainfall peak determined the strength of the inhibitory effect of lucerne residues on seedling establishment. Taken together, these results led us to formulate the following hypothesis: "*the time course of sensitivity of the receptor plant in relation to the time course of residue-mediated inhibitory potential is an important factor determining the level of residue-mediated inhibition of the receptor plant*."

We tested this new hypothesis by re-analysing two additional datasets that were published earlier by Haramoto and Gallandt (2005), specifically testing whether a larger part of the variation in the relative emergence of weed and crop species in *Brassica* residue-amended soil could be explained by taking both seed mass and the T_{50} emergence into account. Implicitly, we assumed that the strength of the *Brassica* residue-mediated inhibitory potential declined continuously with time after incorporation, as was for instance observed in experiment 1.

Consistent with our hypothesis, emergence of later-emerging species was less inhibited by *Brassica* residues than the emergence of earlier-emerging species (Figure 5.4). Interestingly, in both experiments seed mass was not related to the relative emergence when considered on its own, but became a significant factor only after accounting for the T_{50} emergence. When velvetleaf was omitted from the



	Separate intercepts Common slope	Separate intercepts Common slope	Separate intercepts Common slope
CA:	y = 0.63 + 0.014 x	y = 0.54 + 0.019 x	y = 0.25 + 0.033 x1 + 0.030 x2
WO:	y = 0.51 + 0.014 x	y = 0.42 + 0.019 x	y = 0.13 + 0.033 x1 + 0.030 x2
YM:	y = 0.61 + 0.014 x	y = 0.52 + 0.019 x	y = 0.23 + 0.033 x1 + 0.030 x2
	p = 0.118	p = 0.020	x1: p=0.006, x2: p<.001



	Common intercept Common slope	Common intercept Common slope	Common intercept Common slope
	y = 0.62 + 0.008 x	y = 0.23 + 0.064 x	y = -0.30+ 0.045 x1 + 0.099 x2
	p = 0.644	p = 0.005	x1: p=0.009, x2: p<.001

Figure 5.4. Observed and predicted values after linear regression with the average relative emergence of the receptor species as the response variable and 100-seed mass (ln(mg)), T_{50} emergence (days) or both 100-seed mass and T_{50} emergence as the explanatory variables. Data obtained from Haramoto and Gallandt (2005), field experiments in 2002 (a) and 2003 (b). Velvetleaf was omitted from the regression analysis in 2002 and rye in 2003, but represented in the graphs with the grey icons. CA = canola, WO = winter oilseed rape and YM = yellow mustard.

analysis in the 2002 experiment, the R^2 of the multiple regression analysis including both seed mass and T_{50} emergence increased from 29.8 to 50.2 (Figure 5.4). A similar increase in the R^2 of the multiple regression analysis in the 2003 experiment was observed when rye was omitted from the analysis. Omitting rye caused an increase in the R^2 from 20.2 to 32.3 (Figure 5.4). Velvetleaf and rye were the species with the shortest T_{50} emergence in 2002 and 2003 experiment, respectively. Because of the short T_{50}, these species were expected to be largely inhibited by the *Brassica* residues. The fact that this was not the case could be due to the simplification we made when assuming that residue-mediated effects would be strongest directly after residue incorporation and would continuously decline with time. In reality it may have taken a few days before the strength of the residue-mediated effects reached a peak. This could have allowed very early emerging species to 'escape' the inhibitory effects of the *Brassica* residues.

Conclusions

Our findings suggest that residue-mediated inhibition of a receptor plant only takes place when there is an overlap of the time course of sensitivity of the receptor plant and the time course of the residue-mediated inhibitory potential (Figure 5.5). In most

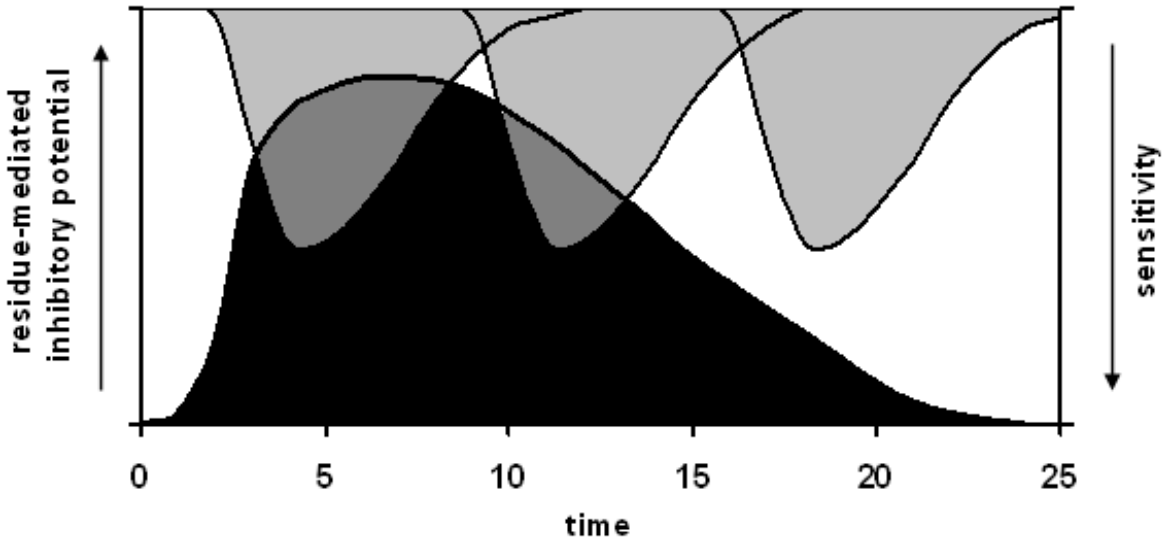


Figure 5.5. Schematic representation of the time course of residue-mediated inhibitory potential (black) and the time course of sensitivity of three different receptor plants (light grey). Where the overlap (dark grey) between the residue-mediated inhibitory potential and the sensitivity of the receptor plant is largest, there is most potential for a residue-mediated inhibitory effect on the receptor plant. In this example the third receptor plant will 'escape' from the residue-mediated inhibitory effect.

cases we found a positive relationship between seed mass and relative emergence, indicating that also under field conditions small-seeded species are more sensitive to residue-mediated effects. However, the results of the analysis of the two additional datasets suggest that seed mass under field conditions only becomes important after accounting for the relative time of emergence. The time course of residue-mediated inhibitory potential depends on residue characteristics, biotic and abiotic soil properties, and environmental factors, and is, therefore, difficult to predict. The results of experiment 2 suggest that the sudden and drastic increase in the residue-mediated inhibitory potential of lucerne residues was due to the occurrence of a large rainfall event 7 to 9 days after residue incorporation. The verification and the mechanisms responsible for this phenomenon are yet to be investigated.

There are two important implications of our proposed mechanistic model of residue-mediated effects including both relative time of emergence and seed mass. From a practical perspective, growers may not be ensured of selective residue effects simply by sowing larger-seeded species. If their emergence is coincident with the greatest potential residue effects, establishment and early growth may be reduced. Secondly, the high degree of variation noted in field studies of allelopathy may be explained, at least in part, by variation in synchronicity of receptor species' sensitivity and potential residue effects.

CHAPTER 6

The influence of rainfall on seedling establishment in lucerne-amended soil

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Summary

In a field study described in Chapter 5, the time course of residue-mediated inhibitory potential of lucerne (*Medicago sativa* L.) residues showed a sudden and large increase, which was tentatively associated with a large rainfall event 7 to 9 days after residue incorporation (DAI). The current paper specifically focuses on the influence of a rainfall peak on the strength and time-course of the lucerne residue-mediated inhibitory effect on seedling emergence. Two subsequent pot experiments were conducted in a climate chamber. Pots received four rainfall treatments, i.e. no rainfall, indicated as basic moisture (BM) treatment, or a 22 mm rainfall peak (RP) applied at 3, 11 or 19 DAI. Pots containing soil without incorporated lucerne residues were used as a control. Lettuce (*Lactuca sativa* L.) was sown at 8 different times after residue incorporation, with 4-day intervals from 0 DAI to 28 DAI, to monitor the time course of the inhibitory potential of the lucerne-amended soil in the different rainfall treatments. Rainfall caused an increase in the residue-mediated inhibitory potential in both experiments, and this effect was stronger in experiment 2. Surprisingly, in experiment 2, also an effect of rainfall was observed in the control soil. This was likely related to the presence of the soilborne pathogens *Rhizoctonia solani* and *Pythium* spp., which were isolated from a number of (seed)lings in all treatments. The absence of a rainfall effect in the control soil of experiment 1 was attributed to the longer period between soil sterilization and the start of the experiment. We hypothesize that in the RP treatments allelochemicals played a less prominent role in the observed

residue-mediated inhibitory effects than in the BM treatment due to leaching of these allelochemicals from the top soil. After a rainfall peak, soilborne pathogens were likely for a greater part responsible for the inhibition of lettuce emergence.

Keywords: *Medicago sativa*, allelopathy, soilborne pathogens, *Pythium*, *Rhizoctonia solani*, ecologically-based weed management, cover crops, green manure

Introduction

Lucerne residues incorporated in the upper layer of the soil have been observed to substantially reduce weed emergence and growth (Xuan et al., 2005; Kruidhof et al., 2008a; 2008b), and could, therefore, make an important contribution to ecological weed management strategies. In a pot experiment in a growth chamber, Xuan et al. (2005) found dried and ground lucerne residues, amended at a rate of 1 ton dry matter ha^{-1}, to reduce barnyardgrass and *Monochoria vaginalis* growth as much as 80–100% during the first 10 days after residue incorporation (DAI), and by 50% between 20–25 DAI. In the field experiments of Chapter 2 and Chapter 5, we observed reductions of 26 to 65% in the establishment of crops and weeds sown directly after lucerne residue incorporation (1.8–5.4 ton dry matter ha^{-1}).

Crop residues can interfere with weed development and growth through an alteration of soil physical, chemical and biological characteristics. Lucerne contains several groups of allelochemicals, including saponins, flavonoids and phenolic acids (Dornbos et al., 1990; Oleszek, 1993; Xuan et al., 2003). These allelochemicals can be leached from the residues into the soil upon tissue disruption, which results from residue decomposition and/or pre-treatment of the residue prior to incorporation. Apart from allelochemicals, also nutrients are released from the residues. Increased nutrient availability stimulates weed germination (e.g. Teasdale and Pillai, 2005). However, soil amendment with residues containing a high C:N ratio can result in nitrogen immobilization (Stevenson, 1986, in Liebman and Mohler 2001). Certain soilborne pathogens can be stimulated after soil amendment with fresh residue material (Dabney et al., 1996; Conklin et al., 2002; Manici et al., 2004). Crop residues can also affect the physical properties of the soil by, for instance, increasing soil moisture retention (Liebl et al., 1992; Teasdale and Mohler, 1993).

Most reports dealing with residue-mediated inhibitory effects on receptor plants state that plant residues decomposing in soil exhibit the most severe inhibition at the early stages of decomposition and mention a decline in phytotoxicity as decomposition proceeds (e.g. Patrick et al., 1963; Ohno et al., 2000; An et al., 2001; Xuan et al., 2005). However, in the field study described in Chapter 5, a continuous decrease of lucerne residue-mediated inhibitory potential over a period of 2 weeks was observed in one experiment, whereas in a second experiment the time course of residue-mediated inhibitory potential of lucerne residues showed a sudden and large increase. This sudden change in temporal dynamics was tentatively associated with a large rainfall event 7 to 9 DAI.

