On the development, environmental effects and human dimension of weed management strategies

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This research was conducted under the auspices of the C.T. de Wit Graduate School "Production Ecology and Resource Conservation".

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Thesis
submitted in partial fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. dr. M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Doctorate Board
to be defended in public
on Monday 7 December 2009
at 4 PM in the Aula.

M.M. Riemens
On the development, environmental effects and human dimension of weed management strategies, 176 pages.

Thesis Wageningen University, Wageningen, NL (2009)
With references, with summaries in Dutch and English

ISBN 978-90-8585-669-6

Contents

CHAPTER 1

General Introduction

Intensification of agriculture

Agriculture has changed tremendously during the last decades, especially in West-European countries such as the Netherlands (Meerburg *et al*., 2009; Henle *et al*., 2008). Nowadays European agriculture is characterized by the use of large amounts of external inputs and high outputs (Hersperger & Bürgi, 2009; Fischer *et al*., 2008; Ten Berge *et al*., 2000). One of the main factors that enabled the intensification of agriculture was the introduction of herbicides (Kropff *et al*., 2008; Bastiaans *et al*., 2000). Before the introduction and availability of herbicides, weed management was one of the major issues determining the design of cropping systems in most agricultural systems. After the introduction of herbicides, however, weeds became to be regarded as solvable side-problems rather than being an important and decisive factor in the design of cropping systems (Macé *et al*., 2007; Bastiaans *et al*., 2000). Today, agriculture is economically still strongly dependent on these chemicals to maintain their crop yields at a certain level (Wilson &Tisdell, 2001; Pimentel, 1997). The strong dependency of agriculture on chemical weed control is considered undesirable (e.g Hyvönen, 2007; Lotz *et al*., 2002; Liebman, 2001). First of all, a strong dependence on herbicides implies the extensive use of compounds with a potential negative side-effect on the environment (Bastiaans *et al*., 2008). Secondly, the risk of the development of herbicide resistance makes herbicide dependent systems vulnerable (Powles, 2008). During the last decades the negative side effects of pesticides on the environment have been reduced in the Netherlands. In the period 1998-2005 the environmental impact as a result of direct emission of pesticides has been reduced with 86%. The use of crop free buffer zones and emission reducing equipment accounts for 75% of this reduction, and the ban of 90 chemicals for the other 25%. At the same time, industry has brought 39 new pesticides on the market with less environmental impact (Van Eerdt *et al*., 2006). Although this is a strong reduction, the goals for 2010: no excedence of the maximum allowable risks (MTR, in Dutch: Maximum Toelaatbaar Risico), will not be met (Van Eerdt *et al*., 2006). As a result, the focus and research on a reduction of herbicide dependency remains necessary.

A reduction of the adverse effects of herbicide dependence

A number of strategies have arisen to reduce the adverse effects of strong herbicide dependence. In the sections below, a short description of those strategies will be given.

1. Ecological weed management

Instead of simply replacing herbicides by other direct control techniques, ecological weed management focuses on the management of weed populations at a time scale extending the current growth season (Kruidhof *et al*., 2008). Bastiaans *et al* (2008) described the basic ecological weed management principles and presented three directions for ecological weed management: A) manipulation of the competitive relation between crop and weed, B) a reduced recruitment of weed seeds from the soil seed bank, and C) gradual depletion of the soil seed bank (Figure 1.1).

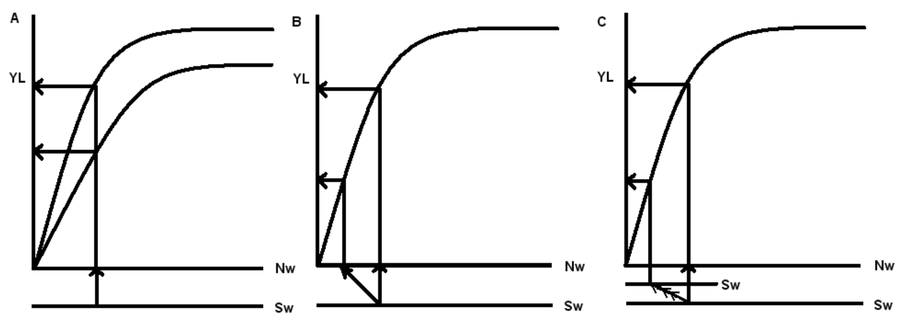

Figure 1.1. After Bastiaans et al. (2008). The hyperbolic yield loss (YL)- weed density relation illustrating three directions for ecological weed management. Nw: the aboveground weed density, and Sw: the density in the soil seed bank. A) manipulation of the competitive interaction between crop and weeds, B) reduced recruitment from the soil seedbank (Sw), and C) gradual depletion of the soil seedbank..

Manipulation of competitive relation crop and weed

Extensive research has been done on the manipulation of the competitive relations between crop and weed. Because of the complexity of this relationship modelling approaches have been used (Kropff & Van Laar, 1993; Kropff & Lotz, 1992; Spitters, 1989; Cousens, 1985). A number of eco-physiological models of competition between weeds and crops have been developed and experimentally evaluated. The principal purpose of these models has been to improve our understanding of competition processes (Vitta & Satorre, 1999; Ryel *et al*., 1992; Kropff, 1988). As a result, these models have given us insight in several important processes and in some cases general guidelines for weed management (Holst *et al*., 2007). A good example is weed competition in rice, for which several eco-physiological model investigations (Caton *et al*., 2003; Asch *et al*., 1999; Kropff *et al*., 1993) have lead to crosses between weed-competitive but low-yielding *Oryza glaberrima* and high- yielding but lower competitive *Oryza sativa*, that have a reduced trade off between competitiveness and yield potential (Dingkuhn *et al*., 1999). Another, somewhat less succesfull example is sugarbeet. For sugarbeet it is known that early covering genotypes with more horizontal leaves, have an enhanced competitive ability against weeds (Lotz *et al*., 1991). The downside of these horizontal leaves is the reduced mechanical weed control possibilities in the crop, which makes it harder to control weeds that were able to establish between the crop rows. So, when selecting for competitive crop varieties, one is often balancing between competitiveness against weeds and crop yield or implications for the agronomic practices in the crop.

Reduced seedling recruitment

To reduce the recruitment of weed seeds from the soil seed bank the germination and emergence of weed seeds should be prevented. Seeds of many species need a short exposure to light to be able to germinate (Andersson *et al*., 1997; Milberg *et al*., 1996; Vleeshouwers *et al*., 1995). A major source of light for buried weed seeds is the short exposure to light received during tillage (Buhler, 1997). In field experiments, dark tillage has been tested as a method of reducing the amount of weed seedlings emerging after cultivation (Botto, 1998; Gallagher & Cardina, 1998; Milberg *et al*., 1996; Jensen, 1995; Ascard, 1994; Scopel *et al*., 1994). Although the results suggest that dark tillage is promising, variation in emergence creates uncertainty as to the effect of such a treatment. Another way to reduce the recruitment of weed seeds from the soil seedbank is the application of a physical barrier that the germinated seeds have to overcome. Promising experiments have been conducted with a thin layer of compost that prevents the emergence of weed seedlings, but allows the emergence of the crop seedlings (Bleeker, 2009). Another barrier that can be used is a layer of mulch, consisting of a layer of decaying fresh plant material. The release of allelochemicals from the decaying layer enhances the inhibitory effect and reduces the recruitment of weed seeds from the soil (Kruidhof *et al*., 2008).

Depletion of the soil weed seed bank

The third direction into ecological weed management is the gradual depletion of the weed seed bank. The weed population emerging after cultivation in some studies is related to the size and composition of the weed seed bank (Zhang *et al*., 1998; Roberts &Ricketts, 1979). In other studies, no relationship was found between seed bank and aboveground communities (Derksen & Watson, 1998), or only for a small number of species (Webster *et al*., 2003). This indicates the complexity of processes determining germination and emergence of weed seeds in the soil. Nevertheless, the soil seed bank is a product of the past and represents the potential future of the aboveground plant community (Figure 1.2a) (Swanton & Booth, 2004). Therefore, a modification of the size of the seed bank will result in changes of the emerging weed populations, and *vice versa*. The seed bank can be reduced by increasing the losses, reducing the input or a combination of both.

Depletion of the weed seed bank: increasing the losses

The sensitivity of seeds of many species to short exposures to light (Andersson *et al*., 1997; Milberg *et al*., 1996;Vleeshouwers *et al*., 1995) can not only be used to reduce seedlings recruitment, but also to increase the losses from the seed bank. The stale seedbed technique makes use of this sensitivity to enhance the germination of seeds in the soil seedbank. During the preparation of a seedbed, seeds are exposed to light and are stimulated to germinate. This initial seedbed preparation is then followed by destruction of the emerging weed seedlings with minimal soil disturbance (Lamour & Lotz, 2007; Mohler, 2001).

Depletion of the weed seed bank: reducing the input

The disadvantage of mechanical weed control techniques such as harrowing is that they cause soil disturbance and thereby light penetration of the soil, possibly causing the emergence of new flushes of weeds. Therefore, a maximization of the effects in of a stale seedbed followed by mechanical control in terms of light exposure and working depth is required if a further decrease in herbicidal use is the objective. Studies with population

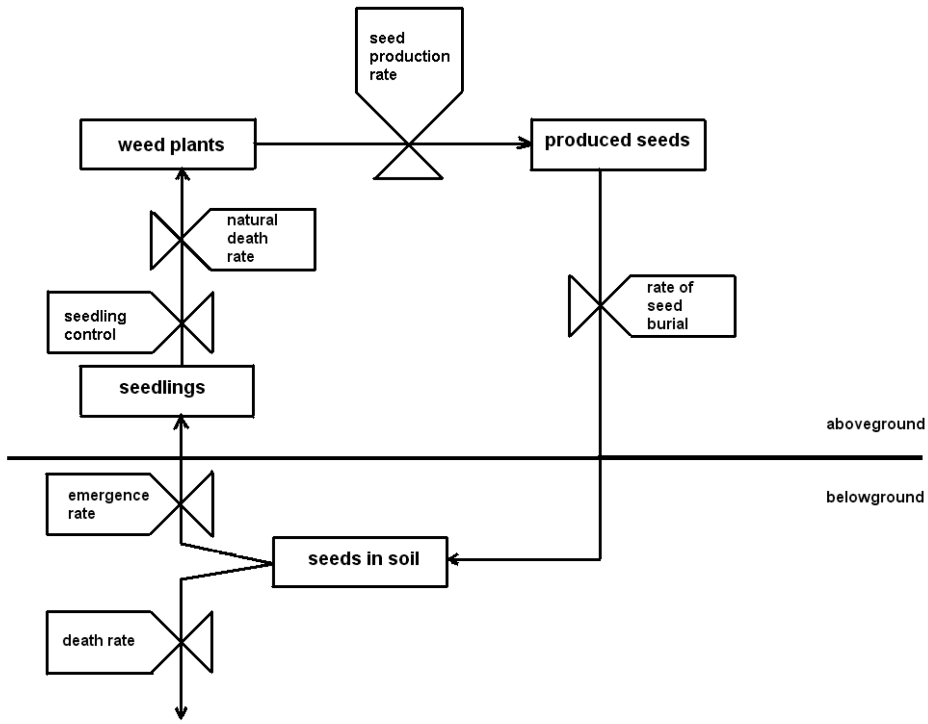

Figure 1.2a Life cycle of annual weeds

dynamic models showed that the seed shed is a critical parameter in the development of weed populations and could be the key to their control (Westerman *et al*., 2006; Wallinga, 1998; Pandey & Medd, 1990). However, evidential data on a practical scale to test this hypothesis is lacking so far.

2. Effective herbicide use

Minimum herbicide doses

Techniques to minimize the dose of herbicides are based on the fact that under optimum conditions, herbicide doses can be reduced below label recommendations and still provide adequate control (Kudsk & Streibig, 2003). Decision support systems to aid farmers have been developed and as a result these techniques are widely adopted by farmers in several European countries such as Denmark (Kudsk, 1999), Sweden (Böstrom & Fogelfors, 2002), Finland and the Netherlands (Riethmuller-Haage, 2006; Kempenaar & Lotz, 2004). Further development of those systems requires a good understanding of pre-spraying weather conditions and herbicide types (Riethmuller-Haage, 2006), and the risk of the development of herbicide resistance.

Site specific weed management

Weed plants often grow in patches in arable fields (Heijting, 2007). Systems that only spray those places where a weed plant is present instead of spraying the whole field, can potentially reduce herbicide use.

Site specific weed management (SSWM) systems are systems that contain three components:

a) A weed sensing system, identifying, localizing and measuring crop and weed parameters;
b) A weed management model that contains decision algorithms;
c) A weed control implement that controls the weeds (Christensen *et al*., 2009).

The possible reduction in herbicide application that can be achieved with SSWM systems increases with increasing spatial resolution of the actual weed control (Wallinga *et al*., 1998). Within a farm four levels of spatial resolution for treatment can be distinguished (Christensen *et al*., 2009):

1. individual plant level,
2. grid level,
3. weed patches or subfields with weed clusters,
4. whole field.

Sensing can either be aerial or ground based. Because of the high importance of resolution, ground based systems currently have more potential. The aerial systems are only applicable of detecting patches larger than 1 by 1 m, and have a higher weather dependency (Brown & Noble, 2005). Two ways of distinguishing crop from weed plants are currently available. The first makes use of knowledge on the distance between crop plants within the row and green pixels within rows (Feyaerts *et al*., 1999), the second one is based on differences in morphological characteristics (Van Evert *et al*., 2009).

The decision algorithms are either efficacy based or population dynamics based (Wiles *et al*., 1996). The first makes use of data on herbicide performance in different crops, with different weeds and growth stages. The second uses knowledge on weed biology and ecology through deterministic models.

Several weed control implements have been developed, based on different mechanisms. For weed control on hard surfaces an automated weed detection system that sprays individual plants is available (Kempenaar & Leemans, 2005). Gerhards and Oebel (2006) developed a system that spatially applies herbicides by selective control of small sections of the spray boom (Gerhards & Oebel, 2006). Currently, direct injection sprayers that can apply different herbicides and dosages using maps of weed species are under investigation (Kempenaar *et al*., 2009).

All these implements are suitable to levels of spatial resolution 3 and 4. Highly accurate implements that are able to work at levels 1 and 2 are several mechanical implements (O'Dogherty *et al*., 2007; Åstrand & Baerveldt, 2002; Wisserodt *et al*., 1999; Bontsema *et al*., 1998), electrical discharge (Blasco *et al*., 2002), and flame weeding (Poulsen, 2006).

Until now, few farmers have adopted SSWM. Several reasons have been suggested for this low adoption rate. Christensen *et al* (2009) suggests that this is due to the limited range of usage of the current systems. The current SSWM systems are able to sense in specific crops and weeds, but unable to sense large number of unknown species while making instantaneous decisions. Therefore, the balance between potential savings and the costs of the system is not yet positive.

3. Curative control measures: biological and mechanical

A third strategy is the development of alternative curative control measures such as biological and mechanical weed control. Although several studies (Scheepens *et al*., 2001; Charudattan, 2000; Kempenaar, 1995) show that biological control can be an effective way to control weeds and some have resulted in commercial registration of the product, bioherbicides were never adopted on a large scale. The main reasons are the lack of reliability, specificity and efficacy under field conditions compared to the reliability and efficacy of herbicides (Hallet, 2005).

Mechanical control has progressed a lot during the past decade (Weide *et al*., 2008a; Melander *et al*., 2005). Developments concerning inter-row weeding have mainly aimed at increasing accuracy without losing capacity. Those developments mainly concern steering systems that differ in their discrimination of their mode of crop row detection, which ranges from detection of the crop row with the human eye to sensing of the crop row mechanically by gliders, camera-based optical sensing of the crop row and detection of the crop row with satellite navigation (RTK-DGPS) (Weide *et al*., 2008b). Inter-row cultivation using tines with hoe blades is the most common method (Weide *et al*., 2008a). The main problem for intra-row weeding is the discrimination between crop and weed plants combined with the ability of tools to get close enough to the crop plant. During the last decade, research has successively focused on harrowing, torsion and finger weeding and weeding using compressed air. The possibilities for using these weeding machines vary according to crop type, crop growth stage and field- and weather conditions and depend on selectivity (i.e. higher sensitivity of the weed plants relative to the crop plants) (Weide *et al*., 2008a). This selectivity is based on differences between weed and crop plants, for example in root anchorage forces, leaf area and/or plant height (Fogelberg & Dock Gustavsson., 1997). Conditions for physical weed control are normally favourable where crop plants are larger than the weed plants (Weide *et al*., 2008a). In spite of the recent improvements of mechanical weed control, it is in many cases not possible to reduce weed populations during crop growth mechanically to the same extent as chemically, especially in relatively open and slow developing crops (Albrecht, 2005; Verschwele & Zwerger, 2005; Hyvönen & Salonen, 2003; Barberi *et al*., 1998a).

Human dimension

Weed management involves the tactical, operational and strategic level of decision making. Farmers need to play a key role in the reduction of herbicide dependence. The before mentioned weed control technologies and strategies being developed by research, can be adopted by farmers in order to contribute to the reduction of herbicide dependence of agriculture. Most research has been focused on the development of new weed control technologies (Barberi, 2002), the development of new weed management strategies in cropping systems (Andrews *et al*., 1990), or the comparison of weed management between different cropping systems such as organic vs conventional systems (Albrecht, 2005). In most studies the assumption is being made that farmers operating within a particular farming system show similar behaviour. Or, in other words, all organic farmers will use the same weed control technology or strategy under comparable circumstances. However, farmers who supposedly operate in the same system are known to respond differently to for instance the market (Nowak & Cabot, 2004) or changes in the availability of farming techniques (Vanclay & Lawrence, 1994). It is therefore likely that weed management behaviour will vary between farms that operate within one type of farming system. Data on the farmers weed management behaviour and beliefs and the effects of these aspects on the on farm weed pressure is still lacking (Mertens *et al*., 2002).

Legislation

Another way to reduce the environmental impact of weed control is through legislation. Governments can decide to ban certain weed control techniques and thereby prevent their adverse effects on the environment. These decisions require knowledge on the type and magnitude of the effects of weed management techniques on the environment. The first major decision in the Netherlands to protect the environment from adverse effects of crop protection products and other chemicals was made in 1962. At that time, the Dutch government established the Pesticide Act, which stated general rules for the trade and use of pesticides, in order to protect the safety of men and those animals of which preservation was desirable.

This act was harmonized for the whole European Union to make registration of pesticides similar in all countries in 1994. The act was changed again in 2002 to accommodate registration of biocides in the EU. Adverse effects of pesticides are not only regarded a problem in the so-called western parts of the world, such as Europe and the US (Lazzaro L *et al*., 2009; Moore *et al*., 2009; Boutin *et al*., 2004), but also in other parts of the world (Chen *et al*., 2009). For example, a registration procedure for pesticides to avoid side-effects is currently being developed in China as well (Chuanjiang *et al*., 2007).

Since weed management activities are designed to control plants, the environmental compartment that is mostly at risk, is the plant kingdom. In practice, this concerns non-target terrestrial plants in margins surrounding agricultural fields. These so-called field margins are part of the National Ecological Network (NEN) in the Netherlands. The emission to non-target plants of non-chemical weed control methods can be considered negligible, so most attention goes to herbicide treatments. Herbicides may have a large effect on the biodiversity of both flora and fauna in agricultural fields and the surrounding field margins. These chemicals can affect the plant species composition, the plant diversity, growth and morphology of plants both in and outside the treated areas. Plants are an important part of the habitat to other organisms such as birds and insects, providing them with food, shelter and an environment to reproduce (Moreby & Southway, 1999; Freemark & Boutin, 1995). Changes in the species composition of field margins due to herbicide applications to adjacent fields have been observed (Jobin *et al*., 1997; Marrs *et al*., 1989).

Several organizations have developed schemes for assessing the risk of a crop protection product to non-target terrestrial plants: the European and Mediterranean Plant Protection Organisation (EPPO), the Organisation for Economic Cooperation and Development (OECD), the US Environmental Protection Agency (USEPA) and the Canadian Wildlife Service (CWS).

These schemes do however not address the most fundamental question in this matter: "What are we trying to protect?" "Do we want to protect all plant species present in a certain area, or only those species that are identified as being important to man or wildlife?'"

And if all species are to be protected, to which level, and which endpoints? And what is the reference vegetation? Do we need to consider species composition prior to the first herbicide applications in the area or is it sufficient to protect species that are currently present? All these questions need to be answered, not only by science but primarily by society as a whole. The role science can play is to fill in gaps in the knowledge required to answer these questions.

At this moment, information is predominantly available for single plant species grown in a greenhouse treated in a single phase of their life cycle. Information on the effect of sublethal doses of herbicides on a plants' life cycle, including its reproduction and recovery, but also the germinability of its seeds is lacking. Furthermore, the effects of herbicides in the presence of surrounding vegetation, either through differences in the effect on their inter- or intraspecific competitive ability, or factors such as shading are not investigated yet. All these factors need to be taken into account before a well-considered decision can be made about “what to protect” and about the criteria for herbicide registration.

This thesis

As the schematic overview in figure 1.3 shows, on farm weed management is influenced by many factors. These factors comprise the development and availability of tools, the environmental impact of tools and the attitude and behaviour of the farmer. This thesis focuses on research questions targeting important gaps in our knowledge for each of these factors. In part A we focus on the development of ecological weed management strategies depleting the seed bank, in part B on the environmental effects of weed management strategies and in part C on the human dimension of weed management.

Part A: Development of weed management strategies depleting the seed bank

In the previous paragraphs of this chapter several strategies that can lead to the development of new weed management technologies have been discussed. Ecological weed management is one of those strategies and has good promise for the development of weed control techniques that can aid in a reduction of agricultures herbicide dependence. On one of the directions for ecological weed management, the manipulation of the competitive interaction between crops and weeds, extensive research has been done. In this part of the thesis we focus on the other two directions: the reduced recruitment from the soil seed bank and the gradual depletion of the soil seed bank.

In **Chapter 2** we describe a study on the optimization of the use of the stale seedbed technique to increase the germination and thereby the losses of the seed bank, and prevent new germination of weed seeds that may occur during the control of the weeds emerging after the stale seed bed application. The part of the weeds’ life cycle that is targeted in Chapter 2 is depicted in Figure 1.2b.

The following research questions were investigated in Chapter 2:

- What is the most effective way to apply a stale seedbed technique with regard to light and timing?
- Which weed control method is most suitable to use after the application of a stale seedbed?
- And how does the efficacy of a weed control methodology based on a stale seedbed strategy relate to the efficacy of chemical control?

In **Chapter 3** the depletion of the soil seed bank by a reduction of the input is investigated (Figure 1.2b). The effects of three organic weed management strategies on the size of the soil seed bank, the emerging weed population and the amount of hand weeding are investigated.

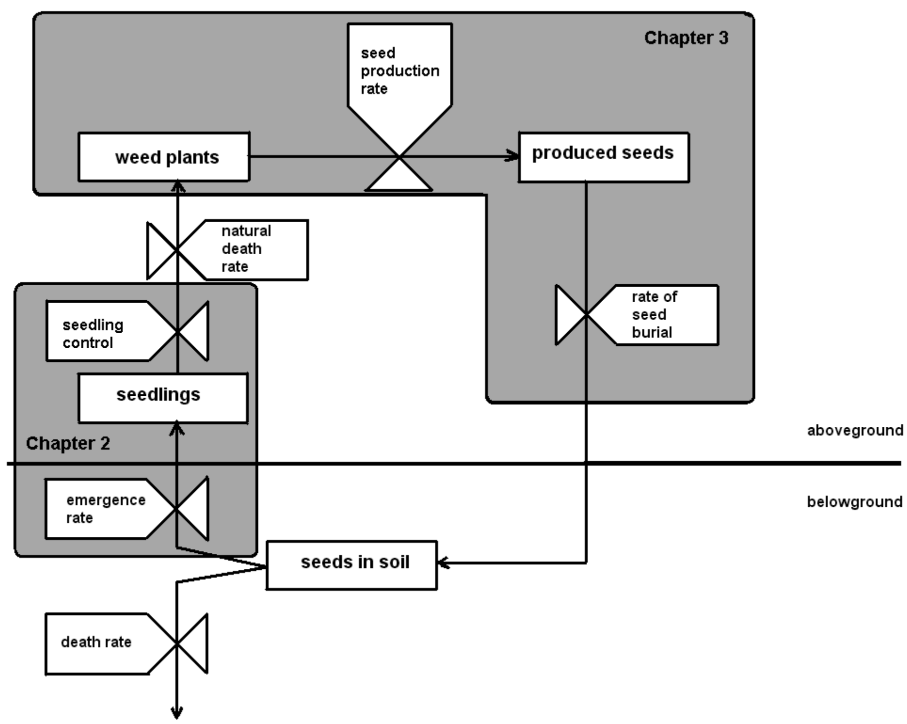

Figure 1.2b Life cycle of annual weeds.

The following research question was studied in Chapter 3:

- Will a strategy, with a high investment in hand weeding to prevent seed return to the soil seed bank during the first years in a rotation, eventually result in lower weed densities and a reduction in the amount of required hand weeding compared to strategies with lower levels of hand weeding?

Part B: the environmental effects of weed management

In this part of the thesis the focus is on the environmental effects of weed management. As we describe in the legislation paragraph, weed management activities are designed to control plants and their effects will be most profound on the plant kingdom. The research in part B aims at research questions targeting the major gaps in our knowledge on environmental effects of weed management: the effects of herbicides on non target terrestrial plants. Several experiments were performed in the greenhouse as well as in semi-field situations with two herbicides: a broad-spectrum (glufosinate ammonium, **Chapter 4**) and a small-spectrum (tepraloxydim, **Chapter 5**).

The following questions were addressed with respect to non target plants in Chapters 4 and 5:

- What is the influence of plant development stage on plant sensitivity and herbicide efficacy?
- How is individual plant sensitivity influenced by the presence of surrounding vegetation?
- What is the effect of sublethal herbicide doses on the biomass, recovery and reproduction of non target plants?
- Is it possible to translate the observed effects of broad-spectrum and small spectrum herbicides on greenhouse grown plants into the effects on field grown plants?

Part C: Human dimension of weed management

Part C concerns the last factor influencing on farm weed management: the human dimension of weed management. As described elsewhere in this chapter, information on the farmers weed management behaviour and its effect on the on farm weed pressure is lacking. In **Chapter 6** we describe an exploratory on-farm study conducted to gain insight in the farmers weed management behaviour in relation to weed pressure within a farming system.

The specific research questions addressed in Chapter 6 were:

- Can differences between farms in weed pressure be related to differences in farmers' weed management behavior,
- Which weed and general management factors are of main influence on the weed pressure,
- What is the influence of farmer's beliefs and knowledge on weed control techniques on the observed weed pressure?

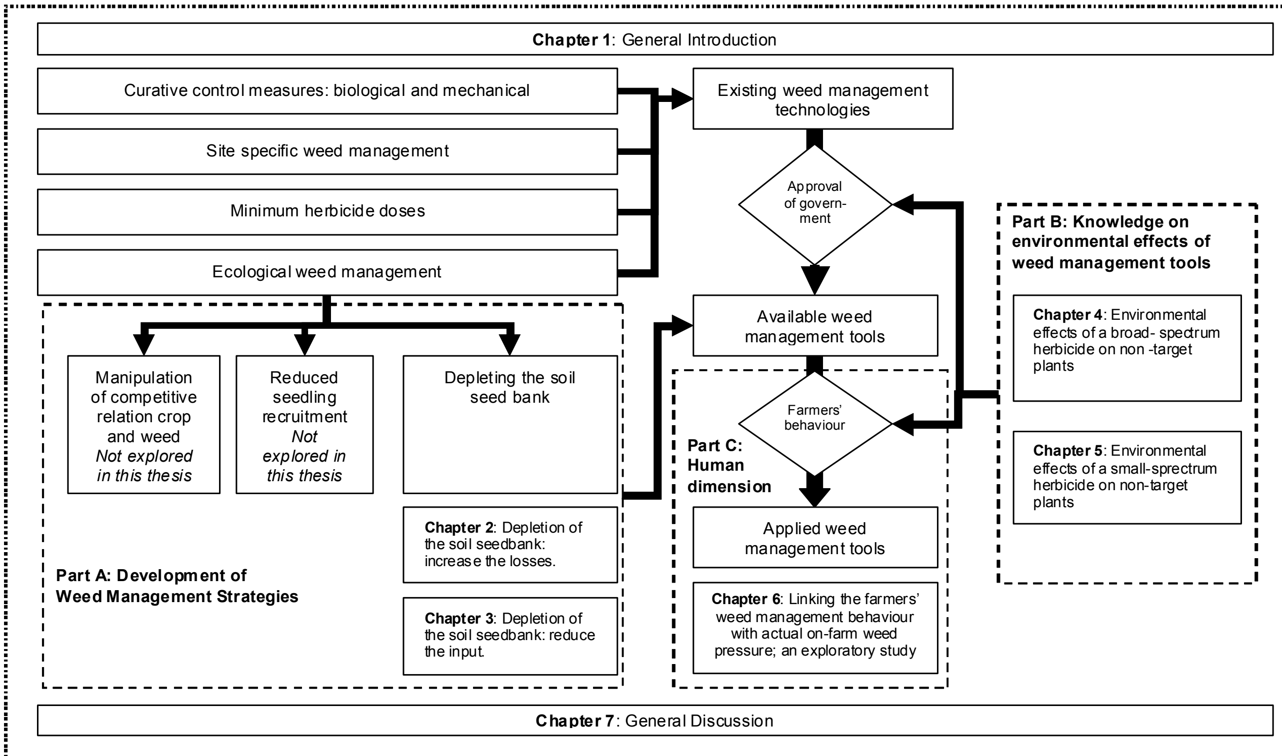

Figure 1.3 Structure of thesis.

CHAPTER 2

Effect of stale seedbed preparations and subsequent weed control in lettuce (cv. Iceboll) on weed densities[1]

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Summary

The effects of stale seedbed preparations and several weed control methods on the emergence of weeds were studied. Specific goal was to evaluate the use of a stale seedbed in combination with chemical or mechanical weed control methods in the field. Depending on location and year, stale seedbed preparations followed by weed control prior to planting reduced the amount of weeds during crop growth by 43 to 83%. Control of the emerged seedlings after a stale seedbed preparation was more effective with glyphosate than with a rotary harrow. Covering the rotary harrow during control improved its effect on the weed density during crop growth in two out of three years. Radiation with far red light (FR) did not reduce the number of emerging weeds in this study. Mechanical control by finger weeder, torsion weeder and hoe was applied without stale seedbed preparations. These measures reduced the weed densities with 88 to 99% compared to the untreated control and were more effective than chemical weed control with carbeetamide and chloorprofam. The results show that the stale seedbed technique in combination with mechanical control of emerging weeds can reduce the weed population during crop growth as effective as chemical control can, and may therefore help reduce the need for herbicides in the future.

