Growth characteristics of several clover species and their suitability for weed suppression in a mixed cropping design

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Growth characteristics of several clover species and their suitability for weed suppression in a mixed cropping design

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Abstract

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Weed management without herbicides is a challenging undertaking and requires reliable alternative strategies, particularly in poorly competing crops, where weeds can cause severe yield losses. Adding a companion crop is one of the means to enhance the weed-suppressive ability of the crop canopy. In this regard, clover is often referred to as an interesting option as, apart from weed suppression, clover species enhance the soil nitrogen status and contribute to pest suppression. Earlier experimental work showed, however, that clover can easily become too competitive, hindering the growth and development of the main crop. It, thus, remains questionable whether clover can be added as a companion cover crop for weed suppression without substantially harming the main crop. In this context, the relevance of the choice of clover species was investigated in the current research project. Next to field experiments, experiments in containers and pots were used to determine various traits of a range of clover species and to relate these traits to competitive and weed-suppressive ability. Differences between clover species were large and it was shown that plant height was the main determinant of the competitive effect on crop plants, while rapid early growth was the trait correlating most strongly to weed-suppressive ability. Persian clover was a good example of a species that suppressed weeds successfully, but also competed too fiercely with the main crop. The performance of the short-growing subterranean clover was unsatisfactory. The slow early growth of this species resulted in poor weed suppression and in addition, the species competed more strongly with the crop than expected based on plant height. This last was shown to be due to a relatively strong ability to compete for below-ground resources. It was concluded that successful introduction of a companion clover crop for enhanced weed suppression will not be possible unless additional management to reduce the competitive damage to the main crop is conducted. Of all the investigated clover species white clover held most promise as a weed-suppressing clover species in a mixed cropping design.

Keywords: Clover, subterranean clover, weed suppression, cover crop, living mulch, smother crop, yield loss.

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Cover design: Nikki Klein Velthuis

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List of Abbreviations

ANOVA	Analysis Of Variance
DAI	Days After Introduction
DAP	Days After Planting
DAS	Days After Sowing
DM	Dry Matter
GLM	Generalized Linear Model
GR	Growth Rate
k	Light Extinction Coefficient
LAI	Leaf Area Index
LI	Light Interception
LSD	Least Significant Difference
LUE	Light Use Efficiency
LWR	Leaf Weight Ratio
NS	Not Significant
PAR	Photosynthetically Active Radiation
RGR	Relative Growth Rate
SLA	Specific Leaf Area
SE	Standard Error
SED	Standard Error Of Difference
SFA	Substance Flow Analysis
WAS	Weeks After Sowing

CHAPTER 1

General introduction

General introduction

The enduring need for sustainable agricultural production

One of the main purposes of agriculture, which began about 10.000 years ago, is the production of sufficient food to meet the demand of the human population. Apart from food, crops are cultivated for other purposes such as fodder, fuel, fibre and medicine (e.g. Elzebroek and Wind, 2008). Agricultural production is characterized by stimulating the growth of useful plants and hinder, or even prevent, the growth of others. For centuries agriculture was extensive, mainly relying on natural conditions as only limited amounts of external inputs were used. Partly as a result, agriculture was labour demanding, and up to 1800 a majority of the European population was actively involved in farming. Yields were low and a relatively large proportion of the annual production was preserved, to be used as seeding material for the next growing season. The rapid growth of the human population in Europe during the industrialization in the second half of the 19^{th} century called for higher crop yields. Eventually, yields increased through the introduction of new crops, the use of manure and composted urban waist and the employment of practices such as crop rotation (Mårald, 1998 in Cordell et al., 2009). Also more sophisticated agricultural tools, such as better quality steel ploughs, became available.

An even more spectacular breakthrough with respect to yield was achieved during the Green Revolution, when chemical fertilizers became broadly available and varieties were introduced that could achieve substantially higher yield levels. Together with technological developments which led to a decreased labour demand, and the introduction of chemical crop protection measures, agriculture became increasingly intensive. Whereas the advantages of these intensive systems were evident, the disadvantages became quickly apparent. High levels of fertilizer use led to eutrophication, as a large quantity of the nutrients were not taken up by the crop, but washed out of the soil column (Lee and Jones-Lee, 2004). Loss of soil structure, soil erosion and loss of species diversity were other setbacks attributable to high fertilizer inputs (Reganold et al., 1987). In addition, the use of herbicides and other chemical control agents led to pollution of air, soil and water, seriously threatening the well-being of our natural living environment (Carson, 1962). Yet another drawback of intensive crop production systems is the strong dependency of farmers on high quantities of external inputs. With relatively low costs for energy, these systems seem appealing as they produce high amounts of food at low costs. In the long run, however, with declining supplies of carbon-based energy sources, it is likely that the sustainability of these systems will be seriously jeopardized.

The call for a further increase in crop production, partly related to the continued growth of the human population (9 billion in 2050), still exists. In production ecology, the concept of different production levels is well-established. Three groups of production factors are distinguished that define the hierarchy in levels of crop production (de Wit and Penning de Vries, 1982; van Ittersum and Rabbinge, 1997):

(i) Growth-defining factors include the physiological and phenological characteristics of a crop that together with site-specific circumstances such as temperature and solar-radiation determine the theoretical *potential yield* of a crop.

(ii) Growth-limiting factors comprise the essential abiotic resources water and nutrient that, in limited supply, cause the yield to decline from the potential to the *attainable level*.

(iii) Biotic factors such as weeds, pests and diseases make up the growth-reducing factors that further reduce growth and define the *actual yield level*.

Losses because of the presence of weeds, diseases and pests can be substantial. Measures to minimize these losses can be focused on managing the size of the pest population to acceptable low densities, or on reducing the negative impact of these harmful organisms on crop production. Current crop protection strongly relies on the use of chemical compounds as these products are effective, not very labour intensive and relatively inexpensive. The negative side effects of these chemical crop protection agents call for environmentally-safe and sustainable alternatives that are attractive to farmers.

Weed problems and curative weed control

Among the biotic production constraints, weeds are considered an important category. Weeds act at the same thropic level as the crop and cause damage because of competition for essential resources such as light, water and nutrients. The outcome of competition between plants is largely determined by the ability of the plants to capture resources (Grace, 1990). How much reduction in crop yield is caused by a weed population depends on the relative competitive ability between crop and weed and on weed density (Cousens, 1985). Weedy plant species that are able to outcompete crop plants are efficient in capturing resources and are often characterized by a high relative growth rate. It is also for this reason, that yield reduction through competition is particularly evident in slow-growing species that reach canopy closure relatively late, thereby leaving much opportunity for weeds to develop (e.g. Rubatzky et al., 1999; Baumann et al., 2001a). Oerke (2006) estimated that worldwide, in the absence of control measures, weeds may cause a potential yield loss of 34% of the attainable yield, making weeds by far the most threatening yield-reducing factor. The estimated yield loss because of weeds was about twice as high as that for both animal pests and pathogens. Actual yield loss estimates were considerably lower and in the same order of magnitude (approximately 8−10%) for the three major categories of biotic production constraints. The large gap between the potential and the actual yield loss estimates for weeds indicates that current weed control is relatively effective. For a large part this is because of the availability and use of herbicides. Chemical control is generally directed towards weed seedlings in the period around crop establishment (Bastiaans et al., 2008). During this stage, the weeds will not yet have laid ground for serious reductions in crop production, whereas the absence of a dense crop canopy enables a well-targeted control operation. Furthermore, controlling seedlings is often far easier than killing or removing taller and more developed weed plants. Weed plants emerging during later stages of crop development are considered less important, as their relative competitive ability is too weak to cause a major reduction in the production of the current crop. Seed production of those weeds contributes to future weed infestations, but in the presence of highly effective, reliable and cheap control options, this is generally not considered a major threat. It is often the availability of chemical control options that allows farmers to envisage the weed problem in such a short-term perspective and in relative isolation from other crop management aspects.

The negative side effects related to the use of chemical herbicides has led to stricter legislations and resulted in a decreased number of available herbicides. The intensive use of a limited number of herbicides creates a situation where herbicide resistance is more likely to develop (Heap, 2011). In turn, herbicide resistance further reduces the range of available herbicides, clearly illustrating the vulnerability of weed management strategies that rely too heavily on chemical control. This is another reason why there is an increasing need for knowledge on the design and functioning of cropping systems that rely to a lesser extent on chemical inputs.

Alternative weed management

Research on alternative curative control strategies largely focuses on biological and mechanical weed control (e.g. Kurstjens, 2002; Hatcher and Melander, 2003). For biological control, research efforts resulted in commercial registration of some bioherbicides, but these never managed to occupy a sizeable share of the market. The main problem is that the reliability of the field efficacy is not at levels comparable with that of herbicides (e.g. Hallett, 2005). Mechanical weed control has received, and is still receiving, significant attention and considerable progress has been made. An important breakthrough has been the development of technologies for intra-row weed control (e.g. Kurstjens and Bleeker, 2000). Still, the strong dependency on weather conditions, as well as damage to soil structure associated with frequent application of mechanical control limits the possibilities of this technology. Hand weeding is a further option, but both labour availability as well as the high costs related to labour restrict the application of hand weeding.

Another alternative is a more systems-oriented approach, in which the design or the management of a cropping system is adjusted such that weed populations are kept at relatively low levels and the negative impact of weeds on crop production is minimized (Barberi, 2002). This approach referred to as ecological weed management is a typical component of integrated crop management, where the focus is not only on maximization of crop production, but also the optimization of resource use and the minimization of external inputs is considered (Harwood, 1990). Bastiaans et al. (2008) distinguished three major principles as a basis for ecological management: (i) a reduced recruitment of weeds from the soil seed bank; (ii) alteration of crop–weed competitive relations to the benefit of the crop; and (iii) a gradual reduction or depletion of the weed seed bank in the soil. Rather than only focusing on the seedling phase, weeds are tackled in different stages throughout their life cycle. Various cultural measures can be used to implement the above-mentioned principles, like the use of mulches for reducing the establishment of weed seedlings and the use of transplants or more competitive cultivars to benefit the crop in its competition with weeds. In this thesis, the introduction of cover crops as a means to enhance the weed-suppressive ability of the cropping system is investigated.

Cover crops for weed control

One option is to introduce the cover crop during the period that the main crop is absent. Inclusion of cover crops in a crop rotation than introduces two important mechanisms through which the development of weed populations might be hampered (Kruidhof et al., 2008). The successful introduction and establishment of cover crops will prevent germination, growth, development, and most importantly, seed production of weeds through niche pre-emption and competition. Additionally, cover crop residue incorporation before the next crop is planted may suppress or retard weed emergence and growth because of allelopathic effects, stimulation of soil-borne pathogens or a combination of these two.

Cover crops can also be used in a mixed cropping design in which the cover crop, also referred to as smother crop, is added as a companion crop to the main crop (e.g. Liebman and Dyck, 1993; Moore et al., 1994; de Haan et al., 1994; Abdin et al., 2000). It is this option which is investigated in the research described in this thesis. In this case, the introduction of the cover crop is primarily aimed at obtaining a fast closing and more competitive mixed canopy that is able to prevent or inhibit germination and establishment of weeds and provides a more competitive environment for those weeds that manage to establish. Using cover crops in this manner seems particularly relevant for slow-growing species, such as leeks, carrots and onion, that leave a large part of the soil bare during a considerable part of the growing season. Apart from weed suppression, the use of undersown or companion crops can have additional benefits. Introduction of *Trifolium* spp. in between crop rows of Brassica species for instance reduced the abundance and damage because of herbivorous insects (Theunissen et al., 1995). Cover crops have also been reported to reduce disease related crop damage (e.g. Kinane and Lyngkjær, 2002; Hiddink et al., 2005) and soil erosion (Wall et al., 1991). Furthermore, leguminous cover crops can enhance soil fertility, by fixing nitrogen from the air (Brown et al., 1993 in Abdin et al., 2000).

A major drawback of combining a cover crop with a main crop is the competition that the cover crop exerts on the main crop. Particularly cover crops with a strong weed-suppressive ability have been found to compete strongly with the main crop (Nicholson and Wien, 1983; Lotz et al., 1997; Kleinhenz at al., 1997; Brandsaeter et al., 1998). In this thesis, emphasis is put on the importance of cover crop species selection as a means to minimize the competitive pressure on the main crop, while maintaining adequate weed suppression. The question is posed whether cover crops can successfully suppress weeds, while leaving the main crop nearly unaffected.

A pilot study on mixtures of Brussels sprouts and malting barley

In 2000, an integrated project on increased biodiversity at the field level, with special emphasis on the role of cover crops, was initiated. The purpose of the study was to investigate pest, disease and weed-suppressive effects of a cover crop added as a minor component in mixture with a main crop. Brussels sprouts (*Brassica oleracea gemmifera*) as main crop combined with malting barley (*Hordeum vulgare*) as added cover crop was selected as model system. The selected species differ substantially in physiology and morphology and were considered suitable for carrying out experiments on the effects of mixed cropping on the abundance of herbivorous insects and their predators and on below-ground disease suppressiveness. Within the framework of the integrated project, a pilot study was done that focused on the weed-suppressive ability and agronomical features of a mixed crop consisting of Brussels sprouts and malting barley. Pure and mixed stands of Brussels sprouts and malting barley were planted in large-sized plots of 9 m × 6.75 m. Brussels sprouts were planted at a fixed row distance of 0.75 m and three plant densities (4.44, 6.67 and 8.89 plants m^{-2}) were created by modifying the interplant distance in the row. Mixed plots were formed by adding either one, two or three rows of malting barley in between the Brussels sprouts rows, corresponding to barley densities of 37, 74 and 111 plants m^{-2}, respectively. As all density combinations were included, a total of 9 mixtures was investigated and compared to the performance of the pure stands. Weed-suppressive ability was visually assessed and for Brussels sprouts both the shoot dry matter of whole plants as well as the harvestable sprout yield was determined. Although the yield of malting barley was determined, it is not contained in this short description of the pilot study.

First and foremost, it was observed that introduction of malting barley, even at the highest density, was by no means able to restrain the severe weed infestation. The establishment and further growth of weeds, mainly *Chenopodium album*, was not prevented by undersowing the Brussels sprouts with malting barley (Bastiaans et al., 2007). With respect to shoot dry matter of Brussels sprouts, results clearly showed that malting barley competed strongly with the main crop (Figure 1). In mixtures with three rows of barley, this resulted in stunted growth of Brussels sprout plants. Moreover, severe losses in sprout yield were observed, even in mixtures that only contained one row of malting barley in between two rows of the main crop (Figure 1).

The pilot study revealed that, from a weed management perspective, a mixed cropping system consisting of Brussels sprouts and malting barley did not offer any advantage compared to a pure stand of Brussels sprouts. On the contrary, some important disadvantages were observed that clearly illustrated some of the aspects that need to be taken into consideration when the aim is to successfully introduce a second crop to a main crop in order to reduce the weed problem.

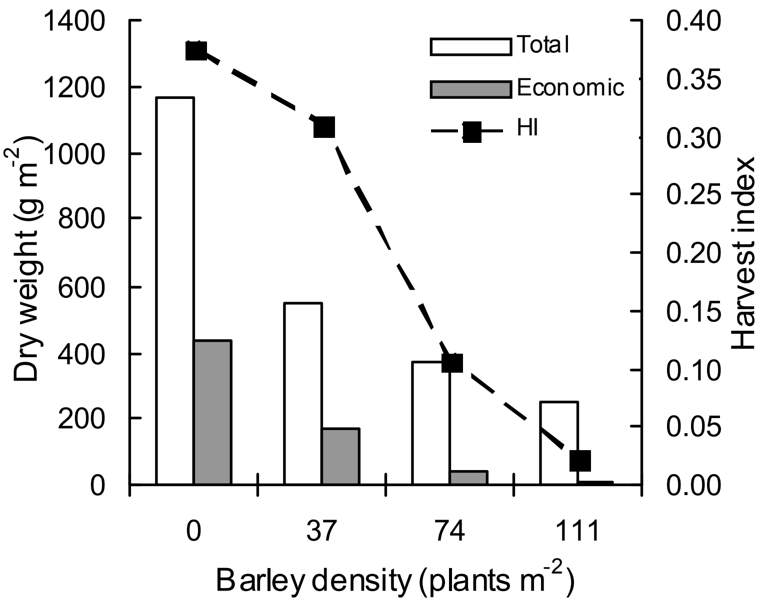

Figure 1. Total yield, economic yield and harvest index (HI) of Brussels sprouts grown in pure stand and in mixture with different plant densities of barley. Results represent averages obtained with Brussels sprouts grown at 4.4 and 6.7 plants m^{-2} (after Bastiaans et al., 2007).

First, the pilot clearly illustrated that the introduction of a smother crop is not a suitable option for every main crop. The results on Brussels sprouts illustrated what could be classified as a ‘worst case’ scenario. Apart from a reduced growth of the main crop, the competition from malting barley had a dramatic effect on the harvest index. Consequently, sprout yield was double affected. This indicates that the yield of Brussels sprouts is particularly sensitive to competition (Bastiaans et al., 2007) and it would be better to restrict the use of smother crops to main crops of which the harvest index is not affected by competition. Secondly, the results demonstrated that not every crop is a suitable companion crop. Malting barley planted between rows of the main crop obstructed the use of mechanical weed control, whereas it competed insufficiently with weeds. At the same time, it competed strongly with Brussels sprouts. Clearly, malting barley qualifies as an unsuitable companion crop for weed suppression.

Problem definition and objective

The pilot study convincingly showed that the introduction of a cover crop might combine poor weed suppression with severe damage to the main crop. The one positive conclusion that can be drawn from this observation is that the characteristics responsible for weed suppression and the characteristics responsible for the competitive damage to the main crop are not necessarily the same. This suggests that, theoretically, cover crops which combine adequate weed suppression with minimal negative effect on the main crop might exist. The study reported in this thesis, therefore, focused on identifying cover crops, as well as the morphological and physiological characteristics behind, that combine these two positive traits. This search was restricted to clover species (*Trifolium* spp.). The reason is that the additional benefits of clover species, particularly nitrogen fixation and pest suppression (e.g. Theunissen and Schelling, 1996), might help in creating a more positive balance between the advantages and disadvantages of the use of cover crops. Additionally, within the *Trifolium* genus there is a large variety in characteristics, such as canopy height, growth rate and seed size which enhances the prospects of finding a suitable species. For these reasons, the experimental work that is presented in this thesis focuses on the potential of clover species as cover crops for weed suppression.

Approach and outline of the thesis

The study started off with two large-scale field experiments in which a range of clover species was morphologically and physiologically characterized and both weed-suppressive ability and competitive damage to the main crop were evaluated. The results of these experiments are presented in the next two chapters. In Chapter 2, the variability in morphological and physiological traits among the investigated clover species is described. Focus was on those traits that were considered relevant for interplant competition. Chapter 3 contains a comparison of the weed-suppressive ability of the investigated clover species. Additionally, a correlation analysis between clover traits and the competitive damage of the cover crops on the main crop is presented. This analysis led to the hypothesis that among the clover species subterranean clover was a relatively strong competitor for below-ground resources. The pot experiments that were conducted to verify this hypothesis are described in Chapter 4. In Chapter 5, the results of a container experiment to further evaluate the weed-smothering ability of three selected and contrasting clover species are presented. Finally, in Chapter 6, main conclusions are drawn and possible implications for further research on the use of clover species as cover crop for improved weed suppression are formulated.

CHAPTER 2

Clover as a cover crop for weed suppression in an intercropping design
I. Characteristics of several clover species

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Abstract

Weeds often form a major problem in weakly competitive vegetable crops, particularly in low input systems. Undersown cover crops can be used to suppress weeds, but often put too high a competitive pressure on the main crop. Cover crop selection is one of the potential means that can be used to design or optimize these intercropping systems. The objective of the current research was to investigate the variability among a range of clover species in morphological and physiological traits that are considered relevant for interplant competition. To this purpose, field experiments with pure stands of eight clover species (2001) and a selection of three clover species (2002) were conducted, in which regular observations and periodic harvests were taken. Clear differences in the time in which full soil cover was obtained, total accumulated biomass, growth duration, height development and N-accumulation were observed. Persian clover (*Trifolium resupinatum* L.) and subterranean clover (*T. subterraneum* L.) were the two most contrasting species in this study, particularly differing in the period in which full soil cover was obtained. Persian clover's faster soil cover could not be attributed to a single trait, but resulted from a number of intrinsic characteristics, like light extinction coefficient, light use efficiency and specific leaf area that together determine the relative growth rate. The study also demonstrated the importance of differences in relative starting position, caused by, for instance, seed size, seeding rate and fraction establishment, for the analysis of early growth characteristics. Alsike clover (*T. hybridum* L.), berseem clover (*T. alexandrinum* L.) and crimson clover (*T. incarnatum* L.) developed slower than Persian clover, but all produced a higher amount of accumulated dry matter, due to a longer growing period. Clear differences in height and height development between species were observed. These differences were not associated with dry matter accumulation, as the tallest (red clover; 80 cm) and the shortest species (subterranean clover; 12 cm) produced similar amounts of dry matter. A strong positive correlation between early soil cover development and N-accumulation was observed. The large variability among clover species indicates that species selection is a very important aspect of the development of cropping systems that include clover as a cover crop.

Keywords: Clover, undersown cover crop, RGR, living mulch, light interception, soil cover, LUE, LAI, SLA

Introduction

The primary objective of weed management is to reduce the negative effects of weeds on crop production. Herbicides have proven to be a reliable and highly effective method to control weeds at relatively low cost. Consumers of the industrialized world however, increasingly demand food products that are safe, of high quality and have been produced with a minimum use of synthetic inputs. For that reason, weed management has to rely on other control measures. Achieving an adequate weed control without the use of herbicides is often reported to be difficult (Whitworth, 1995; Kropff and Walter, 2000). Mechanical weed control is often less effective, and moreover a heavy reliance on mechanical control is undesirable, because of damage to soil structure, increased risk of frost damage and the strong dependency on weather conditions. Removing weeds manually is often restrained by labour availability and is above all costly. Prevention of weed problems is another alternative, for which three mechanisms can be distinguished (Bastiaans et al., 2002). Firstly, the number of seeds that are present in the weed seed soil bank can be reduced. This can be achieved through increased seed mortality or a reduced seed production. Secondly, the fraction of seeds that develop into a weed seedling can be reduced through prevention of germination or emergence. Thirdly, growth and development of weed seedlings can be retarded to reduce the competitive ability of the weed relative to that of the crop, leading to a reduced negative effect on crop production.

Undersown cover crops may potentially reduce weed infestation through each of the discerned mechanisms (Phatak, 1992). The effects of the cover crop are achieved by a rapid occupation of the open space between the rows of the main crop, which prevents germination of weed seeds and reduces the growth and development of weed seedlings. Germination of weed seeds may be inhibited by complete light interception (Phatak, 1992) by the cover crop or by secretion of allelo-chemicals (White et al., 1989; Overland, 1966). After establishment of weed seedlings, resource competition becomes the main weed-suppressing mechanism of the cover crop (Teasdale, 1998).

The use of undersown cover crops for weed management is particularly relevant for crops that are not very competitive. Slow-growing crops like leek and onion, with upright leaves, hardly form a closed canopy and are, therefore, not able to suppress weeds adequately (Baumann et al., 2001a). Clover possesses good potential as a weed suppressor and apart from that has other advantages, like nitrogen fixation and the reduction of pests and diseases in a number of crops (Finch, 1993; McKinlay et al., 1996; Theunissen and Schelling, 1996). Ideally, the main crop and the cover crop should differ to a high degree in the way they explore resources, thus avoiding competition between both species to at least some extent (Vandermeer, 1989). The addition of clover as a cover crop, however, has often been reported to result in severe competition between the cover crop and the main crop (Bottenberg et al., 1997; Brandsæter et al., 1998; Lotz, 1997; Weber et al., 1999). The subsequent yield losses are regarded as a serious constraint for using clover as an undersown cover crop (Liebman and Dyck, 1993; Brandstæter et al., 1998; Hartwig and Ammon, 2002).

In conclusion, the beneficial effect of clover as a cover crop with respect to pest, disease and weed management can only be exploited if yield reduction resulting from competition can be reduced. Several attempts have been made to reduce the competitive strength of the undersown clover while maintaining its weed-suppressing ability. Brandstæter and Netland (1999) focused on temporal complementarity by separating periods of vigorous growth of the cover crop (subterranean clover) and the main crop, while Vrabel (1983) used chemical control of the cover crop to reduce yield losses. Brainard et al. (2004) evaluated different options, particularly, cover crop species, time of seeding, use of supplemental nitrogen and herbicide regulation. Ross et al. (2001) conducted mechanical control of the cover crop and combined this with a screening of different cover crops, including clover species. The screening revealed clear differences in the ability to suppress weeds among cover crops. Brandsæter et al. (1998) demonstrated clear differences in competitive ability between different cultivars of subterranean and white clover and found that yield reduction of the main crop was positively correlated with biomass production of the clover species. To aid the selection of species for a particular crop and aid decisions about crop–weed management, Ross et al. (2001) remarked that a greater understanding of the growth characteristics of clover species is required. In this respect, several studies have indicated at the importance of (early) soil cover development (e.g. Nelson et al., 1991; Brandsæter et al., 1998; Brandsæter and Netland, 1999; Baumann et al., 2000). In crop–weed competition research early growth, particularly early leaf area development (e.g. Kropff et al., 1992), early height growth rate and final plant height (e.g. Bastiaans et al., 1997) have been identified as important characteristics determining the competitive ability of species. It is obvious that these traits are similarly important for strengthening the weed-suppressive function of cover crops. Simultaneously, these characteristics determine the potential yield loss of the main crop resulting from competition with the introduced cover crop.

The current study focused on a comparison of clover species. Main objective was to determine the variability in morphological and physiological traits that are considered relevant for interplant competition. If sufficient variation is available, species selection is likely to be one of the important means to optimize intercropping systems that contain clover for weed suppression. Competitive ability and the relation between the presented characteristics and competitive ability are covered in Chapter 3.

Materials and methods

In 2001, an experiment with eight clover species was conducted. Based on the results of this experiment three contrasting clover species were selected for further study in the 2002 experiment (Table 1).

Experiment 2001

A screening experiment with eight clover species was laid out on a heavy clay soil, in Wageningen, the Netherlands. The site was fertilized with 300 kg ha^{-1} 12-10-18 NPK two weeks before sowing. The experimental design was a fully randomized complete block design in six replicates and treatments consisted of eight clover species. On May 7, the clovers were sown at a rate of 20 kg ha^{-1} in plots of 6 m × 7 m. Seeds were sown with a seed drill of which the pipes were positioned 30 cm above the soil surface to mimic broadcast sowing. After sowing, the soil was rolled with a Cambridge roller to compress the soil. Hand weeding of the clover plots was carried out at the end of June.