Rainfall may interfere in several ways with the mechanisms responsible for cover crop residue inhibitory effects on receptor plants (Figure 6.1). Rainfall can directly

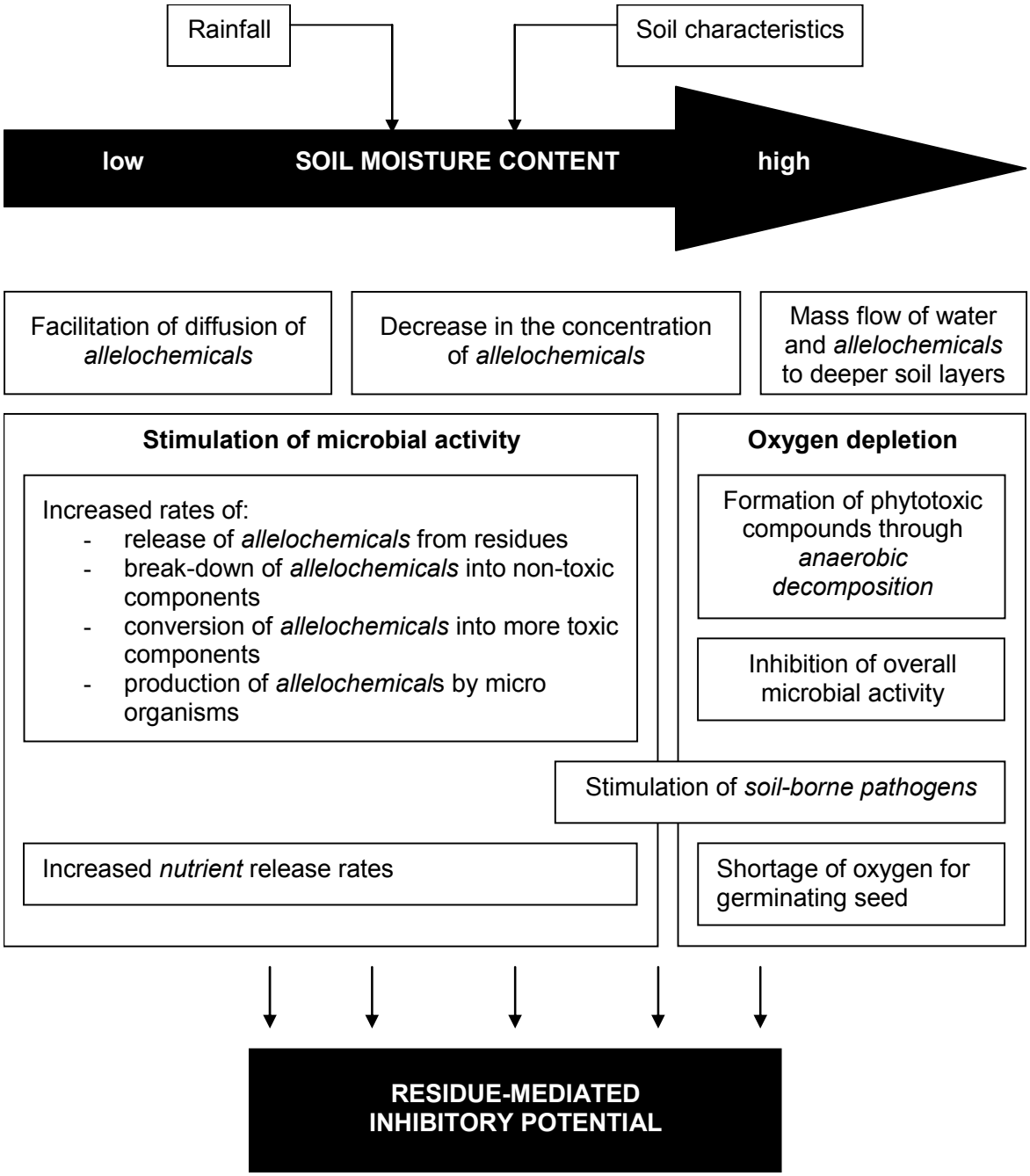

Figure 6.1. Conceptual framework of the influence of rainfall on residue-mediated inhibitory potential.

influence the amount of allelochemicals available for uptake by the receptor plant or exert an indirect influence through an alteration of microbial activity. An increase in the soil moisture content from low to intermediate levels may facilitate the diffusion of water-soluble allelochemicals. However, at the same time it will cause a decrease in the concentration of these allelochemicals in the soil water. At higher moisture levels a mass flow of water likely results in leaching of allelochemicals beyond the receptor

plant root zone. Through a positive effect on microbial activity, soil moisture can cause an increase in the release, production, conversion and break-down rates of allelochemicals, and may also speed up nutrient release. However, at soil moisture levels above field capacity anaerobic decomposition sets in, and overall microbial activity is reduced. Anaerobic decomposition can lead to the production of volatile fatty acids which have phytotoxic effects (e.g. Lynch, 1978; Wallace and Elliott, 1979; Chapman and Lynch, 1983). Reduced O_2 concentrations may also have a direct detrimental effect on the germinating seed (Drew and Lynch, 1980). Furthermore, reduced O_2 concentrations can give some soilborne pathogens, including *Pythium* spp., a competitive advantage over other saprophytes (Kuan and Erwin, 1980). High soil moisture also causes an increase in the size of the spermosphere, stimulating germination of pathogen propagules at greater distances from the germinating seed. For zoospore forming pathogens such as *Pythium* spp., the potential of dispersal increases with increasing soil moisture due to the increased motility of zoospores (Martin and Loper, 1999).

For the application of lucerne residues as a tool for ecological weed management it is essential to obtain knowledge about the factors that influence the time course of residue-mediated inhibitory potential. The current paper therefore focuses on the influence of a rainfall peak on the strength and time-course of the lucerne residue-mediated inhibitory effect on seedling emergence.

Materials and methods

Experimental set-up

Two subsequent pot experiments were conducted in a climate chamber. Pots received four different rainfall treatments, i.e. no rainfall (basic moisture level (BM)), or a 22 mm rainfall peak applied at 3, 11 or 19 (RP1, RP2 and RP3, respectively) days after lucerne residue incorporation (DAI). Pots containing soil without incorporated lucerne residues were used as a control. Lettuce (*Lactuca sativa* L.) was sown at 8 different times after residue incorporation, with 4-day intervals from 0 DAI to 28 DAI, to monitor the time course of the inhibitory potential of the lucerne-amended soil in the different rainfall treatments. A total of 320 pots was organized in a complete randomized block design with 5 blocks and 64 treatment combinations (4 rainfall treatments, 2 soil types (with or without incorporated lucerne residues) and 8 lettuce sowing times). Because rainfall did not alter the emergence of lettuce sown at 7 days or more before rainfall application in the first experiment, these sowing times were removed from the second experiment.

Pots were filled with soil collected from the organic farm “Droevendaal” located in

Wageningen, the Netherlands, which was sieved through a 4-mm (experiment 1) or 10-mm screen (experiment 2). The soil was sandy (97% sand, 2% silt and 1% clay) and had an organic matter content of 4.3%. One part of the soil was sterilized with gamma rays (10 kGray) to kill weed seeds. To stimulate the recovery of microbial activity, sterilized soil was mixed with 1% non-sterilized soil, and stored in open pallets while kept moist for a period of 6 weeks (experiment 1) or 2 weeks (experiment 2) prior to the experiment. The lower part of each pot was filled with 1500 g non-sterilized soil, whereas the top-soil consisted of 2500 g (control pots) or 2450 g (lucerne-amended pots) sterilized soil. Pot dimensions were: height 16.5 cm; top surface 15x15 cm^2; bottom surface 11×11 cm^2 and total volume 2.6 liters.

For both experiments, above-ground material was harvested from the same lucerne stand at the organic experimental farm "OBS" in Nagele, the Netherlands, where lucerne cv. Gulia had been sown on April 15, 2005 on a clay soil with a silt content of 30% without additional fertilization. For experiment 1, lucerne was harvested on May 18, 2006 and for experiment 2 on October 26, 2006. One month prior to harvesting residue material for the second experiment, lucerne had entered the flowering stage and was mown. For each pot, 60 g fresh weight (approx. 3100 g fresh weight m^{-2}) lucerne residue was cut into pieces of 3 cm length and mixed through the upper 10 cm of the soil. Moisture content of the lucerne was similar for both experiments and on average 88%.

Lettuce was used as an indicator species, because of its rapid germination and its sensitivity to allelochemicals (Macias et al., 2000). Lettuce seeds from a non light-sensitive variety were obtained from Rijk Zwaan®. The seeds were surface-sterilized by shaking them for 5 min in a 0.5% NaOCl solution obtained from commercial bleach, followed by rinsing for 2 min with de-ionized water. Forty-nine seeds were sown per pot at 1.5 cm depth at distances of 1.5 cm.

Climate chamber conditions were chosen such that they represented the average natural climatic conditions of April and the first half of May in the Netherlands, which is the time when lucerne residues used for weed suppression need to be incorporated into the soil (see Chapter 1 of this thesis). The light/dark ratio in the climate chamber was 14 h /10 h, the mean day temperature 15 °C, the mean night temperature 12 °C and the average relative humidity 87%.

The basic moisture level was kept constant at a soil water content of approximately 7–8% (w/w; experiment 1) or 8–10% (experiment 2). All pots were compensated for evaporation every second day. The evaporation rate was determined by the weight loss of pots of the basic moisture level, which was on average 15–20 mg pot^{-1} day^{-1} (equal to 0.75–1.00 mm rainfall day^{-1}). The water application method was aimed to imitate rainfall and water was applied through a sprinkler (WRTeeJet, 11003VS valve) driven

by air pressure adjusted to 2 bars. The pots were placed on a rotator, which turned with 27 s revolution^{-1}.

Observations and measurements

Newly emerged lettuce seedlings were counted daily and directly removed (experiment 1) or left untouched for further observations and measurements (experiment 2). In experiment 2, a total of 204 emerged seedlings, 59 germinated seeds and 41 ungerminated seeds were harvested at 10 DAS from pots belonging to the different sowing times and rainfall treatments. Seed(ling)s (approximately twice as many seed(ling)s from lucerne-amended soil compared to control soil) were sampled to determine presence of *Pythium* and *Rhizoctonia* spp. They were rinsed carefully in sterile water and placed on water agar (Oxoid, 15 g l^{-1}) plates. Agar plates were incubated at 20 °C for 3–10 days prior to observation for presence of the soilborne pathogens. The poor isolation medium selects especially for the fast-growing damping-off caused by *Pythium* spp. and *Rhizoctonia solani*. Also the number of wilted seedlings in each pot was scored regularly, after which all wilted seedlings were sampled for infection by *Pythium* and *Rhizoctonia*. Seedlings without visible symptoms were allowed to grow until 22 days after sowing (DAS). At this time, those seedlings that had emerged at either 6 or 7 DAS were cut at ground level and dried at 70 °C for 2 days, after which the dry weight per seedling was determined.

In experiment 2, blocks 1–4 contained extra pots for additional soil-related observations. In these extra pots, the electrical conductivity (EC), the pH, the soil water content (% w/w), the available NO_3^- and NH_4^+, and the concentration of volatile fatty acids (VFAs) were determined at different times after residue amendment. Soil cores to a depth of 10 cm were taken on 5, 13, 21, 29, 37 and 52 DAI. For each sampling date a separate pot was used. Lucerne residue was removed from the soil samples and all samples, except those used for VFA measurements, were dried at 37°C during 5 days. Dried samples were stored in plastic jars in the dark at room temperature until analysis. For the EC and pH measurements, 8 g of loose, dry soil was dissolved in 40 ml dematerialized 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 Autoanalyser II) after extraction with 0.01 M $CaCl_2$. In experiment 1, the soil moisture content was determined at 0, 21 and 37 DAI.

Soil samples for VFA measurements were stored at –20 °C immediately after sampling. Samples were allowed to thaw at 4 °C for one night, after which one part of each sample was used for extract preparation, and another part for water content

determination. Extracts were prepared by dissolving 8 g of loose, dry soil in 40 ml dematerialized water. Solutions were placed on a rotary shaker for 30 min and centrifuged at 7500 RCF for 20 min. An equal amount of supernatant and 6% formic acid were mixed, and stored at –20 °C until analysis. The concentration of different volatile fatty acids was determined by gas chromatography (GC) using a HP 5890 (Amstelveen, the Netherlands) equipped with a 2 m × 6 mm × 2 mm glass column packed with Supelco-port 100–120 mesh, coated with 10% Fluorad 431. The flow rate of the carrier gas (nitrogen, saturated with formic acid) was 40 ml min^{-1}. The column temperature was 130 °C, the injection port and flame ionization detector were at 200 °C and 280 °C, respectively.

The CO_2 concentration in the soil was determined daily from 3 to 32 DAI in the control and lucerne-amended soil of the BM treatment. Additionally, measurements were taken from 17 to 32 DAI in control and lucerne-amended soil of the RP3 treatment. A small air chamber was created in each allocated pot, by burying a 5 cm long and 1.6 cm diam. PVC tube in the soil at a depth of 4 to 9 cm. Attached to this PVC tube was a hollow wire with a rubber on top, which protruded above the soil surface and from which air samples were taken with a syringe. Immediately after sampling, the CO_2 concentration in the air samples was analysed by gas chromatography using a Micro-GC CP 2002 (Chrompack, Middelburg) equipped with a HayeSep A column.

Data analysis

Firstly, the total number of emerged lettuce seedlings in the control pots was compared between sowing times and rainfall treatments. Subsequently, the relative emergence was calculated by dividing the number of emerged seedlings in a lucerne-amended pot in each block by the number of seedlings emerged in identically treated control pots, averaged over all blocks. Comparisons were made using the generalized linear model (GLIM) procedure in the Genstat 9 statistical package (Payne et al., 2006). Pairwise t-tests were used to test for significance between treatments. For comparisons between the control treatments, the GLIM procedure was adjusted for binomial data.

Because no differences in the relative emergence were found between the BM, RP1 and RP2 treatment for the first 2 sowing times, and between the BM and RP3 treatment for the first 4 sowing times, the conclusion was drawn that rainfall did not alter the emergence of lettuce sown, if lettuce was sown at least 7 days before rainfall application.

Comparison of seedling emergence (control soils) or of relative emergence (lucerne-amended soils) between treatments was carried out in a few consecutive steps. First a comparison between sowing times within the BM treatment was made.