Keywords: stale seedbed**,** far red light, finger weeder, torsion weeder, hoe, rotary harrow, glyphosate

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[1] *Weed Research 47 (2007), 149-156.*

Introduction

The awareness of government, consumers and farmers of possible adverse side effects of chemicals has increased over the past decades. This has resulted in research programmes on weed control in which the emphasis is mainly on the development and improvement of strategies that reduce or exclude herbicidal use. In spite of the recent improvements of mechanical weed control, it is in many cases not possible to reduce weed populations during crop growth mechanically to the same extent as chemically, especially in relatively open and slow developing crops (Albrecht, 2005; Verschwele & Zwerger, 2005; Hyvönen & Salonen, 2003; Barberi *et al*., 1998). The development of preventive methods may contribute to the reduction of the amount of weeds in the field during crop growth and thereby reduce the need for herbicides (Kropff *et al*., 2000).

An example of a preventive measure is the stale or false seedbed technique. This technique involves the preparation of a seed- or plantbed to promote germination of weeds a number of days or weeks before the actual sowing or planting of the crop. One major reason for the enhanced germination of the seeds in the soil during the preparation of a seed- or plantbed is the exposure to light. Seeds of many species are sensitive to short exposures to light at a certain moment in the life cycle of the seed (Andersson *et al*., 1997; Milberg *et al*., 1996; Vleeshouwers *et al*., 1995). This initial seedbed preparation is then followed by destruction of the emerging weed seedlings with minimal soil disturbance (Mohler, 2001). The control of emerging weed seedlings is mostly done with herbicides (Heatherly *et al*., 1993; Oliver *et al*., 1993), although some studies included the use of non chemical control methods such as flame weeding and harrowing (Rasmussen, 2004; Caldwell & Mohler, 2001).

The disadvantage of mechanical weed control techniques such as harrowing is that they cause soil disturbance and thereby light penetration of the soil, possibly causing the emergence of new flushes of weeds. Therefore, an optimization of the effects of a stale seedbed followed by mechanical control is required if a further decrease in herbicidal use is the objective. One strategy that aims to avoid the exposure to light is performing soil cultivations at night, also called photocontrol (Juroszek & Gerhards, 2004). In previous studies in Germany (Hartmann & Nezadal, 1990), Argentina (Botto, 1998), the USA (Buhler *et al*., 1998; Scopel *et al*., 1994) and Denmark (Jensen, 1995), it was found that a reduction up to 97.5% of the amount of emerging weeds by night tillage is possible,

although the amount of reduction was highly variable with the weed species, year, location and soil type. The effect of conducting photocontrol of weeds with a light-proof cover during daytime could work in a similar way (Juroszek & Gerhards, 2004).
The aim of the research described in this paper was to compare the effects of different stale seedbed strategies on the weed density in planted lettuce among each other and with treatments without stale seedbeds. Weeds emerging after stale seedbed preparations were either controlled chemically or mechanically. Mechanical weed control involved rotary harrowing, hoeing, hoeing with a torsion weeder, a finger weeder or a covered rotary harrow to prevent the germination of new weed cohorts.

Materials and Methods

Experimental site

Field research was carried out from 1999 until 2001 at three different experimental fields in Lelystad (The Netherlands, 52.30N, 5.26E). The research was carried out on one experimental field per year. At all locations lettuce (cv. Iceboll) was grown on a clay soil. More details are given in Table 2.1.
Ten treatments involving stale seed bed preparations, and chemical or mechanical weed control methods were tested for their efficacy on the control of weeds. Plots were 4 by 10 m and arranged in a randomized block design and replicated four times. Not all treatments were tested for their effect on the number of emerging weeds during each year. An overview of the treatments is given in Table 2.2. All plant beds and all stale seedbeds were prepared with a rotary harrow (working depth 3-4 cm deep). Stale seedbeds were prepared four weeks before the crop was planted; on May 14, May 18 and May 9 in 1999, 2000 and 2001, respectively. The lettuce was grown in paper pots of 4 x 4 cm, which were planted as soon as the plants reached the 6 leaf stage.
The number of emerging weeds after every treatment was counted on July 7 1999, July 13 2000 and June 12 2001, unless otherwise mentioned. Counts were made in 1999 in two 0.525 m^2 (0.725 m x 0.725 m) quadrates, in 2000 in two 1.0 m^2 (1.0 m x 1.0 m) quadrates and in 2001 in two 1.0 m^2 (2.0 m x 0.5 m) subplots, all randomly located in each plot. Numbers were converted to numbers of weeds per 1 m^2.

Table 2.1 Main soil characteristics and cultivation measures in the experimental field trials in Lelystad.

	year		
Characteristic	1999	2000	2001
% clay	16	14	14
% organic matter	2.0	1.9	1.8
pH-KCL	7.2	7.6	7.6
Water soluble P_2O_5 per liter soil (mg/L)	31	34	42
Exchangeable K_2O (mg/100 g soil)	25	24	20
N (kg/ha)		23.4	17.4
Preceeding crop	Winter wheat	Winter wheat	Summer barley
Planting date	June 15	June 9	June 11
Harvest date	July 26	August 1	July 30
Interrow distance (cm)	35	35	35
Intrarow distance (cm)	37	37	37
Field size (m)	154 x 20	172 x 20	136 x 20
Fertilisation:			
K_2O	Autumn 1998 200 kg	Autumn 1999 194 kg	Autumn 2000 195 kg
P_2O_5	Autumn 1998 200 kg	Spring 2000 146 kg	
N	June 2 1999 202 kg	May 23 202 kg	May 7 162 kg
MgO	Autumn 1998 100 kg	Autumn 1999 154 kg	Autumn 2000 97 kg

The effect of a stale seedbed

To test the effect of a stale seedbed followed by weed control on the emerging weed population, treatments A and G (Table 2.2) were compared. Treatment A was the untreated control, in which the only action was the preparation of a regular plant bed with a 2m wide rotary harrow from Lely West NV, Maassluis, The Netherlands (http://www.lely.nl). The same rotary harrow was used in all treatments. Treatment G involved the preparation of a stale seedbed with a rotary harrow four weeks prior to planting the crop, followed by weed control with a rotary harrow just prior to planting. The rotary harrow treatment in A coincided with the rotary harrow treatment in G. The length of the period between the preparation of a stale seedbed and the actual planting, four weeks, was based on practical experience under Dutch weather conditions. Weeds that are stimulated to germinate during stale seedbed preparation need some time to emerge. Four weeks was found to be optimal; the majority of the weeds have emerged and are still small enough to control mechanically. No weed control was applied after planting in either treatment. Both treatments were applied in every year and at each site.

Stale seedbed followed by chemical or rotary harrowing control

Two treatments (treatment F and G) were applied to test the effect of either chemical control or control by rotary harrowing, both applied prior to planting the crop to control the weeds germinating and emerging after a stale seedbed application. Both treatments started with the preparation of a stale seedbed with a rotary harrow four weeks prior to planting the crop on the above described dates. Subsequently, the emerging weeds were either controlled with glyphosate (Round up®, Monsanto Europe NV A.G. Benelux, 1080 g a.i./ha) (treatment F) or controlled with 3-4 cm deep rotary harrowing (treatment G). These treatments were applied in every year and at each site. No additional weed control was applied after planting the crop.

Table 2.2 Applied (stale) seedbed treatments from 1999-2001 in Lelystad at three different fields.

Trtmnt	Years	Stale seedbed	Seed bed preparation	Weed control prior to planting	Weed control after planting
A	1999-2001	-[$]	rotary harrow	-	-
B	1999-2001	-	rotary harrow	chemical∗	-
C	1999-2000	-	rotary harrow	-	torsionweeder
D	1999-2000	-	rotary harrow	-	fingerweeder
E	1999-2000	-	rotary harrow	-	hoeing
F	1999-2001	4 weeks[#]; rotary harrow	-	chemical♣	-
G	1999-2001	4 weeks; rotary harrow	-	rotary harrow	-
H	1999-2001	4 weeks; rotary harrow	-	covered harrow	-
I	2000-2001	4 weeks; rotary harrow	-	covered harrow FR Radiation.	-
J	2000-2001	-	covered harrow	-	-

Prior to planting: carbeetamide (Legurame, 1500 g a.i. ha^{-1}, Feinchemie Scwebda GMBH) + chloorprofam (Brabant Chloor-IPC, 600 g a.i. ha^{-1}, Agrichem).

♣ Glyphosate (Round up®, 1080 g a.i. ha^{-1}, Monsanto Europe NV A.G. Benelux).

[#] The stale seedbed was prepared 4 weeks before the final seed bed.

[$] - indicates that a treatment (A-J) did not comprise this action (columns).

Cover of machinery

The effect of covering the rotary harrow on the germinating and emerging weeds was tested with two comparisons in which four treatments were involved. First, the effect of a rotary harrow covered with black plastic during plant bed preparation (treatment J) was compared with the preparation of a plant bed with a rotary harrow without cover (treatment A). Treatment J was applied in 2000 and 2001.Second, the effect of weed control after a stale seedbed preparation with an uncovered rotary harrow (treatment G) was compared with the effect of weed control after a stale seedbed preparation with a covered rotary harrow (treatment H). The harrow was covered with black plastic and two blankets and had a working depth of 3-4 cm. No weed control was applied after planting the crop. Both treatments were applied in every year and at each site.

Far red light

The effect of far red light on the emergence of weeds was tested during the last two years of the experiments. In 2000 and 2001, four weeks after the preparation of a stale seedbed, the germinating and emerging weeds were controlled with a covered rotary harrow. Three bulb lamps with far red light (75 Watt) were placed under the black plastic and the two blankets that covered the rotary harrow (treatment I). The light spectrum of the lamps was measured in an experimental chamber in which interference from visible light was excluded using an Ocean Optics 2000-series spectrophotometer. The spectrum of the lamps ranged from 695 nm up to 1100 nm, with an optimum around 780 nm. This treatment was compared with treatment H, which followed the same procedure but without FR radiation.

Mechanical weed control after planting and chemical weed control prior to planting

In the first two years (1999 and 2000), three mechanical weed control methods applied after planting were tested for their efficacy on the control of the emerging weeds. The plant bed for the lettuce was prepared with a rotary harrow for all treatments. The tested mechanical control methods were control with a torsion weeder from Frato, Wijchen, The Netherlands (http://www.frato.nl) (treatment C), with a finger weeder from Kress & Co GMBH, Vaihingen/Enz, Germany (http://www.kress-fingerweeder.com) (treatment D) or with a regular hoe from K.A. Havelaar en Zn B.V., Moerkapelle, The Netherlands

(http://www.havelaar.biz) (treatment E). Mechanical weed control was carried out on July 8, 1999 and June 23, 2000. Counts were made the next day in above described plots.
The effect of the chemical weed control on the emerging weeds was tested every year and involved the application of a mixture of carbeetamide (1500 g a.i./ha) and chloorprofam (600 g a.i./ha) just before planting. In this treatment no weed control was applied after planting.

Statistical analysis

The effects of the mechanical weed control methods, chemical treatments or stale seedbed combinations on the emerging weed populations were analyzed per year with a one-way ANOVA (analysis of variance) in a randomized block design followed by a comparison of means (Fisher's Least Significant Difference) using the 8th edition of the Genstat statistical program (Payne *et al*., 2005). Average densities of the total number of weeds per treatment are presented together with standard errors (s.e.), the standard error of the difference (SED) and degrees of freedom (df) calculated within years in Table 2.3.

Results & Discussion

Emerging weed population

The average number of weeds per m^2 in the untreated plots varied over years from 45.5 to 78.4 (Table 2.3). The dominant weed species were, in 1999 *Chenopodium rubrum*, *Solanum nigrum*, *Veronica chamaedrys* and *Capsella bursa-pastoris*, in 2000 *Chenopodium album*, *Stellaria media*, *Poa annua* and *Solanum nigrum* and in 2001 *C. album*, *S. media*, *S. nigrum*, *Senecio vulgaris* and *C. bursa-pastoris*. In general, densities of individual species were too low to detect significant differences between treatments.

The effect of a stale seedbed

The effect of a stale seedbed prepared with a rotary harrow and subsequent weed control (treatment G) was compared with the untreated control in which the only action was the preparation of a regular planting bed with a rotary harrow (treatment A). The density of weeds during crop growth was significantly ($P<0.001$) reduced by treatment G compared to treatment A in all three years (Table 2.3). The preparation of the regular plant bed in treatment A coincided with the preparation of the final plant bed in treatment G. Previously, stale seedbed practices followed by shallow tillage also reduced the size of aboveground weed populations compared to treatments without stale seedbeds in peanut (Carroll Johnson & Mullinix, 1995), maize (Cloutier & LeBlanc, 2002) and fodder beet (Rasmussen, 2003). Weeds emerging after the first seedbed preparation were controlled by shallow tillage such as rotary harrowing, resulting in a depletion of the seed bank and lower weed densities during crop growth. However, weeds surviving the stale seedbed preparation and subsequent weed control in a study in Denmark had a slightly larger biomass than weeds germinating in the same treatment without a stale seedbed (Rasmussen, 2004). The surviving weeds germinated already during the stale seedbed preparation, and were larger than the weeds that started germination during the final seedbed preparation.

Table 2.3 Average number of total weeds and percentage weed reduction compared to the untreated plots in 1999, 2000 and 2001.

	Total (n) ± s.e.			Reduction (%)		
Treatment	1999	2000	2001	1999	2000	2001
A	$53.3 \pm 8.42^{a*}$	78.4 ± 3.39^{a}	45.5 ± 6.53^{a}	0	0	0
B	21.7 ± 4.50^{bc}	23.1 ± 3.32^{d}	14.2 ± 3.46^{bc}	59.3	70.5	68.9
C	2.4 ± 0.60^{e}	2.8 ± 0.45^{ef}	N.T.	95.5	96.4	N.T.
D	0.7 ± 0.35^{e}	0.6 ± 0.18^{f}	N.T.	98.7	99.2	N.T.
E	6.2 ± 0.31^{de}	5.8 ± 0.56^{e}	N.T.	88.4	92.6	N.T.
F	16.4 ± 4.61^{cd}	25.5 ± 1.43^{cd}	11.8 ± 2.29^{bcd}	69.2	67.5	74.0
G	30.3 ± 2.63^{b}	31.4 ± 1.34^{b}	13.3 ± 4.34^{bcd}	43.2	59.9	70.7
H	14.0 ± 3.05^{cd}	22.6 ± 1.87^{d}	9.0 ± 3.08^{cd}	73.7	71.1	80.2
I	N.T.	22.6 ± 1.32^{d}	7.8 ± 0.98^{d}	N.T.	71.1	82.8
J	N.T.	28.9 ± 1.141^{bc}	17.5 ± 2.85^{b}	N.T.	63.1	61.5
SED (df)	5.16 (7)	2.494 (9)	3.488 (6)			

%= percentage weed reduction relative to the untreated (A) plot.

[*] values in the same column followed by a different letter are significantly different from one another at the 0.001 level. N.T.: not tested.

Stale seedbed followed by chemical or rotary harrowing control

Two types of weed control were applied after stale seedbed preparations and prior to crop growth: chemical control (treatment F) and rotary harrowing (treatment G). Application of glyphosate after a stale seedbed reduced the number of weeds in the crop by 69, 68 and 74 % in 1999, 2000 and 2001, respectively, compared to the untreated control (treatment A). Rotary harrowing after a stale seedbed on the other hand reduced this number by 43, 60, and 71 % in 1999, 2000 and 2001, respectively (Table 2.3). The control of the weed seedlings with the rotary harrow was significantly less effective ($p<0.001$) than the chemical control with glyphosate in two out of three years. The rotary harrow disturbed the soil up to a depth of 3-4 cm and may have brought seeds to the surface. These seeds may have germinated and emerged, and resulted in a higher weed density in the rotary harrow treatment. Although the weed densities remaining in the plots treated with glyphosate were lower compared to the densities in other plots, their total biomass was significantly higher than the biomass of weeds remaining in the plots treated in another way, including the untreated plot (data not shown). At the moment weed control was performed, germinating but not yet emerging weeds were probably present in the top layer of the soil as a result of the preparation of a stale seedbed. Glyphosate did not control these un-emerged weeds, rotary harrowing (and mechanical control in general) (Gunsolus, 1990) did. As a result, weeds emerged earlier in the glyphosate treated plots and therefore obtained a higher biomass than the weeds emerging in the rotary harrow treated plots.

Previously, four week stale seedbed preparations followed by glyphosate treatments were more effective than the same stale seedbed preparations followed by treatments with either a tine weeder or a spring toot harrow (Caldwell & Mohler, 2001). However, the control with the tine weeder and spring tooth harrow were not effective compared to the control treatment. The tine weeder penetrated the soil with a depth varying between 0 and 10 cm, the spring tooth harrow penetrated the soil regularly up to a depth of 13 cm. The rotary harrow used in the present study is therefore a better option to control the residual weeds. Due to its working depth of 3-4 cm it affects weeds more than the tine weeder, and brings less seeds to the surface than the spring tooth harrow used in the study of Caldwell & Mohler (2001).

Cover of machinery

Covering the rotary harrow with black plastic and blankets significantly ($p<0.001$) improved the control of weeds emerging after a stale seedbed preparation. Control went up from 43 and 60% (treatment G) to 74 and 71% (treatment H) in 1999 and 2000 respectively (Table 2.3). Covered treatments were more effective than the same treatments without cover, although these differences were not significant in 2001. A similar result was found for the covered and uncovered treatments that were not preceded by a stale seedbed preparation. Covering the rotary harrow during plant bed preparation (treatment J) significantly reduced the amount of weeds with 63 and 62% in 2000 and 2001, respectively compared to the uncovered preparation (treatment A). Results from this study are in agreement with previous studies performed in fallow fields in Sweden (Ascard, 1994) Germany (Hartmann & Nezadal, 1990), the USA (Gallagher & Cardina, 1998; Scopel *et al*., 1994), Denmark (Jensen, 1991 & 1995) and Argentina (Botto, 1998). They compared the effects of cultivation in the dark, during the day and with a covered harrow during daytime, on the number of emerging weeds. Tillage at night or with a covered harrow significantly reduced the number of weeds in a crop-free field, although reduction was, variable between years, locations, weed species and soil types. Part of the observed variation in those studies and the observed variation in the present study may be explained by differences in light requirements of seeds in time (i.e.. degree of dormancy). Differences in light requirements are the result of differences in dormancy state of the seeds. Dormancy state is a seed characteristic determining the range of environmental requirements needed for germination. Germination will be induced when actual environmental conditions, such as light, overlap the dormancy state (Vleeshouwers *et al*., 1995). The degree of dormancy state is determined by temperature during seed development when these are still attached to the plant (Bouwmeester, 1990) and other environmental conditions such as nitrogen concentration and moisture content of the soil experienced by the maternal plant (Luzuriaga *et al*., 2006). Different temperature ranges during seed development may cause different dormancy states of seeds and thereby different responses to environmental conditions such as light. Differences in the effect of cover on the emergence of weeds between years and locations observed in our study may therefore be (partly) caused by differences in temperature and other environmental conditions during seed development and treatment. In addition, the three locations differed in their relative density of the different weed species

(data not shown). Seeds of different species may have had different dormancy states and may also have contributed to the observed differences between locations and thus years.
Another possible explanation for the different responses of seeds between locations and years are the differences in temperature ranges experienced by the seed bank in those years and locations. Changes in the light requirements of buried seeds were found to be correlated to temperature in several burial experiments (Batlla & Benech Arnold, 2005; Derkx & Karssen, 1993).

Far red light

It is known that the germination of seeds of many species can be influenced by exposure to light at a certain moment in the life cycle of the seed (Andersson *et al*., 1997; Milberg *et al*., 1996; Vleeshouwers *et al*., 1995). The light response of the seeds can be attributed to a family of chromoproteins called phytochromes. Two stable photoconvertable forms of phytochromes exist: the inactive form P_r which absorbs light of around 665 nm, and the active form, P_{fr} which absorbs light of around 735 nm. Exposure to red light (R) (around 665 nm) can convert P_r into P_{fr}, which will trigger germination if concentrations of P_{fr} are high enough. Subsequent radiation with far-red light (FR) (around 735 nm) will convert P_{fr} back to P_f and prevent germination (Frankland & Taylorson, 1983). This response of the seed is called the Low Fluence Response (LFR).
The effect of FR on the emergence of weeds was tested by placement of three far red lamps under a covered rotary harrow during the control of weeds that emerged after a stale seedbed preparation (treatment I). Although one can expect a reduced emergence of weeds after irradiation with FR on the basis of the LFR, this was not the case in our study (Table 2.3). Previous laboratory research in which seeds of more than 64 species were tested for their sensitivity to light showed that responses to light are species and population dependent (Andersson *et al*., 1997; Milberg *et al*., 1996). For instance, germination of *Origanum vulgare* seeds increased with 61% after an exposure to FR of 10 minutes whereas germination of seeds of *Plantago major, Plantago lanceolata* (Pons, 1991) and *Galeopsis speciosa* (Karlsson *et al*., 2006) increased slightly or not at all compared to germination in the dark. Germination of species or populations present in the seed bank in this study might have been less inhibited by FR than expected. Alternatively, the absence of a reduction in the number of emerging weeds as a result of FR might have been caused by an increased

sensitivity to light of the buried seeds. Burial of seeds is known to increase the sensitivity of the seeds to light. Seeds of many species are known to respond to relatively very low percentages (<2%) of P_{fr} after burial (Scopel *et al*., 1991; Kendrick &Cone, 1985; Mandoli &Briggs, 1981). This response, the Very Low Fluence Response (VLFR), will result in germination of these seeds after exposure to very low fluences. As a result of soil cultivation burial of weed seeds is very common in agricultural land and a large part of the weed seed bank in agriculture is sensitised (Hartmann & Mollwo, 2000). According to these authors all spectral colours from ultra-violet at 300 nm to near-infra-red at 800 nm are within the range required for the VLFR and therefore all light within this spectrum is able to cause germination of sensitised seeds. This implies that although radiation with FR (around 735 nm) will convert a large amount of the P_{fr} into P_r, the remaining percentage P_{fr} will always remain high enough to induce germination in sensitised seeds (Takaki, 2001). Furthermore, the longer seeds remain buried, the larger the percentage of seeds that will germinate after radiation with FR (Botto, 1998). The seeds in the top soil layer in this study may have been buried for a long period and may have responded according to the VLFR at the very low percentages of P_{fr} generated by the far red lamps.

Mechanical weed control after planting and chemical weed control prior to planting

The mechanical weed control methods, i.e. the finger weeder (treatment D), the torsion weeder (treatment C) and the hoe (treatment E), significantly reduced the total amount of weeds during crop growth by 88 to 99 % compared to the untreated control (treatment A) (Table 2.3).In addition, the torsion weeder reduced weeds more than the hoe in one year. In a previous study the torsion weeder was found to perform better than the finger weeder in terms of weeding and cost effectiveness (Melander *et al*., 2005) but the slight tendency in our experiment was not significant. By hoeing (treatment E), the amount of weeds was significantly reduced by 88 and 93% compared to the untreated control, in 1999 and 2000 respectively. The effect of this method on the number of weeds was not significantly different from the effects of the finger weeder and torsion weeder in 1999. In 2000, the density of weeds that remained after using the finger weeder was significantly lower than the density after application of the hoe. Compared to chemical weed control with carbeetamide (treatment B), the use of the finger weeder, torsion weeder and the hoe all resulted in a significantly better control (Table 2.3).

The chemical control (treatment B) was equally effective as the stale seedbed combinations (treatments F, G, H and I) in 2000 and 2001, except for the treatment with far red light (treatment I) in 2001 which was more effective at that time. The chemical control (treatment B) was more effective than control of weeds with a covered rotary harrow without a stale seedbed (treatment J). Several previous studies already concluded that mechanical intra-row control methods can be as affective as herbicides in those cases in which weeds were not very sensitive to the post-emergence herbicides applied and several cultivations were performed (Mulder & Doll, 1993).

Conclusions

No differences could be detected amongst treatments at the individual species level, except for the difference with the untreated control. The number of weeds per species may have been too low to be able to detect differences between other treatments. Nevertheless, differences were found at the whole vegetation level.

Of the control methods tested, the mechanical weed control with the torsion weeder, the finger weeder, and the hoe were the most effective in reducing total weed densities during crop growth. Although less effective than the use of the torsion weeder and finger weeder, the effect of a stale seedbed preparation and subsequent weed control was similar to or better than the effect of chemical control with carbeetamide and chloorprofam. Weed reductions up to 83% were obtained as a result of the preparation of a stale seedbed and subsequent weed control. Of the control methods tested after a stale seedbed preparation, the application of glyphosate and the use of a covered rotary harrow were most effective. Less effective were tillage practices combining burial and radiation of seeds with FR. Seeds are probably sensitised by burial and will display the irreversible VLFR and consequently the germination and emergence of those weed seeds will not be prevented. The results show that the stale seedbed technique in combination with mechanical control of emerging weeds can reduce the weed population during crop growth as effective as chemical control can.

CHAPTER 3

Effects of three organic weed management strategies on the seed bank, weed emergence and subsequent weed control effort over seven years [2]

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Summary

The effects of three different weed management strategies on the required input of hand weeding in organic farming systems over several years, the weed seed bank in the soil, and the emerging weed seedling population were studied from 1996 to 2003. The strategies were (1) control of weeds as carried out in standard organic farming practice, (2) control of all residual weeds that grow above the crop, and (3) prevention of all weed seed return to the soil. Under all strategies the size of the seed bank increased after the conversion from conventional to organic farming systems. The increase under strategy 3 was significantly smaller than the increase under the other strategies. From 1999 onwards, the weed densities in plots treated with strategy 3 became significantly lower than the weed densities in plots treated with the other strategies. The number of manual weeding hours required to prevent weed seed return in addition to the number in standard organic farming practices was reduced during the study. Results show that a management strategy aimed at the prevention of seed return can reduce the increase of the seed bank size which is usually observed after transition from conventional to organic farming.

Keywords: seed bank, weed control effort, manual control, management strategies, organic farming, population dynamics.

[2] *Weed Research 47 (2007), 442-451.*

Introduction

During the past three decades the reduction of pesticide use has become an important objective in both policy making and agricultural research. Partly as a result of this, organic farming has received a lot of attention. One of the key problems in organic farming is the amount of effort required for weed control. Mechanical weed control alone is usually not sufficient and additional manual control is necessary to achieve the level of weed control that is possible with herbicides. Labour is expensive and usually not available for the purpose of hand weeding in many areas of the world. Therefore, a strong reduction of the amount of manual labour for weed control in organic farming systems is essential.

A reduction of manual labour requirements may be achieved by the improvement of weed management strategies. The use of strategies aimed at the prevention of addition of weed seeds to the soil seed bank may result in a declining seed bank (Sjursen, 2001). The weed population emerging after cultivation in some studies is related to the size and composition of the weed seed bank (Zhang *et al*., 1998; Roberts & Neilson, 1981; Roberts & Ricketts, 1979). In some studies, no relationship was found between seed bank and aboveground communities (Derksen & Watson, 1998), or only for a small number of species (Webster *et al*., 2003). This indicates the complexity of processes determining the germination and emergence of weed seeds in the soil. Nevertheless, the soil seedbank is a product of the past and represents the potential future of the aboveground plant community (Swanton & Booth, 2004). Therefore, a modification of the size of the seed bank will result in changes of the emerging weed populations, and *vice versa*. In this paper, we hypothesize that strategies aimed at the prevention of seed additions to the soil seed bank, will result in lower weed densities and will reduce the amount of manual labour required.

Several previous studies evaluated the effects of different management strategies based on different tillage regimes (Barberi & Cascio, 2001; Menalled *et al*., 2001; Clements *et al*., 1996; McCloskey *et al*., 1996; Froud-Williams *et al*., 1983), several crop rotations (Benoit *et al*., 2003; Menalled *et al*., 2001; Barberi & Cascio, 2001; Cardina *et al*., 1996), fertilizer applications (McCloskey *et al*., 1996) and herbicide applications (Grundy *et al*., 2005; Menalled *et al*., 2001; Barberi *et al*., 1997; Jones & Maulden, 1999; McCloskey *et al*., 1996; Lotz *et al*., 1993; Ball, 1992;) on the seed bank or emerging seedlings or both. They all showed that, depending on the specific circumstances of the study, the actual weed population varies with different management strategies in which the effects of one or more of these factors are investigated. However, none of these studies compared the effects of different organic weed management strategies on both the weed population as well as on the effort to control this population.

The present study was undertaken to test the hypothesis that weed management strategies that focus on a reduction of the weed seed bank eventually require less labour for hand weeding. To do so, we studied the effects of three organic weed management strategies on the size of the soil seed bank, the emerging weed population and the amount of manual labour. The three strategies were (1) control of weeds as carried out in standard organic farming practice, (2) control of all residual weeds that grow above the crop, and (3) prevention of all weed seed returns to the soil.

Materials and Methods

Experimental farm

Field research was carried out between 1996 and 2003 at the organic experimental farm "Dr. H.J. Lovinkhoeve" located in Marknesse, The Netherlands (52°42'N, 5°53'E) where a seven year rotation was established. The size of each of the seven rotation fields was 168 m x 300 m. Soil properties were: a coarse sandy clay, with pH-KCl of 7.3, an organic content of 2.2% and a $CaCO_3$-level of 9%. After being a conventional farm for many decades, this farm was converted to an organic farm in 1996. The ploughing depth in the organic farming system was kept similar to the ploughing depth of 20 cm during the conventional farming period. The applied crop rotation scheme was: a 2-year lucerne crop, sugar beet, spring wheat, potato, maize and tulip. In 1996, winter wheat was grown instead of spring wheat and maize, onion instead of tulip and oats instead of potato (Table 3.1).

Three weed management strategies

The effects of three weed management strategies on the weed seed bank, the emerging weed population and the required manual weed control effort were studied. The three strategies were (1) control of weeds as carried out in standard organic farming practice, (2) control of all residual weeds that grow above the crop, and (3) prevention of all weed seed return to the soil. Thus, strategies 2 and 3 involved additional manual weed control, on top of the mechanical and manual weed control carried out in strategy 1. Strategies were applied in plots of 12 m x 40 m and replicated twice on each of the seven fields, during seven years. The plots were kept in the same location in each field during the experiment. The standard organic farming practices for each crop is given in Table 3.2.

Effects on the weed seed bank

At the start of the experiment in 1996 and at the end in 2003, the size of the weed seed bank was determined for the 0-20 cm top soil layer of each plot. Sampling occurred at the end of March, before seed- or plantbed preparations. Twelve soil cores of 20 cm depth (3 cm diameter) were taken from the centre (30 m x 8 m) of each plot at regular intervals. The 12 samples per plot were pooled to form one sample of 3 kg fresh weight. This sample was mixed thoroughly. Next, a subsample of 300 gram was taken from the 3 kg sample for determination of seed numbers. The subsamples were all taken at one day to prevent differences in subsample weight due to moisture loss. To separate the seeds from the soil particles, an elutriator (Wiles *et al*., 1996) was used. Subsequently, seed numbers and species were determined. The total number of seeds per plot was counted, converted to numbers per m^2 and averaged over the fields for each of the management strategies

Table 3.1 Crop rotation scheme at the experimental farm "Dr. H.J. Lovinkhoeve" during this study.