In each plot, the number of emerged clover plants was counted daily in two squares of 0.50 m × 0.50 m until no further increase in plant number was observed. Soil coverage was assessed weekly by estimating the soil cover in each of the 16 squares (0.125 m × 0.125 m) of a frame of 0.50 m × 0.50 m. Canopy height was determined weekly, at three positions per plot. Starting from one month after sowing, two squares of 0.50 m × 0.50 m were harvested weekly from each plot. The clover plants were cut just above soil level. A subsample was taken and used to determine the fraction leaf and stem. Leaf area was measured using a LI 3100 Area Meter (LI-COR, Lincoln, Nebraska, USA). All samples were dried for 24 hours at 70 °C and weighed subsequently. Leaf area index (LAI) was used to calculate the fraction light interception according to:

$$\frac{I}{I_0} = e^{(-k\,\mathrm{LAI})} \qquad [2.1]$$

where, I/I_0 is the fraction intercepted light, k is the extinction coefficient (determined in a separate experiment; see below), and LAI is the leaf area index of the canopy. Daily values for light interception were obtained by fitting a logistic function through the calculated fraction light interception on the various sampling dates. Daily radiation interception (MJ m^{-2} d^{-1}) was calculated by multiplying the fraction light interception by the daily incoming photosynthetically active radiation as measured on a nearby weather station. Integration of the daily values resulted in the cumulative radiation interception (MJ m^{-2}). Simple linear regression analysis was carried out to investigate the relationship between accumulated dry matter and the cumulative amount of intercepted light. The slope of the line represents light use efficiency (LUE; g MJ^{-1}).

The *k*-values used for calculating the light interception of 2001 were assessed in an additional experiment in 2003. In this experiment, clover species were sown at May 7 in plots of 5 m × 3 m at a density of 20 kg ha^{-1}. The clover plots were kept weed free throughout the experiment. Light interception, using a SunScan Canopy Analysis System (Delta T Devices Ltd, Cambridge, UK) was measured in an area of 1 m^2, after which half of this area was harvested. A subsample was taken and used to determine the fraction leaf and stem. Leaf area of the subsample was assessed using a LI 3100 Area Meter. Samples were weighed after drying for 24 h at 70 °C. For the eight clover species this was done on average four times between June 4 and August 28.

Experiment 2002

From the eight clover species that were evaluated in the 2001 field trial three contrasting clover species were selected for further evaluation (Table 1). Plots of 3 m wide and 5 m long were laid out on a sandy soil in Wageningen, the Netherlands. The experimental design was a split plot design in four replicates with N rate (0, 50 and 150 kg N ha^{-1}; N1, N2, N3, respectively) as main factor and clover species as subfactor. K and P fertilization was applied one month before sowing (100 kg ha^{-1} 60% K_2O and 200 kg 46% P_2O_5). Mechanical weed control was carried out 1 day prior to seed bed preparation. Inoculated (SelfStick Legume Inoculant; MicroBio Limited, Herts, UK) clover was sown on May 21 at a rate of 20 kg ha^{-1} using the same procedure as used in 2001. Hand weeding was carried out on June 13 and 14 in all plots and a second hand weeding was carried out on June 26.

Clover establishment, soil coverage and height were assessed using the same methodology as in 2001. Light interception measurements were done on June 19 (29 DAS), June 26 and 27 (36 and 37 DAS), July 19 and 20 (59 and 60 DAS), and August 2, 4, 5 and 7 (73, 75, 76 and 78 DAS). Two squares of 50 cm × 50 cm were harvested from the clover plots on July 1, July 15, August 12 and August 27 (41, 55, 83 and 98 DAS). The harvested material was treated in the same way as was done in 2001. At 41 and 83 DAS, the observations on shoot dry matter development were extended by taking eight soil cores of 30 cm deep with a diameter of 5 cm to assess root dry matter. The soil cores were cut in three parts of 10 cm each and pooled layer wise. Soil was washed out until clean roots remained. The roots were dried for 24 h at 70 °C. Abundance of root nodulation of the various clover species was assessed in the first week of August. A relative scale with five levels, ranging from very poor to very abundant was used. Additionally, activity of root nodules was investigated by slicing the nodules in half. Nodules with a red content were considered active while white nodules were considered in-active.

Table 1. Clover species used in the experimental work of 2001 and 2002 (marked with an asterix).

Clover species	Cultivar	Common name	1000-Grain weight	2001		2002	
				Seedlings	Ratio	Seedlings	Ratio
T. hybridum	Dawn	Alsike clover	0.83 f	177 bc	0.07 e		
T. alexandrinum	Alex	Berseem clover	3.17 b	256 b	0.41 b		
T. incarnatum	Contea	Crimson clover	2.94 c	235 bc	0.35 c		
*T. resupinatum**	Accdia	Persian clover	1.48 e	671 a	0.50 a	715 b	0.53 a
T. pratense	Violeta	Red clover	2.04 d	203 bc	0.21 d		
*T. subterraneum**	Mount Baker	Subterranean clover	6.28 a	115 c	0.36 bc	136 c	0.43 b
*T. repens**	Aran	White (c1) clover, large leaved	0.71 f	181 bc	0.07 e	873 a	0.31 c
T. repens	Riesling	White (c2) clover, medium leaved	0.78 f	170 bc	0.06 e		
SED			0.117	60.3§	0.028§	50.66§	0.018§

1000-Grain weight (g), density of established seedlings (seedlings m^{-2}) and ratio between seedlings and sown seeds of the clover species used in the field experiments of 2001 and 2002. Standard error of difference (SED) is presented for non-transformed data while the highest standard error of difference (SED_{max}) of the various pair-wise comparisons is presented for transformed data. Different letters (a–f) within a column indicate significant differences at the 0.05 level; NS: not significant. § SED maximum.

Statistical analysis

A Generalized Linear Model (GLM) with a log link function was used for analysing the number of established seedlings. The seedling density as a fraction of sown seeds was analysed using a GLM with a logit link function. Approximate values of the standard error of difference (SED) and least significant difference (LSD) (p = 0.05) were calculated. ANOVA was performed and LSD (p = 0.05) was calculated for the dry matter data of each harvest. For 2001, dry matter accumulation, soil cover and the fraction intercepted light were fitted to a logistic model,

$$y_t = \frac{c}{1 + \mathrm{e}^{(-b(t-m))}} \qquad [2.2]$$

where, c is the upper asymptote, t the time in days after sowing (DAS), m the time at which y obtained 50% of c, and b is the maximum relative growth rate. For 2002, only soil cover was fitted to the logistic model. For dry matter accumulation the 50% point (m) was estimated by linear interpolation of the two harvests nearest to the 50% point.

A weight factor (1/standard error; S.E.) was included in the fitting procedure of the biomass data of 2001, to correct for heterogeneity of variance. Binomial distribution was assumed for soil cover data and normal distribution was assumed for the calculated light interception data. Pair wise comparisons were made between the estimated parameters of different species by calculating the t-statistic. Root dry matter and root dry matter as fraction of the total dry matter were analysed by performing ANOVA and calculating the LSD. All statistical procedures were carried out using the Genstat 7 statistical package (Payne, 2003).

Results

Establishment

In the 2001-experiment, at the first observation date at 5 days after sowing (DAS), plots with Persian clover already contained a considerable number of emerged plants, whereas in all other plots no emergence was observed. After 8 days no further increase in number of emerged plants was observed for Persian clover (Table 1). The other species reached their maximum number of emerged seedlings at about 11 DAS, except for red clover which obtained its maximum number at about 13 DAS. The fraction establishment varied considerably and ranged between 0.06 for white (c2) clover and 0.50 for Persian clover. In 2002, emergence of the selected species was more rapid, Persian clover reached maximum establishment at 6 DAS, white clover at 8 DAS and subterranean clover at 10 DAS. For Persian clover, the fraction establishment was almost identical to that in 2001, while the number of subterranean clover seedlings was slightly higher than in 2001. White (c1) clover had a much better establishment than in 2001, as the fraction established seedlings increased from 0.07 to 0.31.

The different fertilization rates in the 2002-experiment had no effect on emergence and establishment. Of the other traits only specific leaf area (SLA) at the first harvest (41 DAS) and shoot N-content were affected. For that reason only the average response of the various clover species at the three nitrogen rates was presented.

Dry matter accumulation

For 2001, the dry matter accumulation in time for each of the clover species could be adequately described by a logistic model. In Figure 1, this is illustrated for Persian, subterranean and white (c1) clover, while the estimated parameters for all species are

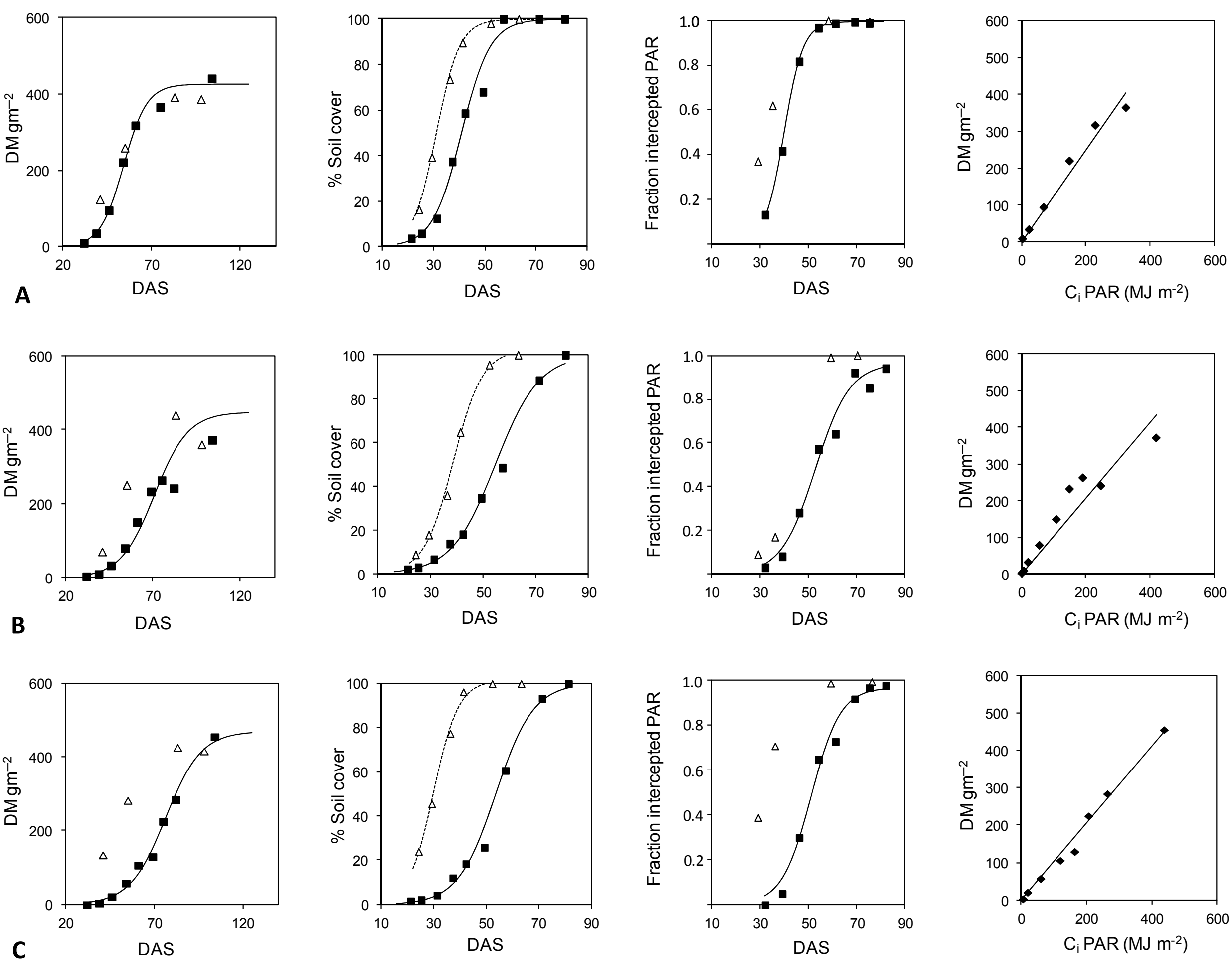

Figure 1. Fitted and observed values for (from left to right) accumulated dry matter, soil cover, fraction intercepted photoactive radiation (PAR) and light use efficiency for Persian clover (row A), subterranean clover (row B) and white (c1) clover (row C) for 2001 (solid line) and 2002 (open triangle).

Table 2. Estimated parameter values obtained by fitting a logistic function to observed values for shoot dry matter of various clover species for 2001.

Species	*b*	GR_{max}	*m*	*c*
2001				
Alsike clover	0.082 (0.009) b	15.90	84 (3) a	773.5 (46.3) a
Berseem clover	0.114 (0.012) ab	15.28	67 (2) c	535.1 (31.6) bc
Crimson clover	0.092 (0.009) b	14.63	76 (2) ab	633.7 (36.8) b
Persian clover	0.152 (0.016) a	16.12	54 (2) d	425.5 (26.1) d
Red clover	0.111 (0.013) ab	14.43	71 (2) bc	520.0 (33.0) cd
Subterranean clover	0.103 (0.013) b	11.55	71 (3) bc	448.5 (33.7) cd
White (c1) clover	0.100 (0.017) ab	11.70	76 (3) ab	469.7 (36.5) cd
White (c2) clover	0.113 (0.021) ab	12.04	74 (3) bc	424.9 (34.3) d
2002				
Persian clover			48	391.0
Subterranean clover			53	439.0
White (c1) clover			48	427.2
SED				NS

b: relative growth rate (RGR; d^{-1}), *m*: moment at which 50% of the maximum dry matter has been obtained expressed in days after sowing (DAS); *c*: maximum accumulated dry matter (DM; g m^{-2}). The maximum growth rate (GR_{max}; g d^{-1}) was derived from the estimated parameters. For 2002, *m* was derived by plotting 50% of the maximum obtained dry matter linearly between the first and the second dry matter measurement and *c* was the maximum dry matter obtained. Different letters (a–d) within a column indicate significant differences at the 0.05 level; values in parentheses denote S.E.; NS: not significant; RGR: relative growth rate; GR: growth rate.

presented in Table 2. The estimated maximum shoot dry matter varied between 425 g m^{-2} (white (c2) clover) and 774 g m^{-2} (alsike clover).

The highest maximum growth rates (about 16 g m^{-2} d^{-1}) were obtained by Persian clover and alsike clover. Whereas Persian clover had the highest relative growth rate (RGR; 0.15 d^{-1}), alsike clover was among the species with the lowest RGR (0.08 d^{-1}). At the same time alsike clover had a much longer growing period than Persian clover and reached its maximum growth rate 30 days later than Persian clover. This resulted in a final accumulated dry matter (774 g m^{-2}) of nearly twice the amount produced by Persian clover (426 g m^{-2}). Red clover, crimson clover and berseem clover had maximum growth rates between 14 and 15 g m^{-2} d^{-1} and their final accumulated dry matter varied between 520 g m^{-2} (red clover) and 634 g m^{-2} (crimson clover).

Subterranean clover and both white clover varieties had clearly the lowest maximum growth rates (about 12 g m^{-2} d^{-1}). Still those species produced nearly similar amounts of shoot dry matter as Persian clover. This is mainly because Persian clover had a relatively short growing period and obtained 50% of its total accumulated dry matter already at 54 DAS. Subterranean clover and the white clover species had a much longer growing period, reflected in a 50% point which was reached 17–22 days after Persian clover.

In 2002, when three clover species were selected for further analysis, final amounts of accumulated shoot dry matter of Persian, subterranean and white (c1) clover were not significantly different from each other and lower or equal to that obtained in 2001. Subterranean and white (c1) clover in particular reached the 50% point more rapidly than in 2001. For Persian clover the difference between both years was negligible. In 2002, root dry matter was assessed on 41 DAS and 83 DAS. Total root dry matter, the fraction root and the vertical root distribution in the upper 30 cm of the soil are presented in Table 3. Total root dry matter of white (c1) clover was significantly higher than the total root dry matter of Persian clover and subterranean clover, both at 41 and 83 DAS. At 41 DAS, each species had invested nearly 20% of their total accumulated amount of dry matter into root growth, whereas at 83 DAS white (c1) clover had allocated a significantly higher fraction to the root (15%) than Persian (10%) and subterranean clover (9%).

Table 3. Root dry matter (g m^{-2}), the fraction root dry matter (DM) of the total dry matter and the vertical root distribution (fraction) over the upper 30 cm of the soil at 41 and 83 DAS.

Species			Root distribution		
	Root DM	Fraction root DM	0–10 cm	10–20 cm	20–30 cm
41 DAS					
Persian clover	23.9 b	0.18	0.75 ab	0.18	0.08 ab
Subterranean clover	15.3 c	0.18	0.68 b	0.18	0.13 a
White (c1) clover	31.4 a	0.19	0.79 a	0.15	0.06 b
SED	3.32	NS	0.040	NS	0.022
83 DAS					
Persian clover	41.1 b	0.10 b	0.71	0.17	0.12
Subterranean clover	46.8 b	0.09 b	0.69	0.19	0.13
White (c1) clover	75.6 a	0.15 a	0.75	0.16	0.08
SED	11.09	0.020	NS	NS	NS

Different letters (a–c) within a column indicate significant differences at the 0.05 level; NS: not significant; DAS: days after sowing.

At both dates small species differences in vertical root distribution were observed. At 41 DAS, white (c1) clover had a higher fraction (0.79) of its root material in the top soil layer (0–10 cm) than subterranean clover (0.68). Subterranean clover in turn had a higher fraction root dry matter in the lower zone. A similar trend was observed at 81 DAS, but then differences were not significant. The distribution of Persian clover held an intermediate position and did not differ significantly from either of the two species.

Leaf area development, soil cover and light interception

In 2001, at 39 DAS, the fraction leaf dry matter ranged from 0.57 (berseem clover) to 0.80 (alsike clover) (Table 4). Also at the later harvests it was obvious that alsike clover invested a relatively large proportion of its shoot dry matter into the leaves. Other species that invested a high amount of shoot dry matter into their leaves were white (c1 and c2) clover, red clover and crimson clover. The low fraction leaf dry matter of berseem clover indicates that a large fraction of the above-ground material was invested in the stem. The same was observed for Persian clover and subterranean clover. The specific leaf area (SLA; cm^2 g^{-1} leaf DM) of berseem and Persian clover were among the highest for each observation date. Alsike clover showed a clear increase in SLA over time, whereas the ranking of the other species remained fairly constant. Both white clover varieties and subterranean clover had the thickest leaves throughout the growing period. In 2002, the fraction leaf dry matter was generally lower than in 2001. As in 2001, white clover invested a higher fraction of its dry matter in leaves than subterranean clover, whereas Persian clover invested the smallest fraction. Subterranean clover produced thicker leaves than Persian and white (c1) clover, except for the last harvest at 83 DAS, when this difference had disappeared.

In 2001, Persian clover had a more rapid increase in LAI than the other clover species (Table 4). It reached an LAI of 3 at least 10 days before the other clover species. Persian clover's rapid increase in LAI corresponds to its rapid increase in shoot dry matter, and was realized despite its relatively low Leaf Weight Ratio (LWR). Berseem clover reached a maximum LAI of 3.7, which was lower than the LAI of the other three clovers that accumulated high amounts of shoot dry matter (alsike, 5.0; crimson, 5.1; red clover, 4.5). This was due to differences in allocation pattern, with berseem clover investing a relatively high fraction of its shoot dry matter into stem material. Subterranean clover and white clover (both varieties) showed a slow initial leaf area development, but continued to increase in LAI after 81 DAS, when the LAI of all other clovers started to decline. Still, subterranean clover only reached a maximum LAI of 3.7.

Table 4. Leaf weight ratio (LWR; g leaf DM g^{-1} total above-ground DM) and specific leaf area (SLA; cm^2 g^{-1} leaf DM) at three moments (DAS).

Species	LWR			SLA			$T_{LAI=3}$	LAI_{max}	$T_{LAI=max}$
	39 DAS	54 DAS	75 DAS	39 DAS	54 DAS	75 DAS			
2001									
Alsike clover	0.80 a	0.61 a	0.44 ab	201.0 cd	362.5 b	390.3 b	65	5.0	75
Berseem clover	0.57 c	0.36 c	0.30 c	292.2 a	422.7 a	347.5 d	68	3.7	75
Crimson clover	0.77 a	0.63 a	0.49 a	207.1 cd	339.3 bc	359.2 c	62	5.1	75
Persian clover	0.64 b	0.36 c	0.30 c	241.2 b	425.6 a	423.3 a	52	4.5	75
Red clover	0.75 a	0.60 a	0.41 b	222.4 bc	335.5 bc	337.9 cde	65	4.5	75
Subterranean clover	0.66 ab	0.47 b	0.33 c	172.7 e	287.8 c	260.9 f	79	3.5	82
White (c1) clover	0.76 a	0.63 a	0.45 ab	187.0 de	306.3 bc	312.5 e	71	4.4	82
White (c2) clover	0.78 a	0.61 a	0.44 ab	173.9 e	294.1 c	328.2 de	73	4.6	82
SED	0.026	0.028	0.026	12.77	28.22	14.47			
	LWR			SLA			$T_{LAI=3}$	LAI_{max}	$T_{LAI=max}$
	41 DAS	55 DAS	83 DAS	41 DAS	55 DAS	83 DAS			
2002									
Persian clover	0.50	0.27 c	0.20 c	507.2 a	494.7 a	370.6	40	3.6	55
Subterranean clover	0.57	0.31 b	0.26 b	350.0 b	343.4 b	377.0	62	4.9	98
White (c1) clover	0.53	0.36 a	0.32 a	480.4 a	507.5 a	395.1	37	5.4	83
SED	NS	0.017	0.019	19.16	20.26	NS			

The moment at which a leaf area index (LAI) of three was reached $T_{LAI=3}$ (DAS) and the maximum leaf area obtained (LAI_{max}) and the moment at which LAI_{max} was obtained $T_{LAI=max}$ (DAS) for various clover species for 2001 and 2002. Different letters (a–f) within a column indicate significant differences at the 0.05 level, DM: dry matter; DAS: days after sowing; NS: not significant.

Table 5. Estimated parameter values obtained by fitting a logistic function to observed values of soil cover and light interception (LI).

Species	*b* (soil cover)	*m* (soil cover)	*b* (LI)	*m* (LI)	*k* value
2001					
Alsike clover	0.15 (0.012)	52 (1.0) ac	0.22 (0.014) ab	49 (0.8) bc	0.83
Berseem clover	0.15 (0.007)	50 (0.5) bc	0.22 (0.034) ab	45 (0.3) d	0.97
Crimson clover	0.13 (0.016)	51 (1.5) ac	0.17 (0.031) b	46 (0.3) d	0.86
Persian clover	0.18 (0.024)*	41 (1.1) d	0.25 (0.008) a	40 (0.1) e	1.04
Red clover	0.13 (0.012)	52 (1.2) ac	0.21 (0.018) ab	48 (0.5) c	0.79
Subterranean clover	0.12 (0.011)*	54 (1.3) a	0.14 (0.027) b	56 (1.7) a	0.80
White (c1) clover	0.14 (0.011)	54 (1.0) a	0.17 (0.031) b	51 (1.3) ab	0.94
White (c2) clover	0.14 (0.014)	55 (1.3) a	0.15 (0.024) b	53 (1.3) a	0.90
SED	NS				NS
2002					
Persian clover	0.22 (0.006)	31 (0.2) a			
Subterranean clover	0.18 (0.019)	39 (0.7) b			
White (c1) clover	0.21 (0.018)	30 (0.4) a			
SED	NS				

b: relative growth rate (RGR; d^{-1}); *m*: moment at which 50% of the maximum was reached (DAS) for various clover species for 2001 (soil cover and LI) and 2002 (LI). Estimated extinction coefficient (*k* value) for various clover species based on field observations collected during the 2003 growing season. Different letters (a–e) indicate significant, within column, per season, differences at 0.05 level; values in parentheses denote S.E.; NS: not significant. * Significantly different at 0.10 level.

In 2002, Persian clover even had a faster initial leaf area development than in 2001, though its maximum LAI remained lower. White (c1) clover and subterranean clover also had a faster development than in 2001. White (c1) clover even reached an LAI of 3 before Persian clover. Both white (c1) clover and subterranean clover reached a higher maximum LAI than in 2001.

Observations on soil cover were conducted between 21 and 87 DAS. For all species full soil cover was reached and the development of soil cover over time could be accurately described by a logistic function. Persian clover reached full soil cover at 57 DAS, well before the other clover species (Table 5). This rapid soil cover development was most clearly expressed in the early moment (41 DAS) at which 50% soil cover was obtained. This was between 9 and 14 days earlier than for the other clover species. No significant differences between red, crimson, alsike (52, 51 and 52) and white (c1 and c2) and subterranean clover (54, 54 and 55) were obtained for the time to achieve 50% soil cover. Berseem clover however, reached this point significantly faster than subterranean and both white clover varieties. In 2002, soil cover measurements were conducted between 24 and 63 DAS, and again the development in soil cover could be well described by a logistic function. Persian, white (c1) and subterranean clover reached full soil cover at or before 63 DAS (Figure 1) which was faster than in 2002. Subterranean clover and white (c1) clover in particular had a much faster increase in soil cover in 2002 than in 2001. Soil cover development of white (c1) clover was almost identical to that of Persian clover, while that of subterranean clover lagged behind.

Light interception for the various clover species in 2001 was based on observed LAI data and the experimentally determined light extinction coefficients (k). Values of k ranged from about 0.8 (red, subterranean and alsike clover) to around 1.0 (Persian clover), but differences were not significant (Table 6). The logistic model gave an accurate description of the light interception data (R^2 > 0.97). Light interception of Persian clover increased more rapidly than that of the other species, whereas the slowest increase in light interception was obtained for white clover and subterranean clover (Table 6). The light interception measurements of 2002 confirmed the observations on soil cover. Light interception of Persian clover and white (c1) clover developed as fast as light interception of Persian clover in 2001. Light interception of subterranean clover developed faster than in 2001, but still lagged behind white (c1) and Persian clover.

Figure 2 illustrates that soil cover and light interception were highly correlated. It is clear that with an increase in soil cover more light was captured. For some species, like subterranean clover, the rate of increase in light interception was nearly proportional to the rate of increase in soil cover. For other species, such as berseem

Table 6. Canopy height (cm) at 4 moments for various clover species in 2001 and 2002.