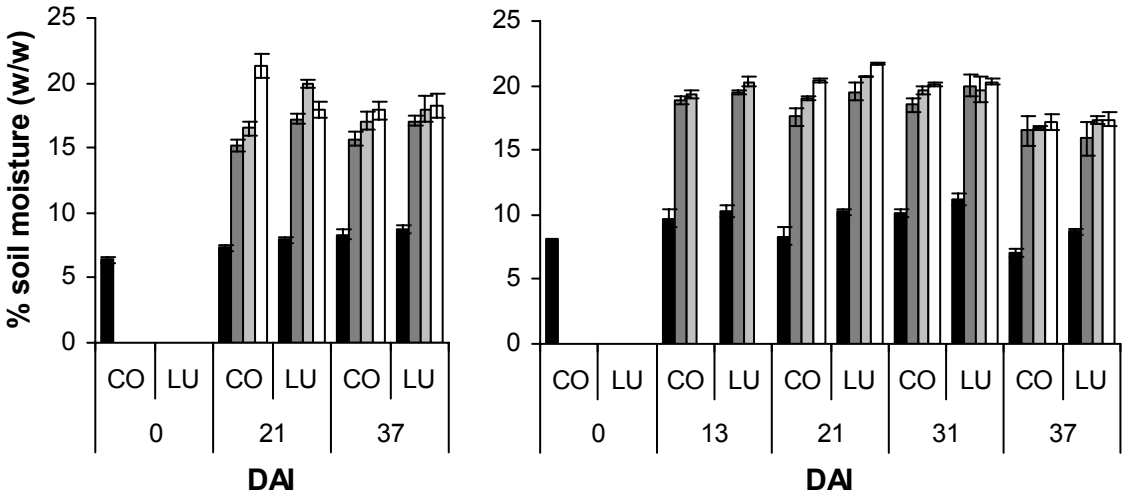

Figure 6.2. Soil water content measured at different times after residue incorporation in control soil (CO) or lucerne-amended soil (LU) in different rainfall treatments of experiment 1 (left) or experiment 2 (right). Basic Moisture = black, Rainfall Peak 1 (RP1) = dark grey, RP2 = light grey, RP3 = white. Vertical bars indicate mean value ± SE.

Thereafter, subsequent comparisons were made between the BM treatment and each of the three rainfall treatments. When comparing the BM-RP1 treatments, all sowing times were taken into account, whereas when comparing the BM-RP2 treatments, only sowing times 3–8 were included in the analysis and when comparing the BM-RP3 treatments, only sowing times 5–8 were included in the analysis.

The time needed to reach 50% seedling emergence (T_{50} emergence) was determined by fitting a three parameter logistic curve through the emergence data, assuming a binomial distribution and maintaining the upper limit fixed to the total number of emerged seedlings. When the total number of emerged seedlings in a unit was 5 or lower, the T_{50} emergence was not determined. Differences in T_{50} emergence were analysed in the same way as differences in relative emergence.

The relative biomass of lettuce seedlings was calculated by dividing the dry weight per seedling of pots with lucerne-amended soil by the average dry weight per seedling in the corresponding control pots. When the number of emerged seedlings at 6 or 7 DAS was lower than four, the dry weight per seedling was not determined. Because only few seedlings emerged shortly after a rainfall peak, no value for relative seedling biomass could be obtained for some of the sowing times in the rainfall treatments. To investigate the effect of a rainfall peak on the relative biomass of healthy seedlings, the dataset was classified into two categories: “basic moisture” and “rainfall”. Accordingly, the earlier described GLIM procedure was used for analysis. The GLIM procedure was also used to compare the concentration of VFAs in the BM treatment and at 2 and 10 days after each rainfall peak.

Results

The soil moisture content of the BM treatment was 6.4–8.7% in experiment 1 and 8.2–11.2% in experiment 2 (Figure 6.2). In the lucerne-amended soil the soil moisture content was slightly higher than in the control soil. Shortly after a rainfall peak the soil moisture content increased to approximately 20%, after which it slowly decreased and stabilized at around 16–18%.

In both experiments, the fraction emergence in the control soil of the BM treatment was independent of sowing time and was on average 0.96 (Figure 6.3). In experiment 1, the rainfall peaks only caused slight reductions in the fraction emergence of the control soil (4–7%), whereas in experiment 2 this reduction was much larger. In the RP1 and RP2 treatments for instance, emergence of lettuce sown at 1, 5 and 9 (RP1), and at 1 and 5 (RP2) days following the rainfall peak, was severely inhibited (17–76%). For RP3, only a reduction in the relative emergence at 9 days after the rainfall peak was observed.

In experiment 1, lucerne residues in the BM treatment inhibited the emergence of lettuce sown at 0 DAI by 32%, after which the inhibitory effect increased to 79% for lettuce sown at 4 DAI and decreased to 49% for seedlings sown at 8 DAI. No or only very slight inhibitory effects were observed for sowing times at 12 DAI and later. In experiment 2, the inhibitory effect of lucerne residues in the BM treatment was less severe than in experiment 1. Only for lettuce sown at 8 (43%) and 16 (36%) DAI inhibitory effects were observed.

A rainfall peak applied at 3 DAI reduced the average relative emergence of all sowing times in both experiments to a similar extent (28–32% reduction). The second rainfall peak, which was applied at 11 DAI, had a longer lasting and stronger effect in experiment 2. In this experiment the relative emergence of all sowing times after the rainfall peak was strongly reduced (on average 79%), whereas in experiment 1 only the relative emergence of lettuce sown at 1 and 9 days after the rainfall peak was reduced (on average 47%). For the last rainfall peak, which was applied at 19 DAI, relative lettuce emergence in both experiments was reduced for all sowing times following the rainfall peak, although to a much larger extent in the second experiment (on average 80%) compared to the first experiment (on average 30%).

In the control soil of experiment 1, the time needed to reach 50% lettuce emergence (T_{50} emergence) was on average 6 days, and this result was irrespective of rainfall treatment. T_{50} emergence of lettuce was approximately 2–3 days longer for the first three sowing times of the BM treatment in the lucerne-amended soil. This was also observed for the first sowing time of RP1 treatment and the first three sowing times of RP2 and RP3 treatments. These first sowing times of the rainfall treatments

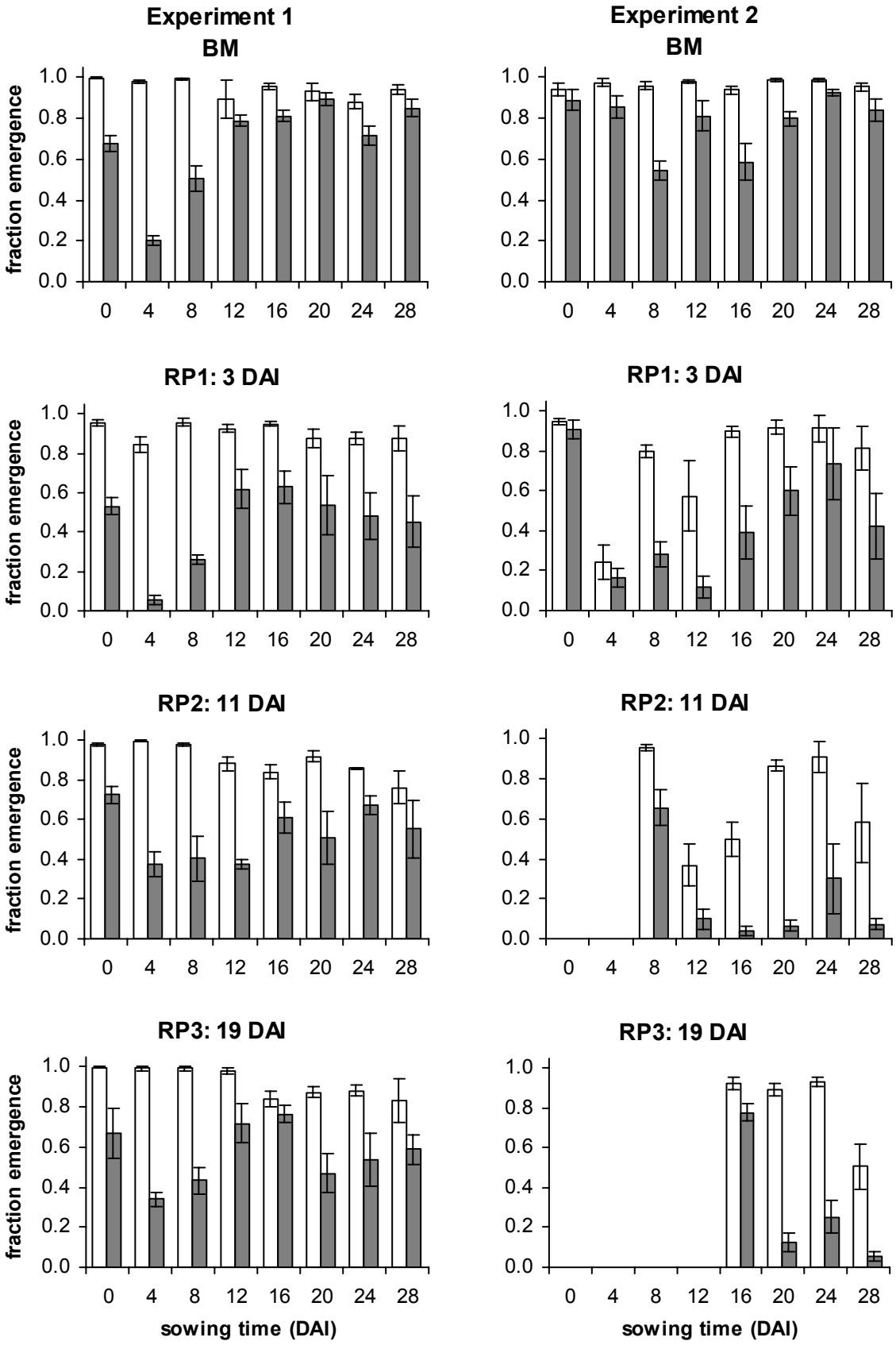

Figure 6.3. Fraction emerged lettuce seedlings in the control soil (white bars) and the lucerne-amended soil (grey bars) of the different sowing times in the four rainfall treatments of experiment 1 and 2. Vertical bars indicate mean value ± SE.

were comparable to first sowing times of the BM treatment, as they occurred before rainfall application. In experiment 2, the severe inhibition of seedling emergence frequently hindered a proper determination of T_{50} emergence, particularly in the RP2 treatment. In this experiment no differences in T_{50} emergence between control and lucerne-amended soil were detected.

In experiment 2, the dry weight of lettuce seedlings that emerged at 6 or 7 days after sowing (DAS) was determined at 22 DAS, to find out whether the addition of lucerne-residue material had a growth reducing effect on lettuce seedlings. If sufficient lettuce seedlings had emerged at the proper time, the ratio between lettuce plant dry weights for lucerne-amended and control soils was determined for different rainfall treatments and lettuce sowing times. For the BM treatment, no consistent time pattern could be determined and the average relative biomass was 0.7 (Figure 6.4). For the different rainfall treatments the average relative biomass was close to 1, which was higher than the relative biomass in the BM treatment ($p<0.001$).

In experiment 2, special attention was given to infection of seedlings by the soilborne pathogens *Pythium* and *Rhizoctonia solani*. Both randomly selected seed(ling)s harvested at 10 DAS and seedlings with apparent symptoms (wilted seedlings) were sampled. In the lucerne-amended soil approximately 9% of the emerged seedlings wilted, which was 3 times as high as in the control soil, where only 3% of the seedlings showed wilting symptoms. No influence of rainfall treatment or sowing time could be detected. On 53% of the wilted seedlings put on agar plates, pathogens were detected. In most cases *Rhizoctonia solani* was observed (32%-points), *Pythium* was detected on 19%-points of the agar plates and on 2%-points of the agar

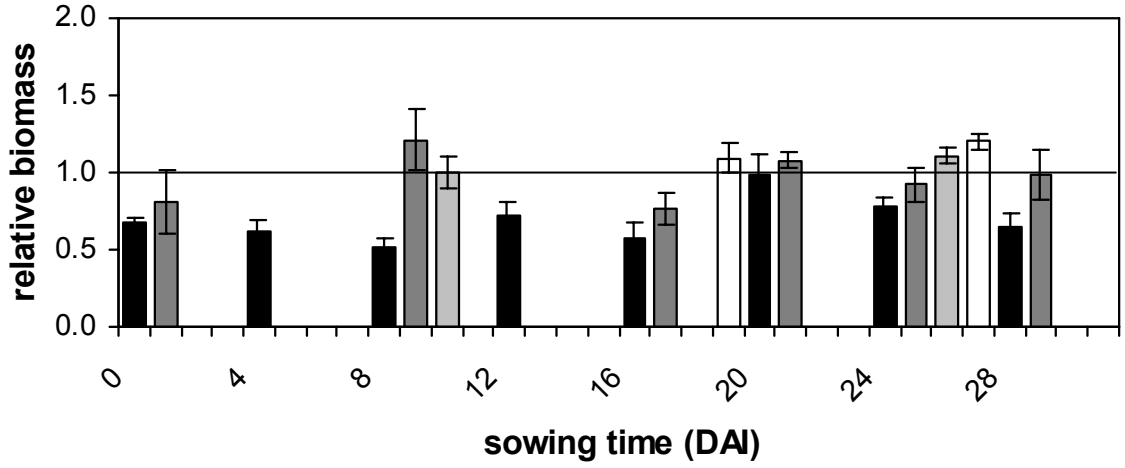

Figure 6.4. Relative biomass of lettuce on a per seedling dry weight basis in different rainfall treatments and sowing times of experiment 2. BM = black, RP1 = dark grey, RP2 = light grey, RP3 = white. Vertical bars indicate mean value ± SE.

plates both pathogens were present. From the randomly selected seed(ling)s harvested at 10 DAS, *Pythium* spp. (on average 34%-points) was detected in higher amounts as *Rhizoctonia solani* (on average 5%-points). No difference was observed between lucerne-amended and control soil. On 73% of the emerged seedlings, 54% of the germinated seeds and 66% of the ungerminated seeds, *Pythium* nor *R. solani* was detected. Differences between sowing times and rainfall treatments could not be tested, due to the low number of replicates and the unbalanced design.