	Year						
field	1996	1997	1998	1999	2000	2001	2002
1	Lucerne	Lucerne	Sugar-beet	Spring wheat	Potato	Maize	Tulip
2	Sugar-beet	Spring wheat	Potato	Maize	Tulip	Lucerne	Lucerne
3	Winter Wheat	Tulip	Lucerne	Lucerne	Sugar-beet	Spring wheat	Potato
4	Winter wheat	Potato	Winter Wheat	Tulip	Lucerne	Lucerne	Sugar-beet
5	Onion	Lucerne	Lucerne	Sugar-beet	Spring wheat	Potato	Maize
6	Lucerne	Sugar-beet	Spring wheat	Potato	Maize	Tulip	Lucerne
7	Oat	Winter Wheat	Tulip	Lucerne	Lucerne	Sugar-beet	Spring wheat

Effects on the emerging weed population

The number of emerging weed seedlings was determined each year at the end of June or at the beginning of July after the last mechanical weed control. Weeds were counted in six 0.375 m^2 quadrates (0.50 m x 0.75 m) randomly located in the centre (30 m x 8 m) of each plot.

Effects on the required manual weed control effort

To be able to compare the amount of effort dedicated to manual weed control between the strategies, the total required amount of manual labour (h) per ha was determined every year for each plot during the whole growing season.

Statistical analysis

Weed seed bank

The counted seed numbers per plot were converted to numbers per m^2. As the background populations from which the soil seed samples were drawn cannot be assumed to be normally distributed (Dessaint *et al*., 1991), variance can not assumed to be constant and tests such as the analysis of variance can not be applied (Anonimous, 1988). Therefore, the dataset was analysed by a IRREML procedure (Iterative Reweighted Residual Maximum Likelihood) assuming a Poisson distribution using Genstat statistical program 8th edition (Payne *et al*., 2005). 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 change in seed numbers per m^2 in the top layer in the period between 1996 and 2003 were calculated, for all species together and for the most abundant species separately. The differences in those changes between the strategies were, after the appropriate checks for normality, analysed using a two way ANOVA and a Fisher's L.S.D. using Genstat statistical program 8th edition (Payne *et al*., 2005).

Emerging weed population

The counted weed numbers per plot were converted to numbers per m^2 and analysed by a IRREML procedure with a Poisson distribution using Genstat statistical program 8th edition

(Payne *et al*., 2005). 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. In the first year after conversion, organic farming was relatively new to the co-workers at the experimental station and mechanical weed control methods were not optimal in this first learning year yet. Therefore, the dataset from this year, 1996, was excluded from the analysis.

Required manual weed control

The total required amount of manual labour (h) per ha was analysed by a REML procedure (Restricted Maximum Likelihood) using Genstat statistical program 8th edition (Payne *et al*., 2005). 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.
Than, the manual weed control hours per ha in strategy 2 and strategy 3 that were required in addition to the manual weed control hours per ha in strategy 1 were calculated:
add21= (manual weed control hours per ha per year in strategy 2)- (manual weed control hours per ha per year in strategy 1), and
add31= (manual weed control hours per ha per year in strategy 2)- (manual weed control hours per ha per year in strategy 3). These additional manual weed control hours were than analysed for differences between crops and years with a REML procedure, followed by a Wald test to test for main and interaction effects and t tests to test for significant difference between treatments, using Genstat statistical program 8th edition (Payne *et al*., 2005).

Results & Discussion

The weed seed bank

The seed bank size increased during seven years of organic weed management practices from about 3083 seeds per m^2 in 1996 to almost 9627 seeds per m^2 in 2003 ($P < 0.001$), averaged over all strategies. The seed bank size determined at the start of the experiment in 1996 was the result of conventional, chemical weed control since 1950.

Table 3.2 The standard organic farming practices for each crop and their average date of application at the experimental farm "Dr. H.J. Lovinkhoeve" from 1996-2002.

crop	Lucerne, 1st year	Lucerne, 2nd year	sugarbeet	spring wheat	potato	maize	tulip	winter wheat	onion	oats
ploughing (previous year)	December		December	December	December	December	December	October	December	December
stale seedbed (rotary harrow)	end of March		1st week of April	1st week of April	1st week of April**	3rd week of April			1st week of April	
seedbed preparation (rotary harrow)	1st week of May		4th week of April	3rd week of April	4th week of April	2nd week of May	October till December of previous year	2nd week of October previous year	3rd week of April	4th week of March
cutting	2nd week of June end of August 1st week of September	2nd or 3rd week of May 1st week of July 2nd or 3rd of August 1st week of October								
fingerweeder*			2nd week of May 3rd week of May							

hoeing	3^{rd} or 4^{th} week of April 3^{rd} week of May	3^{rd} week of May	4^{th} week of May	2^{nd} week of June 3^{rd} week of June 1^{st} week of July			1^{st} week of May 2^{nd} week of May	
layer of straw					one week after planting			
milling			3^{rd} week of May					
ridging	2^{nd} week of June							
harrowing		2^{nd} week of May				1^{st} week of April		1^{st} week of April
flame burning			2^{nd} or 3^{rd} week of July					
disk harrowing						1^{st} week of May 2^{nd} week of May 4^{th} week of May	3^{rd} week of July 2^{nd} week of August	1^{st} week of May 2^{nd} week of May
stubble treatment (cultivator)						2^{nd} week of September		2^{nd} week of September

**) fingerweeder was bought in 2000 and applied from 2001 onwards.*

***) only stale seedbed in potato in 1998 and 1999.*

The seed bank size in 2003 was the result of this conventional history plus the organic farming practices over the seven years in study. The observed increase in the number of seeds in the soil seed bank from 1996 to 2003 may therefore be caused by the transition to an organic farming system.

Similar effects after transition from a conventional to an organic farming system on the seed bank size were observed in other studies. Albrecht and Sommer (1998) observed an increase from 4050 seeds per m^2 to 17320 seeds per m^2 three years after the conversion, Verschwele and Zwerger (2005) found an increase from 4188 in the first year to 11528 seeds per m^2 in the ninth year after conversion. In a Norwegian study an increase from 4000 to 10000 seeds per m^2 was observed during the first six years after conversion (Sjursen, 2001).

The absence of herbicides probably plays an important role in the increase after conversion. Several studies reported that seed banks are much larger under organic management systems than under chemical systems (Hyvönen & Salonen, 2003; Barberi *et al*., 1998a;; Roberts &Neilson, 1981). More seedlings are able to survive and produce seeds in systems without or with reduced herbicide application, resulting in a larger weed seed bank (Hyvönen & Salonen, 2003; Jones & Maulden, 1999). Albrecht (2005) argues that besides the absence of herbicides, a decline in crop cover due to the increase in cultivation of less competitive crops and reduced nutrient availability may play a role in weed seed banks after conversion.

The size of the weed seed bank was strongly influenced by the main effects of field, year and strategy ($P < 0.001$). The interaction terms were not found to be significant ($P > 0.05$). Table 3.3 shows that the seed bank in 2003 was larger than the seed bank in 1996 for each strategy and that variability is very high. This high variability probably causes the absence of a significant interaction term. Therefore, the increase in seed numbers per m^2 from 1996 to 2003 was compared between strategies. Under standard organic farming practices (strategy 1), the average increase in seed bank size was more than 10000 seeds per m^2 over a period of seven years (Table 3.4). The average increase in seed bank size under strategy 2 was similar with about 9500 seeds per m^2 over the same time period (Table 3.4). So, removal of weeds growing above the crop after standard organic weed control (strategy 2) did not affect the seed bank size compared to the standard organic farming practices.

Table 3.3 Mean seed numbers to 20 cm depth before and after seven years of different weed management strategies.

	Size of seedbank (#/m^2)		
Strategy	1996	2003	SED* (df=2)
1	3460	12874	1665
2	3242	11932	960
3	2581	5411	1901

**back transformed SED values to indicate the range of the SED.*

Strategy 1: standard organic farming practice, 2: removal of weeds growing above the crop, 3: prevention of all weed seed addition to the soil. No significant differences at the 0.05 level were observed.

The number of weeds growing above the crop after standard organic farming practices was low, as a result of which no differences between the two strategies could be observed. The increase in number of seeds in plots treated with strategy 3 was on average 3048 (Table 3.4). It was expected that prevention of seed shedding would lead to a decline in the size of the soil seed bank due to germination and mortality of seeds already present in the soil and not to an increase. The aimed full control before weeds were able to produce seeds was probably not completely accomplished with this strategy. Nevertheless, the increase in seed bank size with this strategy (3) was significantly ($P < 0.002$) smaller than the increase observed under the standard organic farming practices (strategy 1) (Table 3.4).

The most abundant weed species in the seed bank in 1996 were the same as the most abundant in 2003: *Poa annua, Stellaria media, Chenopodium album and Polygonum persicaria.* The size of the seed bank of these species was not found to be affected by the applied strategies, year or field ($P > 0.05$). The species and number of species in the seed bank was in 2003 the same as in 1996. In this study we used 12 soil cores per plot to sample the weed seed bank. However, from previous research we may assume that the seeds are distributed following the Poisson or the Negative Binomial distribution.

Table 3.4 Mean changes in seed numbers to 20 cm depth after seven years of different weed management strategies.

Strategy	change in seed number per m^{-2}
1	10381^{a*}
2	9592^{a}
3	3048^{b}
SED (df)	1680 (2)

**) different characters indicate significant differences at the 0.002 level.*

Strategy 1: standard organic farming practice, 2: removal of weeds growing above the crop, 3: prevention of all weed seed addition to the soil.

This means that it probably would require more than 12 soil cores per plot to detect or estimate the seed numbers of species with a low density (Luschei, 2003; Ambrosio *et al*., 1997). Based on the mean number of seeds per sample, it would have required at least 18 soil cores per plot to estimate the number of seeds of the species with low densities accurately in this study (Dessaint *et al*., 1996). This means that the counted total number of seeds in this study may have a certain deviation of the actual number. However, since the deviation is due to species occurring at low densities that are not of main interest in this study with respect to the manual weed control effort and seed production, the estimation of the seed bank size was adequate enough for our purposes. In this study we used one of two main categories of methods for weed seed bank analysis: a method that directly extracts seeds from soil and identifies and counts them. The other method, that allows seeds present in soil to germinate and emerge for a certain period of time prior to identification and counting, is thought to estimate the species composition of a weed seed bank more accurate (Barberi *et al*., 1998b; Miele *et al*., 1998; Gross, 1990). Small seeded species are in some cases not detected with the direct seed extraction method (Barberi *et al*., 1998b) and changes in the number of seeds or the introduction of these species as a result of the applied strategies may therefore not have been observed in this study. Nevertheless, both methods are thought to estimate the total seed bank size over a soil layer equally well (Barberi *et al*., 1998b).

The emerging weed population

The number of weeds emerging after the last mechanical control treatment was not influenced by crop ($P = 0.512$) or year ($P = 0.420$). Crop type and rotation was found to influence the weed density in some previous studies (Hyvönen & Salonen, 2002 & 2003; Blackshaw *et al*., 2001; Doucet *et al*., 1999). Crops differ in their competitive ability, shading conditions, weed management, fertilization application and sowing times, and may therefore affect the species composition of weed communities (Kegode *et al*., 1999). In this study, weed densities were determined after the last mechanical control, meaning that it may not have been possible to observe differences in weed densities between crops. However, weed density was influenced by the interaction term strategy*year ($P < 0.001$). In the first years of the experiment the weed densities in plots treated with strategies 1, 2 and 3 were not significantly different from each other ($P > 0.05$). From 1999 onwards, the weed densities in plots treated with strategy 3 (that aimed at the total prevention of weed seed return), became significantly lower than the weed densities in plots treated with the other strategies (Table 3.5). The decrease in the weed densities from 1997 to 2002 was not significant for any of the strategies ($P > 0.05$).

The most commonly found weed species at the start were the same as the most abundant species during the whole study: *P. annua*, *S. media*, and *Sonchus oleraceus.* However, no differences could be detected amongst years or strategies at the individual species level ($P > 0.05$). The number of weeds per species may have been too low or the variability may have been too high to be able to detect differences between years and strategies.

The required manual weed control effort

The amount of manual labour was strongly influenced by the main effects of strategy, crop and year and the interaction terms strategy*crop and crop*year ($P < 0.001$). There was no significant effect of strategy*year ($P > 0.05$). Therefore, we analysed the manual labour in strategies 2 and 3 that was required in addition to the manual labour in strategy 1. There was no significant difference in the amount of hand weeding between strategies 1 and 2 in any of the years ($P > 0.05$). This was probably due to the low number of weeds growing above the crop after the last mechanical weed control treatments.

Table 3.5 Mean weed density m^{-2} per year for the different weed management strategies.

Strategy	1997	1998	1999	2000	2001	2002
1	15.86 ^{a*}	22.92^{a}	10.48^{a}	5.25^{a}	10.32^{a}	7.16^{a}
2	19.72^{a}	18.78^{a}	8.47^{a}	6.16^{a}	14.8^{b}	7.10^{a}
3	16.05^{a}	22.34^{a}	4.77^{b}	2.75^{b}	8.36^{a}	3.00^{b}
SED-range** [min; max]	[1.16; 2.47]					
mean SED	2.20					
df	10					

[*]values in the same column followed by a different letter are significantly different from one another at the 0.05 level, [**]back transformed SED values to indicate the range and average of the SED.

Strategy 1: standard organic farming practice, 2: removal of weeds growing above the crop, 3: prevention of all weed seed addition to the soil. No significant differences at the 0.05 level were observed.

The number of manual hours required to prevent weed seed return in addition to the manual weed control hours in standard organic farming practices was reduced from 21.14 (h ha^{-1}) in 1997 to -1.24 (h ha^{-1}) in 2001 (Table 3. 6). However, in 2002 the additional amount of weeds in strategy 3 was increased to 23.82 h ha^{-1}. This could completely be ascribed to the manual weed control effort in tulip in that year. Under strategies 1 and 2 no manual weed control was applied in tulip, while under strategy 3 around 100 hours of manual weed control were used to prevent seed shedding. Prior to growing tulip, the field was ploughed about 5 cm deeper than usual at the farm. This resulted in an increased emergence of small weeds, mainly *P. annua*. These weeds were not regarded as a threat to the yield of the tulip and not manually controlled under standard organic farming practices (strategy 1), nor under the strategy aiming at the removal of large weeds (strategy 2). Under strategy 3 however, all weeds were to be prevented from seed shedding and a lot of manual effort was required to remove these weeds.

Table 3.6 The additional manual labour required in strategy 3 (add31) compared to labour in strategy 1.

year	add31($h*ha^{-1}$)
1997	21.14[a*]
1998	32.91[b]
1999	10.45[c]
2000	-7.06[d]
2001	-1.34[d]
2002	23.82[a]
SED (df)	3.120 (5)

[*] *values in the same column followed by a different letter are significantly different from one another at the 0.05 level.*

Strategy 1: standard organic farming practice, 3: prevention of all weed seed addition to the soil.

Species diversity and composition

Previous studies report of a density increase of herbicide-sensitive species after the avoidance or reduction of herbicides (Hyvönen & Salonen, 2003; Menalled *et al*., 2001; Albrecht & Sommer, 1998) or a density increase in insensitive species after repeated application of a specific herbicide (Ball, 1992). Verschwele and Zwerger (2005) also found a small increase in the number of species in the seed bank nine years after conversion, although the species that were most abundant at the time of conversion were still the most abundant species nine years later. It was therefore expected that the diversity of species in the present study would increase after the transition, or that the species composition would be altered by the absence of the herbicides, but they were not. In the previous studies the herbicide sensitive species, although in lower densities, were already present in the seed bank before the experiments started. These results suggest that an increase in species diversity and composition may take a long time after conversion, probably more than the seven years that the present study lasted. Furthermore, the introduction of new species will depend on the environment of the farm and factors such as the presence of seeds in fertilizer. The presence of nature reserves, conventional farms or other organic farms in the vicinity of the organic farm will determine the source and variation of the species that can be introduced in a relatively short time period.

Overall

The conversion from a conventional farming system to an organic farming system resulted in an increase in the amount of seeds in the soil. This increase will probably have been the result of a reduced control of the emerging weeds in the organic system, compared to the previously applied conventional system that included herbicide application. However, we did not determine the weed density prior to weed control during the cropping season, nor did we determine the weed density prior to conversion, and can not determine whether the density of the aboveground weeds was increased after conversion or not. In previous studies it did increase after conversion (Verschwele & Zwerger, 2005). We therefore hypothesize that weed densities in the organic system were higher than in the conventional system in the present study as well.

If the seed bank was higher in 2003 than in 1996, why didn't the density of weeds increase from 1997 to 2003? The explanation lies in the improved mechanical control during the experiment. In the first years after conversion, organic farming was relatively new to the co-workers at the experimental station and mechanical weed control methods were not applied in an optimal manner (pers. comm. Andries Siepel, manager of the experimental farm "De Lovinkhoeve"). Therefore, the weed densities, which were determined after the last mechanical control, were relatively high in the first years after conversion. During the experimental period, the application of the mechanical weed control methods was improved. Due to this improved mechanical control the number of weeds remaining after the last mechanical control treatments could be maintained at a constant level, in spite of an increasing seed bank.

The weed density in the plots where strategy 3 was applied became significantly lower than the densities in plots where the other two strategies were applied from 1999 onwards. This is most likely due to the larger increase in seed bank size in strategies 1 and 2 compared to strategy 3. The amount of seeds in soil in plots where the aim was to prevent all seed shedding was lower than in plots under the other two strategies. This resulted in lower densities of emerging weeds prior to mechanical control. With similar mechanical control methods applied under all strategies, with equal efficiency, the weed density remaining after this control will be lower under strategy 3 than under strategies 1 and 2. The lower weed density under strategy 3 compared to strategy 1 resulted in a reduction of the additional amount of manual weed effort in strategy 3 compared to the standard organic farming practice (strategy 1) (Table 3.6).

Concluding remarks

Results from this study show that a management strategy aimed at the prevention of seed return can reduce the increase of the seed bank size which is usually observed after transition from conventional to organic farming. To achieve a substantial reduction of the seed bank with such a strategy, seed return will have to be prevented completely. This study shows that it will be very difficult, if not impossible to prevent seed return for a 100% in practice and reduce the seed bank size with this strategy. According to Swanton and Booth (2004) the seed bank can only be managed successfully if not only seed production is reduced, but other parts of the plant's life cycle are affected as well. A successful management system should also increase the seed mortality, manipulate the germination and emergence of weeds and remove sufficient amounts of the above-ground biomass. However, this will require a lot of effort and will sometimes be impossible to achieve due to external factors, such as unfavourable weather conditions.

Acknowledgements

We thank the manager of the experimental farm "Dr. H.J. Lovinkhoeve", Andries Siepel, and co workers for their assistance. Authors also like to thank Jacques Withagen for his statistical support.

CHAPTER 4

Predicting sublethal effects of herbicides on terrestrial non-crop plant species in the field from greenhouse data[3]

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Summary

Guidelines provided by OECD and EPPO allow the use of data obtained in greenhouse experiments in the risk assessment for pesticides to non-target terrestrial plants in the field. The present study was undertaken to investigate the predictability of effects on field-grown plants using greenhouse data. In addition, the influence of plant development stage on plant sensitivity and herbicide efficacy, the influence of the surrounding vegetation on individual plant sensitivity and of sublethal herbicide doses on the biomass, recovery and reproduction of non-crop plants was studied. Results show that in the future, it might well be possible to translate results from greenhouse experiments to field situations, given sufficient experimental data. The results also suggest consequences at the population level. Even when only marginal effects on the biomass of non-target plants are expected, their seed production and thereby survival at the population level may be negatively affected.

Keywords: Non-crop terrestrial plants, Glufosinate ammonium, Biomass, Seed production, Life cycle.

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[3] *Environmental Pollution 155 (2009), 141-149.*

Introduction

During the past two decades, the interest for vegetation on edges immediately surrounding arable fields has increased significantly. As a consequence, a great deal of concern has arisen regarding effects of pesticides on these margins, in The Netherlands (De Snoo, 1995), other parts of Europe (Marrs *et al*., 1993), Canada and the United States (Boutin *et al*., 2004). Herbicides in particular may have a large effect on such networks. These chemicals may alter biodiversity as they can affect plant species composition, diversity, development, growth, or morphology. Plants are an important part of the habitat in relation to other organisms such as birds and insects, providing them with food, shelter, and an environment to reproduce (Moreby & Southway, 1999; Freemark & Boutin, 1995). Changes in the species composition of field margins due to herbicide applications to adjacent fields have been observed in previous studies (De Snoo, 1999; Jobin *et al*., 1997; Marrs *et al*., 1989). Issues such as herbicide doses and allowable distances to field margins for spraying are currently being discussed (De Jong *et al*., 2008; De Snoo, 1995). However, insufficient knowledge is available on the effects of sublethal doses of herbicides on non-target, non-crop terrestrial plants required to estimate these distances and doses. The European and Mediterranean Plant Protection Organization (EPPO) Council has provided a standard for the environmental risk assessment of plant protection products such as pesticides to non-target terrestrial higher plants with a tiered approach (European and Mediterranean Plant Protection Organization, 2003). They present a definition of a non-target plant that we will use in this paper: a non-crop plant located outside the treatment area. However, a number of factors that need to be dealt with during the collection of data required for the risk assessment are not described in this approach. Risk assessments of herbicide phytotoxicity are often performed with data obtained from greenhouse experiments with single plant species, which may over- or underestimate effects. The advantage of greenhouse experiments is that they can be easily standardized. To date, very few studies have investigated the possibilities of directly predicting effects in the field from greenhouse data (Wright & Thompson, 2001; Breeze *et al*., 1992; Fletcher & Johnson, 1990). Those few used greenhouse and field data that originated from different studies in which either the plant species or origin of the plants differed. A direct comparison was not made in any of these studies. Such knowledge may be very useful during the development of risk assessment protocols. The present study was undertaken to investigate the predictability of effects on field-grown plants using greenhouse data and to investigate the effects on vegetation assemblages, so-called mesocosms, in a greenhouse. In addition, the sensitivity of plants during different developmental stages, their recovery and effects on the next generation (seed production and germination) were addressed.

Materials and Methods

Experiment 1: Sublethal effects of glufosinate ammonium on four non-crop species

To test the effects of glufosinate ammonium on the aboveground biomass, seed production, seed germination and recovery of different species grown in the greenhouse and in the field, seeds of *Chenopodium album*, *Stellaria media*, *Poa annua*, and *Echinochloa crus-galli* were obtained from a commercial seed supplier (Medigran, Hoorn, The Netherlands, http://www.medigran.nl). Seven hundred and sixty-eight 0.5-L pots were filled with a peat:sand mixture (2:1). Seeds of the four species were scattered over the soil surface (one species per pot) and covered with a thin layer of sifted soil. The species were sown in such a manner that emergence of all species would coincide. Half of the pots were randomly arranged in four blocks in a greenhouse (day/night temperature 18/12 °C and a 16/8 h light/dark period) and watered in trays. The other half was randomly placed in four blocks in a field adjacent to the greenhouse located in Wageningen, The Netherlands. The experiment started in the first week of May 2005. After emergence the number of plants was thinned to four per pot. Half of the pots from both the greenhouse and the field were sprayed 2 weeks after emergence (WAE) with glufosinate ammonium and the other half at 4 WAE. Four pots per treatment remained unsprayed as control. After treatment, the plants were placed back in the position they were in prior to treatment. Glufosinate ammonium is a broad-spectrum contact herbicide and is used to control a wide range of weeds after crop emergence, or for total vegetation control on noncultivated land. It inhibits the activity of the enzyme glutamine synthetase which causes the accumulation of ammonia, leading to cell destruction and inhibition of photosynthesis. Field-grown plants of all species were of a similar size as greenhouse-grown plants at 2 WAE. Dosages were 0, 0.04, 0.2, 0.4, 2, and 4 L Finale per ha (which corresponded with doses of 0, 6, 30, 60, 300 and 600 g glufosinate ammonium per ha). The recommended field dose for this herbicide ranges from 3 to 5 L per ha, depending on the crop. The expected deposition of glufosinate ammonium when Finale is applied at 3 L per ha, varies from 111.0 g active ingredient (a.i.) per ha at 1 m, 36.2 g a.i. per ha at 4 m to 3.86 g a.i. per ha at 10 m from the field edge (calculated with the IDEFICS model (Holterman *et al*., 1997)). The herbicide was applied in a 4 m wide by 2 m deep spray chamber. The sprayer consisted of a 1 m wide movable spray boom with three Teejet XR11004 (class Medium spray quality) flat fan nozzles (Spraying Systems Co., Wheaton, IL, USA, http://www.teejet.com) that delivered 400 L per ha. The height between nozzle and the soil surface of the pots was 50 cm. Fresh weight was used as effect parameter. A preliminary comparison between the effect of glufosinate ammonium on the fresh and dry weight of plants did not show significant differences (Riemens *et al*., 2004). Furthermore, the effects of sublethal doses on fresh and dry shoot weight of *Brassica napus* in a previous study carried out in both greenhouse as well as field were highly correlated and the coefficients of variance were similar for dry and fresh weight, (De Jong & Udo de Haes, 2001). The aboveground fresh weight of all species was determined at 4 weeks after treatment (WAT) and at seed setting (SS). For *C. album* and *S. media* plants seed setting already occurred at 4 WAT, so all plants were harvested at that moment, both in the field as well as in the greenhouse. Seeds were collected and counted per pot. After storage at 10 °C in a dark room in which they were shielded from light and moisture, four lots of 20 seeds were randomly chosen per pot for a germination experiment. Germination tests were conducted in a greenhouse at day/night temperatures of 24/12 °C and a 16/8 h light/dark period. Each seed lot was allowed to germinate in a plastic pot (6 x 5 x 5 cm) filled with sterilized soil. Germinated and emerged seeds were regularly counted and removed from each pot for 21 days. Experiments were conducted from May 2005 until February 2006.

Experiment 2: Effects of glufosinate ammonium on mesocosms

Mesocosms were composed of eight species in 5 L pots filled with a peat/sand mixture (2:1). Each mesocosm consisted of four monocotyledons, *P. annua*, *E. crus-galli*, *Elymus repens*, *Panicum milliaceum*, and four dicotyledons, *Solanum nigrum*, *S. media*, *C. album*, and *Centaurea cyanus*. All seed reproducing species were seeded in such a manner that emergence of the species would coincide. Since *E. repens* reproduces vegetatively, cuttings of the root system were placed into the soil in such a manner that its emergence would coincide with the emergence of the other species. Monocotyledons and dicotyledons were placed alternately in the pots and thinned to eight plants per species per pot after emergence. The experiment started in May 2004. The pots were randomly arranged in a greenhouse with a day/night temperature of 18/12 °C and a 16:8 h light:dark period, and were watered in the trays. At 4 weeks after emergence the pots were sprayed with the same herbicide and doses and in the same manner as in Experiment 1. The first visual symptoms of herbicide injury were recorded at 2 days after treatment based on four categories: no visible injury (1), yellow spots or leaftips (2), yellow spots or leaftips and wilting of the plant (3), and necrosis of plant tissue (brown coloration) and wilting of the plant (4). After 4 weeks, the total fresh weight of the eight plants from each species per pot was determined.

Statistical analysis

Experiment 1: Sublethal effects of glufosinate ammonium on four non-crop species

Fresh weight

The aboveground fresh weight reduction compared to the control per dose was calculated for each plant species and analyzed using nonlinear regression analysis with a logistic growth curve: $y = c + (d - c)/(1 + e^{-b(\log(dose) - \log(e))})$ (Seefeldt *et al*., 1995) with four parameters b, c, d, and e. The lower limit, c, was set at 0. The upper limit (d), the slope (b) and the ED50 (dose at which an effect of 50% can be observed) (e) were estimated. Because the fresh weight reduction compared to the control was plotted on the y-axis the values of the upper limit, d, were always estimated around 1 and not significantly different between treatments. Regressions were performed using the statistical program R (Team RDC, 2005, http://www.R-project.org), as described by Nielsen *et al*. (2004), and Ritz & Streibig (2005). Parameter estimates were compared using a two way analysis of variance using Genstat 8th edition (Payne *et al*., 2005). Fisher's Least Significant Difference test was used to compare means.

Comparison of effects on field and greenhouse-grown plants

The estimated ED10, 20,.,90-values of the greenhouse grown plants were log-transformed and plotted against the log-transformed ED10,20,.,90-values of the field-grown plants. The relationship was analyzed with linear regression analysis using Genstat 8th edition (Payne *et al*., 2005) for all species together and for each species separately.

Seed production and emergence

The percentage seed production and the percentage seedling emergence, both relative to the control, were calculated per dose for each plant species, location and age if seed production was sufficient for analysis. The percentages were arcsine transformed (Sokal & Rohlf, 1981). After the appropriate checks for normality, a two-way analysis of variance with a randomized block design was used. Fisher's Least Significant Difference test was used to compare means. Box plots were made with the number of seeds per plant fresh weight (g) on the y-axis and the glufosinate ammonium dose (g a.i./ha) on the x-axis to compare the reduction in seed production with the reduction in plant fresh weight per dose.

Experiment 2: Effects of glufosinate ammonium on mesocosms

The aboveground fresh weight reduction per dose was calculated for each plant species, the total vegetation, the dicotyledons and the monocotyledons in the mesocosms and analyzed using nonlinear regression analysis as described above for fresh weight in Experiment 1. The effect of surrounding vegetation was studied for plants sprayed with glufosinate ammonium at 4 weeks after emergence and harvested at 4 weeks after treatment. The doses at which plants show a certain effect level (the ED10, 20,.,90- values) were compared between individually greenhouse-grown plants and plants of the same species grown in the vegetation assemblages. In order to make this comparison, selectivity indices (Ritz & Streibig, 2005), defined as the ratio between the effective dose for a species in a vegetation and the effective dose for the same species grown individually, were calculated for each species and plotted against the corresponding effect level. If this ratio is larger than 1, the species will benefit from the surrounding vegetation, if the ratio is smaller, the effects of the surrounding vegetation on the sensitivity of the species will be negative.

Results

Experiment 1: Sublethal effects of glufosinate ammonium on four non-crop species

Effect of plant development stage on sensitivity

The ED50-values of the dose-response curves of field-grown plants sprayed in an earlier developmental stage (2 WAE) were significantly ($p < 0.05$) smaller than the ED50-values of field-grown plants sprayed in a later stage (4WAE) for *S. media*, *E. crus-galli* and *C. album* (Table 4.1). This indicates that field-grown plants are more sensitive to glufosinate ammonium when treated in an earlier developmental stage than when treated in a later developmental stage. However, there was no difference between the ED50-values of *P. annua* field grown plants sprayed in different developmental stages (Table 4.1). Greenhouse-grown plants showed no effect of plant development stage on their sensitivity; the ED50-values between plants sprayed at 2 WAE and 4 WAE did not significantly differ (Table 4.1).