Species	Canopy height			
	38 DAS	46 DAS	54 DAS	84 DAS
2001	1.9 d	5.8 d	12.1 d	37.3 d
Alsike clover				
Berseem clover	14.6 a	25.6 a	51.1 a	49.5 b
Crimson clover	4.1 c	6.3 a	9.3 e	47.6 bc
Persian clover	9.6 b	13.8 b	31.0 b	46.5 c
Red clover	5.5 c	9.7 c	17.2 c	80.0 a
Subterranean clover	0.9 e	1.0 e	3.6 f	11.6 g
White (c1) clover	1.9 d	4.9 d	10.4 e	23.7 f
White (c2) clover	2.1d	4.7 d	9.2 e	27.7 e
SED	0.86	1.49	0.60	1.38

Species	Canopy height			
	41 DAS	52 DAS	61 DAS	79 DAS
2002				
Persian clover	25.4 a	37.9 a	45.1 a	42.9 a
Subterranean clover	10.0 c	18.7 c	25.6 c	21.4 c
White (c1) clover	20.7 b	24.8 b	29.5 b	31.2 b
SED	1.42	1.46	1.16	1.41

Different letters (a–g) within a column indicate significant differences at the 0.05 level; DAS: days after sowing.

clover, light interception developed slightly faster than soil cover. It is clear that species that grew tall (e.g. berseem clover; see below) showed a relative faster increase in light interception compared to soil cover than species that remained low (e.g. subterranean clover). The relation between light interception and soil cover for the two white clover species was similar to that of subterranean clover. The remaining species all showed a similar relationship as was found for berseem clover.

Light use efficiency

Data for 2001 were used to determine the light use efficiency (LUE). For this purpose, the amount of accumulated dry matter during the exponential and the linear growth phase was related to cumulative intercepted PAR by simple linear regression. The slope of the fitted line represents LUE. For Persian clover only observations up to 75 DAS were used, as senescence for this species started soon after this date. For all other

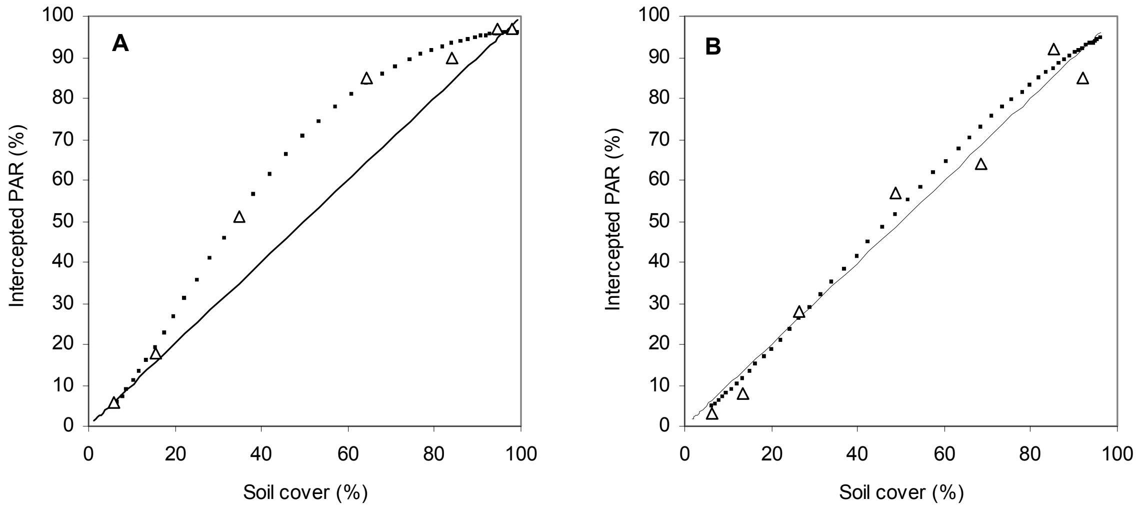

Figure 2. The relation between the percentage of intercepted PAR and the percentage soil cover for berseem clover (A) and subterranean clover (B) for 2001.

species observations until 104 DAS were included. A strong linear relation was found for all species and examples for three clover species are given in Figure 1. LUE varied from 1.03 g DM MJ^{-1} for subterranean clover till 1.25 g DM MJ^{-1} for Persian clover.

Height development

In Table 6, height of the various clover species is presented for four different dates. In 2001, berseem clover was by far the fastest growing species in height as it reached its maximum height (51 cm) well before any of the other clover species. This observation corresponds well with the observation that berseem invested a large fraction of shoot dry matter into stem material. However, a heavy shower directly after the measurement on 54 DAS reduced its height to 31 cm. At 84 DAS, red clover, which reached a maximum of 80 cm, was the tallest species. Observations on height were carried out until this date, as strong showers directly after the measurement on 84 DAS considerably reduced the height of all species except that of subterranean clover and both white clover varieties. Next to berseem clover, Persian clover had a rapid height development, whereas subterranean clover and both white clover cultivars had the slowest development in height. Both Persian clover and subterranean clover invested a relative high fraction of their shoot dry matter into the stem. For Persian clover, this resulted in a rapid height development while for subterranean clover an extensive lateral development was observed. Bulk densities determined at 54 DAS confirmed these findings and were highest for subterranean clover (2.60×10^{-3} g cm^{-3}) and lowest for berseem clover (0.22×10^{-3} g cm^{-3}). In 2002, at around 60 DAS, subterranean clover and white (c1) clover were about 20 cm taller than in the 2001 growing season. Persian clover also grew taller more rapidly in 2002 but did not obtain a larger maximum height.

Nitrogen accumulation

In 2001 (75 DAS) and in 2002 (55 DAS), clover samples were analysed for total nitrogen (N) content of the shoot (Table 7). In 2001, alsike clover and Persian clover accumulated the highest amounts of N (10.8 g m^{-2} and 12.0 g m^{-2}, respectively). These species were also among the clover species with the highest N content (3.73% and 3.36%). Berseem clover accumulated nearly as much N as Persian and alsike clover

Table 7. Clover dry matter (DM) (g m^{-2}), N content (%) and the amount of total accumulated N (g N m^{-2}) at 75 DAS (2001) and 55 DAS (2002) as dependent on species and N-fertilization rate.

Species	DM	N content	Total N
2001	293.0 b	3.73 a	10.77 a
Alsike clover			
Berseem clover	359.2 a	2.52 b	9.18 ab
Crimson clover	291.2 b	2.59 b	7.59 bc
Persian clover	354.4 a	3.36 a	12.00 a
Red clover	286.0 b	3.37 a	7.59 bc
Subterranean clover	254.6 b	2.26 b	5.53 c
White clover (c1)	225.8 b	3.45 a	7.83 bc
White clover (c2)	222.4 b	3.49 a	7.78 bc
SED	48.44	0.248	1.46
2002			
Persian clover	260.1	2.87	7.46
Subterranean clover	250.4	2.66	6.66
White clover (c1)	282.8	2.86	7.78
SED	NS	NS	NS
N1 (0 kg N ha^{-1})	301.9	2.56 b	7.60
N2 (50 kg N ha^{-1})	237.7	2.71 b	6.36
N3 (150 kg N ha^{-1})	253.7	3.13 a	7.94
SED	NS	0.189	NS

Different letters (a–c) within a column indicate significant differences at the 0.05 level; NS: not significant.

Table 8. Correlation coefficients (r) between a number of traits determined for eight clover species/cultivars in 2001.

	$b_{soil\ cover}$	b_{LI}	b_{DM}	$T_{LAI=3}$	$DM_{61\ DAS}$	DM_{max}	$Height_{max}$	N-total
$b_{soil\ cover}$	1	0.79**	0.67	–0.75*	0.72*	0.06	0.15	0.94***
b_{LI}		1	0.46	–0.81**	0.72*	0.25	0.61	0.87***
b_{DM}			1	–0.50	0.75*	–0.69	0.21	0.43
$T_{LAI=3}$				1	–0.78*	–0.18	–0.58	–0.80**
$DM_{61\ DAS}$					1	–0.15	0.32	0.63
DM_{max}						1	0.21	0.27
$Height_{max}$							1	0.28
N-total								1

RGR for soil cover development ($b_{soil\ cover}$), light interception (b_{LI}) and dry matter production (b_{DM}), moment at which a leaf area index of 3 was reached ($T_{LAI=3}$), the obtained dry matter at 61 days after sowing ($DM_{61\ DAS}$), the estimated maximum obtained dry matter (DM_{max}), the maximum canopy height ($Height_{max}$) and the total amount of accumulated nitrogen (N-total).

* $p < 0.10$, two-tailed.

** $p < 0.05$, two-tailed.

*** $p < 0.01$, two-tailed.

but had a much lower N content (2.52%). Crimson clover and subterranean clover also had a relatively low N content (2.59% and 2.26%). Subterranean clover accumulated the lowest amount of N (5.53 g m^{-2}).

In 2002, no significant differences between species were observed for both the amount of accumulated N and N content. N fertilization had a significant effect on the N content, resulting in a significantly higher N content at the highest fertilization rate.

Correlation analysis

For a number of relevant traits a correlation study was conducted (Table 8). As expected, RGR's of soil cover and light interception, were strongly positively correlated ($p < 0.05$). The RGR of shoot dry matter accumulation was not significantly correlated with these two measures. This might be due to differences in allocation pattern (Table 5). Two other measures that represent early growth (the moment at which a leaf area index of 3 was reached ($T_{LAI=3}$) and the obtained dry matter at 61 days after sowing (DM_{61DAS})) were closely correlated with the RGR's of soil cover and light interception. No significant correlation between height and any of the other traits was detected. Total nitrogen accumulation correlated strongly and positively ($p < 0.01$) with the RGR's of soil cover, light interception and $T_{LAI=3}$. This suggests that nitrogen accumulation benefits from a fast development, resulting in pre-emption of below-ground resources.

Discussion

In this study, a comparison of various clover species was made. A number of growth characteristics was determined with the aim to investigate the variability among species. Particular attention was given to those characteristics that are expected to affect the suitability of clover as an undersown cover crop for weed suppression in full field vegetable production. The variability among species determines the importance and opportunities of species selection as a component in the design of a suitable weed management system. A prerequisite for the selected species of this study was their commercial availability and adaptation to the temperate climate of Western Europe. In 2001, seven different clover species were selected to obtain a wide range of characteristics. Also within clover species clear differences in growth characteristics do exist (e.g. Brandsæter et al., 1998; Holland and Brummer, 1999). But as the aim was to obtain a wide range of characteristics the emphasis was put on a comparison between species, and only for white clover two cultivars, differing in leaf size (large leaved: Aran; medium leaved: Riesling) were selected. It should thus be realized that the presented results are typical for the selected cultivar within a species. In 2002, three out of the eight clovers were selected for further evaluation (Persian clover, white clover (Aran) and subterranean clover). The three selected clover species were differing strongly in characteristics such as early growth, soil cover and height development that are relevant for competitive ability.

Weed suppression benefits from a rapid soil cover, as this reduces the germination and establishment of weeds as well as the relative competitive ability of established weed seedlings (Ross and Lembi, 1985). Differences in soil cover development do not only depend on species differences in morphology and physiology. The relative starting position, determined by, for instance, seed size, seeding rate and date of emergence, is another major factor in this respect. For the species selected in this experiment seed size differed with nearly a factor ten. To account for these differences in seed size, all clover species were sown at a fixed seeding rate of 20 kg germinable seeds ha^{-1}. A germination test in Petri-dishes revealed that for all species the percentage germination exceeded 97%. Despite the results of this test, a nearly eight-fold difference in fraction established seedlings was observed between Persian clover (0.50) and the small-seeded clover species (white and alsike clover; 0.06–0.07). As the relative growth rate for soil cover of the small-seeded species (0.14–0.15 d^{-1}) corresponds to a doubling time of 4.6–4.9 (=ln(2)/RGR) days, this lower fraction establishment corresponded to a delay of about 2 weeks. These figures illustrate that comparisons between species in initial development should consider establishment, or should focus on traits, like RGR, that are independent of establishment. At the same time, the results of 2001 suggest that small-seeded species are more sensitive to conditions that might cause a poor establishment. This was confirmed by comparing the results of 2001 and 2002. Whereas differences in establishment between years were very small for both Persian clover and subterranean clover, a remarkable improvement in establishment was observed for white (c1) clover. Not only did seedlings emerge about 3 days earlier, also the fraction of established seeds was considerably higher in 2002 (31% versus 7%). Most notable differences between both years were soil type (clay versus sand), the accumulated amount of precipitation in the 2 weeks around sowing (3 mm in 2001 and 18.2 mm in 2002) and a heavy shower at 7 DAS in 2001 (25.7 mm), which may have washed seeds away. As only white clover had a higher fraction of established seedlings, it is suggested that for establishment this species and perhaps more in general small-seeded species are more sensitive to environmental conditions.

Persian clover had the fastest soil cover development of all species. To some extent this was due to its good starting position. Persian clover emerged 2–5 days before the other species and also had the highest fraction establishment. Further analysis, however, showed that this species also had the highest intrinsic growth rate. This was not only observed for soil cover ($p < 0.10$), but also for light interception and dry matter accumulation. The RGR reflects the increase of characteristics such as soil cover and dry matter accumulation during early development, when growth is still exponential. In this period, a strong positive feedback exists between radiation interception, crop growth and leaf area formation resulting in exponential growth (Blackman, 1919). The relative growth rate of a plant species is, thus, affected by its light capturing ability, by the efficiency by which it converts light into biomass and by the fraction of newly produced biomass which is invested in leaves. For the first two characteristics, the light extinction coefficient (k) and the light use efficiency (LUE), a wide range of values was obtained, though no significant differences between species were observed. Values for k ranged from 0.79 to 1.04, whereas LUE ranged between 1.03 and 1.25 g DM MJ^{-1}, values that correspond well to values reported for two white clover cultivars (Nassiri, 1998; 1.02 g DM MJ^{-1} for Alice and 0.96 g DM MJ^{-1} for Gwenda). Persian clover was characterized by high values for both traits. For the third characteristic, leaf area ratio (LAR = LWR × SLA), a moderate to high value was obtained. Values for SLA were always relatively high for Persian clover, but LWR was relatively low right from the start and declined faster than for most other species. This observation might be related to the rapid phenological development of Persian clover, which was expressed by, for example, early flowering, as plants often show a reduced LWR when ageing (Woolhouse, 1967). Compared to Persian clover, subterranean clover was the only species that was characterized by a significantly lower RGR for soil cover ($p<0.10$), dry matter accumulation and light interception. The low RGR's for subterranean clover are not surprising, as for all characteristics that together constitute the RGR (k, LUE, LWR and SLA) the value of subterranean clover was always amongst the lowest of all species. Of the two white clover cultivars, cultivar 'c1' (Aran) was used in both seasons. In 2001, white (c1) clover developed relatively slow for soil cover, light interception and dry matter accumulation, whereas in 2002 it developed as rapid as Persian clover. This difference could be explained by its much better starting position caused by a much higher fraction emergence, resulting in a nearly five times higher number of emerged seedlings. Furthermore, also its RGR was considerably higher than in 2001. This last observation was true for all three species and might be related to the higher average air temperature in the first month after emergence (14.2 °C for 2001 and 16.0 °C for 2002). In this study, white clover showed to be a more rapid developing species than subterranean clover which contradicts statements of Frame et al. (1998), who described the vigour of white clover seedlings as slow growing during early establishment, whereas the vigour of subterranean clover was described as moderate to high. Brandsæter et al. (1998) concluded, based on experiments with undersown white and subterranean clover, that white clover had a slower soil cover development than subterranean clover. The sowing density of subterranean clover was, however, much higher than that of white clover, resulting in a different starting position. No information on establishment was provided, while our findings clearly show the importance of seedling density.

Clear differences were obtained in maximum amount of accumulated biomass. Alsike and crimson clover produced the highest amount of biomass. Biomass production of Persian clover, being the fastest developing species, was among the lowest, mainly due to its relatively short growth duration. For weed competition and weed suppression, earliness has been reported an important characteristic (de Haan et al., 1994). Particularly for competition for light, which is asymmetric (Weiner, 1986), obtaining a good starting position seems highly relevant. From that perspective the RGR seems to be a more important characteristic than the maximum accumulated amount of biomass.

Apart from soil cover development, height is an important characteristic, determining competition for light (e.g. Berkowitz, 1988). This effect was quantified by Kropff and van Laar (1993) in a simulation study. For the species represented in this study, no clear relation between total dry matter accumulation and maximum height was observed (Table 8). Alsike clover, being the species with the highest amount of accumulated dry matter, only reached a maximum height of nearly 40 cm, resulting in an intermediate position with respect to height. Furthermore, red clover, being the tallest species, and subterranean clover, being the shortest species, accumulated similar amounts of dry matter. Berseem clover also took a special position, as it was the species with the fastest height development during early growth stages. This observation was in line with the allocation pattern of berseem clover, which clearly invested a higher fraction of its shoot dry matter into stem material.

The overall competitive ability of a plant species does not only depend on its ability to compete for light but also on its ability to compete for water and nutrients (Tilman, 1988) of which nitrogen is of particular importance for plant growth. Obviously, the nitrogen accumulation of clover is realized by both N_2 fixation from the air and by uptake of nitrate from the soil. It is, therefore, difficult to attribute a low or high amount of accumulated nitrogen solely to a better or poorer below-ground competitive ability. That these two systems for nitrogen accumulation are present at the same time in clover may very well explain why the different amounts of nitrogen fertilization in 2002 did not affect the total amount of accumulated nitrogen for any of the clover species. Griffith et al. (2000) observed that at higher levels of available mineral soil nitrogen, the nitrogen fixation of white clover decreased, in other words, the available mineral nitrogen substitutes the nitrogen otherwise captured from the air. One other reason why the nitrogen fertilization may have had little effect on nitrogen uptake of clover, is because all nitrogen was applied 1 day after sowing well before the nitrogen demand of the growing plants was at its maximum. As the experiment was conducted on sandy soil and a number of heavy rain showers occurred during the first weeks after sowing, leaching of nitrogen may have taken place, resulting in much smaller differences in available soil mineral nitrogen than was intended. Even though fertilization did not result in differences in accumulated nitrogen, increases in shoot N-content and SLA were observed. An increased specific leaf area following higher N-fertilization is more often observed (e.g. green cabbage by Li et al., 1999).

In 2001, clear differences in N-accumulation between species were observed. The two species which had accumulated most nitrogen at 75 DAS, Persian clover and alsike clover, were characterized by a very distinct growth pattern. Persian clover had a short growth period, combined with a rapid initial growth, whereas alsike clover combined a long growth period with a slow initial growth. This implies that, at least in pure stand, a fast initial development is not a prerequisite for the accumulation of a high amount of nitrogen. Still the correlation analysis revealed a strong positive association between total N-accumulation and the RGR's of soil cover, light interception and the moment at which a LAI of 3 was reached. This is not surprising, as all other clover species, except for berseem clover, accumulated a significantly lower amount of nitrogen and were characterized by a relatively slow initial development. For subterranean and crimson clover the low amount of accumulated nitrogen was combined with a low N-content, indicating that for those species N-uptake might have been a growth limiting factor.

Aim of this study was to evaluate growth characteristics of a range of clover species. Specific attention was given to a number of traits like relative growth rate, total accumulated biomass, height development and N-accumulation that are expected to affect the suitability of clover as an undersown cover crop for weed suppression in full field vegetable production. The results showed that for most of these characteristics clear differences between species were present. Moreover, a correlation analysis revealed that hardly any correlations were present between the groups of traits that represent various aspects of competitive ability, like earliness, final amount of accumulated dry matter, height development and N accumulation. As the various group of traits are not intertwined, competition experiments conducted with this group of selected species offers good opportunities to identify which traits are specifically responsible for differences in competitiveness. Results of such experiments are discussed in Chapter 3.

CHAPTER 3

Clover as a cover crop for weed suppression in an intercropping design
II. Competitive ability of several clover species

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Abstract

Undersown cover crop species introduced for weed management purposes should ideally combine adequate weed suppression with only marginal negative competitive effects on the main crop. The aim of this research was to identify the growth characteristics of clover species that determine weed-suppressive ability and competitive ability against the main crop. In addition, the variation in these characteristics among clover species was determined, to identify whether species selection is an important component of the optimization of living mulch systems.

In 2001, a field experiment was conducted in which weed suppression and competitive ability against leek of eight clover types was determined. Based on the results, three contrasting clover types were selected and their weed-suppressive ability was further investigated in a field experiment conducted in 2002. Results of both experiments were related to growth characteristics that were simultaneously determined and described in Chapter 2.

Correlation analysis showed that the competitive effect of clover on transplanted leek was strongly correlated with maximum canopy height, indicating that yield reduction was mainly caused by competition for light. Subterranean clover, being the shortest species, gave inadequate weed suppression, whereas all other species were found to provide at least reasonable weed suppression. Subterranean clover reduced individual leek plant dry weight more strongly (60%) than expected based on its canopy height. Indications were found that this clover species was hindering the uptake of nitrogen by leek. Clover species with superior weed suppression, such as Persian clover, red clover, alsike clover, berseem clover and crimson clover also gave the strongest negative effect on dry matter accumulation of leek (reductions between 70% and 90%). White clover was found to give the best compromise between adequate weed suppression and mild yield reduction, though also with this species reduction in leek plant dry weight was still 60%. It was concluded that for optimization of systems in which clover species are introduced as a weed-suppressing cover crop, species selection is an important element, though additional management to restrict the competitive effect on the main crop remains a requirement.

Keywords: Cover crop, clover, competition, weed suppression, soil cover, light interception, height, allelopathic activity

Introduction

A cover crop (living mulch) may contribute significantly to weed management (Moore et al., 1994). The number of established weed seedlings is reduced through provision of an early soil cover, whereas the harmful effect of the established weeds on the main crop is reduced through competitive suppression. Cover crops may also be introduced in a main crop for a number of other reasons. Pest control (Theunissen and den Ouden, 1982) and the improvement of soil quality (Brandsæter et al., 1998) are important beneficial traits of cover crops especially in systems where pesticides are not used. A reduction in yield of the main crop through competition by the added cover crop has often been recognized as a serious problem (Wiles et al., 1989; Müller-Schärer, 1996; Kleinhenz et al., 1997; Bottenberg et al., 1997; Brandsæter et al., 2002). A number of solutions has been proposed to reduce this negative effect of the cover crop on the main crop. Mechanical and chemical means have been suggested to restrict the growth of the cover crop and through that its competitive effect on the crop (Vrabel, 1983; Wiles et al., 1989; Mohler, 1991; Galloway and Weston, 1996). However, if reduction of herbicides is the main reason for the use of cover crops, growth reduction by chemical means is not an option. Separating the growth periods of the main crop and the cover crop has also been advocated. Müller-Schärer and Potter (1991) proposed a late sowing of the cover crop, after establishment of the main crop, to give the main crop a head-start. Ilnicki and Enache (1992) and Brandsæter and Netland (1999) proposed the use of a winter annual, sown in autumn, as cover crop in spring-sown crops. The autumn sown cover crop (e.g. subterranean clover) establishes and forms a dense mat covering the soil before winter. In early spring, the cover crop resumes its vegetative growth while flowering and senescence of the cover crop begins already in late spring. In such a system, weeds are suppressed early in the growing season while there is little competition between the cover crop and the main crop as senescence of the cover crop and maximum growth of the main crop take place at approximately the same time. Apart from these management options, selection of the most suitable cover crop species was recommended. Most often, a direct screening procedure, in which a number of cover crop species was introduced in a relevant main crop, was used (e.g. Nelson et al., 1991; Galloway and Weston, 1996; Creamer and Bennett, 1997; Abdin et al., 2000; Ross et al., 2001). Suitability was mainly based on the effects of the cover crop on various relevant features such as yield and quality of the main crop and weed abundance. Ross et al. (2001) tried to generalize the outcomes of these screenings, by determining various growth characteristics of the cover crop species, like earliness, maximum height and the time course of dry matter production. Correlation of growth analysis parameters with competitive ability may help to identify characteristics of growth that determine plant-to-plant interactions. This insight might then be used as a guideline for selecting the most appropriate cover crop species.

This research project focused on the evaluation of various *Trifolium* species, with respect to their potential use as a weed-suppressing cover crop in a main crop. True clovers were chosen because several clover species already proved to be effective weed suppressors, either as undersown crop or in rotational cropping (Ilnicki and Enache, 1992; Fisk et al., 2001). Furthermore, clovers have a proven ability to reduce various pests in a number of vegetable cultures (Theunissen and Schelling, 1996; Weber et al., 1999) and are able to add to the nitrogen status of a cropping system through their nitrogen fixation ability. Clover species for the experimental work were selected based on their suitability to the Western European climate and their commercial availability. To obtain a wide range of growth characteristics a wide range of clover species was chosen. Only for white clover two cultivars, a medium leaved (Riesling) and a large leaved (Aran) cultivar, were included. Morphological and physiological characteristics of the various species were determined. The results were presented and discussed in Chapter 2. Through a number of additional experiments and observations, the competitive ability of the species was established. The results of these experiments are presented in this Chapter. Additionally, the suitability of the clover species as a cover crop for weed suppression, as well as the characteristics that determine this suitability, will be discussed.

Materials and methods

Experiment 2001

An experiment with eight undersown clover species (Chapter 2) was laid out on a heavy clay soil, in Wageningen, the Netherlands. The site was fertilized with 300 kg ha^{-1} 12-10-18 NPK 2 weeks before sowing. The experimental design was a fully randomized complete block design in six replicates and treatments consisted of eight clover types and two bare soil reference plots. At May 7, the clovers were sown at a rate of 20 kg ha^{-1} in plots of 4.5 m × 9 m. Seeds were sown with a seed drill of which the pipes were positioned 30 cm above the soil surface. After sowing, the soil was rolled with a Cambridge roller to compress the soil.

One day after the clover was sown one 9 m long row of leek (*Allium porrum* L. cv. Stanley F1 256) was machine planted at 0.75 m from the edge of the plot. The distance between individual leek plants within the row was 0.10 m. A second and a third row of leek were planted 4 weeks (June 3) and 8 weeks (June 26) after the first planting, at a distance of 1.5 m and 3.0 m from the first planted row, respectively. Hand weeding of the plots was carried out at the end of June.