In the control soil the electrical conductivity (EC) was similar for all rainfall treatments and rather stable between sowing times, with an average value of 61 µS cm^{-1} (Figure 6.5). In the lucerne-amended soil a continuous increase of the EC was observed over time. In the BM and RP1 treatment this increase was stronger (295–327 µS cm^{-1} at 52 DAI) than in the RP2 and RP3 treatment (190–215 µS cm^{-1} at 52 DAI). pH-H_2O values, obtained from the same 20% soil extracts as the EC, ranged from 5.6 to 6.8, with an average of 6.4. Determination of the volatile fatty acid content only revealed the presence of acetic acid, the concentration of which was lower than 0.62 mg $liter^{-1}$ in all treatments. In lucerne-amended soil a 72% higher concentration of acetic acid was found at both 2 and 10 days after a rainfall peak ($p=0.011$). In control soil, no increase in the concentration of acetic acid was observed following a rainfall peak.

In the control soil the concentration of NO_3^- was similar to the concentration of NH_4^+, and did not change between 13 and 29 DAI (on average 16 mg kg^{-1}). In the lucerne-amended soil the concentration of NH_4^+, at 13 DAI, had increased to, on

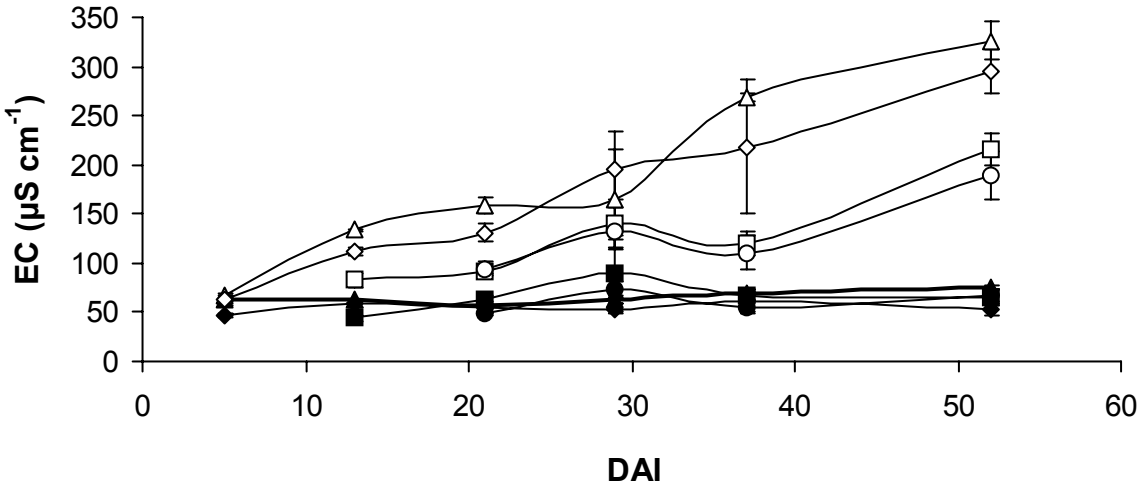

Figure 6.5. Electrical conductivity (EC, µS cm^{-1} at 25 °C) measured in a mixture of 20% dry soil and 80% de-ionized water. Soil samples were taken at different days after residue incorporation (DAI). Closed markers = control soil, open markers = lucerne-amended soil. BM = triangles, RP1 = diamonds, RP2 = squares, RP3 = circles. Vertical bars represent mean values ± SE.

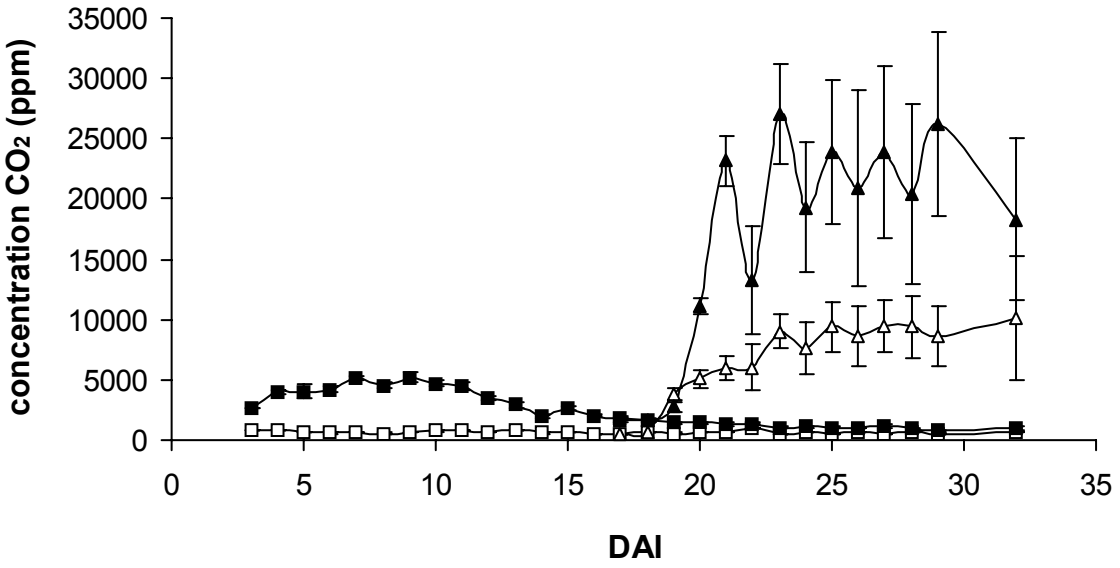

Figure 6.6. CO_2 concentration measured at different times after residue incorporation in control soil (open markers) or lucerne-amended soil (closed markers), with either a basic moisture level (squares) or with a rainfall peak applied at 19 DAI (triangles). Vertical bars represent mean values ± SE.

average, 87 mg kg^{-1}, whereas the concentration of NO_3^- in the same soil was only 9 mg kg^{-1}. At 29 DAI the NO_3^- content of the lucerne-amended soil had increased to 46 mg kg^{-1}, while no further increase in the NH_4^+ content of the soil was observed. No effect of rainfall treatment on the concentration of NO_3^- or NH_4^+ was detected.

In the control soil of the BM treatment the CO_2 concentration fluctuated between 470 and 980 ppm (Figure 6.6). Addition of lucerne residues caused the CO_2 concentration to increase. A gradual increase was observed over the first 7 to 9 DAI, with peak values reaching over 5000 ppm, after which the CO_2 concentration gradually declined to below 1000 ppm at 28 DAI. Additional observations in the RP3 treatment, where the rainfall peak was applied at 19 DAI, showed a marked increase in CO_2 concentration in both the control as well as in the lucerne-amended soil, directly after the rainfall event. Increases in the lucerne-amended soil (max. values around 25,000 ppm) were about 2.5 times higher than in the control soil (max. values around 10,000 ppm). The CO_2 concentrations after the rainfall peak remained high, but showed large daily fluctuations, which were likely to be related to moisture application every second day to compensate for evaporation.

Discussion

In line with most literature on inhibitory residue-mediated effects (Patrick et al., 1963;

Ohno et al., 2000; An et al., 2001; Xuan et al., 2005), lucerne residues in the BM treatment caused the most severe inhibition of lettuce emergence during the early stages of decomposition. This was also the period when the highest CO_2 concentrations in the BM soil were measured, indicating increased microbial activity associated with the decomposition of lucerne residues. Rainfall caused an increase in the residue-mediated inhibitory potential. This is congruent with the observations in the second field experiment of Chapter 5. However, most other publications report a decline in the residue-mediated inhibitory potential after heavy rainfall. Lockerman and Putnam (1979) found that the allelopathic effect of cucumber was less under periods of increased rainfall. Cochran et al. (1977), who investigated the potential phytotoxicity of five crop residue types to winter wheat, found that toxin production was irregular, and appeared only shortly after light rain events with air temperatures above freezing but below 15°C. After heavy rainfall, growth stimulation was observed, which according to the authors may have been caused by a "dilution effect". It is widely accepted that low concentrations of allelochemicals can stimulate plant growth (e.g. Lovett et al., 1989). Similarly, Barker and Bhowmik (2001) reported that high rainfall destroyed the weed-controlling potential of mulched or incorporated cover crop residues.

In experiment 2, the effect of rainfall was stronger than in experiment 1. Surprisingly, the effect of rainfall in experiment 2 was observed in both the control and lucerne-amended soil, whereas in experiment 1 only an effect in the lucerne-amended soil was observed. *Rhizoctonia solani* and *Pythium* spp. were detected in all treatments, though most wilted seedlings were found in the lucerne-amended treatments. The observed rainfall effect in the control soil of experiment 2 may therefore be explained by interference of these soilborne pathogens. The absence of a rainfall effect in the control soil of experiment 1 might be related to the longer period between soil sterilization and the start of the experiment. During this period the sterilized soil, mixed with 1% non-sterilized soil, was stored in open pallets for recolonization by microorganisms. The longer period allocated for recolonization in experiment 1 (6 weeks) compared to experiment 2 (2 weeks) presumably resulted in more competition of antagonistic microorganisms against soilborne pathogens. The suppression of *Pythium* spp., for instance, was found to be well correlated with microbial competition for nutrients (Grunwald et al., 2000). Van Os and van Ginkel (2001) found the percentage of infection in *Iris* caused by *Pythium macrosporum* to be lowest in untreated soil and to progressively increase in sterilized soil amended with 1% compost, fumigated soil (methylisothiocyanate) and sterilized soil.

Allelochemicals leached from lucerne residues into the surrounding soil are likely to be another factor responsible for the observed inhibitory effects on lettuce

emergence. However, it is difficult to separate allelopathic from pathogenic effects. Besides that, lucerne allelochemicals and soilborne pathogens may have acted additively or even synergistically. Phenolic compounds, one of the groups of allelochemicals present in lucerne residues, are known to interfere with cell membrane permeability (Einhellig, 2004), resulting in higher exudation of organic molecules into the spermo- or rhizosphere. As propagule germination and germtube elongation of pathogens, like *Pythium* spp. (Martin and Loper, 1999) as well as direction of growth (*R. solani*) or movement (*Pythium*), is enhanced by the presence of plant exudates, increased exudation resulting from phenolic action may have caused higher seed(ling) infection rates.

Of the volatile fatty acids, phytotoxic compounds produced during anaerobic decomposition, only acetic acid was detected and longer chain, more phytotoxic (Janovicek et al., 1997) fatty acids were absent. Lynch (1977) found that acetic acid at a concentration of 10–26 mM present in a solution of a mixture containing 200 g dry soil, 10 g straw and 600 ml distilled water, which is equivalent to approximately 1000–2600 g liter^{-1}, reduced root extension of barley seedlings by 48–58%. The average concentration of acetic acid detected in our experiment (0.62 mg liter^{-1}) was approx. 1600–4200 times lower than the concentrations that Lynch used. Therefore, in spite of the relatively higher concentrations of acetic acid that were found after a rainfall peak in lucerne-amended soil, indicating the presence of anaerobic micro-sites, the overall effect of acetic acid on lettuce emergence was most probably negligible. This is probably due to the relatively short period of high soil moisture content.

The lower EC measured in the RP2 and RP3 compared to the BM and RP1 treatments indicate leaching of plant components out of the top soil after rainfall application. We indeed observed water dripping out of the bottom of the pots after rainfall application. The reason why the EC was not decreased shortly after the rainfall peak application in RP1 may be that hardly any plant metabolites had been released from the residues within this short period (3 DAI) after incorporation.

In the BM treatment the number of emerged seedlings was higher than after a rainfall peak, but the emergence rate was slower (experiment 1) or the growth rate reduced (experiment 2), indicating that many seedlings were affected but not directly killed. After a rainfall peak most seedlings were killed before emergence, but the few seedlings that emerged were less affected by the residue-mediated inhibitory effects than the seedlings in the BM treatment.

Although it is difficult to unravel the underlying mechanisms of the effect of rainfall on the residue-mediated inhibitory potential of lucerne, we hypothesize that in the RP treatments allelochemicals played a less important role in the observed residue-mediated inhibitory effects than in the BM treatment. After the rainfall peak,

allelochemicals likely leached out of the lettuce root zone, and/or their concentration decreased considerably. This may be the reason why, presumably in absence of significant populations of soilborne pathogens, rainfall was often observed to decrease the residue-mediated inhibitory potential (Cochran et al., 1977; Lockerman and Putnam, 1979; Barker and Bhowmik, 2001). After a rainfall peak in our experiments, soilborne pathogens were likely largely responsible for the inhibition of lettuce emergence. A high soil moisture content is generally stimulatory to soilborne pathogens, because of an increase in the size of the spermosphere, and, in the case of *Pythium* spp., a competitive advantage of the pathogens over other saprophytes at low O_2 concentrations and a higher motility of zoospores (Martin and Loper, 1999). The positive effect of lucerne amendment on *R. solani* is more difficult to explain, since it is thought that *R. solani* is suppressed by specific antagonists (Hoitink and Boehm, 1999). So for this pathogen, an indirect effect of lucerne amendment and rainfall on antagonists of *R. solani* could play a significant role. Soils in the current experiments were sterilized at either six (experiment 1) or two (experiment 2) weeks before the start of the experiment. It remains unclear to what extent soil sterilization has influenced the importance of infection by soilborne pathogens as an underlying mechanism responsible for increased residue-mediated inhibition of seedling emergence after a rainfall peak.