Recovery of plants

Recovery was studied by comparing the fresh weight of the plants harvested at 4 WAT with the fresh weight of plants of the same species at SS. Only the recovery of the monocotyledons, *E. crus-galli* and *P. annua*, was studied since seed setting of the dicotyledons coincided with the first measurement time at 4 weeks after treatment and this required harvesting of those plants. None of these plants were able to recover significantly (Table 4.1). The ED50-values at 4 weeks after treatment did not differ from the ED50-values at seed setting ($p > 0.05$), except for the ED50-values of field-grown *P. annua* plants treated in a later developmental stage for which the effect was larger at SS than at 4 WAT.

Table 4.1 Experiment 1: Fresh weight reduction compared to the control. Parameter estimates ± SE (standard error) of dose response curves of the reduction of the aboveground fresh weight of greenhouse grown and field grown plants sprayed at two and four weeks after emergence (2 and 4 WAE) and harvested at four weeks after treatment (4 WAT) and at seed setting (SS) relative to the control treatment, versus glufosinate ammonium dose. Regression equation: $Y= d/(1+e^{-b(log(dose)-log(e))})$.

		parameter estimate ± SE	
species	treatment	slope (*b*)	ED50 (*e*) (g active ingredient/ha)
Chenopodium album	field 2 WAE 4 WAT	1.93 ± 1.05** ^{b}	72.0 ± 7.36* ^{b}
	field 4 WAE 4 WAT	0.73 ± 0.16* ^{b}	430.1 ± 52.49* ^{a}
	greenhouse 2 WAE 4 WAT	6.60 ± 2.09** ^{a}	38.1 ± 0.85** ^{b}
	greenhouse 4 WAE 4 WAT	0.87 ± 0.10** ^{b}	51.4 ± 3.87** ^{b}
Poa annua	field 2 WAE 4 WAT	0.94 ± 0.54* ^{cd}	294.1 ± 76.53** ^{bc}
	field 2 WAE SS	1.00 ± 0.83* ^{cd}	276.11 ± 182.41** ^{bcd}
	field 4 WAE 4 WAT	0.68 ± 0.21* ^{d}	696.1 ± 19.97* ^{a}
	field 4 WAE SS	1.59 ± 0.61* ^{bc}	405.9 ± 63.13* ^{b}
	greenhouse 2 WAE 4 WAT	2.52 ± 0.69** ^{a}	65.9 ± 2.47*** ^{e}
	greenhouse 2 WAE SS	1.75 ± 0.20** ^{b}	108.8 ± 9.99*** ^{de}
	greenhouse 4 WAE 4 WAT	1.06 ± 0.14* ^{bcd}	211.8 ± 27.28*** ^{cde}
	greenhouse 4 WAE SS	1.35 ± 0.38* ^{bc}	276.4 ± 19.59*** ^{bcd}
Echinochloa crus-galli	field 2 WAE 4 WAT	1.34 ± 0.78* ^{a}	248.3 ± 39.19* ^{b}
	field 2 WAE SS	1.25 ± 0.49* ^{a}	336.8 ± 70.87* ^{b}
	field 4 WAE 4 WAT	1.40 ± 1.05* ^{a}	689.0 ± 86.89* ^{a}
	field 4 WAE SS	1.15 ± 0.68* ^{a}	590.7 ± 10.78* ^{a}
	greenhouse 2 WAE 4 WAT	1.68 ± 0.45* ^{a}	28. 1 ± 2.53**^{c}
	greenhouse 2 WAE SS	1.58 ± 0.22** ^{a}	62.1 ± 14.83** ^{c}
	greenhouse 4 WAE 4 WAT	2.45 ± 0.77* ^{a}	58.36 ± 5.87*** ^{c}
	greenhouse 4 WAE SS	2.10 ± 0.88* ^{a}	56.8 ± 13.23** ^{c}
Stellaria media	field 2 WAE 4 WAT	2.66 ± 1.18*^{a}	180.9 ± 38.53* ^{b}
	field 4 WAE 4 WAT	0.66 ± 0.25* ^{a}	356.1 ± 56.36* ^{a}
	greenhouse 2 WAE 4 WAT	1.08 ± 0.52** ^{b}	23.5 ± 1.81** ^{c}
	greenhouse 4 WAE 4 WAT	0.49 ± 0.07***^{b}	19.8 ± 2.80** ^{c}

$^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$

Different letters within a column within a species indicate significant differences at the 5% level.

Comparison of effects on field and greenhouse-grown plants

The dose-response curves for all species differed significantly ($p < 0.05$) for the plants grown in the greenhouse and the plants grown in the field (Table 4.1). The greenhouse-grown plants had a smaller ED50-value than the field-grown plants and were more affected at high doses than were the field-grown plants. The relation between the log-transformed ED10, 20,.,90 -values from the greenhouse data and the log-transformed ED10, 20,.,90-values of the field data is shown in Figure 4.1. The parameter estimates per species are shown in Table 4.2. These data show that a linear relationship exists between the ED-values of greenhouse and field-grown plants treated with glufosinate ammonium on a logarithmic scale for each species. The results were compared with a previous field study with glufosinate ammonium on established vegetations containing both dicotyledons and monocotyledons. In that study the no observed effect concentration (NOEC) was 256 g.a.i./ha (De Snoo *et al*., 2003). We predicted the greenhouse dose corresponding with this NOEC to be 52 g a.i./ha with a 95% confidence interval of 31-89 g a.i./ha. This is consistent with the actual NOEC value of 68 g a.i./ha for the total aboveground weight. This value lies well within the calculated greenhouse range for the total aboveground biomass from the field data of De Snoo *et al*. (2003).

Table 4.2 Experiment 1: comparison of effects on field and on greenhouse grown plants. Parameter estimates ± SE (standard error) of the relationship between the greenhouse and field ED10,20,….- 90- values for the individual species:
log(greenhouse ED-value)=a + b(log(field ED-value)).

	parameter estimates ± SE		
species	*a (constant)*	*b (slope)*	R^2
Poa annua	0.97 ± 0.12***	0.47 ± 0.05***	0.74
Echinochloa crus-galli	0.26 ± 0.18*	0.55 ± 0.07***	0.89
Chenopodium album	0.39 ± 0.18*	0.56 ± 0.07***	0.80
Stellaria media	1.19 ± 0.20***	1.10 ± 0.08***	0.83

****p<0.001, **p<0.01, *p<0.05*

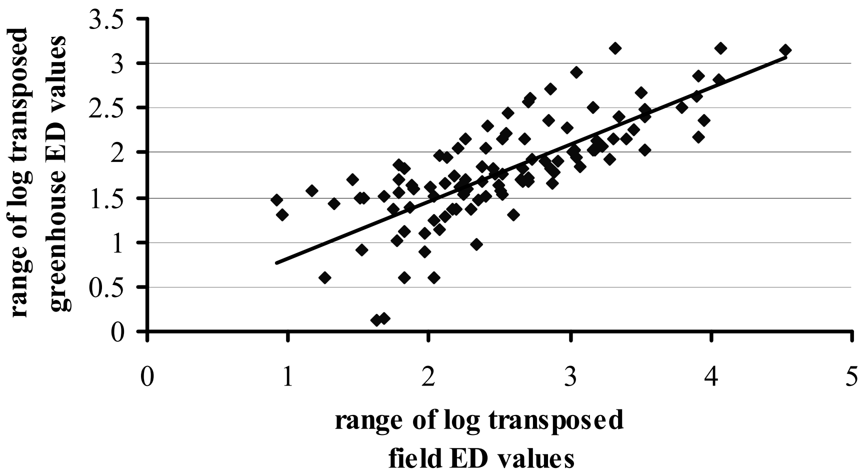

Figure 4.1 The linear relationship between the ED10, 20,., 90-values of greenhouse-grown plants and field-grown plants for all species on a logarithmic scale: log(greenhouse ED-value) = 0.1989 + 0.6314 x (log(field ED-value)), with R^2 = 0.566.

Effects on seed production and seedling emergence

Stellaria media was the only species that produced enough seeds in the greenhouse for analysis. Seed production of young plants (treated at 2 WAE) was similar to that of older plants (treated at 4 WAE) ($p = 0.972$). Therefore, seed production of both groups was analyzed as one. Seed production was strongly affected by glufosinate ammonium dose ($p < 0.001$). Seed production was already reduced at the lowest dose and no seeds were produced at all at the highest dose (Table 4.3). The relative effect (%) on seed production was greater than that on fresh weight (%) (Figure 4.2). Seedling emergence was unaffected for seeds from young treated plants. Seedling emergence was reduced with increasing dose (Table 4.3) ($p < 0.001$) for seeds produced by older treated plants.

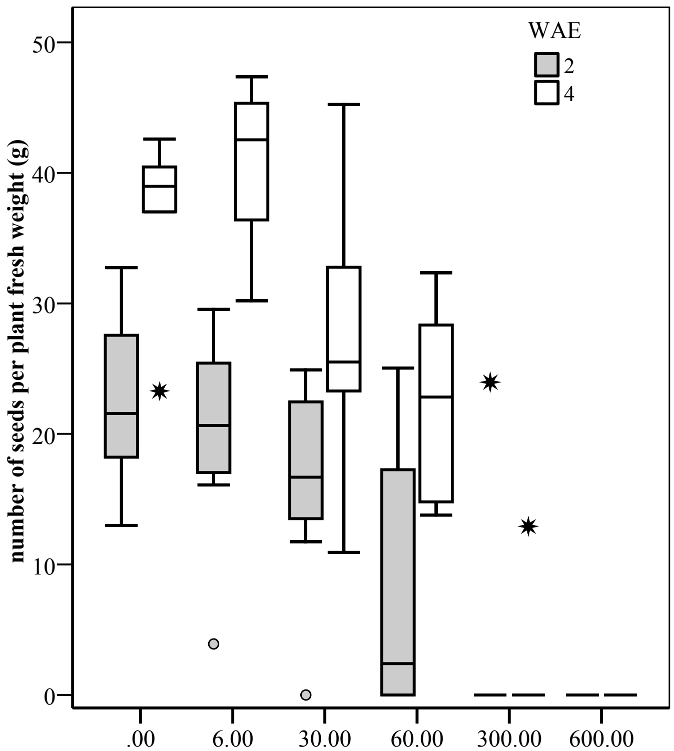

Figure 4.2 Box plot of the number of seeds produced per gram plant fresh weight of the plants sprayed at 2 weeks after emergence(2WAE) and the plants sprayed at 4 weeks after emergence (4WAE) versus sublethal doses of glufosinate ammonium.

Experiment 2: Effects of glufosinate ammonium on mesocosms

Visual symptoms and effects on biomass

The first visual symptoms on the species in the mesocosms were observed at 2 days after treatment. No visual symptoms were observed at the two lowest doses. At 30 g active ingredient/ ha (a.i./ha), *P. annua* showed no visual effects, while *E. repens*, *C. album*, *S. nigrum*, and *S. media* had yellow spots, *P. milliaceum* and *E. crus-galli* had yellow leaftips, and *C. cyanus* had yellow spots and was wilting. At doses of 60 and 300 g a.i./ha all species showed yellow spots and were wilting. At the highest dose applied, necrotic spots appeared on the leaves of all species. The ED50-value of the monocotyledon-curve was significantly ($p < 0.001$) higher than the ED50-value of the dicotyledon- curve (parameters of the dose-response curves are shown in Table 4.4). No significant differences were found for the slopes of the curves ($p > 0.05$), indicating that the monocotyledons in the mesocosms were less affected by glufosinate ammonium treatments compared to the dicotyledons in the same mesocosms. The estimated parameters of the dose-response curves for individual species (Table 4.4) confirm the trend that monocotyledons in a vegetation are less affected by sublethal glufosinate ammonium doses than the dicotyledons in the same vegetation. Except for *S. nigrum* which had a higher ED50 than those of *P. annua* and *E. crus-galli*, the ED50-values of all individual monocotyledons were higher than the ED50-values of the individual dicotyledons.

Effect of surrounding vegetation

The selectivity indices remained constant and below one for *C. album*: the dose before a certain effect can be observed is always five times lower when the species is grown in a mixture, compared to the single species situation (Figure 4.3). Glufosinate ammonium reduces the biomass of dicotyledons more than the biomass of monocotyledons at similar doses. Therefore, it is disadvantageous for *C. album* plants to grow in mixtures with monocotyledons. However, for *S. media* plants it is advantageous to grow in a mixture at low effect levels (that is at low doses), even when monocotyledons are present (Figure 4.3). *S. media* is a small plant that probably receives less of the applied dose due to the shelter provided by the other species when grown in a mixture. At a certain point, the applied dose reaches a threshold above which the provided shelter becomes insufficient and the competitive ability of *S. media* will be reduced compared to that of the monocotyledons. At high doses it will be disadvantageous for *S. media* to grow in mixtures containing monocotyledons. The monocotyledons in the mixture, *P. annua* and *E. crus-galli*, respond in an opposite way; at high doses it will be advantageous to grow in a mixture with dicotyledons, whereas it will be disadvantageous at low dosages. Thus, the ratio for most effect levels was significantly ($p < 0.05$) different from 1, indicating a species-specific response to the habitat, i.e., grown in a vegetation or grown individually (Figure 4.3), indicating that results from single species experiments can not be translated to effects on these species in mixtures.

Table 4.3 Experiment 1: Seed production and emergence. Back transformed percentages of seed production relative to the control treatment per glufosinate ammonium dose for greenhouse grown Stellaria media plants sprayed at two and four weeks together, and back transformed percentages of seedling emergence per glufosinate ammonium dose for seeds from greenhouse grown Stellaria media plants sprayed at two and four weeks after emergence (WAE).

		% seedling emergence	
dose (g active ingredient/ha)	% seed production relative to control	young plants (2 WAE)	older plants (4 WAE)
0	100^{a}	95.42✖	94.28✖
6	66.98^{b}	98.22✖	95.92 ✖
30	41.96^{c}	98.59✖	79.62◆
60	18.89^{d}	95.69✖	81.22◆
300	1.40^{e}	95.63✖	52.44†
600	0^{e}	96.87✖	83.44◆
Fisher's LSD	17.60	13.33	

Different letters indicate significant differences at $p<0.05$ for seed production. Different symbols indicate significant differences at $p<0.05$ for seedling emergence.

Discussion

Comparison of effects on field-grown plants and on greenhouse-grown plants

The current study shows that the aboveground biomass of greenhouse-grown plants is more affected by glufosinate ammonium than that of field-grown plants. The difference in sensitivity between greenhouse and field-grown plants to glufosinate ammonium may be a result of differences in environmental conditions that promote plant growth rate, such as temperature, relative humidity and light intensity (Riethmuller-Haage, 2006; Petersen & Hurle, 2001). Previous studies investigated the influence of these climatic conditions on glufosinate ammonium efficacy on *Galium aparine* and *Brassica rapa* in the greenhouse. Both low relative humidity (Anderson *et al*., 1993) and low light intensity (Petersen & Hurle, 2001) reduced the performance of glufosinate ammonium. The higher efficacy at a high relative humidity may be due to the hydration of the cuticle. Watersoluble compounds such as glufosinate ammonium penetrate the cuticle more easily when it is hydrated (Price, 1982). A low relative humidity, e.g. at field conditions, results in a reduced uptake by the cuticle (Petersen & Hurle, 2001) and hence less efficacy. The influence of light intensity on the efficacy of glufosinate ammonium can be attributed to the production of toxic ammonia during photorespiration that takes place at high light intensities (Wallsgrove *et al*., 1983). The light intensity under field conditions is usually higher than in the greenhouse (Petersen and Hurle, 2001) making glufosinate ammonium more effective outdoors. In their greenhouse study with temperatures ranging from 12 to 24 °C, Petersen and Hurle (2001) found no temperature effect on the efficacy of glufosinate ammonium. However, earlier studies showed a reduced efficacy at temperatures below 10 °C (Mathiassen & Kudsk, 1993; Anderson *et al*., 1993; Langelüddeke *et al*., 1988; Donn, 1982).

Table 4.4 Parameter estimates ± SE (standard error) of the relationship between the aboveground fresh weight of the total vegetation, the monocotyledons, dicotyledons and individual species in the mesocosms sprayed with sublethal doses of Finale versus glufosinate ammonium dose. Regression equation: $Y= d/1+e^{-b(log(dose)-log(e))}$.

	parameter estimates ± SE	
species	slope (*b*)	ED50 (*e*) (g active ingredient/ha)
total vegetation	1.14 ± 0.06***	41.49 ± 4.88***
monocotyledons	1.30 ± 0.11***	91.45 ± 9.71***
dicotyledons	1.23 ± 0.07	25.92 ± 3.57***
Poa annua	0.54 ± 0.19**	52.44 ± 33.59
Panicum milliaceum	1.85 ± 0.36***	58.16 ± 12.45***
Echinogloa crus-galli	1.24 ± 0.29***	45.94 ± 16.49**
Elymus repens	1.99 ± 0.57***	203.66 ± 48.99***
Chenopodium album	0.80 ± 0.23***	5.80 ± 4.79*
Centaurea cyanus	0.78 ± 0.19***	6.87 ± 4.61*
Solanum nigrum	1.57 ± 0.33***	53.36 ± 14.92***
Stellaria media	0.99 ± 0.27***	14.35 ± 7.75*

****p<0.001, **p<0.01, *p<0.05*

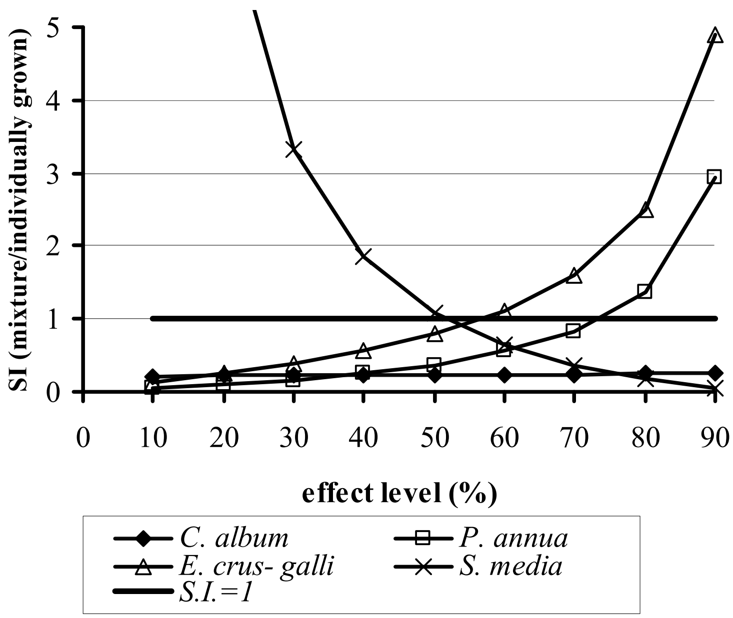

Figure 4.3 Selectivity indices (ratio of effective dose of species grown in artificial vegetations/effective dose of the same species grown individually) versus effect level of plants treated with sublethal doses of glufosinate ammonium.

In the present study, the temperature in the greenhouse varied from 12 to 18 °C (night/day), whereas the temperature in the field reached temperatures well below 10 °C at night, with a maximum temperature of around 18 °C during daytime. Although light intensity was higher under field conditions than under greenhouse conditions in the present study, the relative humidity and the temperature were lower in the field. We hypothesize that the effect of a low relative humidity and temperature in the field had more influence on the efficacy of the glufosinate ammonium than the higher light intensity. Together with a different structure and/or chemical composition of the cuticle of the field-grown plants this may have resulted in a lower efficacy on the field-grown plants compared to the greenhouse-grown plants. A relationship was found between the doses resulting in certain effect levels on aboveground fresh weight of greenhouse- grown plants and the doses corresponding to the same effect levels on the aboveground fresh weight of plants grown in the field (Table 4.2). This relationship was not only found at the individual species level but was also valid for all species tested together (Figure 4.1). In wild plant species, the genetic variability within a species can be large between populations from different locations as well as within a population from specific location. In this study the plants growing in the greenhouse and the field were from the same seed lot, ruling out the genetic variability that may exist between locations, but taking the variability within a location into account. The benefit of this approach is that results are less variable. A main issue for the future is, however, whether it will be possible to use plants from one location in a greenhouse experiment for risk assessment to represent the response of the entire species in the field or that the genetic variability between locations will be too large. The relationship (Figure 4.1) can be used for the total biomass of vegetation composed of several species or for single plants, but not for the prediction of effects on individual species in vegetations. The doses at which certain effects could be observed for species in the vegetations differed from the doses at which the same effects could be observed for those species when grown individually. Depending on the species, these effects increased or decreased with dose (Table 4.1). In previous studies, effects on individual species grown in a vegetation were either difficult to determine in the field (De Snoo *et al*., 2003) or depended strongly on the species composition (Marrs & Frost, 1997) and the herbicide used (Marshall, 1988). Differences between the response of individually grown species and the same species grown in a mixture can have several causes.

First of all, the competitive ability of species present in a mixture can be affected by the herbicide treatments and differ per species. As a result, some species will benefit from a higher competitive ability while others will experience an increased competition from the surrounding species for resources. Secondly, some species may benefit from the sheltering effect of other species present during herbicide application, and thus have a reduced exposure. Thirdly, the presence or absence of monocotyledons in the vegetation is known to influence the response of the dicotyledons in a mixture. Marrs and Frost (1997) found that the dicotyledons in their mesocosms responded differently in the presence or absence of grasses in the mixture. In the present study, the mesocosm experiments show that glufosinate ammonium reduced the aboveground weight of the dicotyledons more strongly than that of the monocotyledons, affecting a shift in the species composition. Larger effects on species composition can be expected for herbicides that have a more specific mode of action, targeting specifically on mono- or dicotyledons. However, since it is impossible to separate the effects of herbicides on the inter- and intraspecific interference with neighbors from the effect of shelter (Marrs *et al*., 1993), it is not possible to determine the contribution of the herbicide to the changes in species composition or changes in the biomass of the individual monocotyledons or dicotyledons. As a result, the prediction of herbicide effects on species in vegetation based on single species experiments is not yet possible.

Plant development stage and reproduction

Plant development stage played a role in the determination of the plant sensitivity on three out of four species in the present study and elsewhere on several other species treated with metsulfuron methyl (Boutin *et al*., 2000), chlorsulfuron (Fletcher *et al*., 1996), glyphosate (Ruiter *et al*., 2000; Marrs *et al*., 1991), MCPA and mecoprop (Marrs *et al*., 1991). Seedlings and young plants were generally more sensitive than older plants. A natural vegetation usually consists of plants in different developmental stages and the balance between young and old plants, the season, and germination period will determine herbicide efficacy. The difference in sensitivity between younger and older plants was significant for field, but not for greenhouse-grown plants. We hypothesize that this is due to cuticle differences of the plants. The cuticle of young plants differs not only in thickness, but also in chemical composition and fine structure from the cuticle of older plants. These components determine the permeability of the cuticle for herbicides and are influenced by environmental conditions. So the cuticle is determined by plant age itself as well as the environment, thereby causing differences in sensitivity to herbicides. For greenhouse-grown plants, age was the only difference between younger and older plants. The leaves of younger and older plants in the field, however, not only differ in age, but also in environmental conditions experienced during development. So differences in sensitivity are easier to detect between field-grown plants of different developmental stages than between greenhouse-grown plants of different developmental stages. To determine effects of herbicides in the long term, reproduction is an important factor (Zwerger & Pestemer, 2000). We were unable to compare the seed production between species or between greenhouse and field-grown plants, because only greenhouse-grown *S. media* plants produced enough seeds for analysis. Glufosinate ammonium strongly reduced the seed production of both young and old *S. media* plants. The seed production was reduced more strongly than the aboveground weight at the same doses, in accordance with previous results on chlorsulfuron (Fletcher *et al*., 1996) and MCPA (Andersson, 1994). These results suggest specific consequences at the population level. Although only marginal effects are to be expected on the biomass, seed production and thereby survival at the population level can be negatively affected. Seedling emergence was also reduced, although not for seeds from plants treated at an early developmental stage. Older plants treated with high doses produced seeds that showed reduced germination and emergence. Results from previous studies support our conclusion that seed production can be affected and that effects on seedling emergence are likely to occur as a result of glufosinate ammonium exposure. These effects were, however, species and herbicide dependent. Andersson (1994) showed that the seed production of *Bilderdykia convolvulus*, *C. album*, *Myosotis arvensis*, and *Thlaspi arvense* was reduced by MCPA, while the seed production of *Chamomilla recutita* and *Galium spurium* remained unaffected. He did not find a reduction in seed size. In another study, fluroxypyr reduced the number of large seeds and increased the number of small seeds produced by *Veronica persica* (Champion *et al*., 1998), possibly due to desiccation. Furthermore, the germination percentage of *V. persica* was found to increase with increasing seed size and therefore decreased with increasing dose (Champion *et al*., 1998). In the present study, the size of seeds from younger treated plants did not differ between doses ($p > 0.05$), whereas the size of seeds from older treated plants decreased with increasing glufosinate ammonium dose ($p < 0.001$), also possibly due to desiccation. Plants treated in an earlier stage may have been able to recover from desiccation before seed production and thus could produce seeds of a normal size.

Ways of exposure of plants to herbicides in non-target areas

Plants in non-target areas can be exposed to herbicides via the air, or via run-off. Because the most likely route for most herbicides is exposure through droplet drift (European and Mediterranean Plant Protection Organization, 2003), we choose to simulate drift exposure by spraying the plants and did not consider vapors. However, under certain circumstances, and for some groups of herbicides, volatilization can play an important role (Wittich & Siebers, 2002; Franzaring *et al*., 2001; Schweizer & Hurle, 1996) and tiered risk assessment protocols for vapor phase toxic compounds have been developed (Dueck, 2003). The effects of a single application were investigated, ignoring possible cumulative effects of repeated exposures. Repeated exposures can be important since some herbicides are applied to the same field more than once during a growing season. We recommend this aspect be investigated in future research.

Conclusion

The risk assessment guideline proposed by the European and Mediterranean Plant Protection Organization (2003) starts with a requirement of exposure studies of six plant species to a single-dose application of a product and then continues with the development of doseeresponse curves, all in the greenhouse. The relationship between the effects on greenhouse and field-grown plants found in the present study, shows that it might be possible to translate results from greenhouse experiments to field situations in the future. At this moment, however, the relationship was only found for total vegetation and for single species, but not for species grown in a mixture. Furthermore, mainly annual species were used in the experiments because of practical considerations. However, arable field boundary vegetation is known to be composed of both annual and perennial species (Kleijn & Verbeek, 2000). Before we can adopt the use of greenhouse data to predict the effects on vegetations in the field, we will have to investigate which endpoints and exposure time are most suitable for the determination of short- and long-term effects on perennial species.

Acknowledgments

We thank André Uffing and Roel Groeneveld for their technical assistance. The research described in this article was carried out within the Research Program ‘‘Environmental Risks and Emission Reduction of Pesticides’’ (Program 416) financed by the Dutch Ministry of Agriculture, Nature and Food Safety.

CHAPTER 5

Sublethal effects of herbicides on the biomass and seed production of terrestrial non-crop plant species, influenced by environment, development stage and assessment date[4]

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Summary

Guidelines provided by the OECD and EPPO allow the use of single species tests performed in greenhouses to assess the risk of herbicides to non-target terrestrial plant communities in the field. The present study was undertaken to investigate the use of greenhouse data to determine effects of herbicides with a different mode of action on the biomass, seed production and emergence of field-grown plants. In addition, a single species approach was compared with a mixed species approach. Effects on the biomass of greenhouse and field-grown plants were found to be related at different effect levels, indicating that it might be possible to translate results from greenhouse studies to field situations. However, the use of single species tests may not be valid. The response of a single plant species to sublethal herbicide dosages differed to the response of the same species grown in a mixture with other species.

Keywords*:* Non-crop terrestrial plants; Tepraloxydim; Greenhouse; Field; Biomass

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[4] *Environmental Pollution 157 (2009), 2306-2313.*

Introduction

It is well known that herbicide application can pose a risk for the vegetation surrounding an arable field (De Jong *et al*., 2008; De Snoo, 1999; Jobin *et al*., 1997; Marrs *et al*., 1989). The availability of methodologies to assess the environmental risk of plant protection products such as herbicides to non-target terrestrial higher plants is currently limited. Non-target plants are non-crop plants located outside the treatment area (European and Mediterranean Plant Protection Organization, 2003). Recently several researchers underline the importance of the development of those methods (Damgaard *et al*., 2008; Olszyk *et al*., 2008). From a practical point of view, the use of greenhouse data in future risk assessment methodologies is preferred since greenhouse experiments can be standardized to a higher degree than field experiments. To date, only a few empirical studies have investigated the effects of herbicides on non-target plants (Boutin *et al*., 2004; Franzaring *et al*., 2001; Zwerger & Pestemer, 2000; Fletcher *et al*., 1996;) and even less have compared the effects of herbicides at sublethal dosages on greenhouse and field grown plants (Wright & Thompson, 2001; Mathiassen *et al*., 2000; Breeze *et al*., 1992; Fletcher & Johnson, 1990). In a previous study we compared the effects of a broad-spectrum herbicide, glufosinate ammonium, on field and greenhouse grown plants of several species (Riemens *et al*., 2008 and Chapter 4) and were able to find a relationship which could be used to calculate effects on field grown plants from greenhouse data for that herbicide. In the present study we hypothesize that this type of relationship will be valid for a small spectrum herbicide as well. We designed two experiments to answer the following research questions: 1) Can a similar relationship between the sublethal effects on greenhouse and field grown plants be found for a small spectrum herbicide? 2) Can plants recover before seed setting? 3) What is the effect of sublethal dosages on the next generation? and 4) What is the effect of surrounding vegetation after application of a small spectrum herbicide on the individual species? To answer the first three questions, greenhouse and field grown plants of several individual species were treated with tepraloxydim in experiment 1. To study the influence of surrounding vegetation, artificial greenhouse grown vegetations were treated with tepraloxydim in experiment 2.

Materials and Methods

Herbicide

Tepraloxydim is the active ingredient of aramo, which is a systemic postemergence leafherbicide used to control annual as well as perennial grass weeds in broad leaf crops. Tepraloxydim belongs to the cyclohexanediones. Their primary mode of action is the inhibition of lipid formation. Since lipids form an important part of membranes and cell walls, the application of aramo causes the prevention of cell wall formation and delay or inhibition of growth.

Individual species (monocultures)

Seeds of *Panicum milliaceum*, *Poa annua*, and *Echinogloa crus-galli* were obtained from a commercial seed supplier (Medigran, Hoorn, The Netherlands, http://www.medigran.nl). Seven hundred sixty-eight 0.5 L pots with a diameter of 10 cm were filled with a peat: sand mixture (2:1). Seeds of the three species were scattered over the soil surface (one species per pot) and covered with a thin layer of sifted soil. The species were sown in such a manner that emergence of all species would coincide. Since *Elymus repens* reproduces vegetative, cuttings of the root system were placed into the soil in such a manner that the emergence of this species would coincide with the emergence of the seed-sown species. The cuttings of *E. repens* were harvested from *E. repens* plants growing on a clay soil in a field near Duiven in the Netherlands.

Half of the pots were randomly arranged in four blocks in a greenhouse (day/night temperature 18 to 12°C and a 16/8 h light/dark period) and watered in trays. The other half was randomly placed in four blocks in a bare field adjacent to the greenhouse located in Wageningen, The Netherlands. Experiments were conducted from May 2007 until February 2008. After emergence the number of plants was thinned to four per pot.