Observations

From each plot, ten leek plants of the first planting were harvested on June 18. At July 25, another 10 leek plants from the first planting were harvested, together with 10 plants from the second planting. At October 1, 10 plants were collected from each planting. At each harvest, a subsample of two plants was taken from the first planting and divided into stem and leaf material. Leaf area was assessed using a LI 3100 Area Meter (LI-COR, Lincoln, Nebraska, USA). The leaf and stem fraction of the subsample as well as the other collected plant samples were dried for 24 h at 70 °C and weighed subsequently. At the last harvest, on October 1, the total nitrogen content of the shoot material of leek plants of the first planting (row 1), was determined by SFA (Temminghoff et al., 2000a). Additionally, the total number of living plants of the second and third planting was counted to assess the fraction survived leek plants. Weed prevalence was determined in all plots in the first week of August, by counting the number of plants of eight weed species. Growth characteristics of the various clover species were determined and described in Chapter 2.

Experiment 2002

Persian clover, subterranean clover and white (c1) clover were selected for further evaluation as the results of the experimental work in 2001 showed that these species were contrasting strongly with respect to their characteristics and competitive strength. Competitive ability of clover was assessed by measuring to which extent each clover species was able to suppress Italian rye grass (*Lolium multiflorum* cv. Bartisimo) and, naturally occurring weed species. Plots of 3 meter wide and 5 meter long were laid out on a sandy soil in Wageningen, the Netherlands. The field was fertilized with P and K one month before sowing (100 kg ha^{-1} 60% K_2O and 200 kg 46% P_2O_5).

The experimental design was a split plot design in four replicates with N rate (0, 50 and 150 kg N ha^{-1}) as main factor. Each main plot was subdivided into seven subplots, containing either a pure stand of Persian clover, subterranean clover, white (c1) clover or Italian rye grass or a mix of rye grass and one of the clover species. Mechanical weed control was carried out 1 day prior to seed bed preparation. Clover species were sown on May 21 at a rate of 20 kg ha^{-1}, using the same procedure as used in 2001. Italian rye grass was hand sown at a rate of 7 kg ha^{-1}. Hand weeding was carried out on June 13, 14 and 26.

Observations

On July 15 and August 27 (55 and 98 DAS), two squares of 50 cm × 50 cm were harvested at fixed positions from all subplots. After sampling, grass and clover of the mixed plots were separated. All samples were dried for 24 h at 70 °C and weighed. On

July 15, total nitrogen content of the shoot material of rye grass and the clover species was determined by SFA (Temminghoff et al., 2000a). Additionally, dry matter of four naturally occurring weed species (*Capsella bursa-pastoris* (L.) Medicus, *Stellaria media* L., *Poa annua* L. and *Chenopodium album* L.) was determined in the plots that contained pure stands of clover. Growth characteristics of the various clover species were determined and described in Chapter 2.

Statistical analysis

The accumulated dry matter of the leek plants of the two reference plots was averaged per replicate to balance the total number of observations per treatment. Leek dry matter and the weed counts were analysed by performing an analysis of variance (ANOVA) or, when a log transformation was required, by using a generalized linear model (GLM) with a log link function. The fraction surviving leek plants of the second and third planting were analysed using a GLM with a logit link function. Approximate values of the SED and least significant differences (LSD) at the 5% level were calculated. ANOVA was performed and standard errors of difference (SED) and least significant differences (LSD) (5%) were calculated for the shoot dry matter of the weeds and for the shoot dry matter, the N content and the total amount of accumulated N for clover and rye grass. Statistical analysis was carried out using the Genstat 7 statistical package (Payne, 2003).

Results

Experiment 2001

Dry matter, specific leaf area and N uptake of leek

At May 8, the first batch of leek plants (individual plant weight 0.50 g) was planted in the clover plots. At June 19, 42 days after planting (DAP), shoot dry matter of leek plants in the reference plots had increased five-fold (2.66 g plant^{-1}) (Table 1). Dry matter of leek plants in the clover plots was not affected, except for leek plants in plots with Persian clover and berseem clover, which had accumulated a significantly lower amount of biomass. For leek plants in plots with berseem clover this reduction was accompanied with an increased SLA (122.7 compared to 102.8 cm^2 g^{-1} in the reference plot). On July 25 (79 DAP), shoot dry matter of leek plants in the reference plot had increased to 14.0 g plant^{-1} while the shoot dry matter of leek plants in all the clover plots was clearly reduced. Reductions in dry matter of leek plants grown in the Persian, berseem, crimson and red clover plots exceeded 50%. SLA of these leek plants was significantly higher than the SLA of leek plants in the reference plot,

Table 1. Specific Leaf Area (SLA), Shoot dry matter (DM; g plant^{-1}) of leek of the first planting growing in various clover plots at June 19 (1st harvest), July 25 (2nd harvest) and October 1 (final harvest).

Plot	First harvest		Second harvest		Final harvest			
	SLA	DM	SLA	DM	SLA	DM	N content	Total N
Reference	102.8 b	2.66 a	106.1 cd	13.96 a	86.1 f	54.25 a	1.10 e	0.58 a
Alsike clover	98.0 b	2.68 a	154.0 b	7.20 c	129.4 bc	15.84 cd	1.81 c	0.28 cd
Berseem clover	122.7 a	2.07 b	174.4 a	4.05 de	122.2 bcd	16.59 c	1.67 cd	0.28 cd
Crimson clover	99.5 b	2.20 a	118.8 c	5.53 cde	140.5 b	13.23 de	1.73 c	0.25 cde
Persian clover	107.6 b	1.90 b	174.4 a	3.23 e	141.4 b	13.96 d	3.05 a	0.38 b
Red clover	101.9 b	2.48 a	157.9 b	5.64 cde	163.9 a	6.58 e	2.32 b	0.12 f
Subterranean clover	101.5 b	2.50 a	102.1 d	11.03 b	105.1 def	21.08 bc	0.94 e	0.21 def
White (c1) clover	102.3 b	2.20 a	120.2 c	7.98 c	100.5 ef	23.51 b	1.52 cd	0.32 bc
White (c2) clover	103.3 b	2.56 a	105.6 cd	7.07 cde	114.0 cde	22.27 bc	1.30 de	0.25 cde
SED	4.60	0.228	7.18	1.770§	9.85	3.348	0.201§	0.046

N content (%) and the total amount of accumulated N (g plant^{-1}) of leek plants at final harvest. Standard error of difference (SED) is presented for non-transformed data while the highest standard error of difference (SED_{max}) of the various pair-wise comparisons is presented for transformed data. Different letters within a column indicate significant differences at the 0.05 level. § SED maximum.

except for leek plants in crimson clover. For leek grown in alsike and the two white clover species the reduction in plant dry weight was between 40% and 50%. Only in alsike clover this reduction was associated with an increase in SLA. Leek plants in plots with subterranean clover clearly suffered the least, as individual plant weight was reduced with only 20% and SLA was not significantly affected. At final harvest (October 1; 147 DAP), accumulated leek dry matter had increased to 54.0 g plant^{-1}. In all clover plots, the reduction in leek plant dry matter surpassed 50%. By far the strongest reduction in accumulated dry matter was found for leek grown in red clover plots (88%) resulting in an individual plant dry weight of 6.6 g. Leek grown in this clover species also had the highest SLA (164 cm^2 g^{-1}), which was nearly twice as high as the SLA of leek in the reference plot. For leek plants grown in crimson, Persian, alsike and berseem clover reduction in individual plant weight was around 70–75%, whereas SLA varied between 122 and 142 cm^2 g^{-1}. The mildest reductions were obtained in plots with white (c1 and c2) and subterranean clover, but also in these plots reductions were still considerable (around 60%). SLA was not significantly different from the SLA in the reference plot, except for leek in white (c2) clover plots.

In Figure 1, the results of these harvests are presented in a different way. The increase in dry matter of the leek plants in three consecutive periods (planting−harvest 1; harvest 1−harvest 2; harvest 2−final harvest) was expressed relative to the increase

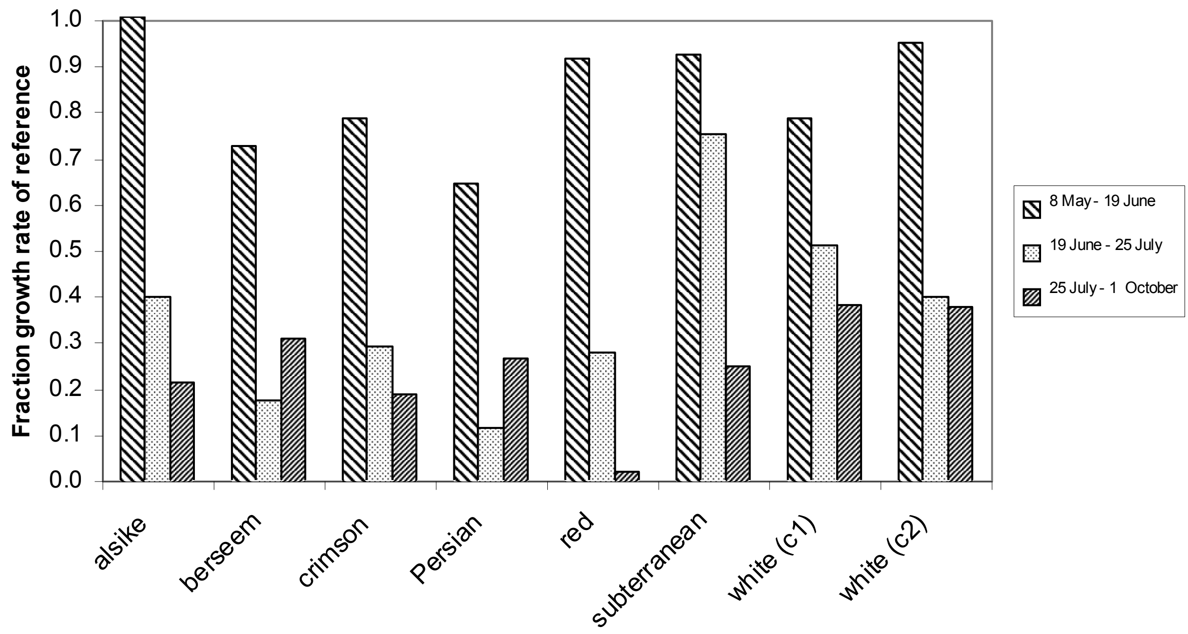

Figure 1. Growth rate of leek affected by various clover species for three consecutive periods in 2001. Growth rate is expressed as a fraction of the growth rate in the reference plots.

of leek plant dry matter in the reference plot. In this way, differences in competitive suppression in the various periods can easily be identified. From Figure 1, it is evident that the strongest reduction occurred in the second and third period. In the second period, subterranean clover suppressed the growth of leek plants to a relatively mild degree, whereas in the third period suppression was at an average level. In this third period, suppression by the two white clover cultivars was less severe than in the other plots. At the same time, red clover developed into the strongest competitor, whereas Persian clover and berseem clover were the only species where growth reduction of leek plants was less strong than in the previous period.

Leek plants of the reference plots accumulated an average of 0.58 g N plant^{-1}, corresponding to a nitrogen content of 1.10% (Table 1). In all the clover plots, the accumulated amount of nitrogen in the leek plants was significantly lower and ranged from 0.38 g N plant^{-1} for leek plants in Persian clover, to 0.12 g N plant^{-1} for leek in red clover. Despite the lower amount of accumulated nitrogen, nitrogen content was either not significantly different (subterranean and white (c2) clover), or significantly higher than the nitrogen content of the leek plants in the reference plot. The highest nitrogen contents were found in plots with Persian clover (3.05%) and plots with red clover (2.32%).

Earlier, various growth characteristics of the clover species that were expected to affect the suitability of clover as an undersown cover crop for weed suppression were determined (Chapter 2). These characteristics were related to early growth, total dry matter production, maximum height and N-accumulation. Correlation analysis between the various characteristics and total accumulated dry matter of leek plants of the first planting demonstrated that only maximum canopy height was significantly correlated with dry matter of leek, suggesting that competition for light was important (Table 5).

With the second and third planting, the competitive strength of some of the clover species became so strong that mortality of the leek plants was significantly higher than in the reference plots (Table 2). This was particularly the case for leek plants in alsike and red clover, with an average survival rate of around 18%. Survival rates in Persian clover (27%) and berseem and crimson clover (47%) were higher but still low. With the white clover species survival rate clearly decreased from 59% with the second planting till 30% with the third planting. Only in subterranean clover plots the mortality rate of leek plants was not reduced compared to the reference plots.

At harvest (October 1) dry weight of the leek plants in the reference plots was 25.7 g plant^{-1} for the second planting and 9.8 g plant^{-1} for the third planting. In clover plots with the highest mortality rate, the dry matter production of the remaining plants was most strongly reduced. For alsike, red, Persian, crimson and berseem clover individual

Table 2. Fraction survival of leek plants of the second and third planting and individual plant weight (DM; g $plant^{-1}$) at final harvest.

Clover species	Fraction survival		Dry matter	
	Second planting	Third planting	Second planting	Third planting
Reference plot	0.79 b	0.88 a	25.70 a	9.79 a
Alsike clover	0.22 g	0.06 e	0.56 d	0.25 d
Berseem clover	0.40 f	0.42 b	1.19 d	0.93 cd
Crimson clover	0.60 d	0.46 b	1.78 cd	0.45 d
Persian clover	0.24 g	0.30 c	0.69 d	0.86 cd
Red clover	0.19 g	0.23 d	0.60 d	0.31 d
Subterranean clover	0.91 a	0.90 a	5.10 b	2.39 b
White (c1) clover	0.49 e	0.28 cd	3.61 bc	0.81 cd
White (c2) clover	0.69 c	0.32 c	3.58 bc	1.45 c
SED	0.034[§]	0.033	1.04[§]	0.413

Standard error of difference (SED) is presented for non-transformed data while the highest standard error of difference (SED_{max}) of the various pair-wise comparisons is presented for transformed data. Different letters within a column indicate significant differences at the 0.05 level. [§] SED maximum.

dry weight of leek plants was less than 10% of the plants in the reference plot. Leek plants in plots of subterranean clover were least affected, but still only weighted 20% (second planting) and 24% (third planting) of the reference plants. White clover held an intermediate position with an individual plant weight of around 15% of the reference plants, except for white (c2) clover, where the leek plants of the third planting weighted less than 10% of the reference plants.

Weed counts

On August 2, the density of eight weed species was counted in each of the clover plots. For the smaller weed species (*Capsella bursa-pastoris*, *Phleum pratense, Lamium purpureum* and *Veronica polita*) abundance was remarkably high in plots with subterranean clover, whereas those species were nearly absent in plots with the other clover species (Figure 2). Only for two of the smaller weed species (*C. bursa-pastoris*, *P. pratense*) moderate numbers were also observed in crimson clover and the two white clover varieties. For the taller weed species (*Chenopodium album, Polygonum persicaria, Euphorbia peplus* and *Echinochloa crus-galli*) differences in abundance were far less extreme. In plots with Persian, berseem, red and alsike clover fewer

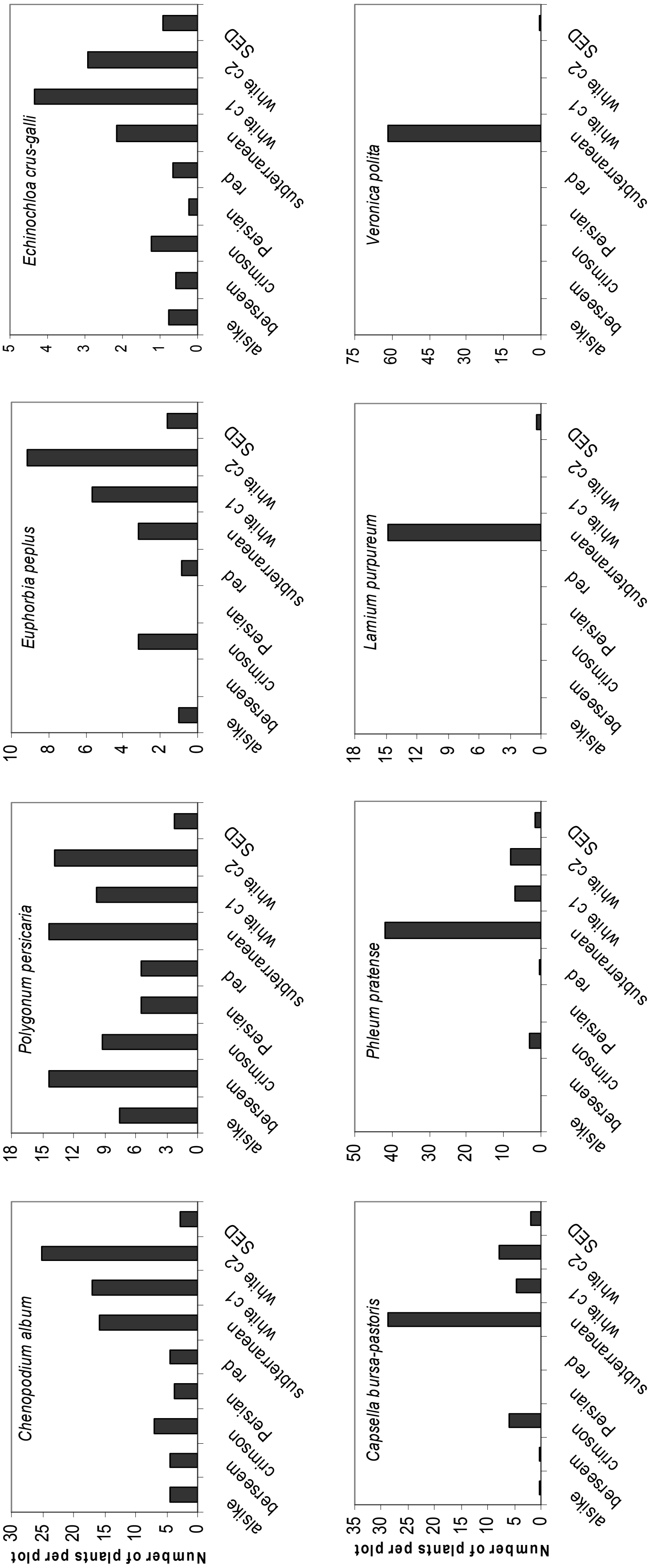

Figure 2. Density of eight naturally occurring weed species (plants plot^{-1}) counted in the first week of August 2001 in plots of various clover species.

C. album, E. peplus and *E. crus-galli* plants were observed than in plots with crimson, white (c1 and c2) and subterranean clover. Persian, alsike and red clover also caused a significant reduction in the number of *P. persicaria* plants.

Experiment 2002

Suppression of rye grass

At 15 July, clover and grass were harvested from the pure stands of clover and rye grass and from the clover – rye grass mixtures. In the pure stands of clover, differences in shoot dry matter between the species were small and not significant (Table 3). In the clover plots to which rye grass was added, shoot dry matter of subterranean clover was only about one-third of the dry matter of white and Persian clover. Between the clover species there were no significant differences in nitrogen content while there was at the same time a clearly reduced amount of accumulated nitrogen in subterranean clover in the subterranean clover – rye grass plots, which was caused by the decreased amount of accumulated dry matter. Dry matter of clover and the total amount of accumulated nitrogen in the pure stands were not significantly affected by nitrogen level. The nitrogen content of the clover species in pure stand and in mixture, on the other hand, was significantly elevated at the highest nitrogen level. No interaction between nitrogen level and clover species was observed.

The poor competitive ability of subterranean clover was also clearly reflected in the shoot dry matter of rye grass in the mixed plots which was about 50% higher in the

Table 3. Total shoot dry matter (DM; g m^{-2}), N content (%) and the total amount of accumulated N (g m^{-2}) for clover in pure stand and in mixture with rye grass.

2002	Pure clover stand			Mixture		
	DM	N content	Total N	DM	N content	Total N
Persian clover	260.1	2.87	7.46	128.9 a	2.93	3.75
Subterranean clover	250.4	2.66	6.66	44.0 b	2.67	0.85
White clover (c1)	282.8	2.86	7.78	149.1 a	2.72	4.12
SED	NS	NS	NS	23.25	NS	0.737
N1	301.9	2.56 b	7.60	112.1	2.52 b	2.83
N2	237.7	2.71 b	6.36	99.6	2.69 b	2.59
N3	253.7	3.13 a	7.94	110.3	3.11 a	3.29
SED	NS	0.189	NS	NS	0.188	NS

Main effects per clover species and N rate are presented. Different letters within a column indicate significant differences at the 0.05 level. NS: not significant.

Table 4. Total shoot dry matter (DM g m^{-2}), N content (%) and the total amount of accumulated N (g m^{-2}) for rye grass in pure stand and in mixture with clover.

	DM	N (%)	Total N
Grass – pure plots	230.5 a	3.10	7.18 a
Grass – Persian	91.8 c	3.35	3.17 c
Grass – subterranean	157.7 b	2.96	4.70 b
Grass – white	100.1 c	3.07	3.17 c
SED	18.91	NS	0.548
N1	122.1 a	2.63 b	3.01 c
N2	148.9 ab	2.89 b	4.32 b
N3	164.2 b	3.83 a	6.33 a
SED	16.38	0.1529	0.474

Main effects per treatment and N rate are presented. Different letters within a column indicate significant differences at the 0.05 level.

subterranean clover plots than in the plots with white and Persian clover (Table 4). Nitrogen content was not affected by clover species and for that reason the differences in shoot dry matter were reflected in the total amount of accumulated nitrogen. Shoot dry matter of rye grass responded significantly to nitrogen level. At the highest nitrogen rate, shoot dry matter was significantly higher than at the lowest nitrogen rate. On top of that, nitrogen content at the highest N level was significantly higher than at the other two N rates. This resulted in clear and significant differences in accumulated nitrogen between all N rates. No interaction between fertilization and clover species were observed.

Regarding the total accumulated dry matter of the pure stand plots and of the mixtures, there were no significant differences between any of the plots. The total amount of nitrogen that was accumulated in the pure stand plots and in the mixtures was not affected by the clover species present but a significantly higher amount of nitrogen was accumulated in the plots with the highest nitrogen fertilization rates. No significant interaction between fertilization rate and plant species was observed.

At 27 August, shoot dry matter of subterranean clover in the mixture with rye grass was much lower than the shoot dry matter of white clover and Persian clover (Figure 3). The opposite was observed for the shoot dry matter of rye grass, as this species was much more productive in the subterranean clover – rye grass mixtures than in the mixtures with white clover and Persian clover. These results clearly show that also in the later stages, white and Persian clover competed much stronger with rye grass than subterranean clover. The combined shoot dry matter of clover and rye grass in the various mixtures were not significantly different from each other and also not significantly different from the total shoot dry matter in the pure stands. Nitrogen fertilization rate had no effect on final shoot dry matter production of clover or rye grass. This was found in pure stands as well as in the mixtures.

Weed dry matter

The weed-suppressing ability of Persian, subterranean and white clover was assessed by determining the dry matter of four weed species (*Chenopodium album*, *Capsella bursa-pastoris*, *Stellaria media* and *Poa annua*). The last two species were harvested together and their combined dry matter was determined. Clear differences in suppressive ability between clover species were observed. Persian clover and white clover suppressed *C. bursa-pastoris* and the combination of *S. media* and *P. annua* stronger than subterranean clover (Figure 4). For *C. album,* the situation was different as no significant differences in accumulated dry matter were found between plots of the various clover species.

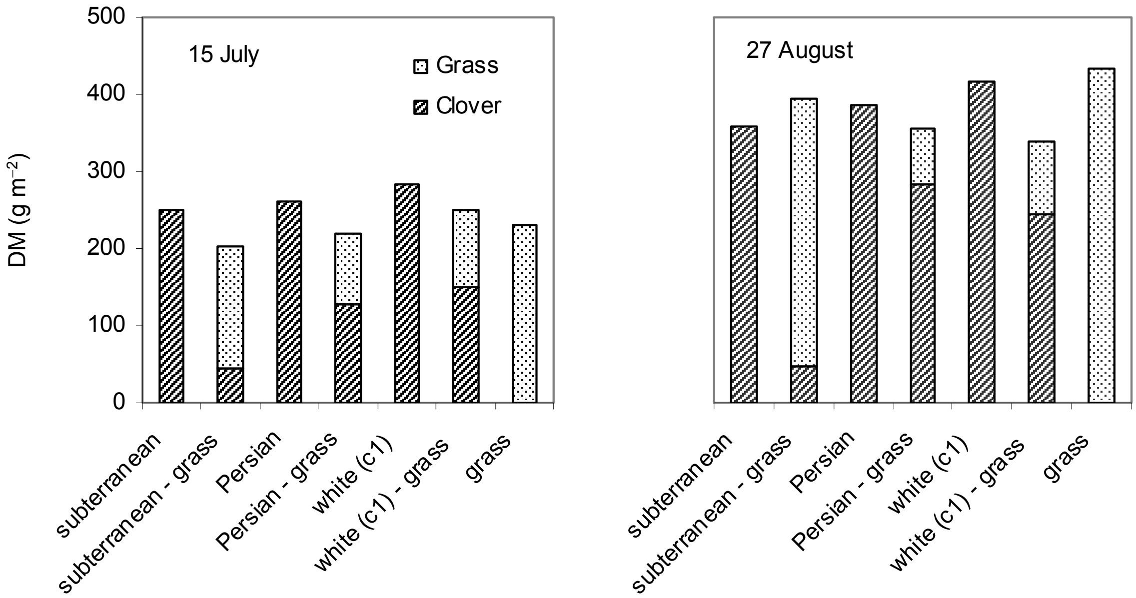

Figure 3. Dry matter (DM g m^{-2}) of rye grass and subterranean, Persian and white (c1) clover harvested at July 15 and August 27 2002 from pure stands and from mixtures of clover and rye grass.

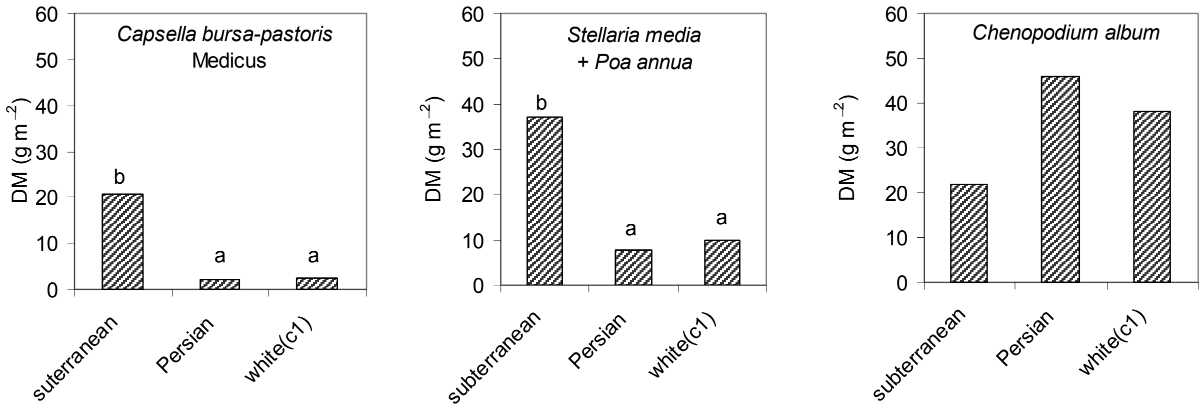

Figure 4. Dry matter (g m^{-2}) of four weed species harvested at July 15 2002, in plots of Persian clover, subterranean clover plots and white clover plots. Different letter indicate significant differences at the 0.05 level.