The possible difference in the underlying mechanism of the observed residue-mediated inhibitory potential in the RP treatments compared to the BM treatment may explain why the growth of seedlings that emerged after a rainfall peak were less affected than seedlings in the BM treatment. New experiments, specifically designed to separate allelopathic from pathogenic effects, should be carried out to verify the underlying mechanisms of lucerne residue-mediated inhibitory effects on seedling establishment before and after rainfall. Furthermore, it would be interesting to assess what would be the effect of rainfall in non-sterilized soil, as it has been observed that disturbed soils are more conducive to soilborne pathogens (e.g. Van Os and Van Ginkel, 2001). Stimulation of soilborne pathogens in lucerne-amended soil after a rainfall peak will not only contribute to the inhibition of weed seedling emergence, but is also likely to have adverse effects on the crop, and might therefore complicate the use of lucerne as an ecological weed management tool. Delayed sowing of the crop, following lucerne residue incorporation, could largely alleviate such problems, but at the same time decreases the head start of crops in their competition with weeds. Good land preparation, resulting in a well-drained seedbed, is another option to reduce crop damage due to damping-off considerably. These observations stress once more that ecological weed management is a matter of systems optimization and successful implementation greatly relies on good farming skills.

CHAPTER 7

General discussion

In the first part of this chapter the contribution of the research presented in this thesis to the understanding of cover crop effects on weeds, as well as the possibilities for optimization of these effects through management, will be discussed. Subsequently, the findings of this thesis will be placed in a broader systems perspective. Here attention will be given to the possibilities of combining various cover crop services and the potential of including cover crops as one of the components in an all encompassing ecological weed management strategy. Finally, at the end of this chapter future research needs regarding cover crop-based ecological weed management will be discussed.

Effects of cover crop residues on weeds

The large variability in results

When comparing the results presented in the different chapters in this thesis, a large variation in inhibitory effects of cover crop residues on weeds becomes evident. Not only did we find cover crop species to differ in residue-mediated inhibitory potential, different experiments also yielded contrasting results for the same cover crop species. For lucerne-amended soil we observed a complete inhibition of various receptor plant species in the second experiment of Chapter 5, whereas in Chapter 2 and in the first experiment of Chapter 5, overall seedling establishment was inhibited by just 26–65%. For incorporated winter rye residues, no effect on seedling establishment could be detected in Chapter 2 and 5, but in Chapter 4 reductions of seedling establishment up to 51% were observed.

This large variation in residue-mediated effects is also observed in literature and reflects the many factors that influence the effects of residues on receptor plants. In Chapter 1, we attempted to visualize this complexity by developing a conceptual model of the allelopathic effect of cover crops on weed establishment and early growth (Figure 1.2). In reality, however, complexity is even larger than presented in this model, for at least two reasons. First, the effect of cover crop residues on the establishment of receptor plants does not only occur through allelopathic interference, but can also be influenced by other mechanisms, such as altered soil nitrogen dynamics, soil physical characteristics and activity of soilborne pathogens. Second, and that is something that was strongly enforced by the results presented in this thesis,

is the prominent role of the temporal dynamics of the system. The results in Chapter 4 indicated receptor plant sensitivity to be related to the emergence time of the species. The results of Chapter 5 strongly suggest that an overlap of the time-course of receptor plant sensitivity and the time-course of residue-mediated inhibitory potential is a prerequisite for residue-mediated inhibition of the receptor plant (Figure 5.5). The largest inhibition thus will be obtained when these two perfectly match, whereas any shift will result in below optimal reductions. Therefore, the high degree in the variation commonly noted in field studies of allelopathy is likely to be the result of, at least in part, variation in synchronicity of receptor species' sensitivity and residue-mediated inhibitory potential. The challenge, therefore, remains to increase our ability to predict the time-courses of both the residue-mediated effects as well as species' sensitivity, as only this will allow us to manipulate both factors and to optimize the residue-mediated inhibitory effect on weeds.

Two important factors: management and environment

Weather conditions strongly influenced the residue-mediated inhibitory potential. In field experiment 2 of Chapter 5, lucerne residue-mediated inhibitory potential strongly increased after a large rainfall peak at 7–9 days after incorporation. This finding was confirmed by controlled experiments in Chapter 6. The difference in the time-course of winter oilseed rape residue-mediated potential between Chapter 4 and the first experiment in Chapter 5 may be explained by temperature differences. In Chapter 5, the inhibitory potential of winter oilseed rape residues cut with a flail mower and incorporated in the upper 10 cm of the soil on April 7, 2005, continuously declined with time and had disappeared after approximately 2 weeks. In Chapter 4, the inhibitory potential of winter oilseed rape residues cut into pieces of 3 cm and incorporated in the upper 10 cm of the soil on March 28, 2006, continuously increased over the first 3 weeks, after which it declined again. The delay of the peak inhibitory effect in the experiment of Chapter 4 may partly be related to the relatively lower temperature. During the first two weeks after incorporation the mean temperature 5 cm beneath bare soil was 7.4 °C for the experiment in Chapter 4 and 10.5 °C for the first experiment of Chapter 5.

Residue pre-treatment prior to incorporation into the soil was shown to have a large influence on the time-course of winter oilseed rape residue-mediated inhibitory potential. Inhibition of seedling emergence occurred earlier with an increased level of tissue disruption: ground winter oilseed rape residues inhibited lettuce seedling emergence only during the first two to three weeks following residue incorporation, whereas inhibition of lettuce seedling emergence by cut residues started after this period (Figure 4.2).

The time of incorporation partly determines cover crop residue characteristics and therefore may have a large influence on the extent of the residue-mediated inhibitory potential. We investigated the allelopathic potential of autumn-sown cover crop plants the next spring, by regularly harvesting plants from March 22 until May 2 and testing the residue extracts in laboratory bioassays (Chapter 3). For winter rye a linear decline in allelopathic activity of residue material was observed during this period, whereas the allelopathic activity of residue material of winter oilseed rape showed a steep decline following the onset of the flowering stage.

It still remains to be further investigated how differences in allelopathic potential established in laboratory bioassays relate to differences in residue-mediated potential in the field. For winter rye in Chapter 4, laboratory and field results pointed in the same direction. We observed one-week older winter rye residues to have a lower allelopathic potential when extracted and tested in a laboratory bioassay, and at the same time have a weaker inhibitory effect on lettuce emergence in a field situation when incorporated at the same rate as the younger residues. This suggests that winter rye residues should be incorporated into the soil in early spring. However, not only the allelopathic activity per unit plant biomass is important for the overall residue-mediated inhibitory potential, but also the amount of cover crop biomass. As cover crop biomass increases over time in spring, this will probably at least compensate for the reduction in allelopathic potential. For this reason, in Chapter 2 the highest allelopathic activity per unit area (biomass × allelopathic activity per unit biomass) of all three cover crop species was found at the end of the sampling period. It would be interesting, however, to assess whether the allelopathic activity per unit area is indeed simply the result of the amount of biomass times the allelopathic activity per unit biomass as measured in a laboratory bioassay. As cover crops mature, fibrous (carbon) plant material increases and protein (nitrogen) content decreases (e.g. Sarrantonio, 1994; Ranells and Wagger, 1992). Because a high C:N ratio has a negative effect on the decomposition rate, also the release rate of allelochemicals may be decreased. A changed release rate of allelochemicals may influence the strength of the residue-mediated effect on different receptor plant species.

Because the timing of residue-incorporation is dependent on the prevailing weather conditions and should be adapted to the sowing/ planting time of the next crop, it would be ideal to be able to optimize the allelopathic potential of the cover crop residues at the time of residue incorporation (Chapter 3). We tried to reach this by stimulating the cover crop to induce synthesis of allelochemicals through mechanical damaging in the period prior to residue incorporation. It was clearly demonstrated that mechanical wounding enhanced the allelopathic activity per unit biomass of all three cover crop species. However, comparing the increase in allelopathic activity per unit

biomass resulting from damaging to the change in this parameter over time, made evident that the impact of damaging is only minor. Generally it was just sufficient to compensate for the loss in plant biomass resulting from mechanical damaging. Therefore it was concluded that the increase in allelopathic activity due to damaging is of little significance for farming practice.

Sensitive period of receptor species

Plants are likely to be most sensitive to residue-mediated inhibitory effects in the early stages of development. In case of allelopathic effects, the concentration of allelochemicals in plant tissue is expected to decline with increasing biomass (Chapter 1). In case of soilborne pathogen effects, infection rates are especially high during the germination process, because of the presence of exudates released by seeds imbibing water (Martin and Loper, 1999). Both are congruent with the observations in this thesis, where the relative time of emergence was found to be an important indicator of species' sensitivity to residue-mediated inhibitory effects (Chapter 4 and 5) and where the time of germination relative to the rainfall peak was thought to be a decisive factor for the strength of the lucerne residue-mediated inhibitory effect (Chapter 5). Further development of models predicting weed emergence patterns (e.g. Grundy and Mead, 2000; Vleeshouwers and Kropff, 2000) is needed to give a good indication of the sensitive period of weed species. Linking these weed emergence models to still to be developed models on the time-course of residue-mediated inhibitory effects may help to estimate the expected inhibition of different weed species by cover crop residues.

Cover crop based ecological weed management in a systems perspective

The many different functions of cover crops

It is not realistic to assume that cover crop choice and related management in organic farming systems can be merely directed towards the maximization of the weed-suppressive function of cover crops. Apart from weed control, cover crops provide many other functions in organic farming systems, for instance through a) conserving soil fertility, soil structure and soil organic matter, b) improving the quality of the environment by preventing leaching of nutrients, c) decreasing dependency on external resources such as fertilizer and d) management of diseases, pests and weeds and e) stimulation of biodiversity (Wijnands and Holwerda, 2003). On the other hand, cover crops can also have adverse effects on the cropping system as several species can act as a host plant for pests, most importantly for nematodes, leatherjackets (larvae of the crane fly), larvae of agriotid beetles and slugs (Wijnands and Holwerda, 2003).

The choice of the cover crop species depends on the relative importance of the

above described cover crop benefits for the specific cropping system, the position in the crop rotation, soil type, the presence of soilborne pests, diseases and nematodes, and climate related factors. How much the cover crop can contribute to conserving soil fertility and soil organic matter and to reducing external fertilizer input depends on the amount of biomass produced, the residue quality and the synchronicity of nutrient release from the residues with nutrient demands of the following crop. These factors in turn all depend on cover crop species and sowing time, weather conditions, nutrient status and structure of the soil, time of residue incorporation, and the sowing/planting time and characteristics of the following crop. Furthermore, cover crops with an extensive root structure, like grass species, contribute most to the improvement of soil structure.

The preceding crop is of main importance for deciding which cover crop species to include in the crop rotation. Firstly, the choice of cover crop species is restricted to those species that can establish well after the harvest time of the preceding crop. Secondly, the nutrient status of the stubble is important for cover crop choice. In a relatively poor wheat stubble a leguminous species will be a better choice, whereas a leguminous species following a nutrient rich stubble will lead to nutrient leaching and a deep rooting and fast growing cover crop species will fit best. On the other hand, the choice of the cover crop species also needs to be adapted to the following main crop, as the nutrient release rate of the cover crop needs to synchronize with the timing of nutrient demands of the main crop. Ideally, cover crop choice and management should be tailored to the specific cropping situation and a cost-benefit analysis should include both the long and short-term objectives of the crop rotation.

Many little hammers

The introduction of cover crops with a strong weed suppressive function in a crop rotation will not be sufficient to obtain adequate weed management. The strength of ecological weed management needs to come from a combination of measures, often referred to as the use of many little hammers (Liebman and Gallandt, 1997). Cover crop-based weed management thus needs to be an integrated part of the overall ecological weed management approach, which should include multiple preventive and cultural weed management tactics (e.g. Liebman and Davis, 2000; Barberi, 2002; Melander et al., 2005). As was described in Chapter 1 and 2, inclusion of cover crops in crop rotations introduces two important mechanisms through which the development of weed populations may be hampered. In late summer and autumn the successful introduction of cover crops can prevent growth, development and, most importantly, seed production of weeds through competition. In springtime, cover crop residues incorporated in the upper layer of the soil may suppress or retard weed

development and growth due to, among others, allelopathic effects.