To compare the effect of tepraloxydim on dicotyledons grown in mesocosms with the effect on dicotyledons grown in monocultures, an additional experiment was carried out from May to June 2008. Ninety six 0.5 L pots with a diameter of 10 cm were filled with a peat: sand mixture (2:1). Seeds of *Solanum nigrum, Stellaria media, Centaurea cyanus* and *Panicum milliaceum* were scattered over the soil surface (one species per pot) and covered with a thin layer of sifted soil. The pots were randomly arranged in four blocks in a greenhouse (day/night temperature 18 to 12°C and a 16/8 h light/dark period) and watered in trays. After emergence the number of plants was thinned to four per pot.

Mesocosms

Artificial vegetations were created by filling forty-eight 5 L pots with a diameter of 20 cm with a peat: sand mixture (2:1). Each mesocosm consisted of four monocotyledons: *P. annua*, *E. crus-galli*, *E. repens*, *P. milliaceum*, and four dicotyledons: *Solanum nigrum*, *Stellaria media*, *Chenopodium album* and *Centaurea cyanus*. All plant species, except *E. repens*, were seeded into the 5L pots in such a manner that emergence of the species would coincide. Since *E. repens* reproduces vegetative, small pieces of the root system of this species were placed in the soil. The seeds of the annuals and the root pieces of the perennial were of the same origin as for the individual species.

Monocotyledons and dicotyledons were placed alternately in the pots. After emergence the number of plants per species was thinned to eight per pot, so in total 64 plants per pot. The experiments started in May 2004. The pots were arranged randomly in a greenhouse with a day-night temperature of 18-12 °C and a light/dark period of 16/8 h. The pots were watered from the bottom.

Treatments

An overview of the experimental schedule is given in Table 5.1. Herbicide treatments took place in a 4 m wide x 2 m deep spray chamber. The sprayer consisted of a 1 m wide spray boom with three Teejet XR11004 (class Medium spray quality) flat fan nozzles (Spraying Systems Company, Wheaton, Illinois, USA, http://www.teejet.com) that delivered 400 l/ha. The nozzle was moved over the pots at a height of 50 cm from the soil surface.

Half of the pots containing the monocotyledon monocultures from both the greenhouse and the field were sprayed two weeks after emergence (WAE) with tepraloxydim and the other half containing the monocotyledon monocultures at four WAE. The dicotyledon monocultures and the mesocosms, which were both grown in the greenhouse, were sprayed

Table 5.1 Overview of experimental schedule for treatments of the mesocosms, the monocotyledon and dicotyledon monocultures.

type of vegetation	species/ pot	species	plants/ species	treatment moment	harvest moment	end point measured	nr of replicates
mesocosm	8	*C. cyanus, S. media, S. nigrum, C. album, E. repens, P. milliaceum, E. crus-galli, P. annua*	8	4WAE	4WAT	fresh weight	4
monocotyledon monoculture	1	*E. repens, P. milliaceum, E. crus-galli, P. annua*	4	2WAE	4WAT	fresh weight	4
					SS	fresh weight + seed number	4
				4WAE	4WAT	fresh weight	4
					SS	fresh weight + seed number	4
dicotyledon monoculture	1	*C. cyanus, S. media, S. nigrum, C. album*	4	4WAE	4WAT	fresh weight	4

at four WAE with the same herbicide. Four pots per treatment remained unsprayed as control. For both the monocultures as well as the mesocosms doses were 0, 0.02, 0.1, 0.2, 1, and 2 L Aramo per ha (50 g tepraloxydim/L).

Measurements

Fresh weight was used as effect parameter. The aboveground fresh weight of half of the pots containing the monocotyledon monocultures was determined at four weeks after treatment (WAT) and the fresh weight of the other half at seed setting (SS). Seeds were collected at SS and counted per pot for all monocotyledon species, except *E. repens*. After storage at 10°C for 4 months in a dark room in which they were shielded from light and moisture, four lots of 20 seeds per pot were randomly chosen for a germination experiment. Germination tests were conducted in a greenhouse at day/night temperatures of 24/12°C and a 16/8 h light/dark period. Each seed lot was allowed to germinate in a plastic pot (6x5x5 cm) filled with sterilized soil. Germinated and emerged seeds were regularly counted and removed from each pot for 28 d.

The aboveground fresh weight of the pots containing the dicotyledons was determined at four WAT. The total aboveground fresh weight of the eight plants belonging to one species in a mesocosm was determined per pot at four WAT.

Statistical analysis

For an interpretation and discussion of the results of the statistical analysis as presented in this section we refer to the results and discussion sections.

Sublethal effects of tepraloxydim on four individual non-crop species

Fresh weight

The aboveground fresh weight was measured for each plant species and analyzed using nonlinear regression analysis with a logistic growth curve: $y = c + (d\text{-}c)/(1+e^{-b(\log(\text{dose})-\log(e))})$ (1) (Seefeldt *et al*., 1995) with four parameters: slope (*b)*, lower limit (*c)*, upper limit (*d)*, and ED50 (*e),* the dose at which an effect of 50% can be observed. The estimations for the parameters are shown in Table 5.2. Regressions were performed using the statistical program R (Team RDC (2005), http://www.R-project.org), as described by Nielsen *et al*. (2004) and Ritz & Streibig (2005). A common parameter was estimated whenever possible. Parameter estimates were compared using a two way analysis of variance. Fisher's Least Significant Difference test was used to compare means. To determine recovery the following definition was used: a plant is able to recover from exposure to Aramo when the ED50 value at SS is equal to or larger than at 4WAT.

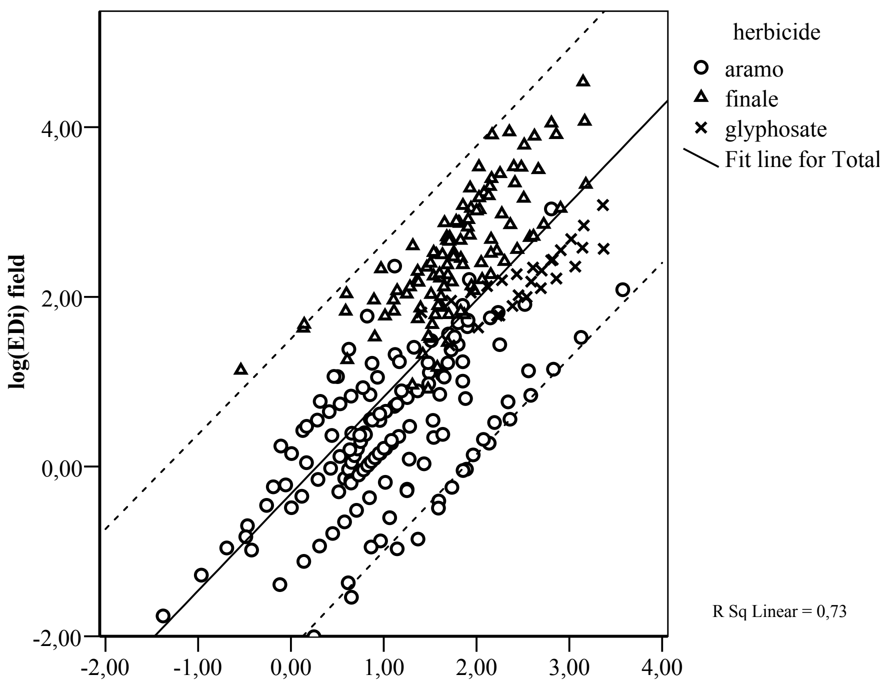

Figure 5.1 Individual species (monocultures). The linear relationship between the log transposed $ED_{10,20\ldots,90}$-values of greenhouse-grown plants and field-grown plants for all species treated with tepraloxydim (circles, Aramo, present study), glufosinate ammonium (triangles, Finale, Riemens et al., 2008 and Chapter 4), and glyphosate (crosses, Gove et al., 2007): log(field EDvalue)=-0.321+1.142 x log(greenhouse EDvalue), R^2=0.73. Dashed lines indicate the 95% confidence interval. Aramo: log(field EDvalue)= -0.454+0.764 x log(greenhouse EDvalue), R^2=0.64; Finale: log(field EDvalue)= 0.914+0.897 x log(greenhouse EDvalue), R^2= 0.57; glyphosate: log(field EDvalue)= 0.626+0.623 x log(greenhouse EDvalue), R^2= 0.72.

Comparison of effects on field and greenhouse-grown plants

The estimated ED10, 20, 90-values of the field-grown plants were log-transformed and plotted against the log-transformed ED10, 20,…, 90- values of the greenhouse-grown plants. The relationship was analyzed with linear regression analysis using SPSS for all species together. Data on the effects of glyphosate on the dry weight of greenhouse and field-grown *Geranium robertianum*, *Primula vulgaris* and *Carex remota* from (Gove *et al*., 2007) was used to fit dose response curves and determine ED values for these species as well. The results for tepraloxydim and glyphosate are plotted together with the results of previous work with glufosinate ammonium (Chapter 4 and Riemens *et al*., 2008) and Chapter 4 in Figure 5.1.

Seed production and emergence

The number of seeds per pot and the number of seeds produced per gram aboveground fresh weight were calculated per dose for each plant species, location and age. After the appropriate checks for normality, an analysis of variance with a randomized block design was used, if seed production was sufficient for analysis. Fisher's Least Significant Difference test was used to compare means for both the number of seeds per gram fresh weight as well as for the number of seeds per pot. *P. annua* and *E. crus-galli* field-grown plants did not produce enough seeds for analysis. The number of *P. annua* seeds produced by early (2 WAE) and late (4WAE) treated plants did not differ significantly and were therefore analyzed together. Results are shown in Figure 5.2.

E. crus-galli and *P. milliaceum* seeds did not emerge in the emergence test for any of the doses, including the control. Therefore, only emergence data for *P. annua* seeds from greenhouse-grown plants were analyzed. The average percentage emergence per dose was calculated for each treatment. After the appropriate checks for normality, an analysis of variance with a randomized block design was applied. Fisher's Least Significant Difference test was used to compare means (Table 5.3).

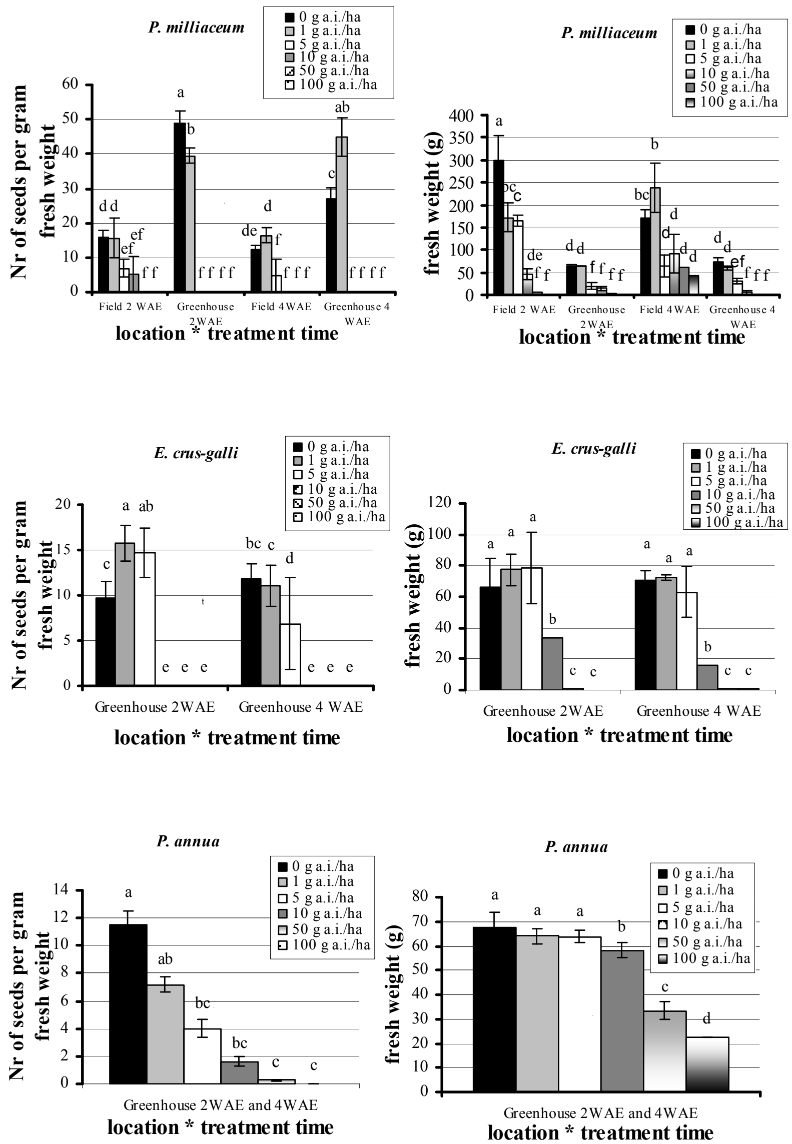

Figure 5.2 Individual species (monocultures). Seed production per gram fresh weight for P. milliaceum, E. crus-galli and P. annua per location and treatment moment. Significant letters indicate significant differences within a species between treatments at the 5% level.

Effects of tepraloxydim on mesocosms

The aboveground fresh weight in each treatment was calculated for each plant species, the total vegetation and the monocotyledons in the mesocosms and analyzed using nonlinear regression analysis as described above for the fresh weight in experiment one. Results are shown in Table 5.4. The aboveground fresh weight per dose of the dicotyledons in the mesocosm was analyzed using linear regression analysis with the equation: $y = a*x + b$ (3). Results are shown in Table 5.5.

The effect of the surrounding vegetation was studied for plants sprayed with tepraloxydim at four weeks after emergence and harvested at four weeks after treatment. The dose response curve of monocotyledonous plant species grown in the mesocosm was compared with the dose response curve of the same species grown individually by calculation of the selectivity indices (SI) (Ritz & Streibig, 2005). The selectivity index is defined as the ratio between the effective dose for a species grown in the mesocosm treated at 4 WAE and the effective dose for the same species grown as monocultures treated at 4 WAE. These indices were calculated for each species and plotted against the corresponding effect level. An SI equal to one, indicates that plants are equally affected in both situations. An SI larger than one indicates that the species will benefit from the surrounding vegetation, an SI smaller than one indicates that the species will experience a negative effect on its biomass from the surrounding vegetation when exposed to tepraloxydim. The SI comparing curves between the individually grown plants and the plants grown in the mixture are shown in Figure 5.3 for *E. crus-galli, E. repens*, *P. annua* and *P. milliaceum*.

Results

Experiment 1: Sublethal effects of tepraloxydim on four non-crop species

Effect of plant development stage on sensitivity

The ED50 values of dose-response curves of field-grown plants sprayed in an early development stage (2WAE) were larger than the ED50 values of those of field-grown plants sprayed in a later development stage (4WAE) (Table 5.1). This indicates that field grown plants are less sensitive when they are treated in an early stage than in a later stage.

Table 5.2 Parameter estimates ± standard error for Y=c+(d-c)/1+e$^{b(log(dose)-log(e))}$ *describing the sublethal effects of tepraloxydim on the fresh weight of four species grown in monoculture in the greenhouse and field, sprayed at 2 and 4 weeks after emergence (WAE), and harvested at 4 weeks after treatment (WAT) and at seed setting (SS).*

		Parameter estimate ± SE			
Species	Treatment	Slope (b)	ED50 (g active ingredient) (e)	upper limit (d)	lower limit (c)
E.crus-galli	Field 2 WAE 4 WAT	2.12 ± 0.255^{a}	1.26 ± 0.254^{a}	9.92 ± 1.407^{a}	0.14± 0.016^{a}
	Field 4 WAE 4WAT	1.29 ± 0.188^{b}	0.22 ± 0.080^{b}	36.24 ± 5.524^{b}	0.45± 0.059^{b}
	Field 2 WAE SS	3.05 ± 0.441^{c}	1.44 ± 0.317^{a}	81.35 ± 12.243^{c}	0.58± 0.059b^{c}
	Field 4 WAE SS	3.78 ± 0.344^{c}	1.13 ± 0.157^{a}	101.28 ± 15.329^{c}	0.29± 0.027^{d}
	Greenhouse 2 WAE 4 WAT	5.62 ± 0.575^{d}	4.84 ± 0.313^{c}	15.77 ± 1.700^{d}	0.17± 0.018^{a}
	Greenhouse 4 WAE 4 WAT	1.37 ± 0.317^{ab}	3.78 ± 1.048^{c}	23.27 ± 3.005^{e}	1± 0.321^{e}
	Greenhouse 2 WAE SS	2.96 ± 0.222^{c}	9.03 ± 1.00^{d}	74.61 ± 7.209^{c}	0.12± 0.039^{a}
	Greenhouse 4 WAE SS	4.03 ± 1.692^{cd}	7.14 ± 1.075^{d}	72.33 ± 8.027^{c}	0.8± 0.099^{c}
P. milliaceum	Field 2 WAE 4 WAT	0.9 ± 0.111^{a}	0.47 ± 0.233^{a}	37.14 ± 7.773^{ab}	0.34± 0.055
	Field 4 WAE 4WAT	0.7 ± 0.094^{a}	2.05 ± 1.166^{bc}	61.27 ± 11.592^{ac}	
	Field 2 WAE SS	2.11 ± 0.157^{b}	5.91 ± 1.116^{d}	232.88 ± 35.602^{d}	
	Field 4 WAE SS	0.6 ± 0.120^{a}	5.37 ± 4.009^{d}	195.41 ± 37.347^{d}	
	Greenhouse 2 WAE 4 WAT	1.88 ± 0.345^{bc}	1.3 ± 0.383^{c}	18.25 ± 3.547^{b}	
	Greenhouse 4 WAE 4 WAT	0.85 ± 0.148^{a}	5.32 ± 3.008^{d}	28.04 ± 5.255^{b}	
	Greenhouse 2 WAE SS	1.79 ± 0.168^{c}	4.05 ± 0.978^{bd}	63.46 ± 10.818^{c}	
	Greenhouse 4 WAE SS	1.32 ± 0.129^{d}	2.2 ± 0.673^{b}	78.98 ± 14.327^{c}	
P. annua	Field 2 WAE 4 WAT	20.2 1 ± 7.959^{a}	20.15 ± 12.186^{a}	16.74 ± 1.312^{a}	0± 0.00
	Field 4 WAE 4WAT	0.71 ±0.264^{b}	4.64 ± 2.699^{a}	20.35 ± 2.511^{a}	

	Field 2 WAE SS	1.15 ± 0.142^{bc}	26.3 ± 3.29^{a}	70.15 ± 2.14^{b}	
	Field 4 WAE SS	0.71 ± 0.166^{b}	1.05 ± 0.423^{b}	38.99 ± 2.630^{c}	
	Greenhouse 2 WAE 4 WAT	1.05 ± 0.570^{bc}	36.42 ± 20.229^{c}	15.58 ± 2.001^{a}	
	Greenhouse 4 WAE 4 WAT	0.83 ± 0.754^{bc}	221.6 3 ± 26.185^{d}	16.49 ± 1.775^{a}	
	Greenhouse 2 WAE SS	4.37 ± 1.126^{a}	54.16 ± 2.392^{e}	58.06 ± 1.313^{d}	
	Greenhouse 4 WAE SS	1.61 ± 0.351^{c}	94.27 ± 8.901^{f}	64.09 ± 1.472^{e}	
E. repens	Field 2 WAE 4 WAT	1.44 ± 0.447a	3.63 ± 1.224a	7.82 ±1.097a	0.35± 0.127
	Field 4 WAE 4WAT	0.3 ± 0.088b	0.49 ± 0.066 b	14.55 ± 1.978b	
	Field 2 WAE SS	2.37 ± 0.414c	6.68 ± 0.862ac	31.41 ± 2.508c	
	Field 4 WAE SS	0.55 ± 0.068b	0.52 ± 0.258b	44.87 ± 4.367d	
	Greenhouse 2 WAE 4 WAT	1.4 ± 0.380cd	8.91 ± 3.230c	7.14 ± 0.962a	
	Greenhouse 4 WAE 4 WAT	0.62 ± 0.166ab	15.89 ± 4.523c	11.37 ± 1.498b	
	Greenhouse 2 WAE SS	1.78 ± 0.382cd	16.81 ± 4.523c	11.28 ± 1.101b	
	Greenhouse 4 WAE SS	0.97 ± 0.216d	16.58 ± 6.778c	12.34 ± 1.413b	

Different letters within a species within a column indicate significant differences at the 5% level.

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However, this difference was not significant (p<0.05) for *E. crus-galli* and *P. milliaceum* harvested at seed setting (SS), and *P. annua* harvested at four weeks after treatment (4WAT). Greenhouse-grown plants showed the same trend, although the difference in ED50 value between plants treated in an early stage and plants treated in a later stage was only significant (P<0.05) for *P. milliaceum* harvested at 4WAT and *P. annua* at both harvest moments (Table 5.1).

Recovery of plants

Field-grown plants were able to recover from the treatments with tepraloxydim. In general, their ED50 values were larger at seed setting (SS) than at four weeks after treatment (4WAT). However, these differences were not significant for *E. crus-galli* and *P. annua* treated in an early development stage (2WAE) or for *E. repens*. Greenhouse grown *E. crus-galli* plants were able to recover, and so were the *P. milleaceum* and *P. annua* greenhouse-grown plants treated in an early development stage. *E. repens* plants showed the same trend, but this was not significant at the 5% level.

P. milliaceum and *P. annua* greenhouse grown plants treated in a later development stage (4WAE) were not able to recover, but showed the opposite response: a stronger effect of the tepraloxydim was observed at seed setting than at 4WAT (Table 5.1).

Comparison of effects on field and greenhouse grown plants

The dose response curves of all species differed significantly in one or more parameters for the plants grown in the greenhouse and the plants grown in the field. The ED50 values of *E. crus-galli* and *P. annua* plants of greenhouse grown plants were significantly larger than of the same plants grown in the field (p=0.05). The same was true for *P. milliaceum* and *E. repens*, except for early treated plants harvested at seed setting, which showed no significant difference, and for *P. milliaceum* plants treated at 4WAE and harvested at seed setting for which the ED50 value of field grown plants was larger (Table 5.1).

The relation between the log-transformed ED10,20…90- values for the greenhouse dose response curves and for the field dose response curves is shown in Figure 5.1, together with the results from a previous study with glufosinate ammonium (Riemens *et al*., 2008 and Chapter 4) and a study with glyphosate (Gove *et al*., 2007). These data show that a linear relationship exists between the ED-values of greenhouse and field grown plants of several species treated with tepraloxydim, glufosinate ammonium and glyphosate on a logarithmic scale.

Effects on seed production and seedling emergence

The influence of location (field or greenhouse) and plant development stage at the moment of treatment on the seed production per pot (Table 5.5) and the seed production per gram fresh weight (Figure 5.2) was studied for *P. milliaceum, E. crus-galli* and *P. annua*.

Seed production per pot was strongly affected by tepraloxydim dose ($p<0.01$). The effect of tepraloxydim on seed production was greater than that on fresh weight (Figure 5.2). Field grown *P. milliaceum* plants produced significantly less seeds per gram fresh weight than greenhouse grown plants did ($p<0.05$). The development stage of the parent plants at the moment of exposure to tepraloxydim did not influence the relative seed production of the plants of *P. milliaceum* and *P. annua*.

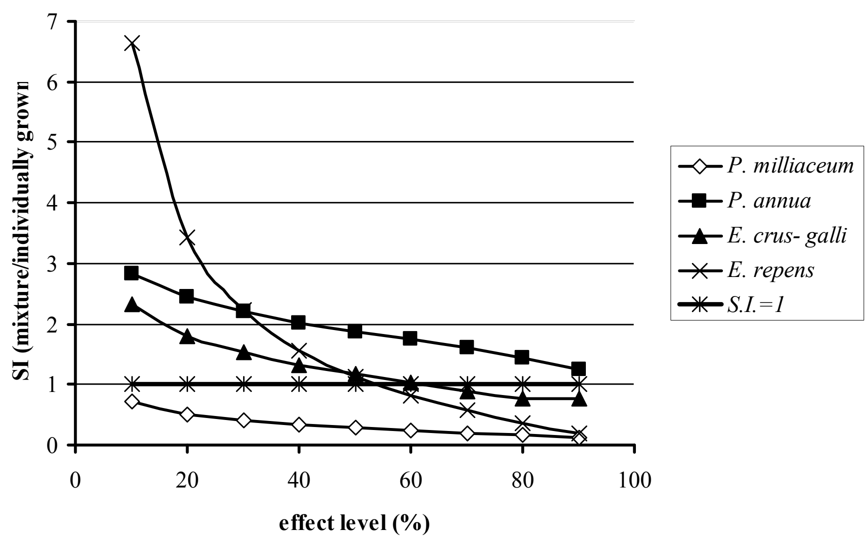

Figure 5.3 Selectivity indices (effective dose in mesocosms: effective dose individually) for *P. annua, P. milliaceum, E. crus-galli and E. repens* versus effect level of plants treated with sublethal doses of tepraloxydim.

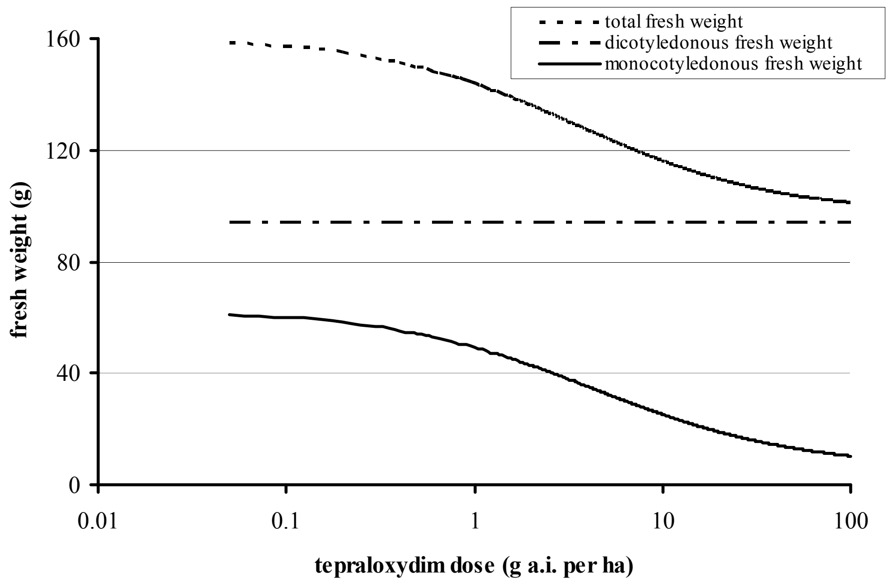

Figure 5.4 Effect of the tepraloxydim dose on the total fresh weight of the mesocosms, and the fresh weight of monocotyledons and dicotyledons in mesocosms.

The greenhouse grown plants of *E. crus-galli* treated in an early development stage were able to produce more seeds per gram fresh weight than the plants treated in a later stage ($p<0.01$). Tepraloxydim dose did not significantly reduce the emergence of *P. annua* seedlings from seeds produced by plants treated in any development stage (Table 5.2).

Experiment 2: Effects of tepraloxydim on mesocosms

Visual symptoms and effects on biomass

The first visual symptoms on the species in the mesocosms were observed at two days after treatment with aramo. At the three lowest doses, no visual symptoms were observed. At 10 g a.i./ha and higher, yellow spots appeared on the leaves of *P. milliaceum*, whereas the other species remained symptom- free.

Table 5.3 Individual species (monocultures). Percentage seedling emergence of P. annua seeds per tepraloxydim dose. Seeds were harvested from plants sprayed with tepraloxydim at 2 and 4 weeks after emergence (WAE).

Species	Treatment	dose (g a.i./ha)	Percentage seedling emergence
Poa annua	Greenhouse 2 WAE	0	38.4
		1	22.6
		5	25.8
		10	20.8
		50	*
		100	*
	Greenhouse 4 WAE	0	19.2
		1	41.4
		5	36.8
		10	24.3
		50	*
		100	*

**) Not enough seeds to perform a seedling emergence test.*

Differences were not significant between treatment at the 5% level.

The fresh weight of the dicotyledons in the mesocosm was unaffected by the tepraloxydim doses. Both the dicotyledon total fresh weight, as well as the fresh weight of the individual dicotyledons was linearly related to the tepraloxydim dose, with the slope not significantly different from 0 ($p>0.05$) (Table 5.4). The total fresh weight of the mesocosms was significantly reduced by the applied tepraloxydim dose ($p<0.05$) (Table 5.3). This was entirely the result of the reduction in fresh weight of the monocotyledonous species in the mesocosm (Figure 5.4).

Effect of surrounding vegetation on individual species

For all species the selectivity indices decreased with increasing effect level (Figure 5.3), indicating that at higher tepraloxydim doses it becomes less beneficial, or more detrimental to be in the mixture for these species. The selectivity indices remained below one for *P.*

milliaceum: the dose before a certain effect can be observed is always lower when the species is grown in the mixture, compared to the single species situation (Figure 5.3). The selectivity indices for *P. annua* remained above one, so this species benefits from the surrounding vegetation when it is exposed to tepraloxydim. At low effect levels, and therefore, low tepraloxydim doses, *E. crus-galli* and *E. repens* benefit from the presence of the other species, but at higher doses they experience a disadvantage of growing in the mixture.

Discussion

Relationship between the sublethal effects on greenhouse and field grown plants

We previously showed that the effective dosages (ED values) obtained in a greenhouse experiment treated with the broad spectrum herbicide glufosinate ammonium were related to the effective dosages from a field experiment treated with the same herbicide, at the same time (Chapter 4 and Riemens *et al*., 2008). We stated that the relationship could be used for the translation of effects on greenhouse grown plants to field grown plants and might be valid for other herbicides and plant species as well. In this study, we fitted dose response curves through data of tepraloxydim on several herbaceous species and data from a previous study with glyphosate (Gove *et al*., 2007), another broadspectrum herbicide, which was applied on greenhouse and field grown plants of three woodland species. The resulting ED values for these herbicides were plotted together with the glufosinate ammonium data in Figure 5.1. The ED values of greenhouse grown plants were linearly related to the corresponding ED values (at the same effect level) obtained from field grown plants for all three herbicides. These results confirm our hypothesis that this type of relationship is valid for herbicides with different modes of action. The line fitted for glufosinate ammonium in Figure 5.1 lies well above the line y=x, which indicates that greenhouse grown plants were more sensitive to this herbicide than field grown plants were. However, the line for tepraloxydim lies well underneath this line, indicating the opposite: greenhouse grown plants were less sensitive to tepraloxydim than the field grown plants. Fletcher and Johnson (1990) previously compared EC50 values from a multitude of studies (in which numerous variables were not the same).

Table 5.4. Monocotyledonous aboveground fresh weight (g) in mesocosms treated with tepraloxydim.