Discussion

The two major requirements of a cover crop which is added to a main crop for improved weed management are: (i) providing a sufficient level of weed suppression and (ii) not having a too strong negative effect on the growth of the main crop. In the current experiments, this last aspect was evaluated by introducing transplants of leek in just sown and well-established plots of eight different clover species. Not causing major damage to the main crop proved difficult, as even subterranean clover and the two white clover species, being the least competitive species, still caused reductions in individual plant weight of just over 50% when introduced 1 day after sowing of clover. One reason for the considerable competitive effect of clover on the leek plants was the fact that in this experiment the leek plants were completely entangled within the clover canopy. Introducing clover in strips in between rows of leek would probably diminish the competitive pressure on leek. Whether such a spatial configuration is acceptable very much depends on the intended purpose. For pest suppression and N-fixation strips of clover might be sufficient, however, it is unlikely that for weed management purposes this is acceptable as it allows the development of weeds in the crop row, where they are most difficult to control by alternative weed-control options like mechanical control. A cover crop may be incorporated for various reasons and the choice of cover crop depends on a variety of factors (Snapp et al., 2005).

In this study, between clover species, clear differences in reduction of individual leek plant weight were present. As a number of growth characteristics, particularly earliness, total dry weight, maximum plant height and N-accumulation, were determined for all of the species (Chapter 2) it was possible to identify which traits were mainly responsible for the reduction in leek yield. This approach was earlier suggested by Wiles et al. (1989) as it helps to pinpoint useful selection criteria for screening purposes. Correlation analysis revealed that maximum canopy height was the only trait that correlated significantly with leek dry matter at final harvest (Table 5). As maximum height was not correlated with any of the other characteristics (Chapter 2), it is obvious that competition for light was the main mechanism through which the clover species competed with leek. This conclusion was confirmed by the very low growth rate of leek in plots with red clover in the last growth period (Figure 1), which coincided with a substantial increase in canopy height of red clover in that period (Chapter 2). Also the observation that reductions in leek plant dry weight nearly always were accompanied by increases in SLA is a strong indication that competition for light was an important mechanism. Finally, the importance of height was again demonstrated by the survival rate of transplanted leek plants of the second and third planting. Survival of young leek plants was the highest in plots with subterranean clover, the lowest clover species. At the same time, only few leek plants survived in those clover species that exceeded the height of the leek transplants.

The above considerations suggest that subterranean clover is likely to be the most suitable cover crop, as in both years the maximum height of this species (2001: 11.6 cm; 2002: 21.4 cm) was significantly lower than that of the other species. Also de Haan et al. (1994) concludes that a relatively short canopy height of the cover crop (lower than 10 cm) is of great importance in reducing yield. It is, therefore, not surprising that in the past subterranean clover has been chosen as cover crop (Illnicki and Enache, 1992; Lotz et al., 1997; Brandsæter et al., 1998). However, additional observations clearly showed that subterranean clover is a poor weed suppressor. This was most clearly demonstrated by the weed counts in clover plots in 2001. All clover species, except for subterranean clover, were able to either completely suppress the four short weed species, or to keep their density at a low level (< 8 plants

Table 5. Correlation coefficients (r) between total shoot dry matter of leek at final harvest (DM Leek) and various estimated parameter values for eight clover species for 2001.

	$b_{\text{soil cover}}$	b_{LI}	b_{DM}	$T_{\text{LAI=3}}$	$\text{DM}_{\text{61 DAS}}$	DM_{max}	$\text{Height}_{\text{max}}$	N-total
DM Leek	–0.10	–0.59	–0.15	0.61	–0.43	–0.32	–0.92***	–0.26

RGR for soil cover development ($b_{\text{soil cover}}$), light interception (b_{LI}) and dry matter production (b_{DM}), moment at which a leaf area index of 3 was reached ($T_{\text{LAI=3}}$), the obtained dry matter at 61 days after sowing ($\text{DM}_{\text{61 DAS}}$), the maximum obtained dry matter (DM_{max}), the maximum canopy height ($\text{Height}_{\text{max}}$) and the total amount of nitrogen that was obtained (N-total).

*** $p < 0.01$. Two tailed.

plot^{-1}). In plots with subterranean clover, the density ranged from 15 plants plot^{-1} (*Lamium purpureum*) till > 60 plants plot^{-2} (*Veronica polita*). For the taller weed species, the density in plots with subterranean clover was among the highest. For this group of weed species also the two white clover species, with the second lowest maximum canopy height, provided a poor level of suppression. In 2002, both the suppression of rye grass in mixed plots as well as the suppression of naturally occurring weeds in pure stands pointed out that subterranean clover was a poor weed suppressor relative to Persian clover and white (c1) clover. The performance of the last two species was nearly identical in this year. Again height might have been a prominent factor, as the maximum height of subterranean clover was 10 and 20 cm lower than that of white clover and Persian clover, respectively. Even though the maximum canopy height of these two species was not the same, this did not affect their relative competitive ability with respect to rye grass and the weed species. This suggests that as long as the clover species are clearly higher than the weeds, differences in height between clover species did not affect the ability to suppress other plants. At the same time, white clover and Persian clover also caused a much more rapid soil cover than subterranean clover. Particularly, if the species have to develop from seed, as was the case for rye grass and the weed species, a rapidly developing soil cover is expected to be an important factor for weed suppression (e.g. Minotti, 1991). The importance of soil cover was also indirectly demonstrated by work of Ilnicki and Enache (1992) and Enache and Ilnicki (1990), who obtained good to excellent weed suppression with subterranean clover in spring when this cover crop was sown in autumn.

Apart from the poor weed-suppressing ability of subterranean clover indications were found that this species is affecting neighbouring plants through other means than just competition for light. The assessment of the dry weight of four weed species in the pure clover stands of the 2002 experiment provide a first indication that below-ground processes might be a relatively important mechanism of plant–plant interaction for subterranean clover. Shoot dry matter of *C. bursa-pastoris*, *S. media* and *P. annua* was far more severely affected by Persian clover and white clover than by subterranean clover. *C. album*, the tallest weed species, on the other hand was at least as strongly suppressed by subterranean clover. As *C. album* grew taller than subterranean clover (data not shown) the competitive effect of subterranean clover on *C. album* did not come about through a reduced availability of light. The hypothesis is that lower weed species also may be affected by a possible below-ground processes but that this negative effect is much smaller than the negative effects of light competition. Another indication was found when the reduction of leek plant dry weight in subterranean clover was more carefully examined. Dry matter of leek in subterranean clover was as severely reduced as in both white clover species, despite the lower height development of subterranean clover. Already during the field season yellowing of leek plants was exclusively observed in plots with subterranean clover. Nitrogen analysis showed that leek in subterranean clover did not only accumulate a low amount of nitrogen but also had a very low nitrogen content. Nitrogen analysis of subterranean clover indicated that this species had accumulated the lowest amount of nitrogen, and consequently the reduced uptake of nitrogen by leek could not be attributed to below-ground competition for nitrogen. Enache and Ilnicki (1990) attributed part of the suppressive effect of subterranean clover on neighbouring plants to allelopathic activity. As our findings could not be explained by competition for nitrogen *per se*, it might be that such an allelopathic activity is interfering with nitrogen uptake by neighbouring plants, thus, explaining the reduced nitrogen uptake of leek plants. Although not significant, a reduction of available nitrogen in soils with subterranean clover was observed by Brandsæter et al. (1998) in an experiment with spring sown white and subterranean clover as cover crops in white cabbage. In this experiment, subterranean clover was found to have a stronger effect on yield of cabbage than white clover. Also Abdin et al. (2000) observed a significantly larger reduction in grain weight of maize in plots with subterranean clover than in plots with a mixture of white clover and rye grass.

Introduction of a cover crop has significant effects on both the main crop and the weed infestation level. In the current experiments, maximum canopy height was identified as the main factor determining the amount of yield loss of the main crop, whereas maximum canopy height and soil cover development rate were found important factors determining the weed-suppressive ability. As canopy height plays an important role in both aspects, it is not surprising that one of the main conclusions from the current experiment is that without additional management it is very difficult to combine adequate weed suppression with an acceptable level of yield reduction in the main crop. Still, large differences were observed between the various clover species. Though far from perfect, white clover was identified as the most suitable clover species.

CHAPTER 4

Is subterranean clover (*Trifolium subterraneum* L.) a better competitor for below-ground resources than other clover species?

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Abstract
The addition of a cover or smother crop to enhance weed suppression in poorly competing main crops is considered a suitable component of ecological weed management strategies. Subterranean clover, because of its prostrate growth form, is often referred to as a suitable companion cover crop. Surprisingly, its competitive influence on the main crop is often stronger than anticipated. It is for this reason, that two pot experiments were conducted to ascertain whether subterranean clover is a relatively strong competitor for below-ground resources. In these experiments, each pot was subdivided in a central and a surrounding area, and in half of the pots both areas were separated by a PVC-tube to prevent root competition. All possible two-species combinations of subterranean, Persian and white clover and rye grass were installed, with one species in the central and one species in the outer area of the pots. Eight weeks after planting shoot dry weight and N-content of the target-species in the central area were determined and used to establish the competitive pressure of the outer species both in the absence and presence of below-ground competition. Rye grass was a better root competitor than the clover species. Subterranean clover was slightly more competitive for below-ground resources than Persian clover, whereas both species were much stronger root competitors than white clover. The results, thus, confirm that subterranean clover is indeed a relatively strong competitor for below-ground resources. It is for this reason, that subterranean clover causes more damage to the main crop than foreseen based on plant height and should be considered a less than ideal companion cover crop.

Keywords: Cover crop, smother crop, root competition, weed suppression, subterranean clover

Introduction

To date, weed management is primarily focused on curative control, as herbicides are highly effective and relatively cheap (e.g. Mortensen et al., 2000). Increasing concerns regarding the negative side effects of herbicides on the environment and the growing interest in organic agriculture have, however, led to a growing demand for alternative weed control measures (Bastiaans et al., 2008). As curative weed control without the use of herbicides is often labour intensive and relatively expensive, there is a growing need for long-term strategies that focus on the prevention of weed problems (Bàrberi, 2002). In this regard, the use of cover crops is one of the possible options.

The addition of a cover or smother crop to the main crop is particularly relevant for poorly competing crops, such as onion, carrots and leek, which grow slow and often not even reach full soil cover at the end of their growing season (Baumann et al., 2000). The cover crop acts as a living mulch that rapidly fills up the open space between the rows of the main crop. Once full soil cover is obtained fewer weeds emerge, while weeds that do emerge remain small through competition. An important concern regarding this strategy is that the added cover crop puts a too strong competitive pressure on the main crop, resulting in lower yields and a poor financial result (Brandsæter et al., 1998; Weber et al., 1999; Chase and Mbuya, 2008; Chapter 3). Various measures for dealing with the competitive pressure of a cover crop on the main crop have been explored. Weakening the relative starting position of the cover crop by transplanting the main crop or by late sowing of the cover crop gave variable results (Nicholson and Wien, 1983; Chapter 3). Mechanical suppression of the cover crop by mowing or vertical root cutting has also been examined (Båth et al., 2008). Additionally, Baumann et al. (2001b) proposed and investigated the use of a cash crop as a companion cover crop, to minimize the financial burden of undesired competition. Cover crops for weed management can also be grown in the period in between two main crops (Kruidhof et al., 2008). Finally, screening and selection of cover crop species that are able to successfully suppress weeds without putting a too high competitive burden on the main crop might offer a solution (e.g. Ross et al., 2001; Chapter 2).

Clover has regularly been put forward as a suitable cover crop (Ilnicki and Enache, 1992; Ross et al., 2001), because it combines good soil cover ability (e.g. de Haan et al., 1994), with additional advantages, such as nitrogen fixation and suppression of certain diseases and pests (e.g. Hofsvang, 1991; Theunissen et al., 1995). As many different clover species exist, field experiments were conducted to identify the most suitable companion cover crop among a group of seven clover species (Chapters 2 and 3). A key outcome was the identification of maximum canopy height as the factor that correlated best to competitive ability. Though this finding suggests that subterranean clover (*Trifolium subterraneum*), being one of the shortest clover species, might be a good option to guarantee a minimal competitive damage to the main crop, the results proved different. With subterranean clover as companion cover crop, the reduction in leek dry matter was quite similar to that of the twice as tall growing white clover species (*T. repens*). Brandsæter et al. (1998), in an experiment with white cabbage, even found a stronger competitive reduction of the crop in subterranean clover plots than in plots with white clover. One possible explanation for these findings is that subterranean clover is a relatively good competitor for below-ground resources. To verify this hypothesis, two pot experiments were conducted in which the below-ground competitive ability of subterranean clover was compared to that of white clover and Persian clover (*T. resupinatum*). In both experiments, Italian ryegrass (*Lolium multiflorum*) was included as a non-leguminous control species. Three nitrogen supply rates were installed in the second experiment, to study the effect of nitrogen availability on the importance of below-ground competition.

Materials and methods

Differences in below- and above-ground competitive ability of three clover species (subterranean clover (cv. Mt. Barker), Persian clover (*T. resupinatum* cv. Accdia) and white clover (*T. repens* cv. Aran)) and Italian rye grass (*Lolium multiflorum* cv. Bartissimo) were investigated in 2003 and 2004. In both years, a pot experiment was carried out using black plastic pots (Ø 20 cm, 25 cm deep, 7.5 litre) filled with a mixture (1:1) of light sandy soil and coarse sand. N-content of this soil was equivalent to 45 kg N ha^{-1}. The soil was inoculated with SelfStick Legume Inoculant (MicroBio Limited, Herts, UK). The surface area of each pot was divided in a central area (Ø 7 cm) containing 15 plants and an outer area containing 107 plants, where the ratio in plant number reflects the ratio in surface area of the central and the outer section. In half of the pots a PVC tube with a length of 23 cm, placed at the bottom of the pot, was used to separate the central area from the outer area.

The experiment of 2003 was a three factorial fully randomized complete block design in six replications, with a total of 240 pots. A first factor was the presence or absence of the 23-cm long PVC tube (Figure 1). In pots without this root separating device, a PVC ring of 1 cm (Ø 7 cm) was placed at soil surface to mark the boundary between central and outer area. A second factor was the plant species in the central area of the pot (four treatments, consisting of the four plant species) and a third factor was the plant species in the outer area (five treatments, consisting of the four plant species and the absence of plants).

In 2004, the design was a four factorial fully randomized complete block design in six replications. This experiment, with a total of 720 pots, was largely identical to that

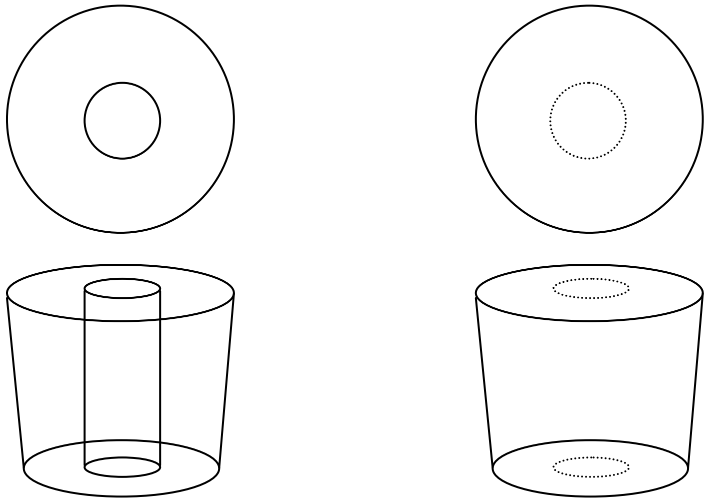


Figure 1. Graphic representation of side and upper view of two pots (Ø = 20 cm) subdivided into an outer area and a central area (Ø = 7 cm). In the pot on the left, both areas are separated by a PVC-tube to prevent root competition.

of the 2003 experiment, except for the addition of nitrogen fertilization rate as a fourth factor (three rates). Whereas in 2003 no fertilization was applied, in 2004 all pots received a P + K + Mg + Fe fertilizer (261 mg P, 622 mg K, 300 mg Mg and 25 mg Fe per pot) at the start of the experiment. In addition, one-third of the pots was given no additional nitrogen (N), one-third of the pots was provided with 170 mg N pot^{-1} (equivalent of 50 kg N ha^{-1}) and the remaining one-third was given 520 mg N pot^{-1} (150 kg N ha^{-1}).

In 2003, the experimental set-up was situated in the open air. Sowing took place on 21 and 22 July. About 22 and 150 seeds were sown in the central and outer area, respectively, and thinning was done on 4 and 5 August. The 2004 experiment was conducted in a greenhouse where seeds were sown on 20 April. Temperatures in the greenhouse ranged between 15 °C and 25 °C. Nitrogen fertilization was conducted on 29 and 30 April (first split) and on 28 and 29 May (second split) and thinning was carried out on 3 and 4 May. The number of seeds that was sown and the number of plants after thinning was the same as in 2003. In both years, pots were watered daily to exclude competition for water. Shoot biomass was determined at around eight weeks after sowing on 2 September, 2003 and on 15 and 16 June, 2004.

Plants in the central area of each pot were cut at surface level and dried (70 °C for 24 h) to determine shoot biomass. Nitrogen analysis of total shoot biomass (substance flow analysis (SFA); Temminghoff et al., 2000b) was done for all samples. Accumulated shoot dry matter and nitrogen content of plants in the central area were analysed by performing an analysis of variance (ANOVA). The analysis was conducted for each species separately and pair-wise comparisons were made between pots with and without PVC-tube, using the Least Significant Difference (LSD).

The ratio between the competitive effect of plants in the outer area on plants in the central area in the absence and presence of a PVC-tube was estimated based on the relationship between dry matter and plant number outlined by Spitters (1983). In this approach the shoot dry weight of plants in the central area (Y_c) is represented by:

$$Y_c = \frac{N_c}{a_{c0} + b_{co-}N_{o-} + b_{co+}N_{o+}} \quad (1)$$

in which, N_c stands for the number of plants in the central area of the pot and N_{o-} and N_{o+} stand for the number of plants in the outer area of the pot in the absence (−) and presence (+) of a PVC-tube, respectively. Parameter a_{c0} (plant g^{-1}) reflects the reciprocal of the dry matter of a plant in the central area of the pot grown in a density of 15 plants per pot, in the absence of any further competition. Parameters b_{co-} and b_{co+} (pot g^{-1}) express the competitive effect on plants in the central area exerted by plants in the outer area in the absence (−) and presence (+) of a PVC-tube, respectively. It is the ratio between these last two parameters (b_{co-}/b_{co+}) that quantitatively expresses the difference in competitive ability in the presence and absence of below-ground competition. Data on shoot dry weight of each species within one year were simultaneously fitted, using non-linear regression. In this way estimates of b_{co-} and b_{co+} for the four species in the outer area were obtained for each of the species in the central area, and accordingly the ratios (b_{co-}/b_{co+}) were calculated. All statistical analyses were carried out using the GenStat statistical package (Payne et al., 2009).

Results

The presence of a root-separating PVC-tube

In a first analysis, the effect of the PVC-tube was examined. Only treatments without plants in the outer area and treatments that contained the same plant species in the outer and central area were included in this analysis. For shoot dry matter a significant three-way interaction between plant species in the central area, the presence of plants in the outer area and the presence of a PVC-tube was observed, both in 2003 and 2004 ($p < 0.001$; Table 1). In the absence of plants in the outer area, significant differences in shoot dry matter of the three clover species were observed, with Persian clover always producing significantly more shoot dry matter than white clover and subterranean clover holding an intermediate position. In 2003, shoot dry matter of rye grass was comparable to that of white clover, whereas in 2004 it was considerably higher and matched that of Persian clover. The presence of plants in the outer area

Table 1. Effect of plant species, the presence of a root separating PVC-tube and the presence of plants in the outer area on shoot dry matter of plants in the central area (g pot^{-1}). The plant species in the outer area of the pots was the same as the plant species in the central area. Results of experiments conducted in 2003 and 2004. For 2004, average values across three N-fertilization rates are presented. Means with the same letter within one year are not significantly different at $p = 0.05$. SED = Standard Error of Difference.

	Shoot dry matter				Shoot N-content			
2003	No plants in outer area		Plants in outer area		No plants in outer area		Plants in outer area	
	Tube	No Tube	Tube	No Tube	Tube	No Tube	Tube	No Tube
Species								
Subterranean clover	4.17 d	11.28 b	3.76 d	3.38 d	2.84 cde	2.84 cde	2.80 de	2.80 de
Persian clover	6.48 c	17.42 a	3.45 d	3.11 d	2.64 ef	2.98 bcd	2.76 def	2.83 cde
White clover	3.73 d	7.41 c	2.64 d	3.00 d	2.51 f	3.67 a	3.17 b	3.08 bc
Rye grass	3.21 d	7.26 c	0.75 e	0.84 e	1.07 g	1.05g	0.85 g	0.84 g
SED	0.772				0.140			
2004								
Species								
Subterranean clover	7.70 c	14.71 a	3.11 e	2.96 e	2.58 cde	2.70 bc	2.58 cde	2.62 cd
Persian clover	10.10 b	16.10 a	2.78 e	2.50 e	2.35 f	2.87 a	2.82 ab	2.82 ab
White clover	5.50 d	7.57 c	2.40 e	2.58 e	2.44 ef	2.81 ab	2.43 ef	2.46 def
Rye grass	10.98 b	14.78 a	2.25 e	2.29 e	1.61 g	1.43 g	1.07 h	1.07 h
SED	0.670				0.088			

nearly always reduced the shoot dry matter production of plants in the central area significantly, whereas the presence of a PVC-tube only caused significant reductions in shoot dry matter if no plants were present in the outer area (Table 1). Both in 2003 and 2004, this reduction was strongest with Persian and subterranean clover. With plants of the same species present in the outer area, no significant effect of the presence of the PVC-tube was observed in any of the two years. Additionally, a second significant three way interaction, that between plant species in the central area, the presence of plants in the outer area and N-fertilization rate, was observed in 2004 ($p < 0.001$; Table 2). This interaction revealed that, both in the absence and presence of plants in the outer area, ryegrass was much more responsive to N-fertilization than any of the clover species.

Also for shoot N-content, a significant three way-interaction between plant species in the central area, the presence of plants in the outer area and the presence of a PVC-tube was observed in both years ($p < 0.001$; Table 1). The shoot N-content of rye grass was consistently significantly lower than that of the clover species. In addition, a significant interaction between plant species in the central area and N-fertilization rate was observed in 2004 ($p < 0.001$; Table 3). This interaction showed that only the shoot N-content of rye grass was affected by N-fertilization rate. The presence of plants in the outer area hardly affected shoot N-content of plants in the central area, except for a consistent reduction in shoot N-content of rye grass in 2004. Just as for shoot dry weight, differences in shoot N-content resulting from the presence of a root-separating PVC-tube were only observed in the absence of plants in the outer area. In this condition, both in 2003 and 2004, the presence of the PVC-tube decreased the shoot N-content of Persian and white clover, whereas that of subterranean clover and rye grass was not affected. With plants of the same species present in the outer area, no significant effect of the presence of the PVC-tube on shoot N-content was observed.

Subterranean clover

To determine the relative ability of the different species to compete for below-ground resources, the shoot dry matter and N-content of treatments containing the same plant species in the central area were analysed and compared. For shoot dry matter of subterranean clover in 2003, both the effect of plant species ($p = 0.101$) and the presence of a root-separating device ($p = 0.212$) were not significant (Figure 2). Only for shoot N-content a significant effect of plant species was observed ($p = 0.04$; data not shown). With rye grass in the outer area the N-content of subterranean clover (2.66%) was significantly lower than in the presence of white clover (2.90%), whereas in the presence of subterranean and Persian clover the N-content was intermediate and not significantly different from any of the other species.