In Chapter 2, it was observed that cover crop species differed in their performance in autumn and spring. Some species, including lucerne and white lupine, were weak competitors in autumn, but provided strong residue-mediated inhibitory effects on weed establishment in spring. Other species, like fodder radish and winter rye, were highly competitive in autumn but their residues did not inhibit weed establishment in spring. Winter oilseed rape performed reasonably well in both situations. The relative importance of a reduction in weed seed production in autumn through cover crop competition, compared to a reduction in weed establishment in spring through residue-mediated inhibitory effects, depends on the position of the cover crop in the crop rotation. If the subsequent main crop has a low competitive ability, like for instance in the case of leek, onion, sugar beet and carrot, the main focus should be on the optimization of residue-mediated inhibitory effects in spring. In this respect it also makes a difference whether the crop is sown or planted, as planting offers the crop a competitive head start over weeds. Planting also mitigates potential negative effects of the residues on the crop. Furthermore, the potential inhibitory effects of cover crop residues on weeds need to be weighted with alternative methods for ecological weed management in the following crop. When the following crop is sown or planted later in spring, establishing a stale seedbed is likely to be highly effective in reducing weed pressure (Riemens et al., 2007b). In slow-germinating row crops such as onion, leek, carrot and corn, flaming before crop emergence also provides a good alternative weed control method (Melander et al., 2005).

Optimal sowing time of cover crops

The best timing of cover crop sowing is species specific and depends on the objectives. If the objective is to get a very high biomass production, in order to maximize prevention of nitrogen leaching and maximize the amount of residue to be incorporated in the next year, then sowing should be performed as early as possible after harvesting of the preceding crop. However, early sowing of certain frost-sensitive species, like for instance fodder radish and yellow mustard, might result in seed production and subsequent problems with volunteer plants if seeds are not removed. Although early sowing of cover crops will increase competitive ability, in experiment C of Chapter 2 earlier seedbed preparation was related to a higher weed pressure and a more competitive species composition as opposed to later seedbed preparation. In this experiment, an additional tillage operation in the control treatment of winter rye and winter oilseed rape, which was conducted one month after tilling the whole field, resulted in a five times lower weed biomass compared to that of the lucerne control treatment, which was only treated once (Table 2.3). Also, the species composition

differed, with the short-statured *Poa annua* and *Stellaria media* being dominant in the winter rye and winter oilseed treatments and a more diverse weed flora being present in the control plots of lucerne (e.g. *Chenopodium album*, *Polygonum spp*. and *Echinochloa crus-galli*). For reducing weed seed production in autumn, it may therefore be beneficial to combine a cover crop that can be sown relatively late with a stale seedbed preparation. Merely delaying soil tillage also has benefits as it can give seed predators the chance to remove a high percentage of newly produced weed seeds in the stubble of the preceding crop (e.g. Westerman et al., 2003).

Future challenges

Although cover crop residues have shown to substantially inhibit weed establishment, there are still a number of challenges that need to be addressed before cover crop residue-based weed management can be successfully implemented in practice. First of all, better insight needs to be gained in the underlying mechanisms of the observed residue-mediated inhibitory effects and their interactions, as well as in how the relative importance of these different underlying mechanisms may shift with changing environmental conditions, with management and with soil characteristics. This knowledge will allow us to better manipulate the strength and the timing of the residue-mediated potential. The residue-mediated inhibition of seedling emergence by lucerne residues, for example, was probably due to both allelochemical effects and stimulation of soilborne pathogens (Chapters 5 and 6). At the end of Chapter 6, we hypothesized that in the rainfall treatments allelochemicals played a less prominent role than in the basic moisture treatment, and that after a rainfall peak soilborne pathogens were likely for a large part responsible for the inhibition of lettuce emergence. This hypothesis should be further investigated. In Chapter 6, we also proposed that the stronger effect of rainfall on the residue-mediated inhibitory potential of lucerne in experiment 2 compared to experiment 1 may have been related to the shorter period between soil sterilization and the start of the experiment. This has raised the question whether increased soil disturbance can cause a shift towards more soilborne pathogen driven residue-mediated inhibitory effects on weed establishment.

Furthermore, future research is required to explore how to optimize inhibition of weed establishment while mitigating adverse effects on the crop. On the basis of observations in laboratory bioassays much was expected from selective weed suppression of relatively small-seeded weeds in larger-seeded crops. Unfortunately, under field conditions seed mass alone could not always explain differences in species' sensitivity to residue-mediated inhibitory effects. On basis of re-analysis of two earlier published datasets of Haramoto and Gallandt (2005) (Chapter 5), we now conclude

that seed mass only becomes important *after* accounting for the time of emergence of the receptor plant in relation to the time course of residue-mediated inhibitory potential. Therefore, seed mass cannot be considered as a single reliable predictor of sensitivity to inhibitory residue effects. For cover crop residue-based weed management to become successful, sowing of large-seeded crops in residue-amended soil should be combined with other tactics that contribute to the avoidance of negative effects on crop growth. Opportunities may be found in residue placement, for example by varying the position of cover crop residues in relation to the position of the crop seeds. Also residue pre-treatment may provide options to reduce overlap in the sensitive period of the receptor plant species and the residue-mediated inhibitory potential. Planting instead of sowing likely provides another solution for decreasing negative effects on crop growth.

Research on cover crop-based ecological weed management has a multi-disciplinary character. Future research projects aiming at disentangling underlying mechanisms of residue-mediated inhibition of receptor plant establishment and early growth will require knowledge and expertise from diverse disciplines, including agronomy, chemistry, microbiology, plant physiology and soil science. A constant iteration of experiments conducted at different levels of complexity, as illustrated in Figure 1.3, will be needed to unravel the many interactions that take place in residue-amended soil. Combining these experiments with modeling may provide a way to gain a better understanding of residue-mediated inhibitory effects on weed establishment and early growth. In all cases special focus should be put on the temporal dynamics of the system. Besides biological/technical studies, social aspects need to be studied as well to ensure that the systems developed fit in the farmer's system. A process of co-innovation can help to ensure the proper prioritization of biological studies and use of the results in the innovation process.

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Summary

In organic farming systems, where the use of pesticides is excluded, weed control is recognized as the foremost production-related problem, and a major reason for conventional farmers not to convert to organic production. Simply replacing herbicides by other direct control measures is inadequate. A heavy reliance on mechanical cultivation is undesirable because of damage to soil structure, increased risk of erosion and frost damage to crops and a strong dependency on weather conditions. Hand weeding is therefore often used, and this requires the availability of sufficient labour and is costly. Consequently, the weed problem cannot just be solved by curative tactics; instead weed management should be seen as a component of integrated cropping systems design. Rather than focusing on the detrimental effects of weeds in current crops, the time horizon of interest should be extended and main emphasis should be given to the management of weed populations. Consequently, systems-oriented approaches to weed management that make better use of alternative weed management tactics, need to be developed.

Cover crops have potential to form an important, pro-active component in such a system-oriented approach. Cover crops are grown for various reasons, like prevention of nitrogen leaching, improvement of soil structure, soil enrichment by nitrogen fixation and control of soilborne diseases, such as nematodes. A promising strategy is to grow cover crops during the period that the main crop is absent. Inclusion of cover crops in crop rotations introduces two important mechanisms through which the development of weed populations may be hampered. In late summer and autumn the successful introduction of cover crops can prevent growth, development and, most importantly, seed production of weeds that remain in the stubble. In springtime, cover crop residues incorporated in the upper layer of the soil may suppress or retard weed development and growth due to, among others, allelopathic effects. Other factors that can be altered through addition of cover crop residues, and that can exert a direct influence on weed development and growth, include soil nitrogen dynamics, soil physical characteristics and soilborne pathogens.

The term allelopathy was introduced by Molisch (1937) to designate the process by which one plant negatively affects another by chemicals means, and is derived from the Greek words ‘allelon’ meaning mutual and ‘pathos’ meaning harm or affection. Rice (1984) considered not only negative but also positive effects on the target organisms to be allelopathic, and in addition included microorganisms (bacteria, fungi and micro-algae) in the definition of allelopathy. In 1996, the International Allelopathy Society (IAS) has defined allelopathy as follows: ‘allelopathy refers to any process

involving secondary metabolites produced by plants, microorganisms and viruses that influence the growth and development of agricultural and biological systems'. In our system there are two possible sources of allelochemicals; allelochemicals can be released directly from the cover crop residues or they can be produced by microorganisms that use the cover crop residues as a substrate.

All experiments were carried out on sandy soil, because of the usually higher weed pressure on these soils and the fact that soil tillage is carried out in spring and not in autumn, as is the case for clay soils. In order to include a broad and balanced range of cover crop species, our initial experiments included both a winter hardy and a frost-sensitive species from each of the families *Brassicaceae, Poaceae* and *Fabaceae*: winter oilseed rape (*Brassica napus* L.), fodder radish (*Raphanus sativus* L.), winter rye (*Secale cereale* L.), Italian ryegrass (*Lolium multiflorum* L.), lucerne (*Medicago sativa* L.) and white lupine (*Lupinus albus* L.). Each plant family contains different groups of allelochemicals. In the *Poaceae*, hydroxamic acids are the main group of allelochemicals. Allelopathy in *Brassica* species has been primarily attributed to the hydrolysis products of glucosinolates, most of which are volatile. Lucerne contains several groups of allelochemicals, including saponins, flavonoids and phenolic acids. White lupine contains quinolizidine alkaloids that act as a herbivore deterrent, but have also been suggested to influence plant-plant interactions. Especially bitter cultivars contain a high level of these alkaloids.

We initiated our study on the field scale with a broad exploration of the potential of a series of cover crop species, sown at different densities, to suppress weed biomass accumulation in autumn and inhibit weed establishment in spring (Chapter 2). Fodder radish, winter oilseed rape and winter rye were found to have the strongest competitive ability in autumn; the competitive strength of Italian ryegrass was intermediate and white lupine and lucerne were poor competitors. Competitiveness was strongly correlated to early light interception. Surprisingly, doubling the recommended sowing density did not increase weed suppressive ability. Although a poor competitor in the fall, after incorporation in spring lucerne had the strongest inhibitory effect on seedling establishment, followed by winter oilseed rape and white lupine. Winter rye and fodder radish did not affect seedling establishment, whereas Italian ryegrass was not evaluated because of re-growth after incorporation. Establishment of the indicator species lettuce and sugar beet reflected the response of *Chenopodium album* in residue-amended soil. Competition in autumn and subsequent residue-mediated suppression of weed establishment in spring varied among the cover crop species evaluated, with winter oilseed rape offering relatively strong effects during both periods.

The results of these exploratory experiments provided a good prospect for the use

of autumn-sown cover crops for weed management and formed a basis for further research on the optimization of this system. Main focus was put on the weed suppressive effect of cover crop residue material in spring and particularly on identifying management options to maximize this effect. To better appreciate the potential of cover crop residue material, the investigations were focused on three aspects, namely allelochemicals in the cover crop, the residence time of the residue-mediated inhibitory potential in the soil and the variability in inhibitory effects on receptor plants. Only winter hardy cover crop species were included in these studies.

In Chapter 3, mechanically damaging plants as a possibility to increase the allelochemical content of the cover crop just prior to residue incorporation was studied. Mechanical damage was used to mimic herbivore damage, which is known to induce the synthesis of allelochemicals, and therefore has potential to increase the allelochemical content of the cover crop. The effect of mechanical wounding of the field-grown cover crops winter rye, winter rapeseed and lucerne on the allelopathic activity of plant residue was studied per unit biomass and per unit area. We investigated how the allelopathic activity of residues of intact and damaged cover crops changed over time. Cover crops were sown in late summer and damage was applied in spring. For lucerne and winter rye, lettuce seedling bioassays were used to determine the allelopathic activity, whereas for winter rapeseed the glucosinolate content was quantified by HPLC. The experiment clearly demonstrated that mechanical wounding enhanced the allelopathic activity per unit biomass of all three cover crop species, but the species differed in the onset and the duration of the response to mechanical wounding. The temporal pattern of allelopathic potential of intact cover crop plant material in spring was characterized by a linear decline for winter rye and a steep decline at the onset of the flowering stage for winter rapeseed, whereas for lucerne no specific pattern was observed. Still, for all three species, the allelopathic activity per unit area (biomass × allelopathic activity per unit biomass) was highest at the end of the sampling period, resulting from an increased amount of biomass. Comparing the increase in allelopathic activity per unit biomass resulting from damaging to the change in this parameter over time made evident that the impact of damaging was only minor. Actually, it often was just sufficient to compensate for the loss in plant biomass resulting from mechanical damaging. Therefore it was concluded that the increase in allelopathic activity due to damaging is of little significance for farming practice.

Release, persistence and distribution of allelochemicals in the soil are other important determinants of efficacy and can be influenced by cover crop residue management. Manipulating the release rate of allelochemicals from the residues, and thereby influencing the time course of allelochemicals in the soil, may change the

effect on the receptor plant. Through its influence on the distribution of allelochemicals in the soil, cover crop residue management can affect the amount of allelochemicals available for uptake by the receptor plant. In Chapter 4, cover crop residue management options to increase the inhibitory effect of cover crop residues on weeds were explored. We evaluated the effect of several ways of pre-treatment and placement of winter rye and winter oilseed rape residues 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 residues, pre-treatment strongly affected the time-course of residue-mediated effects. 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. Whereas residue incorporation gave variable results, placement of winter rye residues on top of the soil inhibited the emergence of all indicator species. In conclusion, the optimal residue management strategy for weed suppression depends both on the cover crop species used and the target weed species.