	Parameter estimate ± SE			
Species	Slope (b)	ED50 (g active ingredient) (e)	upper limit (d)	lower limit (c)
Echinogloa crus-galli	2.26 ± 0.530[a]	4.11 ± 0.758[a]	25.3 ± 2.76[a]	1.09 ± 0.232[a]
Panicum milliaceum	1.77 ± 0.389[a]	1.60 ± 0.522[b]	23.04 ± 3.42[a]	0.99 ± 0.209[a]
Poa annua	3.01 ± 2.670[a]	15.38 ± 5.780[a]	4.56 ± 0.470[b]	1.41 ± 0.259[a]
Elymus repens	2.60 ± 1.517[a]	7.02 ± 1.730[a]	11.24 ± 1.386[b]	2.45 ± 0.432[b]
All monocotyledons	1.83 ± 0.313[a]	4.29 ± 0.502[a]	62.83 ± 2.905[c]	6.02 ± 0.921[c]
Total vegetation	1.88 ± 0.671[a]	3.50 ± 0.813[a]	160.48 ± 5.079[d]	97.38 ± 3.385[d]

Parameter estimates ± SE (standard error) of dose-response curves of the aboveground fresh weight of the individual monocotyledonous species in the mesocosm, the total monocotyledon fresh weight in the mesocosm and the total fresh weight of the mesocosm sprayed at four weeks after emergence and harvested at four weeks after treatment vs. tepraloxydim dose. Regression equation: $Y=c+(d-c)/(1+e^{-b(log(dose)-log(e))})$. Different letters within a column indicate significant differences at the 5% level.

Table 5.5. Dicotyledonous aboveground fresh weight (g) treated with tepraloxydim grown as single species (monocultures) and grown in mesocosms.

	single species		grown in mesocosm	
species	Parameter estimates ± SE		Parameter estimates ± SE	
	a (constant)	b (slope)	a (constant)	b (slope)
Centaurea cyanus	54.06 ± 1.34***	0.090 ± 0.03***	12.50 ± 2.03***	N.S.
Chenopodium album	21.45 ± 1.34***	-0.149 ± 0.03***	10.32 ± 2.03***	N.S.
Solanum nigrum	46.29 ± 1.34***	-0.141 ± 0.03***	30.35 ± 2.03***	N.S.
Stellaria media	87.05 ± 1.34***	-0.130 ± 0.03***	40.25 ± 2.03***	N.S.
Total dicotyledons			94.42 ± 2.03***	N.S.

*Parameter estimates ± SE (standard error) of the equation y =a*x + b, with y being the fresh weight of the dicotyledonous species grown individually (left) or the fresh weight of the dicotyledonous species grown in the mesocosm (right), all sprayed at four weeks after emergence and harvested at four weeks after treatment, and x being the tepraloxydim dose. N.S.: parameter estimate not significantly different from zero, ***: parameter estimate significantly different from zero at the 1% level. R^2 = 95.0 (single species) and R^2 = 0.94 (mesocosm).*

They found that for 55% of the herbicide-plant species combinations they used in their analysis field grown plants were more sensitive, while for 30% greenhouse grown plants were more sensitive and for the remaining combinations the sensitivity was equal (Fletcher & Johnson, 1990). Whether the sensitivity to a certain herbicide will be larger or smaller in the field or greenhouse depends on the herbicide- plant species combination, and the climatic conditions that influence plant growth such as temperature, relative humidity and light intensity (Riethmuller-Haage, 2006).

Effect of surrounding species

While we compared greenhouse and field data on individual species, field margins are in most cases composed of several species, both annuals as well as perennials (Kleijn & Verbeek, 2000). Figure 5.3 clearly shows that species respond in a different way to herbicide exposure when grown in the vicinity of other species. In general smaller species benefit from surrounding vegetation; in both this study with tepraloxydim and the previous study with glufosinate ammonium (Chapter 4 and Riemens *et al*., 2008) *P. annua* plants and the smaller dicotyledonous species such as *S. media* benefited from the other species in the vegetation. The effect of a herbicide not only depends on the applied dosage but also on the exposure to the chemical. In a vegetation the exposure of smaller species is reduced by the presence of other plants. The other three grasses used in this study all respond in a similar fashion; at low dosages it is beneficial to be in a vegetation with other species. The presence of the other, dicotyledonous species, in the mixture reduces their exposure to the herbicide. With increasing dosage it becomes less favorable for these species to be in the mixture, probably as a result of the herbicide induced reduction of their competitive ability. Due to the reduction in monocotyledonous fresh weight with increasing dosages, the dicotyledonous species are expected to benefit from the reduced competition for light, water and nutrients and increase in biomass. However, the aboveground fresh weight of the dicotyledons appears to remain unaffected (Table 5.4, Figure 5.4). We hypothesize that although tepraloxydim is regarded as a small spectrum herbicide that only affects grasses, the dicotyledons are affected by tepraloxydim as well. Tepraloxydim belongs to the cyclohexanedione herbicides which inhibit acetyl-coenzyme A carboxylase, involved in fatty acid an flavonoid biosynthetic pathways. Both monocotyledons as well as dicotyledons use this enzyme in their pathway (Rendina & Felts, 1988). Two forms of this enzyme exist: the eukaryotic form, which is sensitive to cyclohexanediones and the prokaryotic form, which is less sensitive. Monocots lack the prokaryotic form and are therefore highly sensitive to these herbicides, while dicotyledons contain and use the prokaryotic form (Devine & Shukla, 2000). The effect of tepraloxydim on broad leaved species is therefore very small and can be compensated by the relative increase in their competitive ability compared to the monocotyledons. As a result, changes in the fresh weight of the dicotyledons due to tepraloxydim application can not be observed. These results show that the use of single-species tests in the ecological risk assessment of herbicides may not be valid. The presence of surrounding vegetation significantly alters the response of species to herbicide exposure. Recently, Damgaard *et al*. (2008) performed a competition experiment with two species, *C. bursa-pastoris* and *G. dissectum*, and mecoprop-P which supports this conclusion as well. They found that low dosages of the herbicide had significant effects on the interspecific competitive ability of the both species and that results from single species tests can not be used to predict the response in a multiple species study.

Plant development stage and reproduction

Tepraloxydim affected plants in an earlier development stage less than plants of the same species in a later development stage. Previous results showed the opposite: younger plants were more sensitive to glufosinate ammonium (Chapter 4 and Riemens *et al*., 2008), glyphosate (Ruiter *et al*., 2000; Marrs *et al*., 1991), MCPA and mecoprop (Marrs *et al*., 1991) than older plants. These results seem conflicting, but can be explained by the following. All these herbicides, except tepraloxydim, are broad-spectrum herbicides that need to be applied on young plants to be effective. To obtain an optimal control with grass herbicides such as tepraloxydim, cycloxydim and fluazifop-P-butyl it is required to apply them on plants that have a minimum height of 15-20 cm or from the third leaf stage until the formation of new leafs has stopped.

Reproduction is an important parameter in determining long term effects of herbicides on non target plants (Zwerger & Pestemer, 2000). In this study the seed production was stronger affected than the fresh weight at the same dosages, in accordance with previous results on glufosinate ammonium (Chapter 4 and Riemens *et al*., 2008), chlorsulfuron (Fletcher *et al*., 1996) and MCPA (Andersson, 1994). The seed production per gram aboveground weight of field grown plants was more affected than the production of greenhouse grown plants (Figure 5.2, *P. milliaceum*), which is in accordance with the effect on the fresh weight itself: field grown plants are more sensitive than greenhouse grown plants (Figure 5.1). In this study we did not find a significant effect on the emergence of treated plants. Previously studies have shown that effects on seed production and the emergence are herbicide and species dependent (Chapter 4 and Riemens *et al*., 2008; Champion *et al*., 1998; Andersson, 1994).

Conclusion

To assess the risk of side effects of herbicides on non target plants the European and Mediterranean Plant Protection Organization (2003) proposes a guideline. The proposed studies use single species tests in a controlled environment. Our results indicate that the use of effects measured in greenhouse studies on single species may be used to determine the risk of herbicides to single species in the field. However, the relationship between greenhouse and field effects (Figure 5.1) is probably herbicide specific and needs to be assessed for each group of herbicides with a similar mode of action.
The use of single-species tests in the ecological risk assessment of herbicides may not be valid. Exposure of single species to sublethal herbicide concentrations can not be used to predict effects on a mixture of species (this study, Riemens *et al*, 2008 and Damgaard *et al*, 2008). Additional knowledge on the response of common field margin communities to sublethal herbicide dosages is required. Important factors that need to be taken into account are the species composition of those communities (annuals/perennials, monocotyledons/ dicotyledons), mode of action of the herbicides, the development stage of the margin and the choice of endpoint (biomass, reproduction).

Acknowledgments

We thank André Uffing for his technical assistance during the experiments. The research described in this article was carried out within the research program “Environmental Risks and Emission Reduction of Pesticides” (Program 416) financed by the Dutch Ministry of Agriculture, Nature and Food Safety.

CHAPTER 6

Linking the farmers' weed management behaviour with actual on-farm weed pressure; an exploratory study[5]

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Summary

Most studies on weed population dynamics in farming systems have focused on the effects of different farming systems. Those studies usually assume that farmers, operating within a particular system, show homogeneous management behaviour. However, it is likely that weed management behaviour will vary between farms that operate within one system, thereby influencing the weed pressure. In the present study we 1) investigated whether differences between organic farms in weed pressure can be related to differences in farmers' weed management behaviour, 2) explored which weed and general management factors are of main influence on the weed pressure, and 3) investigated the influence of farmer's beliefs and knowledge on weed control techniques and the observed weed pressure. Preventive measures and timing of main soil tillage operation were identified as the weed management factors most influential for weed pressure. With increasing number of preventive measures applied, the weed pressure decreased, with a stale seedbed being the most important preventive measure. The weed pressure increased with the number of days after September 1st on which the main tillage operation was carried out. Field size, rather than weed pressure, determined the number of hand weeding hours per ha. On farms with

[5] *Accepted by Weed Science in revised form*

lower weed pressures a higher percentage of competitive crops were grown than on farms with higher weed pressures. The farmer's beliefs and knowledge on weed control techniques differed between farmers with different weed pressures. It was concluded that exploratory on-farm studies can give us insight in the human dimension, which can lead to a better understanding of the farming systems and to more effective weed management in those systems.

Keywords: Organic farming system, Hand weeding, Beliefs, Weed density, Weed seed production, Management behaviour

Introduction

Weeds are often regarded as one of the largest bottlenecks for organic farming systems as a result of the large amount of labor required for their control (De Buck *et al*., 2001). Although these farming systems have received a lot of attention during the past decades as a result of an increased concern about negative effects of pesticide use, the percentage of organic farms is still rather low: 3,8% on average in Europe and 2,7% in the Netherlands in 2008 (CBS, 2008). Fear of ineffective weed control is often perceived as one of the most important obstacles to conversion from conventional to organic farming (Beveridge & Naylor, 1999).

So far, researchers have used a reductionist approach to study weed management, e.g. focusing only on the comparison between types and adjustments of implements of non-chemical control techniques (Barberi, 2002). Since long time, a systems approach has been regarded as a pillar for the design of real, effective organic crop production systems (Vereijken, 1997; Andrews *et al*., 1990). A few studies have investigated the weed population dynamics in farming systems. Most of them focused on the effects of different farming systems such as conventional and organic systems (Albrecht, 2005; Sjursen, 2001). Although an effort has been made in those studies to keep methods similar to on-farm practices for each type of farming system, the assumption is being made that farmers operating within a particular farming system show homogeneous management behaviour. However, farmers who supposedly operate in the same system are known to respond differently to for instance changes in the availability of farming techniques (Vanclay & Lawrence, 1994) or the market (Nowak & Cabot, 2004). Different weed management strategies within a farming system can influence the weed pressure strongly (Riemens *et al*., 2007a and Chapter 3). It is therefore likely that weed management behaviour will vary between farms that operate within one farming system, thereby influencing the weed pressure.

Exploratory on-farm studies can give us insight in the variation in weed management behaviour as well as weed pressure within a farming system (Mertens, 2002). The objective of this study was to 1) investigate whether differences between organic farms in weed pressure can be related to differences in farmers' weed management behaviour, 2) explore which weed and general management factors are of main influence on the weed pressure, and 3) to investigate the influence of farmer's beliefs and knowledge on weed control techniques and the observed weed pressure.

Materials and Methods

Farms

The research took place at sixteen commercial organic farms distributed over four areas in the Netherlands two on clay soils, and two on a sandy soil. All farms were located between 52° North and 5°East, and 52°North and 10°East. The four farms per region were chosen to represent both farms with high and low weed pressures, relevant crop rotations and the willingness of the farmers to participate in the research.

Measures for behaviour: interview and registration form

Data on the weed management behaviour of the farmer were collected via an interview and a registration form. The interview consisted of a set list of questions regarding the effect of cropping practices on weed population dynamics and soil structure, the effect of weed control activities of the farmers such as preventive weed management tools (e.g. stale seedbed, fallowing, sowing density, stubble treatments, crop choice), grower typology, and the priority of weed management compared to other activities such as pest control and fertilization. The interviews were held at the beginning of the project, spring 2003. Furthermore, each farmer was asked to register activities that took place at the farm each year of the survey (2003-2005) by filling in a registration form on the amount and timing of activities related to a) fertilization, b) cultivation, c) disease control, d) weed control, and e) planting and harvest dates of the crops for each field.

On farm weed pressure

Several parameters were recorded at each of the farms to obtain the on farm annual weed pressure: the weed species, the average density per species (nr of plants m^{-2}), and the average number of viable seeds produced per plant (nr of seeds per plant). Weed density was monitored in 15 quadrates arranged along a diagonal transect in each field. The quadrates had an area of 1 m^2; however different shapes were used in order to maintain the same proportion of crop row to inter-row space found in the field as a whole. The quadrates were placed approximately 10 m apart. Transects were approximately located at the same sites in the fields each year. If weeds were present in two or more of the quadrates, 10 individual plants were taken from the field for seed production measures. Those plants were harvested outside the quadrates. These recordings were done each year (2003-2005), on each farm, on every field, 3 weeks prior to crop harvest. This implies that every field was monitored at least once a year, and in some cases several times a year, due to different succeeding crops in one season. The weed density per species and the nr of seeds produced per plant were used to calculate the total weed density (total nr of plants m^{-2}) and the total seed production (total number of seeds m^{-2}). As a result of measuring at the end of the growing season, the measured weed density and weed seed production were the result of weeds emerging from the viable soil seed banks present in a field (the potential density) and the weeds surviving subsequent weed control during the season.

Statistical analysis

The approach to the analysis was first to identify general patterns in the weed density, weed seed production, and number of weed species between farms by means of summary statistics. The next step was to examine the relationships between various factors (farm size, crop, soil tillage, weed management) and the weed pressure. Finally it was investigated whether farmers with different self-reported beliefs and behaviours on weed management also differed in weed pressure and factors that had an important effect on the weed pressure.

General patterns in weed pressure and weed abundance

The weed density per species was used to calculate the total weed density (total nr of plants m^{-2}) per farm averaged over all years and fields. To investigate which species were most abundant, the weed densities and seed production per species were averaged over three years, farms and fields.

Screening of factors influencing the weed pressure

Two measures were used to represent the on farm weed pressure; the weed density (nr of plants m^{-2}), and the weed seed production (nr of seeds m^{-2}). The relationship between the two measures for weed pressure were analysed by fitting a linear regression model in the Statistical program GenStat (Payne *et al*., 2008) of the seed production vs. the weed density. Prior to fitting the model, the data on weed density and weed seed production was log transformed to meet terms of normality.

Factors influencing the weed pressure were investigated by fitting all possible linear regression models in the Statistical program GenStat (Payne *et al*., 2008) of all *a priori* selected factors that could be influencing the weed pressure (weed density and weed seed production). These factors were hand weeding effort (hours ha^{-1}), timing of main soil tillage (nr of days after September 1^{st}), number of applied preventive weed control measures, field size and soil type. Prior to fitting the models, data was log transformed to meet terms of normality whenever required. The fitted models were compared according to the highest adjusted R square value and the lowest Mallow's Cp. In this way the best regression model containing only the most important factors were selected for the average weed density, and the average weed seed production.

Investigating the effects of factor levels

After the most suitable models were found, the effects of the factors on the weed pressure were investigated by examining the coefficients of the models.

For the factors not included in the models, it was investigated why these were not as influential as was expected. These factors were hand weeding hours, field size and soil type. Because hand weeding hours and field size were correlated, the number of hand weeding hours averaged over the three years (h ha^{-1}) was plotted against the average field size (ha). A logarithmic function was fitted with the statistical program (Payne *et al*., 2008).

Investigating which crops allow high weed pressures

Hierarchical Cluster Analysis (HCA) was used to analyze the seed production and weed density in the crops. HCA is a tool designed to reveal natural groupings within a dataset. We used the agglomerative method, which starts with individual objects (crops) which are combined into groups by collection of objects or groups into larger groups. Grouping is based on similarity between objects/groups. Crops are placed in a multidimensional space (nr of dimensions= nr of crops). Position of crops in the space is based on the measures for weed pressure (density and seed production). Distance between crops is the measure for dissimilarity: Euclidean Distance (ED). Crops were only included in the analysis if they were grown at least three times by different farmers.

Linking farmers' self reported beliefs on weed management with weed pressure

Due to the relatively small sample size (n=16), it was not possible to reduce the number of variables from the questionnaire and perform a PCA (Principal Component Analysis). Therefore descriptive analysis was used to detect differences in the farmers' beliefs on weed management. At several moments during the interview each farmer was asked to tell about their interests. Based on their response during the interview, farmers were categorized as either crop growth or market oriented. This and other variables that were significantly different between farmers were used for comparison with the weed pressure data. For each of those questionnaire variables, the average weed density and weed seed production were calculated. Differences in weed pressure were evaluated with Tamhane's T2 test.

Results and Discussion

Over the survey period of 3 years a total of 20 weed seed producing species were observed (Table 6.1). *Stellaria media* was the most abundant species on each farm and had an average density of 29 plants m^{-2}. Other abundant species were *Chenopodium album, Polygonum convolvulus, Poa annua* and *Polygonum persicaria*, although they were not, like *S. media,* observed at each farm. *Galinsoga parviflora* had the highest average density (36 plants m^{-2}), but was less abundant as *S. media*.

The average weed density on the farms varied from almost 1 weed plant m^{-2} on farm 1 to 26 weed plants m^{-2} on farm 8 (Table 6.2). On a sandy soil the average weed density was, for most farms, higher than the density on a clay soil. Results in Table 6.1 are the average densities of species present in the fields, while average densities in Table 6.2 also include data of fields at which no species were present.

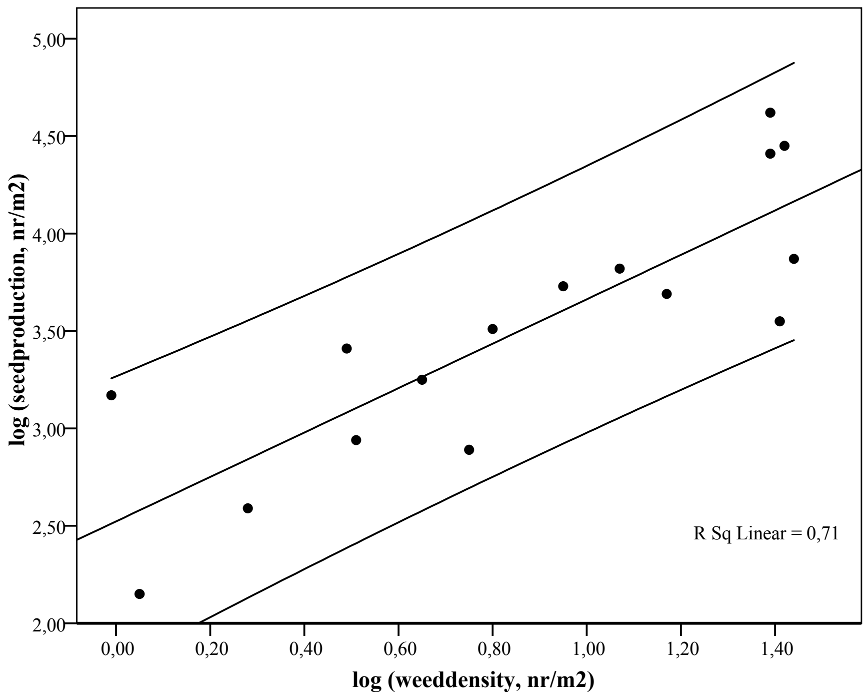

Figure 6.1. Relationship between the log transformed average weed seed production and average weed density, measured at the end of a growing season, three weeks prior to crop harvest. R^2=71.0. Y =2.52 + 1.14 x X, with Y= log(weed seed production), and X= log (weed density).

Factors influencing weed pressure

The two parameters used to represent weed pressure, the average weed density per farm (plants m^{-2}) and the weed seed production per farm (plants m^{-2}), were linearly related when averaged over all years and fields (Figure 6.1). Similar linear relationships were found for the weed seed production and weed density per farm per year (data not shown).

The variation in weed seed production and weed density was best explained by a model with two factors: timing of the main soil tillage treatment (x_1) and the number of applied preventive measures (x_2): $Y = c + a\ x_1 - b\ x_2$, in which a, b, and c are constants and Y is either the log(seed production) or the log(weed density). With increasing number of applied preventive measures, the number of weed seeds being produced and the weed density decrease (Table 6.3). Applied preventive measures were the use of a stale seedbed, a high sowing density, adjusted row distance, stubble treatment, and crop and variety choice. The most influential preventive measure applied was the stale seedbed. A stale seedbed followed by control of the emerging seedlings prior to planting or seeding a crop can reduce the number of weeds during crop growth, compared to a weed control system without a stale seedbed with 80% or more under experimental conditions (Chapter 2 and Riemens *et al*., 2007b).

Table 6.1. Mean density of the individual weed species and the standard error of the mean (s.e.m.), and the mean number of weed seeds produced per species and the standard error of the mean (s.e.m.), averaged over all years, farms and fields.

weed species	weed density (nr m^{-2})	s.e.m.	weed seeds (nr m^{-2})	s.e.m.
Amaranthus retroflexus	2.00	-	569	-
Apera spica venti	15.50	7.500	0	-
Capsella bursa pastoris	11.17	2.555	2217	839
Chenopodium album	25.16	2.806	15018	3790
Echinogloa crus galli	9.04	1.541	1005	204
Elymus repens	12.50	4.010	0	-
Galinsoga parviflora	36.05	6.875	7076	2034
Lamium purpureum	10.40	3.696	143	43
Matricaria chamomilla	5.95	1.186	2329	720
Poa annua	9.75	3.276	5	2
Polygonum aviculare	8.00	2.176	5	2
Polygonum convolvulus	11.17	1.540	182	28
Polygonum persicaria	17.13	4.299	1137	520
Raphanistrum raphanistrum	3.33	1.856	381	212
Senecio vulgaris	8.76	4.120	409	115
Solanum nigrum	10.71	2.339	4138	3052
Sonchus oleraceus	7.25	5.921	228	131
Stellaria media	29.07	2.708	2204	487
Urtica urens	12.40	5.896	2001	1341
Veronica filiformis	12.00	5.862	3942	257

The current results show that the application of a stale seedbed has a positive influence on the reduction of the weed pressure at the farm level as well.

The timing of the main soil tillage operation (e.g. ploughing) influenced the weed density and the number of weed seeds produced on the fields in the season followed by the operation. The number of produced weeds seeds and the weed density significantly increased with the number of days after September 1st on which the main tillage operation was carried out. On fields where the main soil tillage operation was carried out in autumn, the seed production and density were lower than on fields tilled in spring. The average timing of the main soil tillage operation on a clay soil did not significantly differ from the average timing on a sandy soil ($p=0.672$).

Table 6.2. Mean density of the total weed species and the standard error of the mean (s.e.m.), averaged over all years, weed species and fields.

farmer	soil type	weed density (nr m^{-2})	s.e.m.
1	clay	0.99	0.272
2	clay	3.25	1.038
3	clay	1.89	0.195
4	clay	3.06	0.977
5	sand	24.36	4.947
6	sand	1.13	0.830
7	sand	17.34	2.628
8	sand	26.41	10.182
9	sand	14.70	3.801
10	sand	15.84	2.374
11	sand	14.77	5.989
12	sand	8.89	1.773
13	clay	11.64	3.967
14	clay	4.50	0.450
15	clay	6.38	1.485
16	clay	5.63	1.057

Table 6.3. Coefficients of the model $Y = c + a\ x_1 - b\ x_2$, with Y: either the log(seed production) or the log(weed density); x_1: timing of the main soil tillage treatment; and x_2: the number of applied preventive measures.

Y	a	b	c	R^2
log(weed density)	0.72 ± 0.323	0.21 ± 0.069	0.24 ± 0.076	55.8
log(weed seed production)	1.18 ± 0.398	0.22 ± 0.085	1.98 ± 0.935	58.2

Vleeshouwers & Kropff (2000) also observed that late soil cultivation (large number of days after September 1st) results in higher weed densities than early soil cultivation. Three factors influence the differences in seedling numbers after soil cultivation at different dates; the degree of dormancy of the seeds, soil temperature after cultivation and soil penetration resistance after cultivation (Vleeshouwers & Kropff, 2000). In the present study, *S. media* was the most abundant species, occurring at high densities (Table 6.1). Weed management practices affecting *S. media* will therefore have a large effect on the total on farm weed pressure. Under favourable conditions, *S. media* can germinate and emerge all year round in the Netherlands (Sobey, 1981). Seeds of this species emerging in late summer and early autumn, after crop harvest, will be controlled by the main soil tillage operation. When this control takes place in spring, these late-emerging plants are able to grow during winter and produce seeds before the tillage operation takes place in early spring. However, when a treatment is already performed in the autumn, those late-emerging plants will be controlled and will not contribute to the weed pressure in the following season. The weeds emerging during winter after the soil tillage will have a reduced growth and seed production, thereby contributing less to the weed pressure in the following growing season than the weeds emerging in autumn.

Hand weeding hours, average field size and soil type were not included in the models that gave the best fit for one of the weed pressure parameters. The hand weeding effort (that is, the average number of hand weeding hours ha^{-1}) of the farmers was determined by the average field size (ha). The larger the field, the lower the number of hand weeding hours per ha (Figure 6.2). The number of hand weeding hours did not significantly differ between farms on a sandy soil and farms on a clay soil (p=0.129). Earlier studies with multi-year experiments by Van der Weide *et al* (2008a) and Melander &

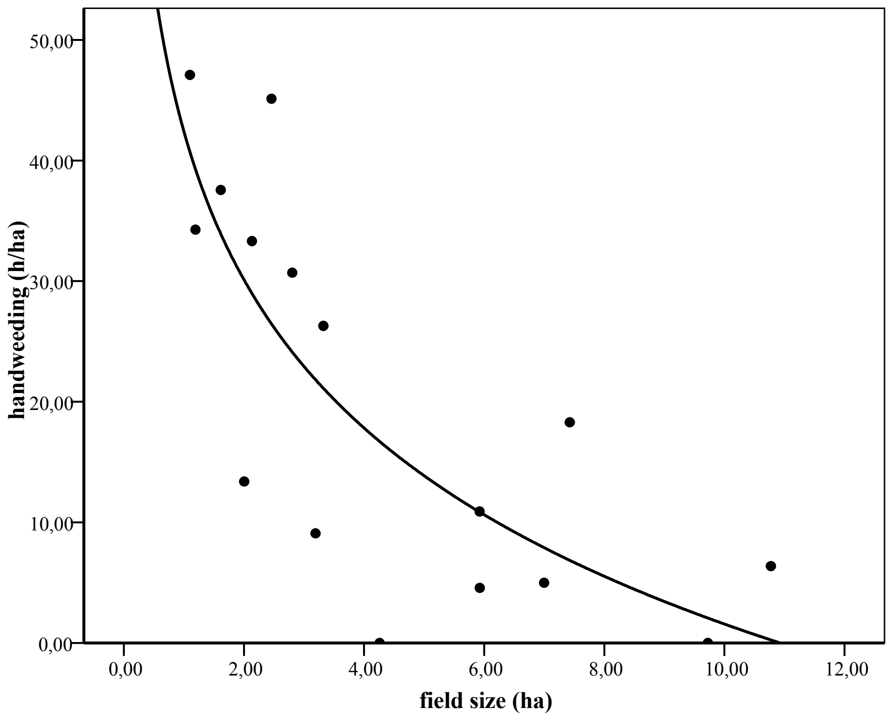

Figure 6.2. Relationship between manual weed control effort (hand weeding hours per ha) and the average field size (ha). R^2=79.2. Y =42.41-17.74log(x) with Y= manual weed control effort (hours per ha), and x= field size (ha).

Rasmussen (2001) showed that the amount of hand weeding (h ha^{-1}) was positively related to the weed density (plants m^{-2}). The difference between those previous studies and the present study can be explained by the farmers' behaviour. The previous studies were carried out on experimental farms and had the objective to manage the weed populations according to best available weed management practices. In contrast, manual weed control on the sixteen farms in this study was related to field size. With increasing field size, the average hand weeding hours per ha decreased. This result suggests that farmers can only dedicate a certain amount of time to manual weed control, independent of the weed densities on the fields. The results further imply that the adoption of preventive weed control measures may provide farmers with an effective way of reducing the weed pressure on their farms. It is well known from previous studies that the use of preventive measures such as the stale seedbed technique (Chapter 2 and Riemens *et al*., 2007b) and the use of a high cropping density (Kropff *et al*., 1993) can significantly reduce weed densities. This is the first study in which the importance of preventive weed control measures for weed pressure is shown in an exploratory study on commercial farms.

Results should be interpreted carefully, because investigated factors were intertwined. An example is the correlation between the applied preventive weed control measures and the soil type. On farms with a sandy soil type the average number of applied preventive weed control measures was 2.88 per farm, which was significantly ($p=0.012$) lower than the average of 4.63 per farm on a clay soil. Due to this correlation, soil type and the number of preventive measures could not be included in one model. The model that best fitted the weed pressure data was the model with the preventive measures, thereby excluding soil type from the model. Soil type did influence a factor that was included in the models; the number of applied preventive measures on a farm.