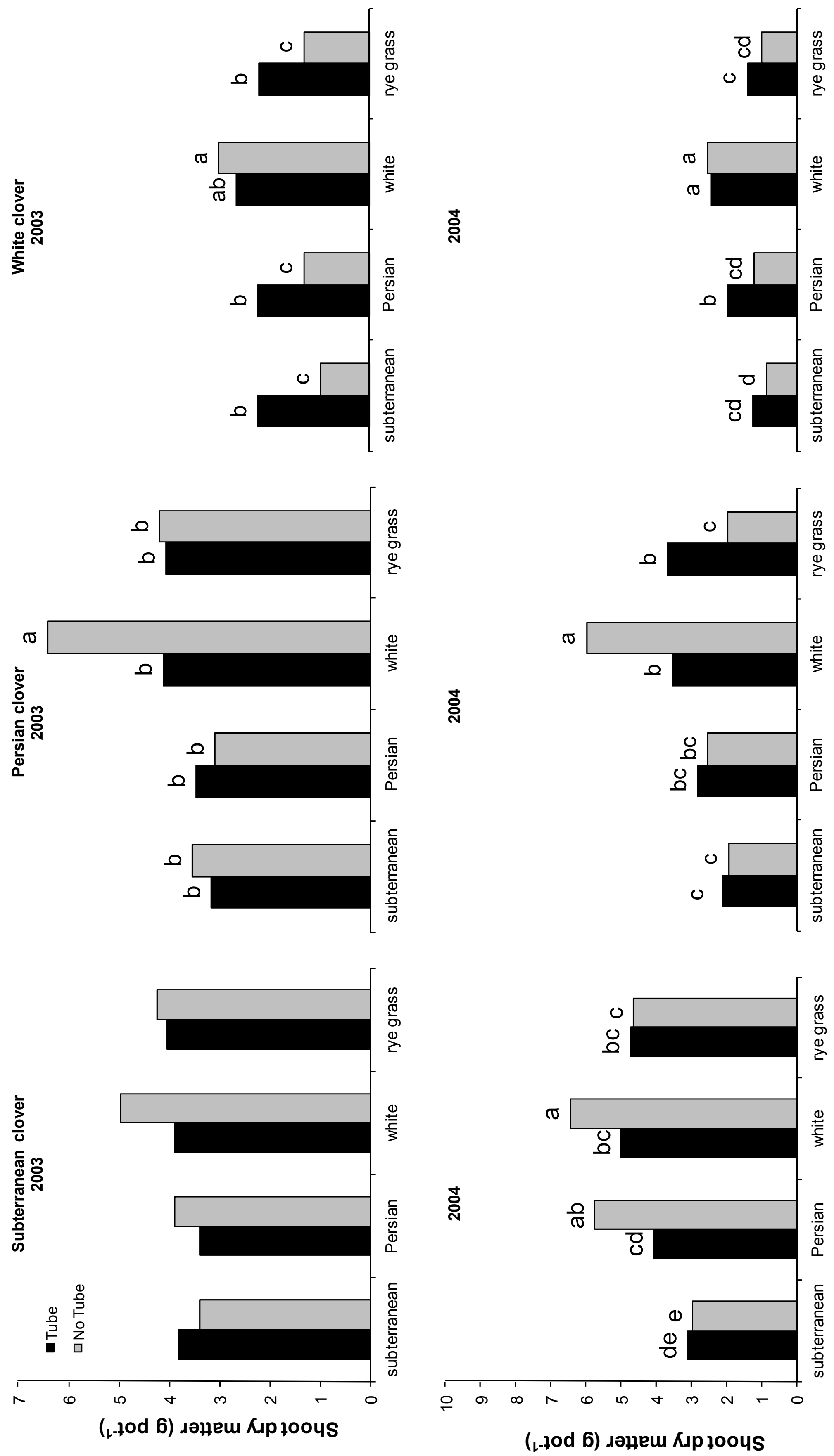
Table 2. Effect of plant species, N-fertilization and the presence of plants in the outer area on shoot dry matter in the central area (g pot^{-1}) in 2004. The plant species in the outer area of the pots was the same as the plant species in the central area. Average values across pots with and without a root separating PVC-tube. N1 = 0 mg N pot^{-1}, N2 = 170 mg N pot^{-1}, N3 = 520 mg N pot^{-1}. Means with the same letter are not significantly different at $p = 0.05$.
SED = Standard Error of Difference.

2004	No plants in outer area			Plants in outer area		
N-rate	N1	N2	N3	N1	N2	N3
Species						
Subterranean clover	9.74 e	12.42 cd	11.46 d	2.78 gh	3.34 g	2.97 gh
Persian clover	14.32 b	13.84 bc	11.14 de	2.92 gh	2.03 gh	2.97 gh
White clover	5.80 f	6.52 f	7.29 f	2.37 gh	2.27 gh	2.82 gh
Rye grass	9.57 e	12.00 d	17.07 a	1.36 h	2.22 gh	3.22 g
SED			0.833			

Table 3. The effect of nitrogen fertilization on shoot nitrogen content (%) of plants in the central area of the pot in 2004. Average values across pots with and without a root separating PVC-tube and across pots with and without plants in the outer area are presented. N1 = 0 mg N pot^{-1}, N2 = 170 mg N pot^{-1}, N3 = 520 mg N pot^{-1}. Means with the same letter are not significantly different at $p = 0.05$.
SED = Standard Error of Difference.

N-rate	N1	N2	N3
Species			
Subterranean clover	2.58 bcd	2.61 bd	2.68 ab
Persian clover	2.68 abc	2.68 ab	2.79 a
White clover	2.49 d	2.53 cd	2.58 bcd
Rye grass	1.07 f	1.21 f	1.61 e
SED		0.0759	

In 2004, a significant interaction between outer plant species and the presence of the tube was found for shoot dry matter ($p = 0.017$; Figure 2). In the absence of a tube, both white clover and Persian clover posed a less severe competitive stress on subterranean clover, whereas the competitive stress of subterranean clover and rye grass was not significantly affected. Shoot N-content of subterranean clover was not significantly affected by any of the treatments and was on average 2.66%.


Subterranean clover
2003
Persian clover
2003
White clover
2003
2004
2004
2004
Tube
No Tube
Shoot dry matter (g pot-1)
Shoot dry matter (g pot-1)
subterranean
Persian
white
rye grass
de e
cd ab
bc a
bc c
c c
bc bc
b a
b c
b b
b b
b a
b b
b c
b c
ab a
b c
cd d
b cd
a a
c cd

Persian clover

For shoot dry matter of Persian clover in 2003, a significant interaction between outer plant species and the presence of a tube was observed ($p = 0.032$; Figure 2). Only with white clover in the outer area, a significant tube effect was found, with the shoot dry matter of Persian clover significantly higher in the absence of a tube. For shoot N-content a small but significant ($p = 0.016$) tube effect was observed. In the absence of a tube the average shoot N-content of Persian clover was slightly higher than in the pots with a tube in place (2.83% vs. 2.70%; data not shown).

Also in 2004, for shoot dry matter, a significant interaction between outer plant species and the presence of a tube was found ($p < 0.001$). A significant tube effect was observed with white clover and rye grass in the outer area. In the presence of white clover, shoot dry matter of Persian clover increased in the absence of the tube, whereas in the presence of rye grass the absence of the tube caused a reduction in shoot dry matter. No significant effect of N-application rate on shoot dry matter was observed ($p = 0.102$). The average shoot N-content of Persian clover was 2.71% and no significant effects of plant species in the outer area ($p = 0.063$), the presence of a tube ($p = 0.673$) and N-application rate ($p = 0.136$) were found (data not shown).

White clover

Both in 2003 and 2004, significant interactions between outer plant species and the presence of a tube were found for white clover's shoot dry matter ($p = 0.003$ and $p = 0.045$, respectively; Figure 2). In 2003, subterranean clover, Persian clover and rye grass caused a stronger reduction in shoot dry matter of white clover in the absence of a tube compared to the situation with a tube. Such a tube effect was not observed with white clover in the outer area. Also for shoot N-content a significant interaction was observed ($p < 0.001$). In the presence of the root-separating tube and with rye grass in the outer area the shoot N-content of white clover was significantly lower (2.14%) than with any of the other treatment combinations (average N-content: 2.98%; data not shown).

Figure 2. Shoot dry matter (g pot^{-1}) of plants in the central area of pots with and without a root separating PVC tube for the 2003 and 2004 experiment. For 2004, average values across three N-fertilization rates are presented. Plant species in the central area are listed at the top, whereas the plant species in the outer area is mentioned on the *x*-axis. Mean values within the same year and for the same species in the central area not sharing the same letter are significantly different at $p < 0.05$.

Table 4. The effect of nitrogen fertilization and species in the outer area on shoot dry matter of white clover and rye grass in the central area (g pot^{-1}) in 2004. Average values across pots with and without a root separating PVC-tube are presented. Means with the same letter are not significantly different at $p = 0.05$. N1 = 0 mg N pot^{-1}, N2 = 170 mg N pot^{-1}, N3 = 520 mg N pot^{-1}. Means with the same letter are not significantly different at $p = 0.05$. SED = Standard Error of Difference.

N-rate	N1	N2	N3
White clover			
Species in outer area			
Subterranean clover	1.05 ef	1.15 def	0.878 f
Persion clover	1.73 c	1.56 cd	1.42 cde
White clover	2.32 b	2.22 b	2.82 a
Rye grass	1.59 cd	1.10 ef	0.817 f
SED		0.2686	
Rye grass			
Species in outer area			
Subterranean clover	3.12 fgh	3.92 ef	5.91 c
Persion clover	2.75 gh	4.94 d	7.40 b
White clover	4.15 de	6.11 c	11.50 a
Rye grass	1.36 i	2.22 hi	3.22 efg
SED		0.4674	

In 2004, with Persian clover in the outer area, the absence of a tube caused a significantly lower shoot dry weight of white clover. In the presence of subterranean clover and rye grass only a marginally significant reduction was observed. Additionally, a significant interaction between plant species in the outer area and N-application rate ($p = 0.031$) was observed. This interaction revealed that with white clover in the outer area shoot dry matter at the highest N-rate was significantly higher than at the two lowest application rates (Table 4). With rye grass in the outer area the opposite was found: shoot dry matter of white clover at the lowest N-rate was significantly higher than at the two highest N-rates. With Persian clover and subterranean clover in the outer area the N-application rate did not have a significant effect on white clover's shoot dry matter. For shoot N-content only a significant effect of plant species in the outer area was observed ($p < 0.001$). In the presence of subterranean clover the shoot N-content of white clover (2.10%) was significantly lower than with any of the other species in the outer area (2.44 – 2.48%).

Rye grass

Also for ryegrass in central area, both in 2003 and 2004, significant interactions between outer plant species and the presence of a tube were observed for shoot dry matter ($p < 0.001$; Figure 2). Significant tube effects were found with the clover species in the outer area. In all instances, shoot dry matter of rye grass increased in the absence of the tube, but with white clover in the outer area this difference was considerably larger. For shoot N-content of rye grass only a significant species effect was observed ($p < 0.001$). With subterranean clover in the outer area, a shoot N-content of 1.32% (a) was found, which was significantly higher than in combination with white clover (1.11%; (b)) and rye grass (0.84% (c)), but not different from the N-content found in combination with Persian clover (1.21% (ab)).

In 2004, with all species in the outer area, except rye grass, a significant increase in shoot dry matter of rye grass in the central area was observed in the absence of the root-separating tube. In addition, a significant interaction between species in the outer area and N-application rate ($p < 0.001$) was found (Table 4). This interaction revealed that the influence of plant species in the outer area on shoot dry matter of rye grass was quite consistent over N-application rate. In the presence of white clover rye grass shoot dry matter was highest, followed by Persian clover, subterranean clover and rye grass. With an increase in N-application rate, the shoot dry matter of rye grass generally increased, irrespective of plant species in the outer area. Only with subterranean clover and rye grass in the outer area no significant increase was observed between the first and the second N-application rate. For shoot N-content, a significant interaction between the presence of a tube and N-application rate was observed ($p = 0.008$). At the highest N-application rate shoot N-content was significantly higher than at the other two rates (Table 5). It was also at this N-application rate that the presence of a PVC-tube had a significant effect on shoot N-content, with a higher shoot N-content in the absence of a tube. In addition, a significant effect of plant species in the outer area was observed ($p < 0.001$). Shoot N-content in the presence of subterranean clover (1.72%) was higher than with Persian clover (1.57%) and white clover (1.55%), whereas the lowest N-content was observed in the presence of rye grass (1.07%).

Ratio of competition indices

The ratios between the competition indices in the absence and presence of a root-separating tube (b_{co-}/b_{co+}) are presented in Table 6. A ratio larger than one indicates that in the absence of a tube the plant species in the outer area was able to pose a stronger competitive influence on the plant species in the central area. With a ratio smaller than 1 the species in the central area was the stronger root competitor.

Table 5. The effect of nitrogen fertilization and the presence of a root separating device on shoot nitrogen content (%) of rye grass in the central area in 2004. Average values across pots with different species in the outer area are presented. N1 = 0 mg N pot^{-1}, N2 = 170 mg N pot^{-1}, N3 = 520 mg N pot^{-1}. Means with the same letter are not significantly different at p = 0.05.
SED = Standard Error of Difference.

N-rate	N1		N2		N3	
	Tube	No Tube	Tube	No Tube	Tube	No Tube
	1.34 c	1.27 c	1.39 c	1.38 c	1.64 b	1.90 a
SED			0.0745			

Table 6. Ratio between competition indices determined in the absence (−) and presence (+) of a root-separating device (b_{co-}/b_{co+}), where each competition index reflect the ability of plants in the outer area to compete on plants in the central area. The background colour reflects whether the shoot dry weight of plants in the central area used for calculating the competition indices were significantly ($p < 0.05$; black grey), marginally significantly ($0.05 < p < 0.10$; grey) or not significantly ($0.10 < p$; white) affected by the presence of the root-separating PVC-tube.

2003	Subterranean clover	Persian clover	White clover	Rye grass
Central area				
Subterranean clover	1.26	0.819	0.667	0.915
Persian clover	0.868	1.14	0.528	0.960
White clover	2.72	2.01	0.817	1.98
Rye grass	0.363	0.379	0.169	0.889
2004	Subterranean clover	Persian	White clover	Rye grass
Central area				
Subterranean clover	1.06	0.596	0.644	1.04
Persian clover	1.10	1.13	0.476	2.21
White clover	1.54	1.82	0.925	1.57
Rye grass	0.383	0.414	0.313	0.983

Basically, these ratios reflect whether the shoot dry weight in the central area differed markedly between situations with and without a tube (Figure 2). If no significant difference in shoot dry weight was observed, the ratio was close to 1 ($0.817 < (b_{co-}/b_{co+}) <$

1.26). In situations where shoot dry weight was significantly affected by the presence of the PVC-tube, the value of this ratio was either larger than 1.82 or smaller than 0.644. In three situations, a marginally significant ($0.05 < p < 0.10$) difference in shoot dry weight was observed and in these situations the ratio was either in between 0.644 and 0.817, or between 1.26 and 1.82. The advantage of using the ratio of competition indices to express the ability to compete for below-ground resources is that absolute differences in shoot dry weight can be properly interpreted. Compared to 2003, the absence of a tube in 2004 caused only small additional reductions in white clover shoot dry weight in the treatments that contained subterranean clover and rye grass in the outer area. The relatively small size of these reductions is related to the fact that both species already reduced white clover quite severely in the presence of the tube. Despite the relatively small absolute reductions, the stronger ability of the species in the outer area to compete for below-ground resources is clearly reflected in the ratio of competition indices (1.54 and 1.57).

Discussion

The methodology for determining the relevance of below-ground competition was based on the 'target technique' described by McPhee and Aarssen (2001). Compared to the divided pot technique, in which pots are subdivided into two halves along a straight line, the target technique does not enable the targeted species to escape from competition. In addition, the set-up warrants that the interface between the two competing species is relatively large, resulting in a situation that allows for a clear expression of the influence of interspecific competition on the dry weight of the targeted species in the centre. In contrast to the single target plant in the set-up described by McPhee and Aarssen (2001), we installed 15 plants in the inner area of a pot. The advantage is that the measured trait is not just reliant on one individual and is likely to be more representative. At the same time, intraspecific competition among plants is introduced, which weakens the expression of interspecific competition. This disadvantage can be overcome through the calculation of competition indices (Equation 1), as even small absolute differences between treatments with and without a tube, were clearly reflected in the ratio between competition indices.

When roots of plants in the central area were allowed to exploit the outer area this resulted in significant increases in shoot dry weight for all species. More relevant for our study was the observation that with plants of the same species present in the outer area no significant differences in shoot dry weight and shoot N-content of plants in the central area were observed for any of the species. This implies that the tube, apart from a physical restriction of the area available for exploitation, did not impose any detrimental effect on plant growth as such.

McPhee and Aarssen (2001) stressed the importance of including reciprocal treatments in experiments focusing on separating competition for above- and below-ground resources. This means that in the experimental set-up the targeted species should serve as surrounding species as well. The results of the current experiment confirm this recommendation. Comparing the results of reciprocal treatments showed that in more than half of the situations the ability of a species to compete for below-ground resources was positively influenced when a species was grown in the central area and served as a target species. Only in one occasion (subterranean clover – white clover; 2003), it was found that root competition was more profound when the species was situated in the outer area. The observation that in most situations below-ground competitive ability of the species in the central area was favoured might be related to the circular shape of the central area. When plants from the central area expand their roots in the surrounding area with a certain distance, the extra area that is gained for resource acquisition is considerably larger than the area gained by plants from the surrounding area that expand their roots with a similar distance into the central area.

Several observations indicate that in 2004 the intensity of competition was higher than in 2003. For instance, in 2004, in the presence of a PVC-tube, the addition of plants to the outer area caused much stronger reductions in shoot dry weight of plants in the central area than in 2003 (Table 1). Additionally, in 2003, it was found that, again in situations where roots were separated by a PVC-tube, the effect of plants in the outer area on shoot dry matter of the clover species in the central area was species independent (Figure 2). Only with rye grass in the central area differences were observed, with subterranean clover and rye grass competing more strongly than Persian and white clover. In 2004, in contrast, clear differences in the competitive ability of plant species in the outer area were observed. In the presence of a tube, subterranean clover, the species with the largest seed size, was always among the most competitive species, whereas the small-seeded white clover was always among the least competitive species. These more prominent species effects also hint at a higher intensity of competition in 2004.

In 2003, the results on shoot dry matter showed that white clover was a poor root competitor while rye grass was identified as a very strong root competitor. Persian clover and subterranean clover held and intermediate position. The more intense competition in 2004 caused this year’s results on shoot dry matter to be more distinctive. Again, white clover and rye grass were identified as the poorest and strongest competitor for below-ground resources, respectively. In addition, subterranean clover was identified as a better root competitor than Persian clover. A direct indication was found with subterranean clover in the central area and Persian clover as competitor in the surrounding area. In the absence of the root-separating tube, subterranean clover produced significantly more biomass than in the presence of the tube, which was reflected in a ratio (b_{co-}/b_{co+}) of 0.596. An indirect indication was found with ryegrass in the outer area, competing with either of the two clover species in the central area. For subterranean clover the competitive influence of rye grass was not affected by the presence of the tube ($b_{co-}/b_{co+} = 1.04$), whereas for Persian clover ryegrass became more than twice as competitive when the tube was absent ($b_{co-}/b_{co+} = 2.21$). In most situations, shoot N-content in the absence of a tube was nearly identical to shoot N-content in the presence of a tube. Consequently, the data on shoot N-content did not provide any additional insights in the ability of the various plant species to compete for below-ground resources.

In the absence of competition, rye grass, being a non-N fixing species, responded positively to an increased N-availability as both shoot dry matter and shoot N-content were significantly increased. These observations are in line with results obtained by Munoz and Weaver (1999). For the clover species the response to an increased N-availability was less pronounced. Also in competition, the strongest response to N was found with rye grass. At the highest N-application rate, regardless of the plant species in the outer area, both shoot dry matter as well as shoot N-content were significantly higher than at the lowest N-application rate. For shoot dry matter this response was not influenced by whether or not below-ground competition was permitted. This observation suggests that the expression of the better ability of rye grass to compete for below-ground resources was independent of N-availability. The higher N-content of rye grass in the absence of a tube, obtained at the highest N-rate, is the only observation that suggests that the superior ability of rye grass to compete for below-ground resources is even more pronounced at high rates of N-availability.

Introduction of a companion cover crop for the suppression of weeds should ideally inhibit the emergence and growth of weeds while leaving the main crop unharmed. Subterranean clover's prostrate growth form (Miller et al., 1989) suggests that this clover species is a poor competitor for light (Evers, 1988), and therefore ideally suited for this purpose. Unfortunately, various papers report that the detrimental effect of subterranean clover is often more pronounced than expected based on its growth form (e.g. Enache and Ilnicki, 1990; Abdin et al., 2000; Chapter 3). The current experiment provides evidence that these findings are related to a relatively strong ability of subterranean clover to compete for below-ground resources. This outcome indicates that the identification of suitable companion cover crops deserves thorough investigation.

CHAPTER 5

Comparing the smothering ability of three clover species

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Abstract

Adding clover species as a minor component in mixture with a main crop for weed-control purposes is only functional if the clover is able to suppress the recruitment and growth of weeds without acting itself as a weed that competes against the main crop. Whereas in earlier research the competitive pressure of clover species on the main crop was studied in detail, the smothering ability of clover species received less attention. For that reason, this research focused on how the smothering ability of *Trifolium resupinatum* L. (Persian clover), *Trifolium subterraneum* L. (subterranean clover) and *Trifolium repens* L. (white clover) advanced over time. Seeds and two-week-old seedlings of *Chenopodium quinoa* Willd. (quinoa) and *Lolium multiflorum* Lam. (Italian rye grass), representing broad-leafed and grassy weed species, respectively, were introduced in 3.5, 5.5 and 8.0 week old clover canopies raised in containers filled with sand. At 20 days after introduction, plant number and dry weight of the test plant species was determined. The smothering ability of Persian clover, and to a lesser extent white clover, was superior and developed faster than that of subterranean clover. The data showed that the smothering ability of all three clover species was closely related to the accumulated amount of shoot biomass, suggesting that Persian clover's better weed-suppressing ability is related to its high initial growth rate. Earlier research revealed that Persian clover is too aggressive towards the main crop to be used as a smother crop. Combined with the poor weed-suppressive ability of subterranean clover this led us to the conclude that, for successful application as smother crop, white clover offers most prospect.

Keywords: Smothering ability, cover crop, clover, competition, shoot biomass, weed suppression, soil cover, height

Introduction

Weeds are a major concern in agriculture, particularly in organic production systems. In slow growing crops, which achieve full canopy closure relatively late, weeds have ample opportunity to germinate and establish (Baumann et al., 2000). Leaving the weeds uncontrolled results in a severe competitive pressure on the crop, which will ultimately be reflected in crop yield loss. The addition of a companion cover crop has regularly been proposed as a means to reduce the weed-infestation level of crops with a slow initial growth rate (e.g. Aldrich, 1984; de Haan et al., 1994; Abdin et al., 2000). The cover crop, also referred to as living mulch or smother crop, helps to realize a faster canopy closure, thereby shortening the period in which weed seedlings are able to establish (Vereijken and Kropff, 1996; Teasdale and Mohler, 2000). Clover species are often recommended as suitable cover crops, as, next to a weed-suppressing function, these species add nitrogen to the system. Specifically in organic systems this is considered an important additional benefit.

One of the complications with the use of living mulches is their competitive effect on the main crop. Among the management options to regulate this competitive pressure are the introduction time of the cover crop relative to that of the main crop (e.g. Müller-Schärer and Potter, 1991; Lotz et al., 1997; Snapp et al., 2005) and the choice of the cover crop species (e.g. Akanvou et al., 2002). For clover species a considerable variation in morphological and physiological traits was observed (Ross et al., 2001; Chapter 2). The study of Chapter 3 revealed that maximum plant height was the clover trait that correlated most strongly to the reduction in dry matter production of the main crop. Of the eight investigated clover species, subterranean clover (*Trifolium subterraneum* L.) and white clover (*Trifolium repens* L.) hindered leek plants the least, whereas Persian clover (*Trifolium resupinatum* L.) was among the species that caused the strongest reduction in leek plant dry weight.

Introduction time of the cover crop is not likely to have much of an influence on weed-suppressive ability. The seed bed preparation prior to seeding of the cover crop not only clears the field, but also initiates a new weed flush, thereby synchronizing the starting point for growth of weeds and cover crop. Choice of cover crop species is likely to have a stronger influence. It is questionable, however, whether maximum plant height, the trait that correlated most significantly to the yield reduction of the main crop, is also the best determinant of the weed-smothering effect. A pilot study in which malting barley was used as a cover crop in Brussels sprouts clearly showed that competitive pressure on the main crop and weed-suppressive ability are not necessarily related. Though the tall growing barley had a strong negative effect on crop yield, it was not able to provide adequate weed suppression (Bastiaans et al., 2007). The question remains whether the opposite, a species less troublesome for the main crop, but with a superior capacity to smother newly emerging weeds in an effective manner, does exist. The main purpose of the current research was, therefore, to investigate differences in the weed-smothering ability of three contrasting clover species.

Persian clover, subterranean clover and white clover were the clover species selected for this experiment, as the species were found to be morphologically and physiologically distinct (Chapter 2) and differed in the competitive pressure they imposed on transplanted leek plants (Chapter 3). Establishment and early growth of a monocotyledonous and a dicotyledonous test plant species were investigated in three to eight week old clover canopies.

Materials and methods

The experiment was conducted in containers (width × length × height = 0.4 × 0.6 × 0.4 m) and carried out in the summer (June to August) of 2003 in Wageningen, the Netherlands. The experimental set-up was a fully randomized block design in four replicates. Treatments consisted of all nine combinations of three clover species (Persian clover, cv. Accdia; subterranean clover, cv. Mt. Barker; large-leafed white clover, cv. Aran) and three clover sowing dates (T1: June 6; T2: June 23; T3: July 7). In each replicate, each treatment was represented by three containers. One of these containers was used as a reference for observations on the growth and development of the clover species. In the other two containers either *Lolium multiflorum* Lam. (Italian rye grass cv. Bartissimo) or *Chenopodium quinoa* Willd. (quinoa cv Carina Red) was introduced to evaluate the effect of the clover species on establishment and early growth of a monocotyledonous and dicotyledonous species. The last two containers were subdivided into two sections in which ten individuals of the specific test plant species were introduced, either as pre-germinated seed or as a two-week-old seedling. Each section within a container was randomly assigned to one of the two stages of the test plant species. Two additional containers without clover were used to complement each block. In these containers, reference plots of either quinoa or Italian rye grass were installed. Also these containers were subdivided into two sections to which the seeds and the seedlings of the test plant species were randomly assigned.

On May 23, the containers were filled with a light sandy soil and the next day a clover inoculant (SelfStick Legume Inoculant; MicroBio Ltd, Herts, UK) was mixed through the top soil layer. For each clover species a plant density was established that corresponded to a seeding rate of 15 kg ha^{-1} (850, 150 and 1100 plants m^{-2} for Persian, subterranean and white clover, respectively). Within a container, plants were sown in rows. The number of rows as well as the number of plants per row was species dependent (12 rows of 17 plants per row for Persian clover, 6 rows of 6 plants for subterranean clover and 12 rows of 22 plants for white clover). To ensure a sufficient number of established clover plants a seeding rate of around 20 kg ha^{-1} was used and the surplus plants were removed one week after germination.

In each container, ten seeds and ten seedlings of either Italian rye grass or quinoa were introduced on July 31 (block 1 and 2) and August 1 (block 3 and 4). Seeds were pre-germinated for either two (quinoa) or three (rye grass) days in Petri-dishes that contained two wetted filter papers. Seedlings were raised in small plastic trays filled with potting soil. Two weeks after sowing, the seedlings were transplanted to the containers. Seeds and seedlings were each placed in two rows of five seeds (or seedlings). These rows were positioned exactly in between two adjacent clover rows, with an interplant distance in the row of 4 cm and an interrow distance of 10 cm.

On August 11, 10 days after the introduction of the test plant species, soil coverage and height of the clovers in all nine treatments (clover species × sowing time) were measured. Soil cover was determined through a grid wise (10 × 10 cm) visual estimation of soil cover in the central area (40 × 30 cm) of a container. Canopy height was measured as the distance between the soil and the top of the canopy. These observations were conducted in the clover reference containers. In the reference containers of the test plant species, emergence of quinoa and Italian rye grass was completed on August 5. On August 20 and 21, 20 days after introduction (DAI), the established quinoa and rye grass plants were counted and harvested. At the same day, the clover plants of the reference containers were harvested. All plant samples were dried for 24 h at 70 °C and weighed subsequently.