In Chapter 5, we wanted to find out whether differences in seed mass could be used to target small-seeded weed species and avoid negative effects on large-seeded crop species. In laboratory bioassays, it has often been observed that cover crop residues inhibit germination and early growth of small-seeded species while, in comparison, large-seeded species better tolerate residue-mediated stress. As weed seeds are often one to three orders of magnitude smaller than most crop seeds, there is potential for selective weed suppression by cover crop residues in large-seeded crops. In this study we assessed to what extent seed mass determines species sensitivity to cover crop residues under field conditions. Two experiments were conducted in which crop and weed species differing in seed mass were sown in control soil (no residues) and soil with recently-incorporated lucerne, winter oilseed rape or winter rye residues. In the first experiment, sowing of lettuce at different times after residue incorporation revealed a continuous decline of residue-mediated effects of lucerne and winter oilseed rape over time. In most cases we found a positive relationship between seed mass and relative emergence, indicating that small-seeded species are indeed more sensitive to residue-mediated effects. However, there were two main exceptions. Results of experiment 1 suggested that carrot emerged better than expected on basis of its seed

mass, which could be due to the late emerging time of carrot in combination with the declining strength of residue-mediated inhibitory potential over time. Results of experiment 2 suggested that for lucerne the time of germination relative to a rainfall peak determined the strength of the inhibitory effect of lucerne residues on seedling establishment and this overruled the effect of seed mass. Taken together, these results led us to formulate the following hypothesis: "*the time course of sensitivity of the receptor plant in relation to the time course of residue-mediated inhibitory potential is an important factor determining the level of residue-mediated inhibition of the receptor plant*." To test this hypothesis, two additional datasets, published by Haramoto & Gallandt (2005), were re-analysed. These data originated from two field experiments that were designed to test the influence of seed mass on seedling emergence in cover crop residue-amended soil in the field. The original analysis indicated that seed mass alone was a poor predictor of a species' establishment. After including the species specific time needed to reach 50% emergence in the analysis a much larger part of the variation in the relative emergence of weed and crop species could be explained. Re-analysis of the datasets thus revealed that seed mass is of secondary importance and only becomes important in determining the strength of the residue-mediated effect *after* accounting for the time of emergence of the receptor plant. Moreover, this outcome strongly supports the earlier hypothesis that residue-mediated inhibition of the receptor plant only occurs when there is an overlap of the time course of the sensitivity of the receptor plant and the time course of the residue-mediated inhibitory potential. A wider extrapolation of this result led us to believe that part of the variation commonly reported in research on residues or allelopathy may be explained by environmental or management effects on the synchronicity of these processes.

Chapter 6 specifically focused on the influence of a rainfall peak on the strength and time-course of the lucerne residue-mediated inhibitory effect on seedling emergence. Two subsequent pot experiments were conducted in a climate chamber. Pots received four different rainfall treatments, i.e. no rainfall, indicated as basic moisture (BM) treatment, or a 22 mm rainfall peak (RP) applied at 3, 11 or 19 days after lucerne residue incorporation. Pots containing soil without incorporated lucerne residues were used as a control. Lettuce (*Lactuca sativa* L.) was sown at 8 different times after residue incorporation, with 4-day intervals from 0 DAI to 28 DAI, to monitor the time course of the inhibitory potential of the lucerne-amended soil in the different rainfall treatments. Rainfall caused an increase in the residue-mediated inhibitory potential in both experiments, but the effect was stronger in experiment 2. Surprisingly, in experiment 2, also an effect of rainfall was observed in the control soil. This was likely related to the presence of soilborne pathogens; *Rhizoctonia solani*

and *Pythium* spp. were detected in all treatments. The absence of a rainfall effect in the control soil of experiment 1 might be related to the longer period between soil sterilization and the start of the experiment. Although it is difficult to unravel the underlying mechanisms of the effect of rainfall on the residue-mediated inhibitory potential of lucerne, we hypothesized that in the RP treatments allelochemicals played a less prominent role in the observed residue-mediated inhibitory effects than in the BM treatment. After a rainfall peak, soilborne pathogens were likely for a greater part responsible for the inhibition of lettuce emergence.

In the final chapter (Chapter 7) the contribution of the research presented in this thesis to the understanding of cover crop effects on weeds, as well as the possibilities for optimization of these effects through management were discussed. Subsequently, the findings of this thesis were placed in a broader systems perspective. Attention was given to the possibilities of combining various cover crop services and the potential of including cover crops as one of the components in an all encompassing ecological weed management strategy. Finally, future research needs regarding cover crop-based ecological weed management were discussed.

Samenvatting

In biologische bedrijfssystemen, waar het gebruik van chemische bestrijdingsmiddelen is uitgesloten, wordt onkruidbestrijding beschouwd als het belangrijkste teelt-gerelateerde probleem. De onkruidproblematiek vormt dan ook een belangrijk obstakel bij de omschakeling van gangbare naar biologische teelt. Het simpelweg vervangen van herbiciden door alternatieve directe bestrijdingsmethoden is vaak niet mogelijk. Een te grote afhankelijkheid van mechanische onkruidbestrijding is niet wenselijk vanwege het grote risico op structuurbederf, bodemerosie en vorstschade aan gewassen. Daarnaast vormt de sterke weersafhankelijkheid van mechanische onkruid-bestrijding een nadeel. Handmatig wieden wordt vaak toegepast als een laatste redmiddel, maar vraagt de beschikbaarheid van voldoende arbeidskrachten en is bovendien erg duur. Derhalve kan het onkruidprobleem in biologische bedrijfs-systemen niet worden opgelost met een louter curatieve aanpak. In plaats daarvan zou onkruidbeheersing moeten worden beschouwd als één van de componenten van een geïntegreerd teeltsysteem. Meer nog dan zich enkel te richten op de nadelige effecten van onkruiden in het huidige gewas, zou de nadruk moeten liggen op het lange termijn beheer van onkruidpopulaties. Dientengevolge is het nodig om systeemgeoriënteerde benaderingen voor onkruidbeheersing te ontwikkelen die beter gebruik maken van alternatieve onkruidbeheersmaatregelen.

Groenbemesters hebben potentieel om een belangrijke en proactieve component te vormen in zo'n systeemgeoriënteerde benadering. Groenbemesters worden geteeld voor verschillende doeleinden, zoals het voorkomen van uitspoeling van nutriënten, verbetering van de bodemstructuur, verrijking van de bodem door stikstofbinding en het beheersen van bodemziekten, zoals nematoden. Een veelbelovende strategie is om groenbemesters te telen gedurende de gewasvrije periode tussen twee hoofdgewassen. Door het opnemen van groenbemesters in de gewasrotatie worden twee belangrijke mechanismen geïntroduceerd waarmee de ontwikkeling van onkruidpopulaties kan worden geremd. In de nazomer en herfst kan succesvolle introductie van groen-bemesters de groei, ontwikkeling en bovenal de zaadproductie van onkruiden voorkomen. In het voorjaar kunnen de in de bovenlaag van de grond ingewerkte gewasresten van groenbemesters de groei en ontwikkeling van onkruidkiemplanten vertragen of onderdrukken, onder andere door allelopathische effecten. Andere factoren die onderhavig zijn aan veranderingen door de toevoeging van gewasresten aan de grond, en welke een directe invloed kunnen uitoefenen op de ontwikkeling en groei van onkruiden, zijn de stikstofdynamiek in de bodem, bodem-fysische eigenschappen en het optreden van bodempathogenen.

De term “allelopathie” is geïntroduceerd door Molisch (1937) om het proces aan te duiden waarmee de ene plant de andere plant negatief beïnvloedt door middel van chemische stoffen. De term is afgeleid van het Griekse woord “allelon”, dat wederzijds betekent, en van het woord “pathos” met de betekenis van schade of affectie. Rice (1984) beschouwde niet alleen negatieve, maar ook positieve effecten op doel-organismen als zijnde allelopathisch, en nam eveneens micro-organismen (bacteriën, schimmels en micro-algen) op in zijn definitie van allelopathie. In 1996 heeft de “International Allelopathy Society” (IAS) allelopathie als volgt gedefinieerd: “allelopathie refereert naar elk proces waarbij secundaire stoffen, geproduceerd door planten, micro-organismen en virussen, betrokken zijn en die de groei en ontwikkeling van landbouw- en biologische systemen beïnvloeden.” In het door ons bestudeerde systeem zijn er twee mogelijke bronnen van allelochemische stoffen: allelochemische stoffen die direct uit de gewasresten van groenbemesters vrijkomen en stoffen die worden geproduceerd door micro-organismen welke de gewasresten van groen-bemesters als voedingsbodem gebruiken.

Al onze experimenten zijn uitgevoerd op zandgrond, vanwege de, over het algemeen, hogere onkruiddruk op deze grondsoort. Daarnaast vindt de hoofdgrondbewerking op zandgrond in het voorjaar plaats en niet, zoals het geval is op kleigrond, in de herfst. Om een zo breed en gebalanceerd mogelijke reeks groen-bemesters op te nemen, zijn de initiële experimenten uitgevoerd met een winterharde en een vorstgevoelige soort van elk van de drie plantenfamilies *Brassicaceae* (winterkoolzaad (*Brassica napus* L.) en bladrammenas (*Raphanus sativus* L.)), *Poaceae* (winterrogge (*Secale cereale* L.) en Italiaans raaigras (*Lolium multiflorum* L.)) en *Fabaceae* (luzerne (*Medicago sativa* L.) en witte lupine (*Lupinus albus* L.)). Vertegenwoordigers van elke plantenfamilie bevatten verschillende groepen allelochemische stoffen. In de *Poaceae* vormen de zogenaamde “hydroxamic acids” de belangrijkste groep allelochemische stoffen. In de *Brassica* soorten wordt allelopathie voornamelijk toegeschreven aan de remmende werking van afbraakproducten van glucosinolaten, welke grotendeels vluchtig zijn. Luzerne bevat verschillende groepen stoffen met een allelochemische werking, inclusief saponines, flavonoïden en fenolzuren. Witte lupine bevat zogenaamde quinolizidine alkaloïden die een afstotende werking op herbivoren hebben, maar waarvan ook wordt vermoed dat ze plant-plant interacties beïnvloeden. Vooral bittere lupinerassen bevatten een hoog gehalte van deze alkaloïden.

We zijn ons onderzoek begonnen op veldniveau met een brede verkenning van een reeks groenbemesters, gezaaid in verschillende dichtheden (Hoofdstuk 2). Bladrammenas, winterkoolzaad en winterrogge kwamen uit dit onderzoek naar voren als de soorten met de sterkste concurrentiekracht in het najaar. Witte lupine en luzerne

waren zwakke concurrenten en de concurrentiekracht van Italiaans raaigras was intermediair. Vroege lichtonderschepping door de groenbemester bleek sterk gecorreleerd te zijn met de concurrentiekracht. Verassend genoeg had het verdubbelen van de aanbevolen zaaidichtheid geen effect op het vermogen onkruiden te onderdrukken. Hoewel luzerne een zwakke concurrent was in het najaar, was deze groenbemester, na onderwerking in de grond in het voorjaar, de soort met het sterkst onderdrukkende effect op de opkomst van kiemplanten. Luzerne werd hierin gevolgd door winterkoolzaad en witte lupine. Winterrogge en bladrammenas hadden geen invloed op de opkomst van kiemplanten. Italiaans raaigras werd niet meegenomen in de analyse vanwege sterke hergroei na onderwerking in de grond. Vestiging van de incidatorsoorten sla en suikerbiet in grond met ondergewerkte gewasresten van de groenbemesters gaven een vergelijkbaar beeld als de respons van *Chenopodium album*. Over het algemeen genomen was het najaars- en voorjaarseffect van groenbemesters op onkruiden afhankelijk van de soort groenbemester en had winterkoolzaad een relatief sterk effect tijdens beide periodes.

De resultaten van de hierboven beschreven verkennende experimenten vormden een goed perspectief voor het gebruik van in het najaar ingezaaide groenbemesters voor onkruidbeheersing en vormden de basis voor verder onderzoek naar de optimalisatie van dit systeem. De algehele focus was hierbij gericht op het onkruidonderdrukkende effect in het voorjaar en daarbij vooral op opties om dit effect te optimaliseren. Het onderzoek richtte zich op drie aspecten: (i) allelochemische stoffen in de groenbemesters, (ii) het tijdsverloop van het onderdrukkende effect van in de grond ingewerkte gewasresten en (iii) de variabiliteit van de onderdrukkende effecten op receptorplanten. Alleen de winterharde groenbemestersoorten werden opgenomen in deze experimenten.