Table 6.4. Farmers' self reported grower typology and farmers' risk perception of soil structural damage as a reason not to control weeds mechanically, in relation to the observed weed pressure (weed density and weed seed production). Different letters indicate significant differences between grower typologies and within the farmers' risk perception of soil structural damage within columns at the 5% level.

		weed pressure (mean ± s.e.m.)	
		weed density (plants/m2)	weed seed production (seeds/m2)
type of grower	crop growth oriented	4.6 ± 1.82[a]	1728 ± 624.2[a]
	market oriented	17.6 ± 3.29[b]	13592 ± 4815.9[b]
how often is the risk of soil structural damage a reason not to control weeds	never	5.0 ± 1.00[a]	2197.0 ± 729.96[a]
	sometimes	8.1 ± 3.18[b]	5510.9 ± 3299.11[a]
	often	22.2 ± 3. 37[c]	16749.1 ± 7406.21[b]

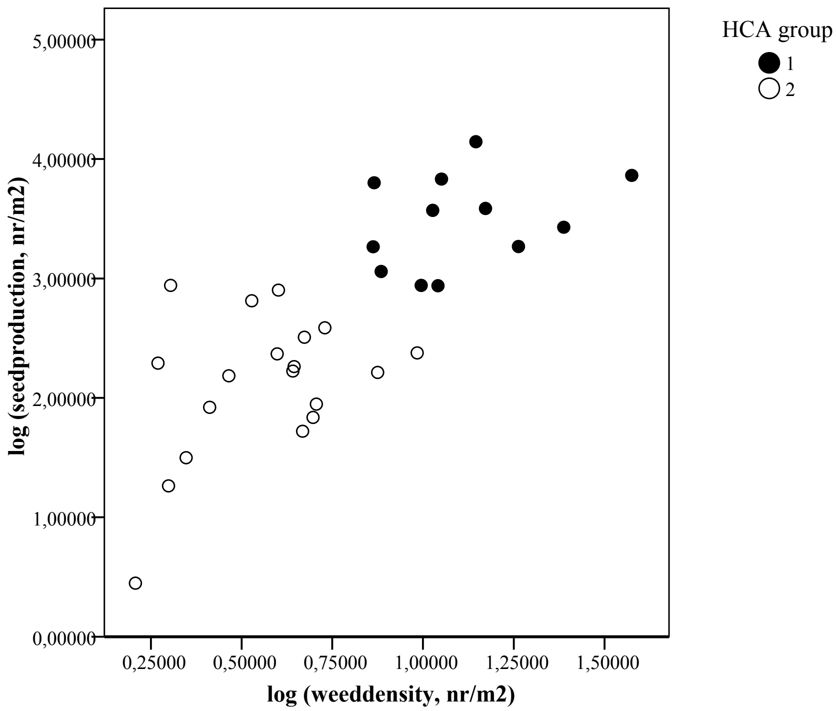

Figure 6.3. Grouping of crops with HCA (hierarchical cluster analysis) based on weed density and seed production. HCA group 1 contains crops allowing high weed densities and weed seed productions, HCA group 2 contains crops that allow lower weed densities and weed seed production.

Crops allowing high weed pressures

The hierarchical cluster analysis showed that crops could be grouped into two groups (Figure 6.3); HCA group 1 with high weed pressure (that is, with high weed density and weed seed production) and HCA group 2 with lower weed pressure. HCA group 1 contains bulb crops (tulip), lettuces (lettuce, endive), onion like crops (e.g. onions, carrots, leek, fennel), sunflower, and vegetable crops (courgette, pumpkin). HCA group 2 contains cabbages (e.g. brussel sprouts, cauliflower, broccoli, chinese cabbage), potatoes, celeriac, cereals (e.g. oat, winter- and summer barley, winter-and summer wheat, rye), grass, and legumes (peas, fresh beans). For each farm we calculated which percentage of the crops that were grown belonged to HCA group 1. Crops with high weed pressures (HCA group 1) are crops with low competitive abilities to weeds. Apparently, this factor has more effect on these farms than the mechanical control possibilities that these crops offer, compared to for instance cereal crops. Again results need to be interpreted with care; the farms located on a sandy soil differed from farms on a clay soil in the percentage of crops grown from HCA group 1 as well. The percentage of crops from HCA group 1 grown on sandy soils was on average 42%, on clay soils it was on average 11% ($p<0.001$). Since soil type determines to some extent the crop types that can be grown, and the crop determines for a large part the management options, factors are intertwined.

Linking farmers' self reported beliefs on weed management with weed pressure

Grower typology (crop growth or market oriented), beliefs on soil structural damage caused by mechanical weed control, and awareness of the influence of crop choice on weed growth were questionnaire variables in which farmers significantly differed. Farmers that regarded themselves more market-oriented growers had a higher average on farm weed pressure (Table 6.4). The mean weed density on farms managed by market oriented farmers was 17.6 plants m^{-2}, while the mean weed density on farms managed by crop growth oriented farmers was 4.6 plants m^{-2}. A similar pattern was found for the mean weed seed production; on farms managed by market oriented farmers the average weed seed production was 13592 weed seeds m^{-2}, while the mean weed seed production on farms managed by crop growth oriented farmers was 1728 weed seeds m^{-2}.

Farmers that were aware of the influence of crop choice on weed growth and took the farm weed situation into account during crop choice, significantly ($p=0.03$) choose less crops from HCA group 1 than farmers who did not take the weed situation into account (37% vs. 16%). Those farmers that believe that soil structural damage can occur when weeds are mechanically controlled and often not control weeds mechanically to avoid soil structural damage had a significantly higher average weed density than farmers that never or sometimes avoid mechanical weed control to prevent structural damage (Table 6.4). The same trend emerged for the weed seed production, although differences between farmers that often avoid the and sometimes avoid mechanical weed control were not significant.

Conclusions and considerations for future research

In this exploratory study we investigated the relationship between weed pressure and weed management behaviour on commercial, organic farms. In addition we explored the possibility of the use of a questionnaire to identify beliefs of farmers regarding their weed management and weed pressure. Differences between organic farms in weed pressure were influenced by differences in farmers' management behaviour. Preventive measures and timing of main soil tillage operation were identified as the most influential weed management factors for weed pressure. The farmer's beliefs and knowledge on weed control techniques differed for farmers with different weed pressures. The sample size we eventually choose (n=16) was a compromise between a minimization of the number of fields for determination of the weed pressure on one hand, and a maximization for the questionnaire variables on the other hand. In future studies, a better view on farmers' beliefs and perceptions on weed management behaviour can be obtained by increasing the number of farmers and reducing the number of fields for weed pressure monitoring. Qualitative aspects such as the perception a farmer has concerning weeds, the strategy the farmer uses to achieve certain goals, the awareness of certain processes in weed biology and the reasons for the use of certain techniques are much more difficult to quantify than the quantitative aspects such as hand weeding hours. Wilson *et al* (2008) presented the mental model approach to identify the motivational and cognitive processes underlying farmer decision making. In future research, the incorporation of the human dimension, in terms of farmers' beliefs, attitudes and behaviour and the underlying processes with the mental model approach, can lead to a better understanding of the (organic) farming systems and lead to more effective weed management in those systems.

Acknowledgements

We would like to thank Saskia Burgers from the mathematical group Biometris of Wageningen University and Research Centre for her statistical support. The research described in this article was carried out within the Research Program ''Non-chemical weed control'' (Program BO-06-397-V) financed by the Dutch Ministry of Agriculture, Nature and Food Safety.

CHAPTER 7

General discussion

On farm weed management is influenced by many factors (Figure 1.3). These factors comprise the development and availability of weed management tools, the environmental impact of these tools and the attitude and behaviour of the farmer. In Chapter 1 we identified major gaps in our knowledge on each of these aspects. Research questions were formulated concerning ecological weed management strategies depleting the soil seed bank, environmental effects of chemical weed management and the human dimension of weed management. In **part A** of this thesis the efficacy of increasing the losses from the soil seed bank (Chapter 2) and reducing the input to the soil seed bank (Chapter 3) was investigated. In **part B** the major gaps in our knowledge on the effects of a broad-spectrum (Chapter 4) and a small-spectrum herbicide (Chapter 5) on non target terrestrial plants were targeted. In **Part C** the relationship between the attitude and beliefs of farmers and its effect on the on farm weed pressure was investigated (Chapter 6).

In this Chapter the contribution of the research described in this thesis to our understanding of seed bank dynamics and weed management at the farm level, the environmental effects of weed management and the human dimension are discussed. Future research needs concerning these topics are formulated.

A1. Understanding seed bank dynamics and weed management

The ecological weed management principle "depleting the soil seed bank" was investigated in Chapters 2 and 3 (part A) of this thesis. In Chapter 2 we showed that the use of a stale seedbed technique in April or May can provide farmers with a tool to increase the losses from the soil seed bank by enhanced germination and emergence of the total weed community. Unfortunately we were unable to show significant differences for the individual species. Nevertheless, we do know that some of the most abundant annual weed species in the Netherlands, such as *Chenopodium album, Stellaria media and Solanum nigrum,* have their peak in emergence in April and May (Colbach *et al*., 2005; Chancellor, 1986; Van den Brand, 1987 & 1985a, b; Roberts, 1964). These species also formed the most abundant species in the fields described in Chapter 2. Furthermore, the application of a stale seedbed in April or May was also found to be an important factor reducing the on farm weed pressure on sixteen organic commercial farms in Chapter 6. The most abundant species on these farms was *Stellaria media*. This implies that the induced emergence and control of these abundant species may be responsible for the observed reduced weed densities during crop growth after application of a stale seedbed. For a further maximization of the efficacy of techniques based on light sensitivity and subsequent germination and emergence of seeds, such as the stale seed bed technique, we need more insight in the seed bank dynamics of weeds in relation to their complete life cycle (Figure 1.2). The gathering of scientific knowledge on the relationship between the soil seed bank and the aboveground weed population began with the development of periodicity tables for common agricultural weeds in the beginning of the 1980s (e.g. Roberts (1981 & 1984)). Since then several attempts have been made to unravel the soil seed bank dynamics of agricultural systems. Two main routes have been followed: an empirical approach and a reductionist approach (Grundy, 2003). The empirical approach requires long term weed emergence data and associated meteorological data. Based on the combined analysis of these datasets the meteorological factor that is most influential for weed emergence is selected and used as main factor dominating the prediction of emergence in relatively simple models. The downside of this approach is that the prediction of low emergence by the models can be either the result of unfavourable meteorological conditions or a large percentage of seeds with a high dormancy state (Grundy, 2003); the models do not provide insight in these processes. The reductionist approach overcomes this problem by partitioning the processes underlying the observed emergence of weeds in the field into three major components: dormancy relief, germination and pre-emergence growth. Although these models have the potential of predicting the emergence of weeds under a range of realistic conditions, very few studies have attempted to combine these three components in one mechanistic model, because of the high complexity. Vleeshouwers & Kropff (2000) provided us with a combined model for *Chenopodium album, Polygonum persicaria* and *Spergula arvensis*, and Van der Weide (1993) with one for *Galium aparine*. One of the major challenges for future development of this kind of models is our limited understanding of dormancy. It is unclear how seeds perceive changes in their environment (such as temperature, moisture and light) and how this perception is used to change their dormancy state. The development of dormancy sub-models, based on data obtained at the molecular physiological level, may provide us with the required insight (Chao, 2002).

Another important aspect is the quality of the input data of these predictive models; the prediction of a model is as reliable as its input data (Grundy, 2003). Data required by emergence models are climatologic data and seed bank estimates (species and number of seeds), sometimes supplemented with data on soil characteristics (Colbach *et al*., 2006). As we discuss in Chapter 3, obtaining a reliable estimate of the size and composition of the viable soil seed bank is still very difficult. Although considerable research effort has been made to determine how the seed bank can best be sampled (Dessaint *et al*., 1996), a final methodology has not been developed yet. The main problems are the non-normal distribution patterns of the seeds, the presence of species in very low numbers, and a quick determination of seed viability after excavation. The use of a model weed to unravel processes determining relief of dormancy, germination and emergence would be helpful to our understanding of the below- and aboveground relationship (Chao *et al*., 2005). Because of its high abundance and densities in agricultural fields, its large geographical distribution (Chapters 2 and 6), (Mertens *et al*., 2002) and competitive nature (Storkey & Cussans, 2007; Lutman *et al*., 2000), *Stellaria media* would be a good candidate species for future research efforts on predictive modeling of emergence from its soil seed bank.

A2. Seed bank dynamics and weed management at the farm level

Organic farming systems

In Chapter 3, three strategies that allowed for different levels of seed return to the soil seed bank were compared: one strategy aimed at a 100% prevention of seed return, one aimed at the prevention of seed return from the largest plants (thus, the plants with most seeds), and a reference strategy, representing standard weed control in an organic system. The strategies were applied for a period of seven years and their effects on the aboveground weed density and the soil seed bank were monitored at the end of this period. The farm was converted from a conventional to an organic managed farm in year one. In general, the size of the seed bank under organic weed management systems is larger than under herbicide-based systems (Sjursen, 2001; Albrecht &Sommer, 1998). Therefore it was expected that the soil seed bank would increase in size under the standard organic strategy, would decrease under the strategy aiming at a 100% prevention of seed shed and decrease or remain at a similar level under the intermediate strategy. However, the soil seed bank increased in size under all applied strategies. This result shows that it is very hard, and perhaps impossible to achieve the desired decrease of the soil seed bank by prevention of weed seed return alone. Weed management strategies depleting the seed bank can only be successful if they not only prevent seed return to the soil, but also target other parts of the weed's life cycle as depicted in Figure 1.2. Based on the results from part A of this thesis, a successful organic management system should manipulate the germination and emergence of weeds and at the same time remove sufficient amounts of the above-ground biomass before seeds will be returned to the soil seed bank. In Chapters 2 and 6 we showed that the use of a stale seed bed can provide farmers with an efficient tool to manipulate the germination and emergence of weeds and reduce the on farm weed densities during crop growth. For a viable and cost effective organic sector, management systems combining these two and the other ecological weed management principles depicted in Figure 1.1 are required. This requires insight in weed population dynamics in time and space, the competitive interaction between crops and weeds, and the influence of agricultural practices on these dynamics (Freckleton & Stephens, 2009).

Integrated farming systems

Preventive measures such as the application of a stale seedbed are not only relevant for organic farming systems, but also for integrated, herbicide based systems. For all systems the phrase "Prevention is better than to cure", is applicable. However, also from an economic point of view the relevance of these measures will increase in the future. Due to European regulations the number of available herbicides is decreasing. Already for some small crops (small meaning a relatively small number of ha world wide, but large economic benefits in the Netherlands) the number of available herbicides is reduced to zero or one. The use of preventive measures, based on ecological principles, can reduce the weed densities during crop growth and the need to control. On top of that, results from Chapter 2 show that the stale seedbed technique in combination with mechanical control of emerging weeds can reduce the weed population during crop growth as effective as chemical control can.

Although the development of preventive measures will continue to be important for integrated systems, the development of precision technologies will be a key factor for a further decrease of herbicide use in integrated farming systems during the next decade. The precise application of available herbicides can save on economic costs (Christensen *et al*., 2009; Kempenaar *et al*., 2009) and reduce environmental impact at the same time. Precision application of herbicides or mechanical implements in time and space requires an accurate detection of large numbers of unknown weed species within different crops while making instantaneous decisions (Christensen *et al*., 2009). This kind of technology can be developed for agriculture, but the current speed of development is slow. According to Kropff *et al*. (2008) the progress could be much faster with enhanced investments. Farmers' income needs to be boosted to create an opportunity to invest in this new technology, other wise these products will keep lacking commercial interest.

In the past, major developments in Dutch agriculture were for a large part induced by the government. Especially after World War II the Dutch government invested heavily in agricultural research to increase the cost-effectiveness and production of agricultural systems. To speed up current developments towards more sustainable agricultural systems with a reduced herbicide (and pesticide) dependence, governments could stimulate farmers towards the use of more technology based agriculture instead of subsidizing farmers to increase production.

B. Environment

An important group of decision makers involved in the reduction of adverse effects of weed management are the regulators. Weed management tools are designed to control plants, and as a result, non target plants are at risk the most when these tools are applied. Current guidelines used by governmental agencies and companies to assess the risk of herbicides to non target plants are very limited in their description of how and what data should be collected and analyzed to asses the risk to non target terrestrial plants. The main reason for this limited guidance is that regulators themselves are not exactly sure about what needs to be protected. Non-target plants are defined as non-crop plants located outside the treatment area. Directive 91/414/EEC does not contain specific data requirements for non target plants. It generally states that there is a need to report all potentially adverse effects and additional studies need to be undertaken where there are indications of such effects. As a result, all herbicide applications should at the moment being turned down by regulators because these compounds will always affect plants. This situation is unwanted by both regulators as well as companies (Füll *et al*., 2000). Currently, two main questions can be formulated. First of all: *what do we want to protect*? And the second one: *how do we test the risk that these compounds pose to that which we want to protect?*

In this thesis we have focused, in Chapters 4 and 5, on aspects that are important for the second question: translation of data from greenhouse to field conditions, from single species to multiple species, recovery of species after exposure and the effects on reproduction. We by all means, do not want to pretend that we have answered all of these questions or provided society with sufficient data to answer them. However, we have provided insight in the responses of wild species to sublethal dosages and contributed to the on going discussion.

Our results from Chapter 4 with the broad spectrum herbicide glufosinate ammonium as well as results from Chapter 5 with the small spectrum herbicide tepraloxydim indicate that the use of measured effects in greenhouse studies on single species may be used to determine the risk of herbicides to single species in the field. However, the relationship between greenhouse and field effects is probably herbicide specific and should be assessed for each group of herbicides with a similar mode of action. For each relationship a safety factor should be developed to account for the variation introduced by climatological conditions, but also genetic and phenotypic diversity amongst the non-target species to ascertain that greenhouse data can be used to protect non-target plants from effects in the field. The use of single-species tests in the ecological risk assessment of herbicides to vegetations may not always be valid. Exposure of single species to sublethal herbicide concentrations can not be used to predict effects of the same species in a mixed vegetation. Important factors that need to be taken into account are the species composition of those communities, mode of action of the herbicides, the development stage of the margin and the choice of endpoint (biomass, reproduction).

The other question, "what needs to be protected" requires insight in the system of which non-target plants are part. Regulators need to make decisions about the protection of system functioning of these margins. They require knowledge on the biodiversity and species abundance of field margins to make their decision. Several studies have focused on the diversity of these margins and how they are affected by agricultural practices (Kleijn & Verbeek, 2000; Kleijn & Snoeijing, 1997). Herbicides strongly influenced the species abundance and composition of the margins in a Dutch study (Kleijn & Snoeijing, 1997). However, insight in the role of plant diversity in the functioning of the ecosystems in those field margins is still needed. On top of that, the species composition of margins differs from country to country. Therefore, the development of a standardized vegetation for risk assessment protocols is an enormous challenge, which may appear to be impossible. A regional risk assessment would be the alternative.

Direct environmental effects of weed management are mainly caused by herbicide drift. Therefore, during the last two decades mechanical weed control has received a lot of attention and is regarded as a more environmental friendly way to control weeds. However, during the development of a more sustainable weed management strategy, indirect effects should not be forgotten. Like other agronomic practices, weed control requires energy. Based on equations described in the Appendix we calculated and compared the energy use of weed management in a conventional, integrated and organic farming system under Dutch circumstances on a clay soil and a sandy soil (Figure 7.1). The energy use of weed control in an organic system on a sandy soil is a factor 1.7-1.8 times higher than in an integrated or conventional system and a factor 2.1-2.5 times higher on a clay soil. Of course, weed management is only part of the total production system, other agronomic practices such as fertilisation should not be neglected. In previous research, fossil energy use of farming systems (including pest control and fertilization) was compared at the crop production level. In general, the total energy consumption of organic production is on average lower than of conventional production (Haas *et al*., 2001). The reduction in energy use varied from 21 to 43 %. When comparing the energy input/output ratio of these systems, data is less clear. For some cropping systems the organic systems have a higher output compared to the input, for others, conventional systems have a better result (Bertilsson *et al*., 2008; Dalgaard *et al*., 2001).

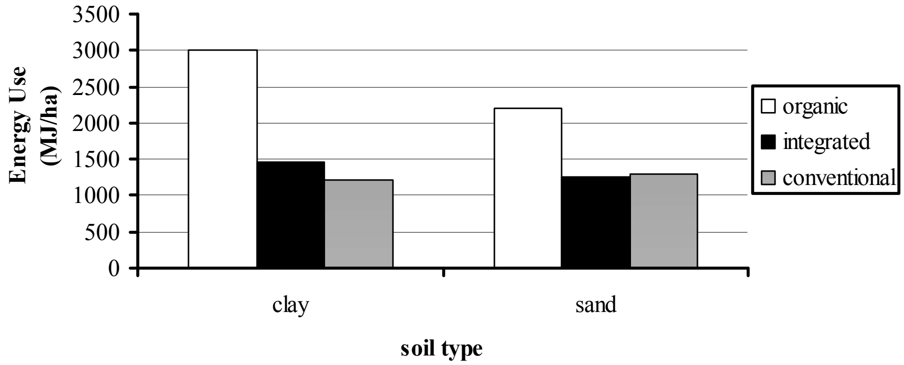

Figure 7.1. Average energy use (MJ/ha) of weed management in an organic, integrated and conventional Dutch farming system, on a clay and a sandy soil.

C. Human dimension

The perception a farmer has concerning weeds, the strategy the farmer uses to achieve certain goals, the awareness of certain processes in weed biology and the reasons for the use of certain techniques are an important part of weed management systems (Chapter 6). The identification of the motivational and cognitive processes underlying farmers' behaviour can therefore lead to a better understanding of weed management systems, organic as well as herbicide-based.

One way to better understand the factors driving decision making is to develop a mental model with respect to the stimulus in question (Morgan *et al*., 1992). The concept of mental models is a well-established theory in psychology and decision science and has been the focus of extensive research (Fischhoff & Downs, 1997; Atman *et al*., 1994; Bostrom *et al*., 1992). Mental models affect how an individual defines a problem, reacts to issues, gathers and processes information, assesses risks and benefits, and makes decisions concerning topics that come to his or her attention through various sources of communications. Mental models define the boundaries of thought and action and tend to prevent people from seeing alternative perspectives. As such, they limit people to familiar patterns of reasoning and action. Effective analyses of mental models can identify *how* different groups of people think about and respond to a variety of topics, including benefits associated with educational activities, and proposals to improve their business lives. Mental models can be assessed through a methodology that includes 1) developing an expert-centered technical model, 2) eliciting an audience-centered mental model through open-ended interviews, 3) conducting a larger confirmatory survey assessment of the target audience, 4) drafting a risk communication message, and 5) evaluating and refining the message. This mental model approach has been applied to determine what people know, and more importantly, what they need to know about issues like global warming (Bostrom *et al*., 1994), radon in homes (Bostrom *et al*., 1992), wildland fire (Zaksek & Arvai, 2004), food safety (Fischhoff & Downs, 1997), and using nuclear energy sources in space missions (Maharik & Fischhoff, 1993). However, the mental models approach has not been widely used in agriculture, despite the abundance of difficult, risk-based decisions that must be dealt with on a regular basis, and the influence that communications from the scientific community and chemical industry representatives could potentially have on farmer decision making. These communication efforts need to be strategically designed and targeted to address the issues known to be most important for strengthening understanding and improving agricultural risk management decisions (Llewellyn *et al*., 2005). Currently, this mental model approach is under investigation in a joint Dutch-US project for weed management (Doohan *et al*., 2009). This approach can be used to develop new and improve existing decision support systems (DSS). Currently, available DSS provide farmers with a tool to reduce the herbicide dose without loosing efficacy. The MLHD technology (Kempenaar & Van den Boogaard, 2004) is a Dutch DSS based on the fact that labels often recommend doses higher than the required doses to control weed populations under standard conditions. The system delivers stage and species dependent recommendations for specific groups of herbicides. The system also provides a handheld MLHD sensor which can be used to measure the efficacy of the applied dose at a moment when effects of the herbicides can not be seen by eye yet. This method of detection has been a key factor in the acceptance of this DSS by farmers (Kempenaar & Van den Boogaard, 2004). DSS providing information on the minimum effective dose on the right place and moment are however still lacking. The main reason is the large amount of factors that need consideration such as the composition of the weed population, the weed and crop development stages, climatic and soil conditions, application technology and additives (Riethmuller-Haage, 2006).

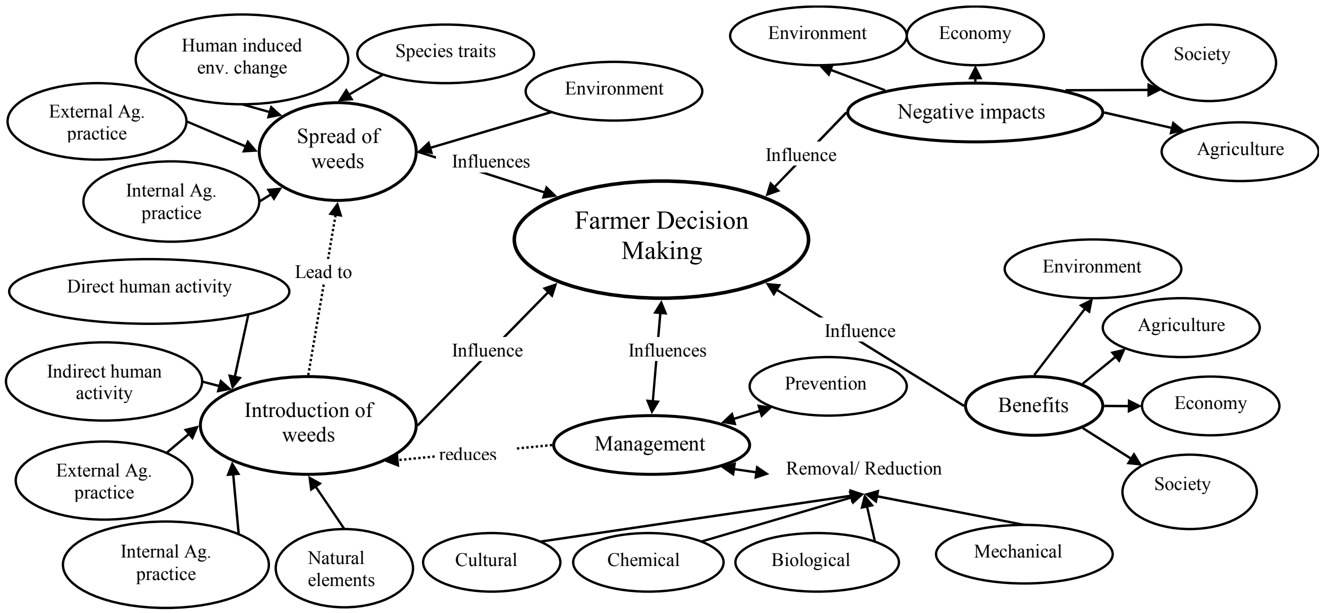

Figure 7.2 Mental model of weed management. Adapted from Wilson et al (2008).

When the development of this kind of DSS becomes possible, or DSS based on ecological weed management, the development of a mental model can provide knowledge on the farmer's behaviour and the risks that they perceive of the new tools, which will be important for a successful adoption of these tools by farmers.

Concluding remarks

As the schematic overview in Figure 1.3 shows, on farm weed management is influenced by many factors (development and availability of tools, environmental impact of tools and the attitude and behaviour of the farmer). For each of these factors we investigated research questions targeting gaps in our knowledge for that specific aspect of weed management. The use of methods that reduce the seed bank proved to be a useful strategy to prevent the frequently observed increase in weed pressure on farms that convert from a conventional to an organic system. Ecological weed management systems should however target more than one stage of the weeds life cycle to reduce weed populations. Many little hammers are needed to reduce the on farm weed populations, and targeting the seed bank is one of them. Ecological weed management systems can be beneficial for organic as well as integrated farming systems. The incorporation of these principles in new weed management strategies can contribute to a reduction of herbicide use and a reduced environmental impact on non targets. The attitude and beliefs on weed management of farmers are very important for the adoption of these new strategies and should be taken into account during its development. Therefore, the combination of natural and social sciences is needed for the further development of sustainable weed management systems.

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APPENDIX

Energy consumption by weed control in a conventional, integrated and organic farming system

To calculate the energy consumption of weed control in a conventional, integrated an organic farming system, we defined a crop rotation, weed management tactics per system and equations for direct and indirect energy use.

Description of a crop rotation and weed management tactics

To compare the energy consumption of weed management in a conventional, integrated and organic farming system, we defined a crop rotation on an arable farm on a clay and on a sandy soil, representative for the Dutch situation. To be able to compare the energy consumption needed for weed control between farming systems, we used one description of a crop rotation for all farming systems (Table A.1). In practice however, the organic farming systems in the Netherlands usually have a wider crop rotation scheme than the conventional or more integrated systems.

Table A.1. Description of a crop rotation and farm size for a sandy and a clay soil.

	soil type	
crop	sand (ha)	clay (ha)
potato	15	15
winterwheat	15	15
sugarbeet	15	15
carrot	7.5	7.5
onion		7.5
	52.5	60

The type and frequency of the applied weed control techniques we used were previously defined by Bleeker *et al*, (2002) for an organic system, and by Bruinsma *et al* (2003) for an integrated and conventional farming system (Tables A.2 and A.3).

Table A.2. Type and frequency of the applied weed control techniques in a conventional, integrated and organic system on a clay soil (according to Bruinsma et al, (2003) and Bleeker et al.(2002).

	conventional	integrated	organic
potato	1x ridging	1x ridging	1x ridging
	1x 1 kg metribuzin	1x harrow	1x harrow
		1x earth up	1x earth up
		1x per 3 years 0,25 kg metribuzin	
winter-wheat	1x 2L isoproturon (500) fall	1x 0,2 L clodinafoppropargyl(240) cloquintoceet-methyl(60) (spring)	5x harrow
	1x 1,5 L bifenox mecopprop-P(308) + 0,5 L fluroxypyr (200) (spring)	1 x 1L bifenox(250) mecopprop-P(308) + 0, 4L fluroxypyr (200)+ 15 g Metsulfuron-methyl(20%) (spring)	
onion	1x 2L MCPA (spring)	pre-emergence: 1x3L glyphosate(360)	pre-emergence: flame weeding
	pre-emergence: 1 x 1,5 L pendimethalin	post-emergence: 2 x 0,15L bentazon(480)+ 0,15L ioxynill(200)	4 x hoe
	pre-emergence: 1 x 3 L glyphosate (360)	2x hoe/ fingerweeder combination	
	post-emergence: 1 x 1L chlorprofam		
	1x 0,15L bentazon (480)+ 0,15L ioxynill(200)		
	1x0,25L bentazon (480)+ 0,25L. ioxynill (200)		
sugar-beet	3 x0,5 L fenmedifam (157)+ 0,5L metamitron (70%)+0,5L ethofumesate(200)+ 0,5L mineral oil(800)	1,5x 0,5L fenmedifam(157)+0,5L metamitron(70%)+0,5L ethofumesate(200)+0,5L mineral oil(800)	2 x harrow
	2x hoe	2 x hoe+fingerweeder	2 x hoe
		1x hoe + earth up	1x hoe + earth up
carrot	3x 0,5L metoxuron+0,5L nonfenol-polyethoxyethanal(250)	1 x0,5L metoxuron (80%) +0,5L nonfenol-polyethoxyetganal(250)	pre-emergence: 1x flame weeding
		2x hoe+ fingerweeder	3x hoe

Table A.3. Type and frequency of the applied weed control techniques in a conventional, integrated and organic system on a sandy soil (according to Bruinsma et al (2003) and Bleeker et al. (2002)).

	conventional	integrated	organic
potato	1x 0,75kg metribuzin	2x harrow	2x harrow
	1x harrow	2x hoe + earth up	2x hoe + earth up
	1x hoe	1x earth up	1x earth up
	1x earth up		
winterwheat	1x 1,5L diflufenican(20)+ ioxynill(100)+ isoproturon(400)	1x 0,2L clodinafop-propargyl(240) cloquintoceet-mexyl(60) (spring)	5x harrow
	1x0,38L fluroxypyr(180)	1 x 1L bifenox(250) mecopprop-P(308) + 0,4L fluroxypyr (200)+ 15 g metsulfuron-methyl (20%) (spring)	
	1x 0,01 kg metsulfuron-methyl (20%)	1x per 3 years 2L MCPA (spring)	
sugarbeet	1x 0,5L quizalofop-P-ethyl (50)	2,5x 0,5L fenmedifam(157)+0,5L metamitron(70%) +0,5L ethofumesate(200)+ 0,5L mineral oil(800)	2x harrow
	4x 2,5L fenmedifam (157)+ 2,5kg metamitron(70%)+ 2,5L ethofumesate (200)+2,5L mineral oil(800)	1x hoe+fingerweeder	2x hoe
		1x hoe+ earth up	1x hoe+ earth up
carrot	1x 0,25L clomazone(360)+ 1L linuron(500)	1x 0,25L clomazone(360)+ 1L linuron(500)	pre-emergence: 1x flame weeding
	3 x0,5 L metoxuron(80%)+0,5L nonylfenol-polyetanal(250)	1x0,5 L metoxuron(80%)+0,5L nonylfenol-polyetanal(250)	3x hoe
		2x hoe+ fingerweeder	

Description of equations to calculate energy use needed for weed control

Energy is defined as fossil energy measured in joule (J). All fuels and electricity are assumed to come from fossil energy sources. Energy use (EU) by weed control is defined as the net energy used for the control of weeds (Hulscher, 1991). Energy use can be divided into direct and indirect EU. Direct EU is defined as the energy input used for weed control that can be converted directly into energy units (eg diesel-fuel). Indirect EU is defined as the energy used for weed control that can not be converted directly into energy units (development and maintenance of machinery, herbicides).