ANOVA was performed on clover characteristics (soil cover, plant height and shoot dry matter). For Italian rye grass and quinoa, ANOVA was conducted on survival and shoot dry weight after data were angular and log transformed, respectively. Means were separated by calculating the least significant difference (LSD) at the 5% probability level. Prevailing weather conditions (temperature and radiation) throughout the experimental period were obtained from a weather station located within 1 km of the experimental site.

Results

Weather conditions

Between the start of the experiment on June 6 and the final observations on August 21 the averages of the daily minimum and maximum temperature were 12.3 and 25.0 °C, respectively. Average daily incoming radiation was 19.8 MJ m^{-2} d^{-1}. Considerable day-to-day variation was observed for all variables (Figure 1) with ranges for minimum temperature between 6.5 and 18.9 °C, for maximum temperature between 17.5 and 34.5 °C and for incoming radiation between 6.4 and 28.9 MJ m^{-2} d^{-1}.

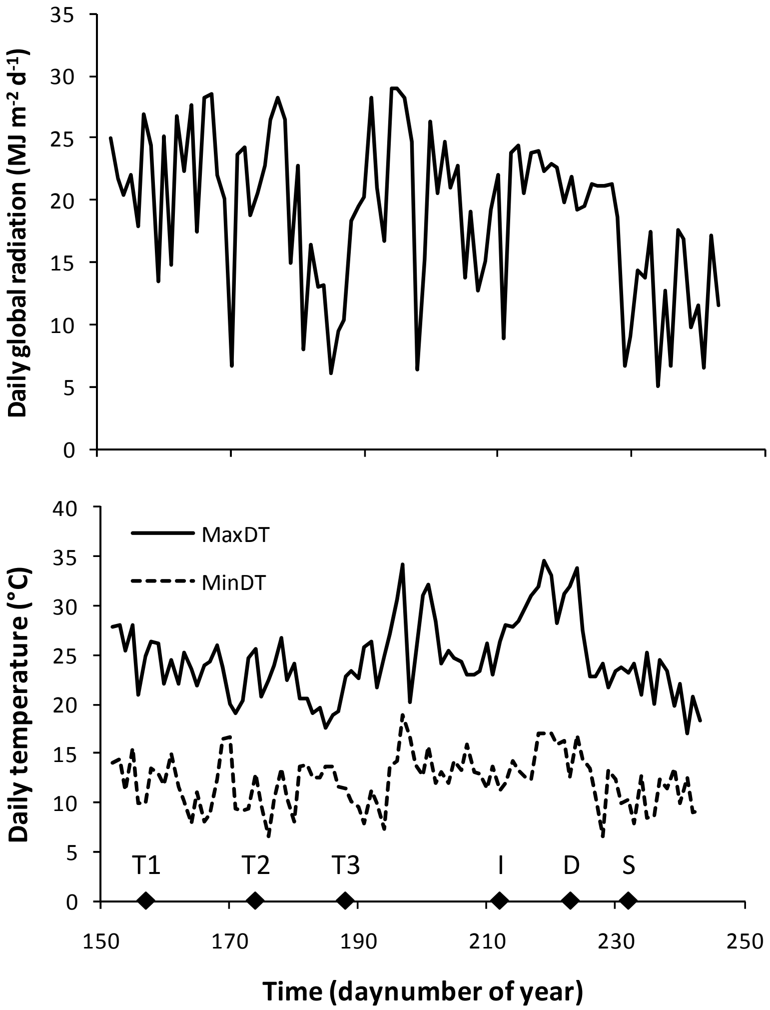

Figure 1. Time course of daily global radiation and minimum and maximum daily temperature in Wageningen, the Netherlands, throughout the months June–August 2003. Sowing times of the clovers (T1, T2 and T3), introduction time of the test plant species (I), determination time of soil cover and plant height (D) and sampling time (S) are indicated on the time-axis.

Clover characteristics

At 11 days after the introduction (DAI) of the test plants, significant effects of clover species ($p < 0.001$) and sowing date ($p < 0.001$) on canopy height were observed. Persian clover was about 9 cm taller than white clover and approximately 18 cm taller than subterranean clover (Figure 2). As expected the earlier sown plants were taller, with a difference of about 7 cm between consecutive sowing dates. For soil cover a significant interaction between clover species and sowing date ($p < 0.001$) was observed. For all clover species of the earliest two sowing dates full soil cover was already obtained at 11 DAI of the test plants (Figure 2). Only for the last sowing date (T3) significant differences between the three clover species were found, with Persian clover (96%) having a higher soil coverage than white clover (83%) and subterranean (55%) clover. Also for shoot dry weight at 20 DAI a significant interaction between clover species and sowing date ($p < 0.001$) was observed. For each clover species, an earlier sowing date resulted in a higher shoot dry matter (Figure 2). At each sowing date, subterranean clover had the lowest shoot dry matter, though at the third sowing date no significant difference with white clover was found. Persian clover had the highest shoot dry weight at the last two sowing dates, but with the earliest sown clovers the shoot dry matter of white clover surpassed that of Persian clover. Correlation analysis showed that all three measured clover traits were significantly correlated (shoot dry matter and height: $r = 0.871$** ($p < 0.05$); height and soil cover: $r = 0.739$** ($p < 0.05$); shoot dry matter and soil cover: $r = 0.684$* ($p < 0.10$)).

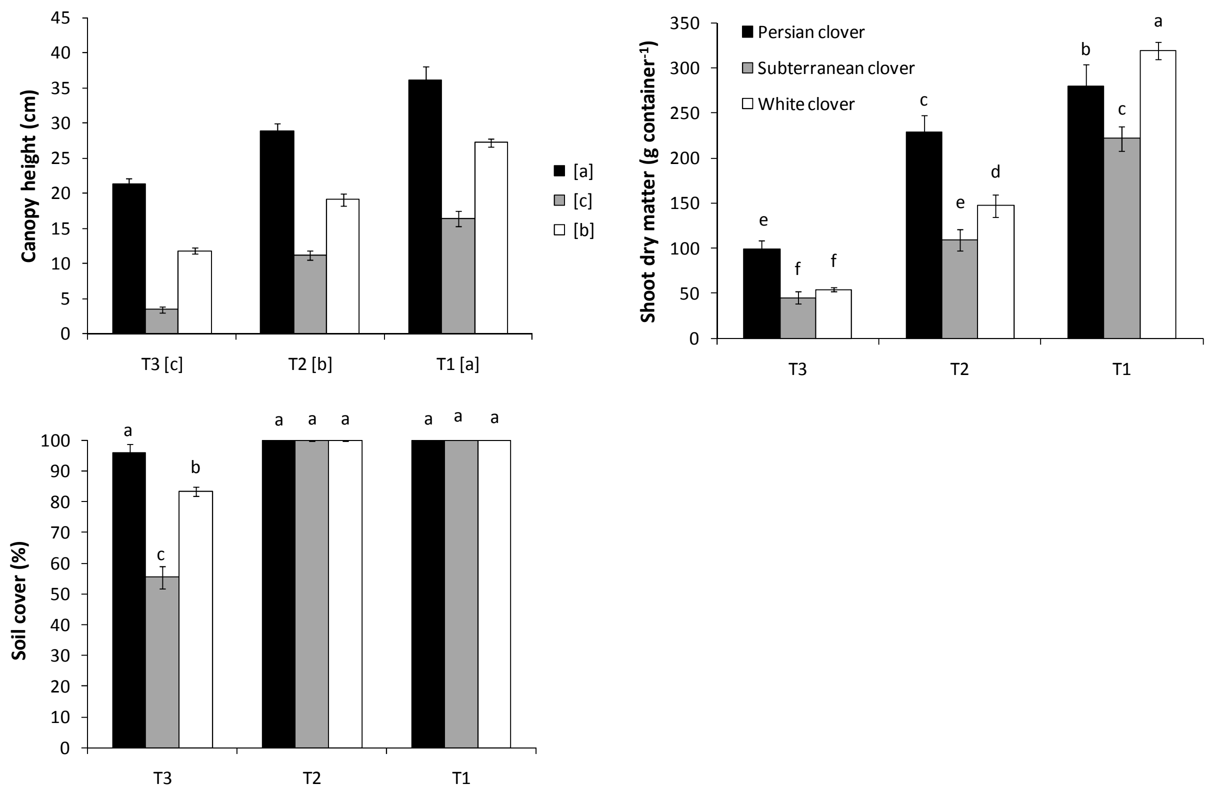

Figure 2. Canopy height, shoot dry matter and soil cover of Persian, subterranean and white clover canopies sown at July 7 (T3), June 23 (T2) and June 6 (T1). Canopy height and soil cover were determined on August 11, at 10 days after introduction of the test plants. Shoot dry matter was determined 10 days later. Vertical bars indicate standard error of means. Bars with the same letter within one graph are not significantly different at $p = 0.05$. For canopy height significant differences at $p = 0.05$ are indicated separately for sowing date and clover species.

Survival and dry weight of test plant species

In Figure 3, the survival of plants from seeds and seedlings is presented. The survival of plants that were introduced as seedling was higher than that of plants that were introduced as pre-germinated seed. Further, the average survival of rye grass plants was slightly higher than that of quinoa plants. For seeds and seedlings of both rye grass and quinoa the combination of sowing time and clover species had a significant effect on test plant survival at 20 DAI ($p < 0.001$). In all cases, the survival in late sown clover plots was either similar or higher than that in earlier sown clover plots. When focusing on the differences between clover species, the situation was less clear-cut. With some sowing dates either all, or nearly all, plants had died (quinoa from seeds in clovers of the first and second sowing date; rye grass from seeds in the first sown clovers), or all plants had survived (seedlings of both species introduced in clovers of the third sowing date), irrespective of clover species. For the other sowing

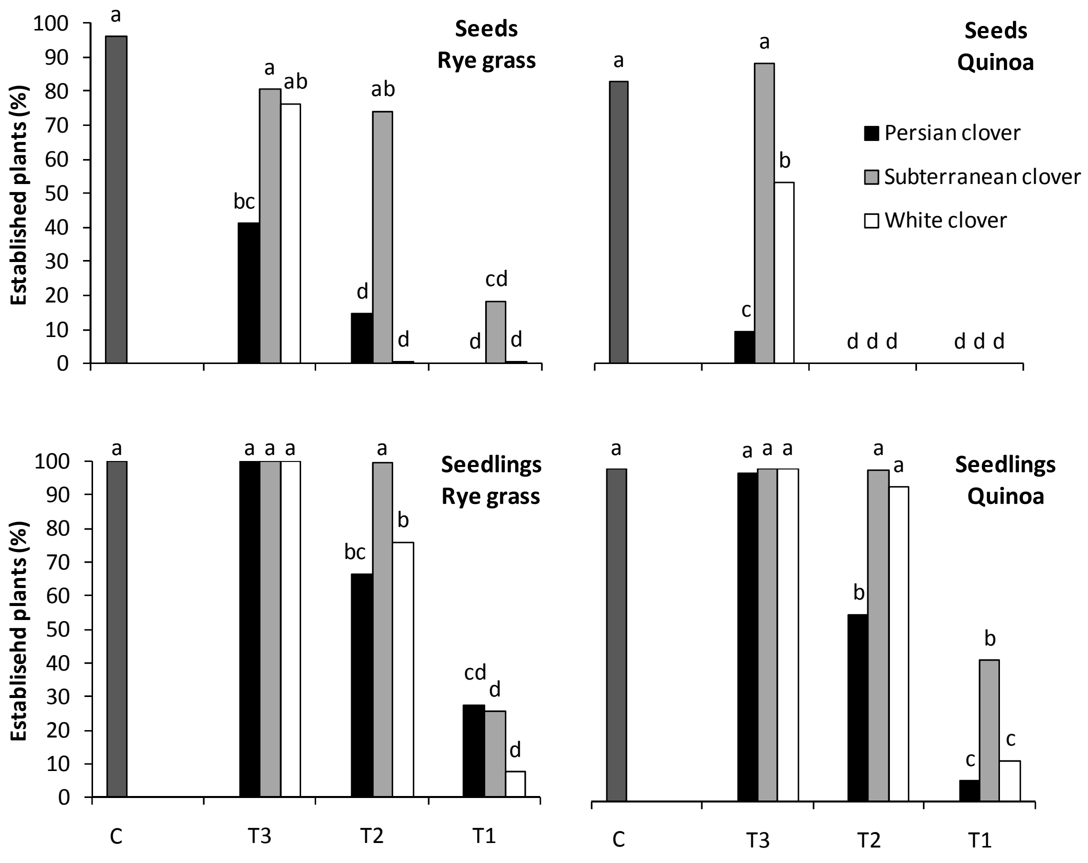

Figure 3. The effect of 3.5 (T3), 5.5 (T2) and 8 (T1) week old canopies of Persian, subterranean and white clover on the establishment of Italian rye grass and quinoa plants, introduced as seeds or two-week-old seedlings, at 20 days after their introduction. C refers to the establishment in the absence of a clover canopy. Different letters (a–d) within one graph indicate significant differences at $p = 0.05$.

Table 1. Total accumulated shoot dry matter (g container^{-1}) of Italian rye grass and quinoa plants, introduced as seeds and two-week-old seedlings, in 3.5 (T3), 5.5 (T2) and 8 (T1) week old canopies of Persian, subterranean and white clover.

Sowing time	Clover species	Rye grass		Quinoa	
		Seeds	Seedlings	Seeds	Seedlings
T1	Subterranean clover	0.006 d	0.130 de	0.000 c	0.574 de
	Persian clover	0.000 d	0.005 e	0.000 c	0.230 e
	White Clover	0.000 d	0.033 de	0.000 c	0.096 e
T2	Subterranean clover	0.027 d	0.897 c	0.000 c	1.48 d
	Persian clover	0.012 d	0.517 cd	0.000 c	0.698 de
	White Clover	0.000 d	0.303 cde	0.000 c	1.22 d
T3	Subterranean clover	0.251 b	6.53 b	0.993 b	29.7 a
	Persian clover	0.033 d	0.758 c	0.021 c	3.53 c
	White Clover	0.107 c	4.19 b	0.126 c	7.04 b
Control	No Clover	0.396 a	12.3 a	1.83 a	38.0 a
	Log - SED	0.0128	0.0824	0.0309	0.1111

Different letters (a–e) within a column indicate significant differences at $p = 0.05$.
SED-values of log-transformed data are presented.

dates a significant effect of clover species on test plant survival was observed. Persian clover was found to be the strongest suppressor and subterranean clover the weakest. White clover held an intermediate position.

The data on shoot dry matter of the test plants (Table 1) show that, in general, quinoa plants accumulated more biomass than rye grass plants, whereas plants that were introduced as seedlings accumulated more biomass than those introduced as pre-germinated seeds. A significant treatment effect on shoot dry weight of the test plants was observed ($p < 0.001$). Differences between the first two sowing dates were only found for plants that were introduced as seedlings. Subterranean and Persian clover of the first sowing suppressed biomass production of rye grass seedlings more than did the same clover species of the second sowing. For white clover such a difference between the first and the second sowing was found with quinoa seedlings. Clovers sown at the third sowing time gave in general a significantly lower suppression of test plant species. The exception was Persian clover that only gave a poorer suppression of quinoa seedlings. For white clover the suppression of quinoa plants from seeds was not significantly reduced compared to that of the earlier plantings. Significant differences between clover species were only observed for the third clover planting. Similar to what was observed for survival, Persian clover gave the strongest suppression and subterranean clover the weakest. Also in this case white clover held an intermediate position.

Relating weed suppression to clover traits

Survival and shoot dry weight of the test plants were negatively correlated with all three clover traits (data not shown). Whereas test plant survival was most strongly correlated with clover shoot dry weight (*r* between −0.792 and −0.936), shoot dry weight of the test plants correlated best with soil cover (*r* between −0.958 and −0.989). Plotting survival and shoot dry weight of the test plants against the measured clover traits revealed that only with clover shoot dry weight on the abscissa a smooth decline in plant survival and shoot dry weight was observed (Figure 4). Non-linear regression

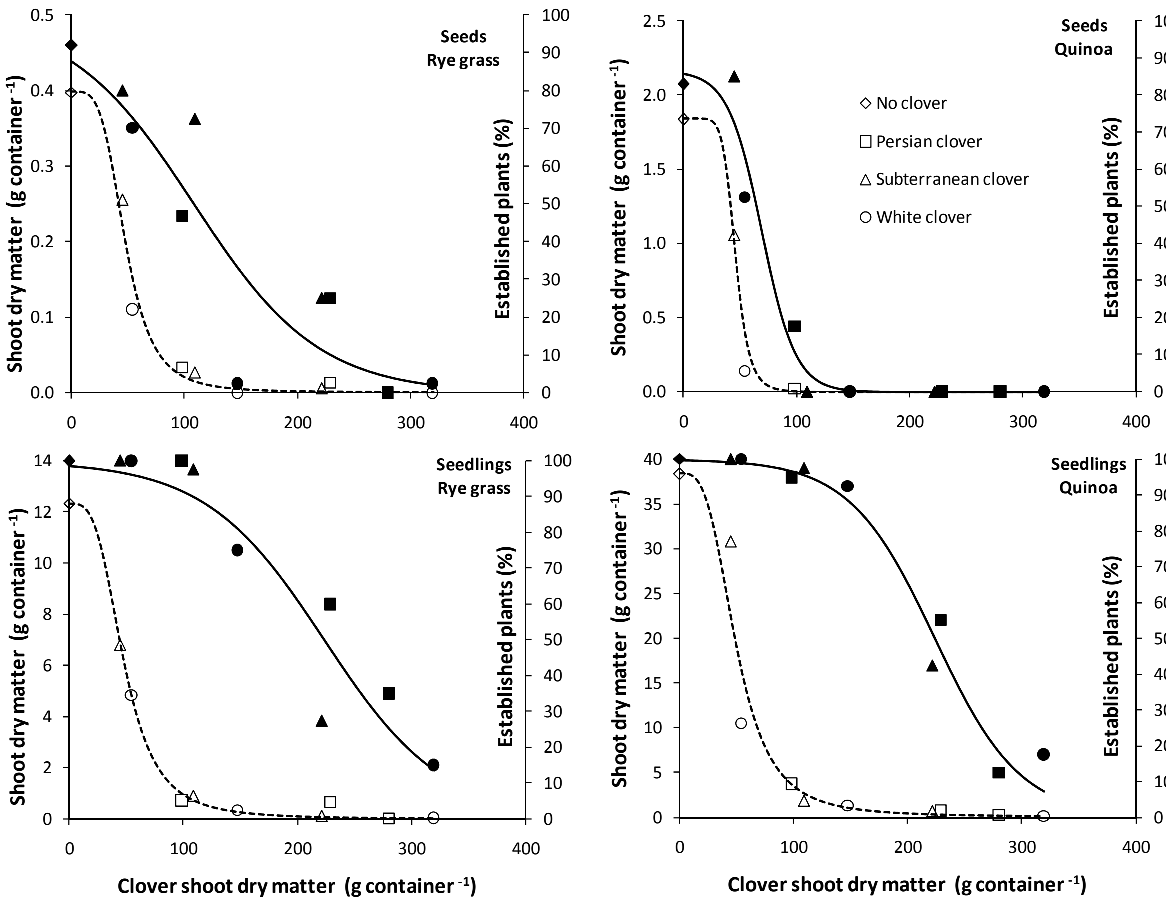

Figure 4. Shoot dry matter (open symbols) and survival (the percentage established plants; closed symbols) of Italian rye grass and quinoa plants, introduced as seeds or two-week-old seedlings in canopies of Persian, subterranean and white clover, plotted against clover shoot dry weight. Curves indicate the best fitting log-logistic (shoot dry matter; dotted line) and logistic (survival; solid line) functions relating clover shoot dry matter to the measured characteristic.

analysis showed that for survival the percentage variance accounted for by a logistic fit ranged between 77 and 97%. The reduction in shoot dry weight was best described with a log-logistic function, resulting in a percentage variance accounted for between 97 and 99%. Already at a low clover dry weight, the shoot dry matter of the test plants dropped rapidly, whereas under these conditions survival was still nearly unaffected. With a further increase in clover dry matter the number of surviving test plants gradually dropped. Both for quinoa and rye grass this reduction in the number of survivors with increasing clover dry weight occurred more rapidly with plants that were introduced as seeds.

Discussion

In this experiment, the differences in growth and development of the three clover species were largely similar to the differences observed in earlier experiments (Chapter 2). Persian clover was the fastest growing species, with nearly complete soil cover already obtained at 5 weeks after sowing (WAS) (T3) and its shoot dry weight at both 6 (T3) and 8 (T2) WAS significantly higher than that of the other two species. Similarly, and in line with previous experiments, Persian clover was also the tallest growing species. The height difference with the other two species was already attained at 5 WAS (T3) and stabilized from there on. Subterranean clover was the slowest growing and shortest species, whereas white clover held an intermediate position. Only at 11 WAS (T1) shoot dry matter of Persian clover was surpassed by that of white clover. Also in earlier experiments Persian clover, because of a relatively short-growing period, combined a fast initial growth with a relatively low final amount of accumulated dry matter (Chapter 2).

The consequences of the cover crops for growth and survival of the test plants were evident. In the younger (T3) and less developed cover crops it was predominantly the growth of the test plants which was hindered, resulting in a lower biomass accumulation. In earlier sown (T2 and T1) and more developed cover crops the reduced biomass accumulation was accompanied with a gradual decline in the number of test plants that survived. For plants, in particular those of quinoa, that were introduced as pre-germinated seed, this even resulted in a complete kill. Clear differences in weed-suppressive ability between clover species were observed. For Persian clover, even the youngest canopy (T3) already gave a strong suppression, though for plants introduced as two-week-old seedlings this was not yet reflected in a reduced survival. A reduced survival was, however, noted with the canopy that had developed from the second sowing (T2). With subterranean clover weed suppression developed much slower. With the youngest canopy (T3) no significant reduction in plant number compared to the control was observed, whereas for quinoa planted as seedling not even a reduction in shoot dry weight was observed. With the canopy of the second sowing (T2) the reduction in shoot dry weight was quite strong, though for rye grass and quinoa introduced as seedlings still no significant reduction in plant number was observed. Only for the subterranean clover canopy of the first sowing (T1) a significantly reduced survival was found for both stages of the two test plant species. White clover held an intermediate position. The weed-suppressive ability of white clover developed faster than for subterranean clover, but not as fast as for Persian clover. This intermediate position was evident from both the data on shoot dry weight and on plant survival. These results are in line with the previously reported field screening of clover species (Chapter 3). Hiltbrunner et al. (2007) also observed that white clover suppressed weeds better than subterranean clover and suggested that this was because of the poor dry matter accumulation of subterranean clover. Enache and Ilnicki (1990) and Ilnicki and Enache (1992) concluded that subterranean clover was a good weed suppressor. However, in their studies the subterranean clover was established in autumn, whereas the weed-suppressing ability was evaluated no earlier than in summer.

Figure 4 suggests that both survival and shoot dry weight of the test plant species are directly related to cover crop biomass. Data regarding the three clover species could be adequately described with a single line, implying that the weed-suppressive effect of the clover cover crop can be captured in a single trait. The observed differences in weed-suppressive ability among clover species can largely be attributed to their differences in accumulated biomass, which in turn stem from differences in initial growth rate. Despite the significant correlations between shoot dry matter, soil cover and plant height, use of the last two traits did not provide a clear pattern. For soil cover this was because of the time of observation. At the time that soil cover was determined, at 10 days after the introduction of test plants, all three clover species of the first and second sowing had already obtained full soil cover. Consequently, the discriminating ability of this trait was marginalized.

The observations imply that the smothering ability of a cover crop is closely related to the amount of biomass produced. Provided that seed rate gives them an equal starting position, clover species with a high initial growth rate will develop their ability to suppress the establishment of weeds more rapidly. This explains why Persian clover, and to a lesser extent white clover, possess a better smothering ability than subterranean clover. In earlier research, it was established that height of the cover crop, rather than initial growth rate, was the most determining factor for the competition-related yield reduction of the main crop (Chapter 3). Consequently, the smothering ability of the cover crop and its competitive pressure on the main crop are not necessarily related. The reason that these factors differ is related to the strong influence of initial size differences on competitive relations (e.g. Weiner, 1986). Smothering refers to a cover crop that prevents an emerging weed plant to establish. Clearly the cover crop has the initial size advantage, and once the cover crop canopy is well developed newly germinated weeds will be suppressed. The competitive relation between cover crop and main crop is of a different kind. Transplanting, regularly applied in vegetable production systems, provides the main crop with a initial size advantage. Particularly in situations where competition is mainly for light, the hinder on the main crop will be largely determined by the height of the cover crop.

In theory, the detachment of smothering ability and the competitive pressure imposed on the main crop is a major advantage. Yet, the clover species that combines a superior smothering ability with a minor competitive pressure on the main crop has not been identified. Whereas Persian clover combines a superior smothering ability with unacceptable damage to the crop, it is the poor smothering ability that makes subterranean clover a less suitable cover crop. Clearly white clover offers most prospects.

CHAPTER 6

General discussion

General discussion

Biodiversity and ecosystem functioning

It is well recognized that biodiversity is a fundamental prerequisite for the existence of human life on earth. This in itself makes it worth putting our efforts in maintaining environments that support a large variety of species. In the previous decades increasing attention was given to the relation between biodiversity and the provisioning of ecosystem services. Within the life support system of the earth there are a large number of ecosystem services that are beneficial for humans, like for instance food production, waste treatment, water supply and biological control (Costanza et al., 1997). Such ecosystem services are linked to ecosystem functions such as primary production and storage and retention of water. Numerous studies have shown that to maintain a well functioning ecosystem, a sufficiently diverse system is needed (e.g. Cardinale et al., 2006; van Ruijven and Berendse, 2009). Larger numbers of species enhance ecosystem reliability, meaning that the ecosystem performs well over a considerable period of time (Naeem and Li, 1997). More recently, the observation that many species are required to maintain ecosystem functioning and services under changing environmental conditions was confirmed by Isbell et al. (2011).

Species richness of cropping systems is generally low and related to that, the functioning of cropping systems is for a large part dependent on the availability of chemical fertilizers and pesticides (e.g. Liebman and Dyck, 1993). These inputs allowed a practice in which the crop is the single species stimulated to grow, while attempts are made to remove all other plant species. The resulting low species diversity is one of the characteristics that differentiate agro-ecosystems from natural ecosystems, but clearly this is not the only one as low species diversity is correlated to a reduced stability (Elton, 1958) and results in a system that is more vulnerable to pests, diseases and weeds (Table 1).