Het onderzoek beschreven in Hoofdstuk 3 was gericht op mechanische beschadiging van groenbemesters. Mechanische schade werd gebruikt als imitatie van aan planten aangerichte schade door planteneters. Van deze laatste is bekend dat zij de aanmaak van allelochemische stoffen induceren. Het doel was om te testen of we door mechanische beschadiging de concentratie van allelochemische stoffen in de groenbemester, net voor het inwerken van de gewasresten, konden verhogen. De onder veldomstandigheden geteelde groenbemesters winterrogge, winterkoolzaad en luzerne werden mechanisch beschadigd. Vervolgens werd het effect hiervan op de allelopathische activiteit van de gewasresten bestudeerd. Ook werd onderzocht in hoeverre de allelopathische activiteit van de gewasresten van beschadigde en onbeschadigde planten veranderde in de tijd. De groenbemesters waren in de nazomer gezaaid en de mechanische schade werd toegediend in het voorjaar. Voor luzerne en winterrogge werd de allelopathische activiteit van het plantenmateriaal getest door

middel van biotoetsen met sla als testsoort, terwijl voor winterkoolzaad het glucosinolaatgehalte werd bepaald door middel van HPLC. De experimenten lieten duidelijk zien dat de allelopathische activiteit per eenheid biomassa werd verhoogd als gevolg van het aanbrengen van mechanische beschadiging. Het tijdsverloop van het allelopathische potentieel van het onbeschadigde groenbemestermateriaal in het voorjaar werd gekenmerkt door een lineaire afname voor winterrogge en een sterke afname aan het begin van het bloeistadium voor winterkoolzaad. Voor luzerne werd geen specifiek patroon waargenomen. Desondanks was de allelopathische activiteit per eenheid oppervlakte (biomassa × allelopathische activiteit per eenheid biomassa) voor alle drie groenbemesters het hoogst aan het einde van de bemonsteringsperiode. Dit tengevolge van een toename in de biomassa. Vergeleken met de veranderingen in allelopathische activiteit per eenheid biomassa over de tijd, is het effect van mechanische beschadiging uiterst gering. In feite was de toename in allelopathische activiteit meestal net voldoende om het verlies aan plantbiomassa, als gevolg van mechanische beschadiging, te compenseren. Zodoende is geconcludeerd dat de als gevolg van mechanische beschadiging verhoogde allelopathische activiteit van geringe betekenis is voor de landbouwpraktijk.

Het vrijkomen, het tijdsverloop en de verdeling van allelochemische stoffen in de grond zijn belangrijke factoren die de mate van onkruidonderdrukking bepalen. Deze factoren zelf zijn mede te beïnvloeden door de wijze van voorbehandeling van gewasresten en de wijze waarop de gewasresten in de grond worden ingebracht. In Hoofdstuk 4 zijn in dit kader verschillende opties voor management van gewasresten van groenbemesters verkend. We hebben onder veldomstandigheden het effect van voorbewerking en plaatsing van gewasresten van winterrogge en winterkoolzaad op de opkomst van kiemplanten onderzocht. Van beide groenbemestersoorten werden twee rassen gebruikt die verschilden in het gehalte aan allelochemische stoffen. Gewasresten die werden ingewerkt in de bovenste laag van de grond hadden een sterk onderdrukkend effect op de opkomst van de relatief vroeg opkomende kiemplanten van sla (*Lactuca sativa* L.) en spinazie (*Spinacia oleracea* L.). Het onderdrukkende effect van de iets later opkomende kiemplanten van *Stellaria media* L. was erg variabel en er werd vaak een stimulerend effect waargenomen op de laat-opkomende kiemplanten van *Chenopodium album* L. Verschillen tussen de rassen van de groenbemesters waren gering. De wijze van voorbewerken van gewasresten van winterkoolzaad had een sterke invloed op het tijdsverloop van de effecten van de gewasresten. Fijngemalen gewasresten waren alleen tijdens de eerste twee tot drie weken na onderwerken in de grond effectief in het onderdrukken van opkomst van kiemplanten. Daarnaast werd gevonden dat in stukjes geknipte gewasresten pas na deze periode actief werden. In het geval van winterrogge was de plaatsing van de

gewasresten een doorslaggevende factor. Terwijl het onderwerken van de gewasresten in de bovenste laag van de grond variabele resultaten opleverde, gaf plaatsing van de gewasresten op de grond een onderdrukkend effect op alle indicatorsoorten. De resultaten van dit onderzoek tonen aan dat de optimale gewasrest-managementstrategie afhankelijk is van zowel de soort groenbemester als van de te bestrijden onkruidsoort.

Het doel van het in Hoofdstuk 5 beschreven onderzoek was om te achterhalen of het verschil in zaadgewicht tussen kleinzadige onkruidsoorten en grootzadige gewassoorten benut kan worden als basis voor selectieve onkruidonderdrukking. In biotoetsen in het laboratorium is vaak waargenomen dat gewasresten van groenbemesters de kieming en vroege groei van kleinzadige soorten onderdrukken terwijl grootzadige soorten de gewasrest-gerelateerde stress beter tolereren. Omdat onkruidsoorten meestal één tot drie ordes van grootte kleiner zijn dan de meeste gewaszaden, is er mogelijk potentieel voor selectieve onkruidonderdrukking door gewasresten van groenbemesters in grootzadige gewassen. In deze studie hebben we onderzocht in hoeverre, onder veldomstandigheden, het zaadgewicht de gevoeligheid van verschillende plantensoorten voor gewasresten van groenbemesters bepaalt. Er zijn hiervoor twee experimenten uitgevoerd waarin gewas- en onkruidsoorten met sterk uiteenlopend zaadgewicht zijn gezaaid in controle-grond (zonder toevoeging van gewasresten) en in grond met recentelijk ingewerkte gewasresten van luzerne, winterkoolzaad en winterrogge. In het eerste experiment liet het zaaien van sla op verschillende tijdstippen na het onderwerken van de gewasresten een continue afname van de gewasrest-gerelateerde effecten van luzerne en winterkoolzaad in de tijd zien. In de meeste gevallen vonden we een positieve relatie tussen zaadgewicht en de relatieve opkomst van kiemplanten, duidend op een relatief hogere gevoeligheid van kleinzadige soorten. Er waren hierop echter twee belangrijke uitzonderingen. De resultaten van het eerste experiment suggereerden dat peen, gekenmerkt door een late opkomst, beter opkwam dan verwacht op basis van het zaadgewicht. De resultaten van het tweede experiment werden vooral beïnvloed door een regenvalpiek. Soorten die voor deze regenvalpiek opkwamen werden beduidend minder geremd dan soorten met een relatief late opkomst. Dit effect deed de invloed van zaadgewicht teniet. Tezamen hebben deze resultaten geleid tot de formulering van de volgende hypothese: *"het tijdsverloop van de gevoeligheid van de receptorplant ten opzichte van het tijdsverloop van het gewasrest-gerelateerde remmende potentieel is een bepalende factor voor de mate van onderdrukking van de receptorplant."* Om deze hypothese te testen hebben we twee additionele datasets, eerder gepubliceerd door Haramoto & Gallandt (2005), geheranalyseerd. Deze data waren afkomstig van twee veldexperimenten die waren opgezet om de invloed van zaadgewicht op opkomst van receptorplanten in grond met ondergewerkte gewasresten van verschillende soorten groenbemesters te onderzoeken.

De oorspronkelijke resultaten gaven aan dat zaadgewicht een slechte voorspellende waarde had voor wat betreft de vestiging van receptorplanten. Na het verdisconteren van de soortspecifieke tijd die nodig was om 50% opkomst te bereiken, kon een veel groter deel van de variatie in de relatieve opkomst van onkruid- en gewassoorten worden verklaard. Heranalyse van deze datasets bracht derhalve aan het licht dat zaadgewicht van secundair belang is en dat dit laatste alleen een rol speelt *nadat* eerst het opkomsttijdstip van de receptorplant in acht is genomen. Dit resultaat lijkt daarmee de eerdere geformuleerde hypothese te ondersteunen: remming van de receptorplant treedt alleen op als de aanwezigheid van het gewasrest-gerelateerde remmende potentieel overlapt met de gevoeligheid van de receptorplant. Extrapolatie van dit resultaat suggereert dat een deel van de variatie die normaliter wordt gevonden in onderzoek naar gewasresten of allelopathie, kan worden verklaard door omgevings- of managementeffecten op de synchroniciteit van deze processen.

Het onderzoek beschreven in Hoofdstuk 6 was specifiek gericht op de invloed van een regenvalpiek op de sterkte en het tijdsverloop van de gewasrest-gerelateerde remmende effecten van luzerne op de opkomst van kiemplanten. Twee opeenvolgende potexperimenten werden uitgevoerd in een klimaatkamer. Potten werden blootgesteld aan vier verschillende regenvalbehandelingen, namelijk “geen regenval”, aangegeven als de “basic moisture” (BM) behandeling, en een 22 mm regenval piek (RP) toegediend op 3, 11 of 19 dagen na het onderwerken van de gewasresten van luzerne. Potten waaraan geen gewasresten waren toegevoegd, werden gebruikt als controle. Slazaad werd op 8 verschillende tijdstippen na onderwerken van de gewasresten gezaaid, met 4-daagse intervallen van 0 tot 28 dagen na inwerken, om het tijdsverloop van het remmende potentieel te volgen. Beide experimenten lieten zien dat regenval een toename in het gewasrest-gerelateerde remmende potentieel veroorzaakte. Het effect bleek sterker in experiment 2 en, verassend genoeg, werd ook een effect van regenval waargenomen in de controlegrond. Dit was waarschijnlijk gerelateerd aan de aanwezigheid van bodempathogenen; *Rhizoctonia solani* en *Pythium* spp. werden in alle behandelingen gedetecteerd. De afwezigheid van een effect van regenval in de controlegrond van experiment 1 zou gerelateerd kunnen zijn aan de langere periode tussen sterilisatie van de grond en de start van het experiment. Hoewel het moeilijk is om alle onderliggende mechanismen van het effect van regenval op het gewasrest-gerelateerde remmende potentieel van luzerne te ontrafelen, lijkt het waarschijnlijk dat, in vergelijking tot de “basic moisture” behandeling, allelochemische stoffen een minder prominente rol hebben gespeeld bij de regenvalbehandelingen. Na een regenvalpiek waren bodempathogenen waarschijnlijk voor een groter deel verantwoordelijk voor de remming van opkomst van slaplanten.

In het laatste hoofdstuk (Hoofdstuk 7) wordt de bijdrage van het in dit proefschrift

beschreven onderzoek aan de kennis omtrent de effecten van groenbemesters op onkruiden bediscussieerd. Daarna wordt ingegaan op de mogelijkheden voor optimalisatie van deze effecten door management en worden de bevindingen uit dit proefschrift in een breder kader geplaatst. Hierbij wordt aandacht geschonken aan de mogelijkheden om verschillende teeltdoelen van groenbemesters te combineren en wordt bediscussieerd in hoeverre het mogelijk is groenbemesters op te nemen als één van de componenten van een alles omvattende ecologische onkruidbeheersstrategie. Als laatste worden mogelijke toekomstige onderzoekslijnen met betrekking tot groenbemester-gebaseerde ecologische onkruidbeheersing bediscussieerd.

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of Literature (5.6 ECTS)
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Laboratory Training and Working Visits (6.8 ECTS)
- Advanced statistics; PE&RC (2005)
- Metabolomics (2005)
- Statistical assessment of dose-response curves with free software (2005)
- Multivariate analysis (2006)
- The art of modelling (2006)

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- Systems analysis, simulation & systems management; PPS (2006)
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- Basic statistics; PE&RC (2004)
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PE&RC Annual Meetings, Seminars and the PE&RC Weekend (1.8 ECTS)
- PE&RC day (2003)
- PE&RC day (2004)
- PE&RC weekend (2004)
- PE&RC day (2006)

International Symposia, Workshops and Conferences (7.4 ECTS)
- 12th International conference on weed biology; INRA/EWRS, France (2004)
- 13th EWRS Symposium; Italy (2005)
- Najaarsvergadering KNPV (2005)
- 7th Workshop of the EWRS working Group: physical and cultural weed control (2007)
- 14th EWRS Symposium; Norway (2007)
- 23rd Annual meeting of the ISCE; Jena, Germany (2007)

Curriculum vitae

Henriëtte Marjolein Kruidhof was born on the 21th of July, 1978 in Almelo, the Netherlands. In 1996, she graduated from secondary education at the "OSG Erasmus" in Almelo. After secondary school she spent one year in Mexico, where she lived with a Mexican family and attended local secondary education as part of a cultural exchange program. In 1997, she initiated her study Biology at Wageningen University and specialized in Population Ecology. For the field work of her first MSc thesis she went to Mali, where she interviewed Malian farmers about their knowledge of the parasitic weed *Striga*, and explored the relation between this knowledge and the application and appreciation of *Striga* control methods by these farmers. This thesis project was based on collaboration between two research groups: Communication and Innovation Studies and Crop and Weed Ecology. Her second MSc thesis was conducted at the Laboratory of Entomology of Wageningen University. For this project, she studied the differences in associative learning in two closely related parasitic wasps, *Cotesia glomerata* en *Cotesia rubecula*, with respect to plant odours that indicate host presence. To study the behaviour of these parasitoids she used different wind tunnel set-ups. In March 2003, she completed her studies "cum laude". In the same month, she started a PhD-project at the Crop and Weed Ecology Group of Wageningen University and Plant Research International, under supervision of Dr. ir. L. Bastiaans and Prof. dr. M.J. Kropff. The project concerned the exploration and optimization of cover crop-based ecological weed management, and is described in this thesis. In February 2008, she started as a postdoc at the University of California, Riverside in the United States. There she is conducting field and laboratory research on the interactions between natural enemies of plant herbivores and host plant resistance mechanisms in the native plant species *Datura wrightii*.