The total energy use for the use of a specific weed control technology (EU_{wc}) can be expressed by equation (1):

$$EU_{wc} = (EU_{direct} + EU_{indirect}) * D \quad (1),$$

in which the EU_{direct} is the direct energy use per hour (MJ/h) and the $EU_{indirect}$ is the indirect energy use per hour (MJ/h), and D is the duration of the activity (h/ha).

The duration of the activity D on its turn is determined by the forward speed s (km/h), the implement width i (km) and the field efficiency e (-):

$$D = \frac{1}{s * i * e} \quad (2).$$

Direct energy use

The direct energy use of a specific weed control tactic is determined by its fuel use per hour F (kg/h), and the energy content of the fuel E_c (MJ/kg):

$$EU_{direct} = F * E_c \quad (3).$$

The fuel use per hour is determined by the required power P (KW/h), the fuel use at full power F_p (kg/h) and the corrected load of the tractor L_c (-):

$$F = P * F_p * L_c \quad (4).$$

The load of a tractor is never 100%. The maximum power that can be developed through the ground is depending on the kind of tracking machine. We assumed that the load of a two wheel driven tractor is 60%, of a four wheel driven tractor is 70% and for self driving vehicles it is 80%. Furthermore, the load is dependent on the type of weed control operation that is carried out. For each weed control operation a correction factor was used to take this into account (Table A.4).

The corrected load (L_c) is calculated as:
$L_c = L - (L * c_f)$ (5),
with Load L, and correction factor c_f.

Indirect energy use
The indirect energy use ($ED_{indirect}$) is determined by the weight of the machinery W (kg), the energy use required for production, maintenance and transport of the machinery EU_{main} (MJ/kg), the life span of the machinery L_s (yr) and the user frequency f (h/yr):

$$EU_{indirect} = \frac{(W * EU_{main})}{(L_s * f)} \quad (6).$$

Table A.4. Load and correction factors for the tractor load per weed control operation.

weed control operation	Load (L)	correction factor (c_f)
herbicide spraying	0,60	0,30
rotary harrow	0,70	0,00
harrow	0,70	0,00
hoe	0,70	0,15
fingerweeder	0,70	0,15
flameweeder	0,60	0,30
earth up	0,60	0,15

Summary

On farm weed management is influenced by many factors. These factors comprise the development and availability of weed management tools, the environmental impact of these tools and the attitude and behaviour of the farmer. In Chapter 1 we identified major gaps in our knowledge on each of these aspects. Research questions were formulated concerning ecological weed management strategies depleting the soil seed bank, environmental effects of chemical weed management and the human dimension of weed management. In part A of this thesis the efficacy of increasing the losses from (Chapter 2) and reducing the input to (Chapter 3) the soil seed bank were investigated. In part B the major gaps in our knowledge on the effects of a broad-spectrum (Chapter 4) and a small-spectrum herbicide (Chapter 5) on non target terrestrial plants were targeted. In Part C the relationship between the attitude and beliefs of farmers and its effect on the on farm weed pressure was investigated (Chapter 6).

Part A Weed management strategies depleting the soil seed bank

In Chapters 2 and 3 (part A of this thesis) the efficacy of weed management depleting the soil seed bank was investigated. The reduction of the soil seed bank by means of increasing the losses as a result of stimulation of germination and emergence of weed seeds was studied in Chapter 2. The effects of stale seedbed preparations and several weed control methods on the emergence of weeds were observed. Specific goal was to evaluate the use of a stale seedbed in combination with chemical or mechanical weed control methods in the field. Depending on location and year, stale seedbed preparations followed by weed control prior to planting reduced the amount of weeds during crop growth by 43 to 83%. Control of the emerged seedlings after a stale seedbed preparation was more effective with glyphosate than with a rotary harrow. Covering the rotary harrow during control improved its effect on the weed density during crop growth in two out of three years. Radiation with far red light (FR) did not reduce the number of emerging weeds in this study. Mechanical control by finger weeder, torsion weeder and hoe was applied without stale seedbed preparations. These measures reduced the weed densities with 88 to 99% compared to the untreated control and were more effective than chemical weed control with carbeetamide and chloorprofam. The results show that the stale seedbed technique in combination with

mechanical control of emerging weeds can reduce the weed population during crop growth as effective as chemical control can, and may therefore help reduce the need for herbicides in the future.
In Chapter 3 weed management strategies decreasing the soil seed bank by reducing the seed input were studied. Three strategies that allowed for different levels of seed return to the soil seed bank were compared: one strategy aimed at a 100% prevention of seed return, one aimed at the prevention of seed return from the largest plants (thus, the plants with most seeds), and a reference strategy, representing standard weed control in an organic system. The strategies were applied for a period of seven years and their effects on the aboveground weed density and the soil seed bank were monitored at the end of this period. The farm was converted from a conventional to an organic managed farm in year one. In general, the size of the seed bank under organic weed management systems is larger than under herbicide-based systems. Therefore it was expected that the soil seed bank would increase in size under the standard organic strategy, would decrease under the strategy aiming at a 100% prevention of seed shed and decrease or remain at a similar level under the intermediate strategy. However, the soil seed bank increased in size under all applied strategies. Nevertheless, the weed densities in plots treated with the strategy aiming at a 100% prevention of seed return became significantly lower than the weed densities in plots treated with the other strategies. The number of manual weeding hours required to prevent weed seed return in addition to the number in standard organic farming practices was reduced during the study. Targeting the seed bank proved to be a useful strategy to prevent the frequently observed increase in weed pressure on farms that convert from a conventional to an organic system. Ecological weed management systems should however target more than one stage of the weeds life cycle to reduce weed populations. Many little hammers are needed to reduce the on farm weed populations, and targeting the seed bank is one of them. Ecological weed management systems can be beneficial for organic as well as integrated farming systems. The incorporation of these principles in new weed management strategies can contribute to a reduction of herbicide use and a reduced environmental impact on non targets.

Part B Environmental effects of weed management

Weed management tools are designed to control plants, and as a result, non target plants are at risk when these tools are applied. Current guidelines used by governmental agencies and companies to assess the risk of herbicides to non target plants are very limited in their description of how and what data should be collected and analyzed to asses the risk to non target terrestrial plants. Guidelines provided by OECD and EPPO allow the use of data obtained in greenhouse experiments in the risk assessment for pesticides to non-target terrestrial plants in the field. In this thesis the use of greenhouse data to determine effects of herbicides with a different mode of action on the biomass, seed production and emergence of field-grown plants was investigated in Chapter 4 and 5. In addition, a single species approach was compared with a mixed species approach. Effects on the biomass of greenhouse and field-grown plants were found to be related at different effect levels, indicating that it might be possible to translate results from greenhouse studies to field situations. The relationship between greenhouse and field effects is probably herbicide specific and should be assessed for each group of herbicides with a similar mode of action. The use of single species tests may not be valid. The response of a single plant species to sublethal herbicide dosages differed to the response of the same species grown in a mixture with other species. The results also suggest consequences at the population level. Even when only marginal effects on the biomass of non-target plants are expected, their seed production and thereby survival at the population level may be negatively affected. Important factors that need to be taken into account are the species composition of those communities, mode of action of the herbicides, the development stage of the margin and the choice of endpoint (biomass, reproduction).

Direct environmental effects of weed management are mainly caused by herbicide drift. Therefore, during the last two decades mechanical weed control has received a lot of attention and is regarded as a more environmental friendly way to control weeds. However, during the development of a more sustainable weed management strategy, indirect effects should not be forgotten. Like other agronomic practices, weed control requires energy. Based on equations described in the Appendix a comparison of the energy use of weed management in a conventional, integrated and organic farming system under Dutch circumstances on a clay soil and a sandy soil was made and is presented in Chapter 7. According to these calculations, the energy use of weed control in an organic system on a sandy soil is a factor 1.7-1.8 times higher than in an integrated or conventional system and a factor 2.1-2.5 times higher on a clay soil. Of course, weed management is only part of the total production system, other agronomic practices such as fertilization make up for a large part of the energy consumption in agriculture and should not be neglected.

Part C Human dimension of weed management

Most studies on weed population dynamics in farming systems have focused on the effects of different farming systems. Those studies usually assume that farmers, operating within a particular system, show homogeneous management behaviour. However, it is likely that weed management behavior will vary between farms that operate within one system, thereby influencing the weed pressure. In Chapter 6 we 1) investigated whether differences between organic farms in weed pressure can be related to differences in farmers' weed management behaviour, 2) explored which weed and general management factors are of main influence on the weed pressure, and 3) investigated the influence of farmer's beliefs and knowledge on weed control techniques and the observed weed pressure. The farmer's beliefs and knowledge on weed control techniques differed between farmers with different weed pressures. Preventive measures and timing of main soil tillage operation were identified as the weed management factors most influential for weed pressure. With increasing number of preventive measures applied, the weed pressure decreased, with a stale seedbed being the most important preventive measure. The weed pressure increased with the number of days after September 1st on which the main tillage operation was carried out. Field size, rather than weed pressure, determined the number of hand weeding hours per ha. On farms with lower weed pressures a higher percentage of competitive crops were grown than on farms with higher weed pressures. It was concluded that exploratory on-farm studies can give us insight in the human dimension, which can lead to a better understanding of the farming systems and to more effective weed management in those systems.

Finally, in Chapter 7 the contribution of the research described in this thesis to our understanding of seed bank dynamics and weed management at the farm level, the environmental effects of weed management and the human dimension was discussed. Future research needs on each of these aspects of weed management are formulated. It was

concluded that the development of future sustainable weed management strategies with a minimum environmental impact would benefit from an approach combining social and natural sciences.

Samenvatting

Het beheersen van onkruiden op de boerderij wordt beïnvloed door een groot aantal factoren. Deze behelzen onder andere de ontwikkeling en beschikbaarheid van onkruidbestrijdings-methoden, de invloed van deze methoden op het milieu, en het handelen van de boer zelf. In hoofdstuk 1 is beschreven beschrijven hoe deze aspecten gerelateerd zijn en welke kennisleemten er voor al deze aspecten zijn. Daarbij zijn onderzoeksvragen geformuleerd betreffende ecologische onkruidbeheersing gericht op het uitputten van de zaadbank, de milieu-effecten van (chemische) onkruidbeheersing en de invloed van menselijk handelen op de onkruidbeheersing. In deel A van dit proefschrift zijn twee ecologische onkruidbeheersingsstrategieën onderzocht: 1) het verhogen van de verliezen uit de zaadbank (Hoofdstuk 2) en 2) het verminderen van de instroom van nieuwe zaden naar de zaadbank (Hoofdstuk 3). In deel B zijn onderzoeksvragen ten aanzien van de effecten van een breedwerkend (Hoofdstuk 4) en een smalwerkend (Hoofdstuk 5) herbicide op terrestrische niet-doelwit planten behandeld. In deel C is ten slotte ingegaan op de relatie tussen de houding en overtuigingen van de boer ten aanzien van onkruidbestrijding en de onkruiddruk op de boerderij.

Deel A Onkruidbestrijdingsstrategieën: uitputten van de zaadbank

In hoofdstuk 2 en 3 (deel A van dit proefschrift) werd de effectiviteit van onkruidbestrijdingsstrategieën die gericht zijn op uitputting van de zaadbank onderzocht. In Hoofdstuk 2 werd onderzocht hoe een vals zaaibed gevolgd door verschillende onkruidbestrijdingsmethoden ingezet kan worden om de zaadbank te verkleinen en de onkruiddruk tijdens de gewasgroei sterk te verminderen. Door het toepassen van een vals zaaibed bleek dat, afhankelijk van de locatie en het jaar, het aantal onkruiden gedurende de gewasgroei met 43 tot 83% gereduceerd kan worden. De bestrijding van de zaailingen die na toepassing van een vals zaaibed opkwamen was effectiever met glyfosaat toediening dan met een rotorkopeg. Door de rotorkopeg tijdens bestrijding af te dekken met zwart plastic kon de onkruiddruk gedurende de daaropvolgende gewasgroei in twee van de drie jaar verminderd. Blootstelling aan ver-rood licht had geen effect op de aantallen onkruiden. Onkruiden werden ook mechanisch bestreden met een vingerwieder, torsiewieder en schoffel zonder toepassing van een vals zaaibed. In vergelijking met de onbehandelde controle kon het aantal onkruiden met deze methoden met 88 tot 99% verminderd worden. Deze methoden waren daarmee effectiever dan de chemische bestrijding met carbeetamide en chloorprofam. De resultaten laten zien dat de toepassing van een vals zaaibed in combinatie met mechanische bestrijding van de zaailingen de onkruiddruk tijdens de daaropvolgende gewasgroei even goed kan bestrijden als chemische bestrijding, en daarmee de noodzaak om onkruiden chemisch te bestrijden kan helpen verminderen.

In hoofdstuk 3 werden onkruidbeheersingsstrategieën gericht op het voorkomen van aanvulling van de zaadbank met nieuw geproduceerde zaden onderzocht. Drie strategieën die elk een verschillende mate van aanvulling van de zaadbank toestaan werden gedurende zeven jaar op een proefbedrijf toegepast. Eén strategie was gericht op een 100% preventie van aanvulling van de zaadbank, één strategie op de preventie van zaadproductie en aanvulling van de zaadbank door onkruidplanten die boven het gewas uitstaken en één strategie diende als referentie voor standaard onkruidbestrijding in een biologisch landbouwsysteem. De effecten van deze strategieën op de bovengrondse onkruid populatie en de zaadbank werden waargenomen. Het eerste jaar waarin het experiment werd uitgevoerd was ook het eerste jaar waarin op de proefboerderij, na meer dan 40 jarig gangbaar en geïntegreerd geboerd te hebben, biologisch geteeld werd. Over het algemeen neemt de omvang van de zaadbank na omschakeling naar een biologisch systeem sterk toe. Het was dan ook de verwachting dat de omvang van de zaadbank onder de referentiestrategie (standaard biologisch) sterk zou toenemen, onder de strategie gericht op 100% preventie zou afnemen en onder de tussenliggende strategie zou afnemen dan wel gelijk blijven. De omvang van de zaadbank nam echter onder alle strategieën toe. Onder de strategie gericht op een 100% preventie werd de onkruiddruk gedurende de zeven jaar van het onderzoek echter wel significant lager dan onder de andere twee strategieën. Het aantal handwieduren dat bij deze strategie extra ingezet moest worden ten opzicht van de referentie, nam gedurende de studie ook af.

De resultaten in deel A van dit proefschrift laten zien dat ecologische onkruidbeheersingssystemen meerdere stadia van de levenscyclus van de onkruiden moeten aanpakken om de onkruiddruk te kunnen reduceren. Het aanpakken van de zaadbank bleek echter wel een goede strategie te zijn om de gebruikelijke toename van de onkruiddruk na omschakeling naar een biologisch systeem te voorkomen, maar een afname van de onkruiddruk bleek niet haalbaar te zijn. Strategieën gericht op het uitputten van de zaadbank, hetzij door een toename van de kieming, hetzij door het voorkomen van nieuwe aanvoer, zijn één van de vele maatregelen die nodig zijn om onkruidpopulaties in het veld te kunnen verminderen. Door toepassing van onkruidbeheersingsstrategieën gebaseerd op ecologische principes kan een bijdrage geleverd worden aan een vermindering van de afhankelijkheid van herbiciden in zowel biologische als geïntegreerde systemen.

Deel B Milieu-effecten van onkruidbeheersing

Onkruidbestrijdingsmethoden zijn gericht op het doden van planten. Als een gevolg daarvan lopen niet-doelwit planten in akkerranden bij toepassing van deze methoden het meeste risico. De huidige richtlijnen die door overheden en het bedrijfsleven gebruikt worden om deze risico's in kaart te brengen, zijn beperkt in hun beschrijving over hoe en welke data verzameld en geanalyseerd moet worden. De OECD en EPPO richtlijnen staan het gebruik van data verkregen uit kasexperimenten toe om de risico's op niet-doelwit planten in het veld te bepalen. In de hoofdstukken 4 en 5 van dit proefschrift werden de effecten van herbiciden met verschillende werkingsmechanismen op de biomassa, zaadproductie en opkomst van kasplanten vergeleken met die van planten die buiten de kas groeiden. Daarnaast werd een vergelijking gemaakt tussen een aanpak gebaseerd op een individuele soort en een aanpak waarbij mengsels van soorten werden gebruikt. Effecten op de biomassa van kas- en veldplanten bleken op meerdere effectiviteitsniveau's gerelateerd, waarmee een vertaling van resultaten uit kasstudie naar veldsituaties perspectiefvol lijkt te zijn. De relatie tussen kas en veldeffecten is waarschijnlijk herbicide-specifiek en zal voor elke groep van herbiciden met een vergelijkbaar werkingsmechanisme bepaald moeten worden. Het huidige gebruik van individuele soorten om het risico op akkerranden te bepalen lijkt niet terecht te zijn. De sublethale effecten van een herbicide op een individuele soort verschilden sterk van de effecten op dezelfde soort wanneer deze in een vegetatie met meerdere soorten groeide. De invloed van sublethale herbicide doseringen op de zaadproductie, en daarmee hun overleving, bleek groter te zijn dan de effecten op de biomassa van de planten. Voor de risico-bepalingen van herbiciden op niet-doelwit planten zijn de soortensamenstelling van de te beschermen vegetatie, het werkingsmechanisme van de herbiciden, het ontwikkelingsstadium van de soorten in de vegetatie en de keuze van beoordelingsparameter (biomassa, reproductie) factoren die meegenomen moeten worden.

Directe milieu-effecten van onkruidbestrijding worden hoofdzakelijk door herbiciden veroorzaakt. Als een gevolg daarvan is de afgelopen decennia veel aandacht besteed aan mechanische onkruidbestrijding, omdat deze als een milieu-vriendelijker alternatief worden gezien. Wanneer men echter een duurzame onkruidbeheerssingsstrategie ontwikkelt, moeten de indirecte effecten niet vergeten worden. Zoals de meeste andere agronomische activiteiten, vraagt ook onkruidbestrijding energie. Op basis van de vergelijkingen in de Appendix werd in dit proefschrift een vergelijking gemaakt tussen het energieverbruik van onkruidbestrijding in een gangbaar, geïntegreerd en een biologisch systeem op een zand en een klei grond onder Nederlandse omstandigheden. De berekeningen wezen uit dat de benodigde energie voor onkruidbestrijding in een biologisch systeem op een zandgrond een factor 1.7 tot 1.8 hoger en op een kleigrond een factor 2.1 tot 2.5 hoger ligt dan in een geïntegreerd of gangbaar systeem. Onkruidbestrijding is echter slechts een klein deel van het totale productiesysteem, andere agronomische activiteiten zoals bemesting maken een groot deel uit van het energieverbruik in de landbouw en moeten zeker niet genegeerd worden.

Deel C Menselijk handelen en onkruidbestrijding

De meeste studies die zijn uitgevoerd naar onkruidpopulatiedynamica in landbouwsystemen hebben zich gericht op de effecten van verschillende landbouwsystemen. Er werd in die studies over het algemeen aangenomen dat boeren, werkzaam binnen een bepaald systeem (hetzij biologisch, hetzij geïntegreerd), een homogeen management-gedrag vertonen. Het is echter zeer goed mogelijk dat het gedrag ten aanzien van onkruid beheersing sterk verschilt tussen boeren binnen een bepaald systeem, en dat daarmee ook de onkruiddruk op een bedrijf verschillend beïnvloed wordt. In Hoofdstuk 6 werden daarom de volgende vragen onderzocht: 1) kunnen verschillen in onkruiddruk tussen verschillende (biologische) bedrijven gerelateerd worden aan verschillend gedrag van de boeren ten aanzien van de onkruidbestrijding?, en 2) welke onkruidbeheersingsmaatregelen en andere agronomische maatregelen hebben de grootste invloed op de onkruiddruk op een bedrijf?, en ten slotte 3) wat is de invloed van de kennis en overtuigingen van de boeren ten aanzien van onkruidbestrijdingsmaatregelen op de onkruiddruk op het bedrijf? De overtuigingen en kennis over onkruidbestrijding verschilde tussen boeren waarbij op het bedrijf verschillende onkruiddichtheden aangetroffen werden.

Het aantal preventieve maatregelen en de timing van de hoofdgrondbewerking werden als belangrijkste factoren geïdentificeerd die de onkruiddruk beïnvloeden. De onkruiddruk nam significant af met een toename van het aantal preventieve maatregelen, waarbij het vals zaaibed als belangrijkste maatregel kon worden aangemerkt. De onkruiddruk nam toe met het aantal dagen na 1 September waarop de hoofdgrondbewerking werd uitgevoerd. De grootte van een akker, en niet de onkruiddruk zelf, bepaalde het aantal handwieduren per ha dat ingezet werd. Op boerderijen met een lagere onkruiddruk werden meer competitieve gewassen geteeld dan op boerderijen met een hogere onkruiddruk. Dit type studies kan ons inzicht verlenen in de invloed van het menselijk handelen op bedrijven en ons helpen effectievere management systemen te ontwikkelen.

Ten slotte werd in hoofdstuk 7 de bijdrage van het onderzoek zoals beschreven in dit proefschrift aan onze kennis over zaad bank dynamica en onkruidbeheersing op boerderij niveau, en de milieu-effecten en menselijke aspecten van onkruidbeheersing bediscussieerd. In dat hoofdstuk worden toekomstige onderzoeksbehoeften geformuleerd voor elk van deze aspecten van onkruidbeheersing. De ontwikkeling van toekomstige duurzame onkruidbestrijdingssystemen met een verminderde milieu-belasting zou baat hebben bij een gecombineerde bèta- en gammawetenschappelijke benadering.

Acknowledgements

A PhD thesis is not something that your write on your own. Many people have made a large or somewhat smaller contribution to the work described in this thesis and to my development as a researcher. When I started my career as researcher “Weed management and Nematology” at PRI, I did not expect to end up in writing a thesis. After one year however, I was given the opportunity to combine my research activities at PRI with a PhD research. I would like to thank my supervisor, Prof. Dr. Martin Kropff, my co-supervisor, Dr. Bert Lotz, and the current and former head of my department at PRI Dr. Jacques Neeteson and Dr. Anton Haverkort for granting me this possibility. Martin and Bert, I would like to thank you for your time, effort and constructive discussions during the writing phase of the several papers. Bert, I would like to thank you for your encouraging words during the completion of this thesis, which I really appreciated .

Next to my supervisors a large number of colleagues were directly or indirectly involved in this PhD project. Roel, since my first day at work, you have introduced me into “the world of weeds”. Your enthousiastic explanations of farming equipment and your knowledge on practical weed control have helped me a lot. Part of the data that we collected during one of our first projects together has resulted in Chapter six. I really appreciate you being one of my paranimphs today!

I further would like to thank Rommie van der Weide, from Applied Plant Research (PPO) in Lelystad, for the very nice cooperation during the past years. Rommie, thank you very much for your contributions to chapters two and six (maybe sometimes even without knowing), and your interest in my progress during these studies. I have come to know you as a pleasant and involved colleague and I hope we can continue our collaboration in the future. Thanks are also due to team- and officemate Corné Kempenaar for his contribution to chapters four and five. Tom Dueck, thank you for your contribution to chapters four and five, and your suggestions to improve my english writing. André Uffing, bedankt voor het opkweken, oppotten en behandelen van alle planten tijdens de verschillende herbicide-proeven.

Several colleagues of PPO have contributed to the completion of this thesis. Piet Bleeker has shared a lot of his extended knowledge on mechanical weed control and contributed to chapter two. Piet, thank you for being a very pleasant colleague and being good company on our business trip to Birmingham. I would also like to thank Wijnand Sukkel and Wiepie van Leeuwen Haagsma for their comments and data input during the research that forms the basis of chapter six. Andries Siepel, bedankt voor het opdiepen van oude gegevens over de Lovinkhoeve voor hoofdstuk drie, hetgeen soms makkelijker leek dan het was. Data analysis would have been very complicated without the statistical support of Jacques Withagen, Saskia Burgers and Christian Ritz. Thank you for your help. Reint-Jan Renes of Communication Sciences: thank you very much for your help with the data analysis of chapter six. Ed Luschei (University of Wisconsin), thanks a lot for your enthusiasm for and discussion on our research on the linkage between farmers' behaviour and on farm weed management. It really was a good stimulus to continue that research. Without the tips and trics of Rina Kleinjan-Meijering this thesis would not have the layout it currently has. Thank you. Without Marjolein Elings and Tanja Speek, the past years at "the institute" would not have been such a nice place to work. Thanks for your interest and joyfull company! Marjolein Kruidhof (NIOO-KNAW), thank you for the good company and discussions in Bari and Hamar during visits to the EWRS meetings. All colleagues of "Gewas" and later on "Agro": thank you for your interest and support during the last years.

Finally, I want to thank my family. De "schone" familie Aveskamp, John, Anne, Swenja and Niek, bedankt voor jullie getoonde interesse. Pap en mam, bedankt voor alle steun en support, niet alleen tijdens dit promotietraject, maar zeker ook tijdens de aanloop daar naar toe. Mede dankzij jullie heb ik me kunnen ontwikkelen tot wie ik nu ben, bedankt! Evelien, sister!, thank you for all the times you realised that it was enough just to listen! I would never have thought that the modern dance scene and research community would have so much in common. Eelco, thank you for your interest and assistance with the field work! This helped me making time to finish the last bits and pieces of the thesis. I certainly hope that all the thistles have not reduced your enthusiasm for biology. And, last, but certainly not the least, Maikel. Thank you for all the editorial work you have done on this thesis and certainly for all the support you gave me during the past years. Your ability to make the sun shine on a cloudy day is worth a million.

PE&RC PhD Education Statement Form

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)

The C.T. De Wit Graduate School PE&RC PRODUCTION ECOLOGY & RESOURCE CONSERVATION

Review of Literature (4.2 ECTS)

- Weed management: strategies and environmental effects (2006)

Writing of Project Proposal (7 ECTS)

- Availability of weed management tools: knowledge and decisions at the environmental and farm level (2006)

Laboratory Training and Working Visits (0.9 ECTS)

- Ecological effects of weed management; Penn State University (2009)

Post-Graduate Courses (10.2 ECTS)

- Survival analysis; FE, PE&RC (2005)
- Community ecology; PE&RC-SENSE (2005)
- Basic & advanced statistics; PE&RC-SENSE (2005/2006)
- Multivariate analysis; Biometris (2006)
- The art of modelling; PE&RC-SENSE (2006)

Deficiency, Refresh, Brush-up Courses (0.3 ECTS)

- Statistical assessment of dose-response curves with free software; EWRS (2005)

Competence Strengthening / Skills Courses (2 ECTS)

- Scientific writing; CENTA (2005)
- PhD competence assessment; WGS (2005)

Discussion Groups / Local Seminars and Other Scientific Meetings (7.4 ECTS)

- KNPV werkgroep onkruidkunde (2003-2009)
- Plant and Crop Ecology discussion group (2004)
- KNPV najaarsvergadering (2004)
- EWRS workshop on weed population dynamics models (2006)
- DEFRA expert meeting on weed biology (2007)
- WeedML and WTDB working group (2007-2009)
- EWRS meeting on perennial weeds (2008)

PE&RC Annual Meetings, Seminars and the PE&RC Weekend (1.8 ECTS)

- PE&RC weekend (2005)
- The ecology of temperature (2005)
- PE&RC days (2005/2006)

International Symposia, Workshops and Conferences (9 ECTS)

Symposia during which an oral presentation was given:

- 17th annual SETAC (Society of Environmental Toxicology and Chemistry) meeting; Porto, Portugal (2007)
- 14th EWRS (European Weed Research Society) symposium; Hamar, Norway (2007)
- Annual WSSA (Weed Science Society of America) meeting; Orlando, US (2009)

Symposia during which a poster presentation was given:

- 13th EWRS (European Weed Research Society) symposium; Bari, Italy (2005)
- 19th annual SETAC (Society of Environmental Toxicology and Chemistry) meeting; Goteborg, Sweden (2009)
- 16th annual SETAC (Society of Environmental Toxicology and Chemistry) meeting; Den Haag, Netherlands (2006)

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Curriculum vitae

Maria Magdalena (Marleen) Riemens was born on the 22nd of July, 1980 in Zwijndrecht, the Netherlands. In 1998, she graduated from secondary education at the "Develstein college" in Zwijndrecht.

In the same year, she initiated her study "Plant Breeding and Crop protection" at Wageningen University. The subjects of her MSc thesis were Virology (Laboratory of Virology, Wageningen University), Nematology (NIOO-KNAW and Laboratory of Nematology, Wageningen University) and Integrated Crop Protection (Plant Research International and the Laboratory of Nematology, Wageningen University).

After graduation in November 2002 she worked at the Laboratory of Nematology of Wageningen University as a supervisor for students during the course "Ecological Crop Protection". In april 2003, she started her work as weed scientist at Plant Research International which is continued until present. Here, she is responsible for the acquisition and management of several research projects on crop protection, weed management and ecology. In 2004, she started her PhD research at the Crop and Weed Ecology group of Wageningen University and Plant Research International, under supervision of Prof. Dr. M.J. Kropff and Dr. L.A.P. Lotz.

Funding

The research described in Chapters 2, 3 and 6 was funded by the Research Program ''Non-chemical weed control'' (Program 349 BO-06-397-V) financed by the Dutch Ministry of Agriculture, Nature and Food Safety.
The research described in Chapters 4 and 5 of this thesis was supported by the Research Program ''Environmental Risks and Emission Reduction of Pesticides'' (Program 416) financed by the Dutch Ministry of Agriculture, Nature and Food Safety.