One key characteristic of agro-ecosystems is a high net productivity, as the growth of the crop is stimulated through the creation of optimal conditions, such as the alleviation of water and nutrient deficiencies and the control of pests, diseases and weeds (van Ittersum and Rabbinge, 1997). As history has taught us, the use of synthetic fertilizers and pesticides has resulted in wide spread and severe damage to the environment (e.g. Leistra and Boesten, 1989; Colborn et al., 1993; Oenema et al., 1998). Increased awareness of these negative side effects has let to stricter regulations on the use of chemical inputs and an intensified search for alternative methods to safeguard a high crop productivity. Consequently, there is a growing interest in the

Table 1. Structural and functional differences between natural ecosystems and agro-ecosystems (modified after Gliessman (1998) in Altieri and Nicholls, 2004).

Characteristics	**Natural ecosystem**	**Agro-ecosystem**
Maturity	Mature, climax	Immature, early successional
Phenology	Seasonal	Synchronized
Temporal permanence	Long	Short
Net productivity	Medium	High
Species diversity	High	Low
Habitat heterogeneity	Complex	Simple
Trophic chains	Complex	Simple, linear
Entropy	Low	High
Stability (resilience)	High	Low
Mineral cycles	Closed	Open
Human control	Not needed	Definite

design and management of agro-ecosystems in which enhanced soil fertility and the control of pests, diseases and weeds is less reliant on synthetic fertilizers and pesticides (Liebman and Dyck, 1993). Even more, as carbon-based energy sources are not unlimited, more sustainable agro-ecosystems need to be developed eventually.

Gliessman (1998; cited by Altieri and Nicholls, 2004) related system characteristics that are the outcome of ecological processes to the degree of successional development of an ecosystem (Table 2). He showed that most characteristics of fully developed natural ecosystems, such as greater stability and greater potential for biological control, are considered favourable for agro-ecosystems. The exception being net primary productivity, which is relatively low in mature ecosystems. He also noted that most of the favourable characteristics are related to a high degree of species diversity. Increasing species richness is, therefore, thought to be one of the critical elements for enhancing the reliance of agro-ecosystems on ecological processes and for minimizing the need for human intervention (Altieri, 1995). Most agro-ecosystems are, however, characterized by their early successional development, which is not congruent with a high species diversity. Clearly, purposeful attempts for cropping system diversification are required to enhance species richness and to promote the delivery of desired ecosystem services. In this pursuit, the main challenge is to enhance species diversity, without reducing crop productivity.

The research presented in this thesis focused on the greater potential for biological control of weeds through diversification of the agro-ecosystem. Biological control of weeds often makes use of species at another trophic level. Examples are herbivorous

Table 2. Ecological characteristics desirable to agro-ecosystems and how these relate to the level of successional development (early, middle and late). A darker shade indicates a stronger expression of the particular characteristic (after Gliessman (1998) cited by Altieri and Nicholls, 2004).

Characteristic	**Early**	**Middle**	**Late**	**Benefit to agro-ecosystem**
High net primary productivity	■			Greater potential for production of harvestable biomass
High total biomass			■	Larger source of soil organic matter
High species diversity		▒	■	Reduced risk of catastrophic crop loss
High complexity of species interactions		▒	■	Greater potential for biological control
Mutualistic interference			■	Greater stability; diminished need for external inputs through for instance plant-mycorrhizae relation
Efficient nutrient cycling		▒	■	Diminished need for external nutrient inputs

insects that target a specific plant species or host plant specific pathogens. The classical method of biological control aims at re-establishing the balance between an invasive weed species and one of its antagonists, by introducing the antagonist from the native region of the weed. Striking progress has, for example, been made in the control of tropical aquatic weed species such as *Salvinia molesta* Mitchell (giant salvinia) or *Pistia stratiotes* L. (water lettuce) by releasing host specific weevils from the original habitat in South America into the invaded habitats of Africa (den Hollander et al., 1999; Pieterse et al., 2003). An alternative method, referred to as the inundative or bioherbicide approach, is to rear and release indigenous host specific plant pathogens to control native weed species. The biological control of weeds in this research project was based on smothering or competitive suppression of weeds by other plant species. Introduction of these plant species, resulting in a more diversified agro-ecosystem, can be realized in a number of ways.

Plant species as agent for biological weed control

One method to enhance diversity is crop rotation, which is defined as growing different plant species in a systematic and recurring sequence on the same land (Yates, 1954; Liebman and Dyck, 1993). Before the introduction of crop rotation schemes, plots were often continuously planted with the same crop and fallow periods were occasionally included as a means to regenerate the soil. The shift from a monoculture cropping system interrupted with fallow periods to a crop rotation system occurred in Western Europe in the 18^{th} century and enabled intensified food production with the maintenance of a good soil fertility and a physical soil structure of high quality (Grigg, 1974; Leighty (1938) in Liebman and Dyck, 1993). Apart from the fore-mentioned qualities, crop rotation is also regarded as one of the most important components of any weed control strategy (Leighty, 1938). Liebman and Dyck (1993) evaluated several studies on crop rotation and concluded that in most cases crop rotation did indeed have a weed-suppressive effect that is stronger than the average suppression of weeds obtained in the monocultures of the respective crops. Kruidhof et al. (2008) showed the value of incorporating a cover crop in the rotation scheme. Two mechanisms of weed suppression were identified. In autumn, after the cover crop had been planted following the harvest of the main crop, weeds were hampered in their growth and eventually produced less seeds. In spring, after the cover crop residues were mixed through the top soil layer, weed seed germination was inhibited and early growth of the weeds was retarded. The results of the experiments showed furthermore that both the residue incorporation method as well as the choice of cover crop species influenced the level to which weed seed germination was inhibited. Where the shallow incorporation of winter rye had a consistent and strong inhibiting effect on weed seedling emergence, the incorporation of finely ground winter oilseed rape led in some instances to an increased rate of weed-seedling emergence (Kruidhof et al., 2008).

Other methods for enhancing species diversity are mixed cropping or the addition of a companion crop to the main crop. Mixed cropping or intercropping is widely practiced by small-scale farmers in developing countries for a variety of reasons. A reduced risk of complete crop failure, an increased yield level through a better exploitation of light, nutrients and water following from niche differentiation of the two crops (Mkamilo, 2004; Molla and Sharaiha, 2010) and an improved control of pests and diseases (e.g. Uvah and Coaker, 1984; Theunissen et al., 1995; Zhu et al., 2000) are some of these reasons. Within the context of a mixed cropping design, Vandermeer (1989) introduced the terms facilitation and competition to discern the two main principles that together determine the functioning of mixed cropping systems. Central in the concept of Vandermeer is the interaction between crops and the environment. A crop is having an influence on the environment and this modified environment causes a response in the other components of the intercrop. Facilitation means that there is a clear positive effect on the other crop, whereas competition stands for a negative effect. In case of a pest-suppressive effect, the added companion crop may attract predator insects that prey on herbivorous insects that are detrimental to the main crop. Clearly the added companion clearly serves as a facilitator, providing a reduced pest pressure for the main crop. On the other hand, competition through the uptake of resources by the companion crop creates a reduced availability of resources for the main crop, resulting in a lowered yield. It is the balance between facilitation and competition that ultimately determines the suitability of the design, as was for instance demonstrated by Zhang and Li (2003) in their research on legume-cereal intercropping systems. In such a system, one of the two crops is more competitive and maturing earlier. After harvesting the early crop, the second crop is left to recover from the negative effects of interspecific competition. Examples of facilitation include maize enhancing the iron uptake of peanut, faba bean improving nitrogen and phosphorus uptake by maize, and chickpea facilitating phosphorus uptake by wheat. At the same time, if the second crop is not able to recover sufficiently from the negative effects of competition, overall yield may be suppressed. Only if the positive effects of facilitation surpass the negative effects of the competitive damage to the other crop such a system will be attractive to farmers

There are numerous examples that show the positive effect of intercropping on weed control (e.g. Saucke and Ackermann, 2006; Paulsen et al., 2006; Bauman et al., 2000). In these cases, the companion crop hinders the establishment and growth of weeds through pre-emption of resources. In a sense, facilitation, or the creation of a weed-free environment for the main crop, is obtained through competition. The dilemma that lies at the heart of this thesis research is that on the one hand the companion crop needs to be sufficiently competitive to have a weed-reducing effect, while at the same time the competition between the companion crop and the main crop should not lead to severe yield losses.

The results of the pilot experiment presented in the first chapter of this thesis illustrate this dilemma as it shows the dramatic outcome of a poorly chosen combination of species. The main crop, Brussels sprouts, is extremely sensitive to competition, as at a too strong competitive stress no buds, or only small, nonmarketable buds are formed (de Wit et al., 1979; Everaarts and Sukkel, 1999). As a consequence, the presence of the companion crop, even at a relatively low density, resulted in a substantial reduction of the harvestable yield of the main crop. At the same time, malting barley, the companion crop, did not produce a closed canopy and was, therefore, not able to suppress tall-growing weed species, such as *Chenopodium album,* adequately. On top of that, the presence of malting barley hindered, or even prevented, the removal of weeds by hand or mechanical means (Bastiaans et al., 2007). The results of this pilot experiment thus clearly showed that a companion crop which is competing fiercely with the main crop does not necessarily compete strongly with weeds. The one positive conclusion that can be drawn from this is that competitive damage to the main crop and weed suppression are not necessarily related to the same traits of the companion crop. This implies that it might also be possible to identify companion crops which are able to successfully suppress weeds without severely harming the main crop. The research presented in this thesis was aimed at identifying whether a suitable companion crop could be identified among clover species (*Trifolium* spp.).

Selecting a proper companion crop among clover species

There are many plant species that could potentially be used as a companion or cover crop in a mixed cropping design. In this research project, the focus was put on representatives of the genus *Trifolium*. The reason for choosing clover species was that clovers are known to have additional benefits, like nitrogen fixation and in some instances the suppression of pests and diseases (Theunissen et al., 1995; Finch and Kienegger, 1997; Grafton-Cardwell et al., 1999). Because of these multiple benefits, it is more likely that the introduction of clover will result in a positive balance between facilitation and competition, which adds to the acceptance of clover as a suitable cover crop for weed suppression. In addition, a large variety of morphologically different clover species exists. The experiments described in this thesis were aimed at comparing the performance of different clover species and were conducted in the field, in containers and in pots, under outdoor and more controlled greenhouse conditions. Studying the various clover species under field conditions is important as such trials generate information on the performance of the species under the conditions that they may eventually be used in. Still, finding answers to more specific questions, such as the contribution of below- and above-ground competition demands for more controlled conditions. Pots in which root barriers were introduced (Chapter 4) allowed for this comparison, and it is obvious that this kind of comparison is very difficult to carry out under field conditions.

A good basis for determining the ideal companion crop for weed suppression (smother crop) would be to assess the influence of several individual traits in isolation. Ideally one would like to vary a single trait (e.g. plant height), while all other characteristics remain identical. Mechanistic modelling of crop growth and competition is a good method for making such comparisons as single trait changes can easily be made and their effects evaluated. Bastiaans et al. (1997) used this approach for identifying the most suitable traits for increasing the weed-suppressive ability of rice genotypes. Single trait differences can also be investigated *in vivo*. Much of the research on *Arabidopsis thaliana* (L.) Heynh. is based on the creation of mutants that often only differ in a single trait from the wild type plant (e.g. Koornneef et al., 1989).

The research presented in this thesis started off with a group of eight clover types (seven species and two cultivars of one species), which varied from one another in a range of traits. Correlation analysis was used to identify which trait, or group of traits, was most closely related to the specific characteristics under study, being the weed-smothering ability and the competitive pressure on the main crop. Clearly, the suitability of the chosen approach greatly depends on the number of entries, and in this regard eight species might not seem extremely high. Difficulty with a low number of species is that the species might not contain the entire variability that is present for a specific trait. Additionally, it might be that in this set of species two or more traits are confounded, making it difficult to identify the actual trait responsible for a certain characteristic. The labour and area required for conducting the detailed observations that were performed and the number of commercially available and suitable clover species for the temperate climatic conditions in Western Europe, were major determinants of the relatively low number of investigated species. In hindsight, it can be concluded that for most of the traits substantial variation among the selected species was present. Compared to other work on the same topic (e.g. Creamer and Bennet, 1997; Biazzo and Masiunas, 2000; Ross et al., 2001) the research project that is presented in this thesis holds a unique position as it combines detailed information on individual species traits with the overall competitive ability of the clover species.

Differences in traits between clover species

The results of the first field trials using the eight different clover species immediately revealed that significant differences between species characteristics were present. Persian clover, for example, stood out as the fastest developing species, with underlying traits such as a high light extinction coefficient (1.04), high light use efficiency (1.25 g DM MJ^{-1}) and a high specific leaf are (425 cm^2 g^{-1}). Subterranean clover, on the other hand, was the slowest developing species with a much lower light extinction coefficient (0.80), lower light use efficiency (1.03 g DM MJ^{-1}) and a much lower specific leaf area (175 cm^2 g^{-1}). Also height development and maximum canopy height were characteristics that brought a clear discrimination between species. Berseem clover was the fastest developing species regarding height (51 cm at 7.5 weeks after sowing (WAS)), but was not the species with the highest maximum plant height. Red clover was only 17 cm tall at 7.5 WAS, but grew up to become the tallest species with 80 cm at 12 WAS. Subterranean clover remained the shortest species throughout the experiment (3.6 cm at 7.5 WAS and 11.6 cm at 12 WAS). Differences in accumulated shoot dry matter were also apparent. Persian clover had the highest relative growth rate, but, because of a relatively short-growing period, it only accumulated 426 g m^{-2}. This was considerably lower than the 775 g m^{-2} of alsike clover, the clover species that accumulated the highest amount of dry matter.

Correlation analysis showed furthermore that dry matter accumulation and maximum plant height were not correlated. Not surprisingly, strong and positive correlations were observed between traits such as soil cover, light interception and the moment at which a leaf area index of three was reached, all of which represent early growth.

Differences in competitive ability

Next to the characterization of clover traits, the competitive effect of clover species on weeds as well as on crop plants was investigated. Also for these aspects, clear differences between clover species were observed. For example, in plots with Persian, red and alsike clover tall-growing weeds such as *Chenopodium album*, *Echinochloa crus-galli* or *Polygonum persicaria* were much better suppressed than in plots with subterranean and white clover. White clover performed much better in an experiment where rye grass was used as model weed. Persian and white clover were both suppressing rye grass strongly while, in contrast, subterranean clover, the third clover species in this experiment, was not able to suppress rye grass. All clover species caused substantial competitive damage to transplanted leek. However, also in this case clear differences were evident. Red clover caused a yield reduction of 87%, Persian, alsike, berseem and crimson clover a reduction of about 70%, and both white clover cultivars and subterranean clover a reduction close to 60%.

Most of the plant traits of clover that were determined were obtained at the population level. This was also the case in the experiments directed at assessing the effects of competition of clover on weeds and crops. This was done purposely, as the value of cover crops is typically related to their performance at population level. It is, however, important to realize that some of these population traits, apart from the genetic make-up of the species, are strongly influenced by starting condition. Early growth and soil cover development, for example, are not only influenced by the intrinsic growth rate of a species, but also depend on the amount of plant material that is present at the start of the experiment. In this regard, the criteria for choosing the proper amount of seeding material become crucially important. If one wants to start with the same number of seedlings per unit area, the implication will be that, particularly if seed size varies a lot among species, the initial biomass will differ substantially. This was particularly relevant for this research project as subterranean clover seeds were about nine times the seed weight of white clover. Alternatively, one might start with the same amount of seed (in kg ha^{-1}), as this might be considered a more equal starting position. In this case, the seeds with the largest seed size might have a disadvantage, as their initial biomass will be less equally distributed over the field. The importance of an even distribution of plant material for weed suppression was emphasized by, for instance, Olsen et al. (2005). A more practical option would be to stick to the recommended amount of seed material for that particular species. However, it is not always clear on which criteria these recommendations are based, whether the same criteria are used for all clover species and whether the recommendations also hold for use of the clover species as cover crop. For experiments that are focused on early development, striving for similar amounts of biomass at the start of the experiment is preferred. Traits such as soil cover development are directly influenced by the starting condition of each species and sowing equal numbers of seeds per surface area would thus give an enormous advantage to subterranean clover, particularly compared to small-seeded clover species such as white clover.

Of course there are other factors that affect the amount of biomass at the beginning of the experiment. Differences in rate of emergence are important as well, as relative time of emergence has frequently been identified as a main determinant of the competitive relations between plant species (e.g. Kropff and van Laar, 1993; Bastiaans et al., 2008; Mercer et al., 2011). In our experiments, Persian clover established about two days before the other clover species. An important question then is whether this earliness is a species trait or whether it is related to seed quality. The fraction germinated seeds is another factor affecting the starting position. Despite attempts to correct for germination rate, this is not always easy and straightforward. For each of our experiments germination tests were conducted. Although similar and high germination rates (over 90%) were obtained for all species under laboratory conditions, large differences in emergence between species were observed under field conditions. White and alsike clover, for example, had extremely low emergence rates in the first field trials in 2001 (only 7%). Weather conditions may have played a role. In this case, the poor emergence of white and alsike clover, the species with the smallest seeds, may have been caused by heavy rainfall just after sowing. Also Ross et al. (2001) reported a reduction in clover emergence rate that could possibly be attributed to heavy rainfall. In 2002, emergence was much more similar (53%, 43% and 31% for Persian, subterranean and white clover, respectively), but still considerably lower than indicated by the preliminary germination tests.

Clover traits and competitive ability

The pilot study showed that weed-suppressive ability of the cover crop and the competitive damage that is imposed on the main crop are not necessarily related to the same traits of the companion crop. This is an important observation, as it implies that it might be possible to identify cover crop species that adequately suppress weeds without harming the main crop. In Chapter 3, the competitive strength of various clover species was compared by studying the effect of competitive stress on model weed plants that emerged more or less simultaneously with the cover crop and on transplanted crop plants.

In Chapter 5, the focus was on weed-smothering ability. Correlating the observed competitive effects with the traits of the clover species that were used as companion crop showed that weed-suppressive ability was strongly correlated to traits that expressed a fast early growth, whereas damage to the main crop was most strongly correlated to plant height. These findings were confirmed by results obtained by Uchino et al. (2011) who, with a different set of cover crop species, showed that the negative effects on the main crop were most strongly correlated to plant height, while rapid soil coverage was of great importance for an adequate suppression of weeds.

In a study on the competitive relations between seven plant species, Freckleton and Watkinson (2001) concluded that differences in maximum plant size of plants grown in isolation, was the best predictor for the outcome of competition. Also model studies revealed that maximum plant height, among other factors, is an important determinant for the outcome of plant competition, particularly when competition is mainly for light (Kropff and van Laar, 1993). This result fits to the observations in this research project, where it was found that clover plant height correlates positively to the damage imposed on the main crop.

The experiment presented in Chapter 5 shows that species with a fast early growth are most effective in hindering weed seedling establishment. In Figure 1, weed-suppressive ability and damage to the main crop are presented on the abscissa and the ordinate, respectively. The ideal cover crop species, one that suppresses weeds adequately without harming the main crop, is positioned in the lower right corner.

Based on the current research, species that combine a rapid soil cover development with a relatively low canopy height would fit into the ideal zone. Clearly, malting barley that was used as a companion crop for weed suppression in the pilot study, contrasted strongly with a species that would fit in the ideal zone. Barley was unable to suppress weeds adequately, but it did have an enormous negative effect on crop yield.

Unfortunately, also the clover species did not match the description of an 'ideal zone' species. The rapid early growth of Persian clover gave it a competitive advantage over weeds which resulted in complete smothering of species such as rye grass and quinoa (Chapter 5). Both light interception and light use efficiency contributed to this fast early growth. Surprisingly, Persian clover did not stand out as a strong weed suppressor in experiments of Uchino et al. (2011). This may be explained by a poor emergence (18.5%) resulting in a relatively poor starting condition. In the experiments presented in this thesis, Persian clover had, apart from a high relative growth rate, also a good starting position relative to the other clover species as it germinated faster and had a high rate of establishment. Despite its good weed-

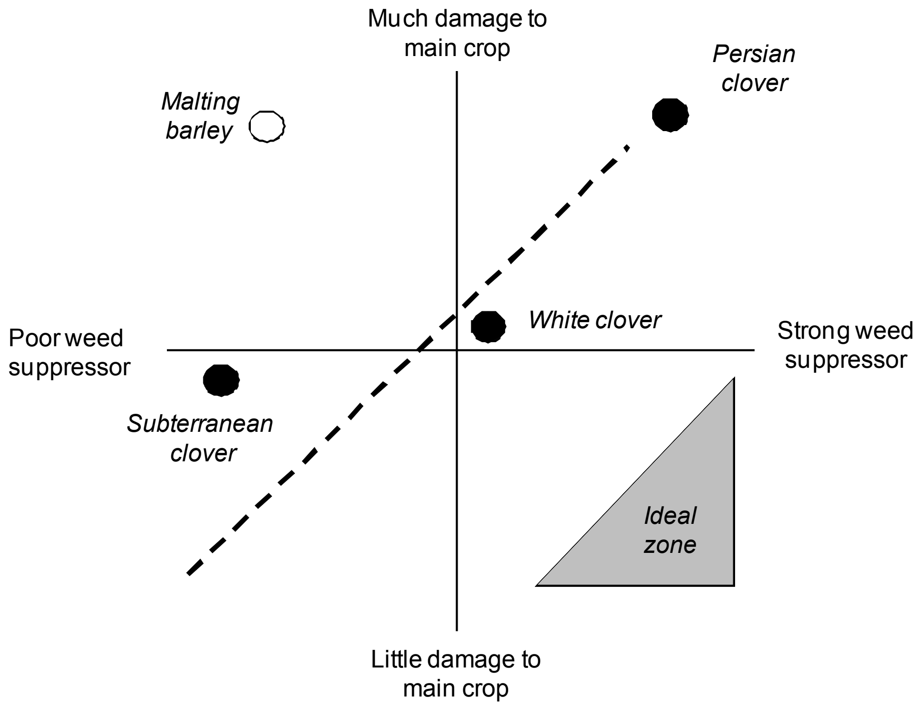

Figure 1. Smothering ability (abscissa) and damage to the main crop (ordinate) for Persian clover, white clover, subterranean clover and malting barley expressed on relative scales and estimated based on the experiments described in this thesis. The dashed diagonal line represents the position of species for which weed suppression and competitive damage are closely linked, since both characteristics are determined by the same underlying traits. The grey zone represents the position of cover crops that combine a strong weed-suppressive function with only little damage to the main crop. In this case, weed suppression and competitive damage to the main crop are disconnected and, thus, based on different underlying traits.

suppressive function, Persian clover did not fit the ideal zone as it caused too much damage to the main crop. This negative effect on the main crop was attributed to its rapid height development and tallness. Persian clover is an example of a species that combines good early development in terms of soil cover and shoot dry matter with a rapid height development and is for that reason placed in the upper right quadrant of Figure 1, in which species can be found that are good weed suppressors but are unsuitable as a companion crop as the damage to the main crop is too high.

As mentioned above, subterranean clover’s height development was slow and it remained low. As plant height of the cover crop was identified as one of the main factors affecting damage of the main crop, cover crops with a low plant height are expected to cause less yield loss (Lotz et al., 1997). Surprisingly, subterranean clover, being the shortest species, caused more yield loss in leek than was expected based on its height. It is interesting to note that the traits of subterranean clover are, in a natural environment, fitting to plant species that are both stress tolerant and competitive in an unproductive environment (Pelzer and Wilson, 2001). It was hypothesized that the unexpected high degree of yield loss was due to the fact that, apart from maximum plant height, also competition for below-ground resources is a factor that can bring serious damage to the main crop.

Competition for below-ground resources

This aspect was investigated in Chapter 4 and results showed that subterranean clover indeed competed relatively strong for below-ground resources. The results of Chapter 3 showed that although neighboring leek appeared to be hindered in the uptake of nitrogen, subterranean clover did not accumulate more nitrogen compared to the other clover species. Visual inspection showed that nodulation had developed well for all species indicating that all species were able to fix nitrogen from the air. It is unlikely that some clover species suffered of nitrogen shortage and had to compete stronger for nitrogen. Allelophatic activity of subterranean clover, however, may be a plausible explanation as evidence of allelopathic activity was found in extracts of clover leaves and in dead residue by Else and Ilnicki (1989). Subterranean clover may have hindered leek's growth by releasing natural toxins (allelochemicals) which possibly interfered with the uptake of nitrogen by leek.

In low-input agricultural systems, competition for below-ground resources is of great importance. Teasdale and Cavigelli (2010), for example, showed that in organically grown corn, competition for nitrogen easily developed into the major growth-limiting factor. Even more, since below-ground competition is size symmetric, also relatively small plants that hardly compete for light with taller plants, may have a serious impact on the main crop (Berger et al., 2008).

The use of companion crops for weed suppression

The starting point of this thesis was to investigate whether the choice of the companion crop would greatly influence the performance of a mixed cropping system, particularly with regard to realizing adequate weed suppression without giving in too much on the yield obtained for the main crop. The results of the research conducted within the framework of this PhD-project clearly underline the importance of the choice of the companion crop. Choosing subterranean clover as a companion crop will undoubtedly have a different impact on weed control and the yield of the main crop than choosing Persian clover. A clover species fitting in the 'ideal zone' was, however, not identified. The ideal companion crop would have to grow as rapid as Persian clover, or at least as fast as white clover, to guarantee a sufficient weed-smothering ability. At the same time it should remain at least as low as subterranean clover, though preferably with a lower ability to compete for below-ground resources. The implication that follows from the absence of the ideal cover crop is that, in addition to seedbed preparation and planting, the introduction of a companion crop will always require additional management, either to restrict the competitive influence of the companion crop on the main crop or to provide supplementary weed control to the companion crops with a lower smothering ability.

Possible management options that would fit in low-input agriculture consist of mechanical methods such as mowing, harrowing and horizontally or vertically cutting of roots (e.g. de Haan et al., 1994; Garibay et al., 1997; Biazzo and Masiunas, 2000). Another possibility is to introduce the cover crop much earlier or somewhat later than the main crop. Although in a different context, the work of Akanvou et al. (2002) showed the impact of varying introduction time of a companion crop on the performance of the system as a whole. The consequence of altering the introduction time of a companion cover crop would be that the growth of the main crop is not hindered by the growth of the cover crop as the cover crop has already died off or is not yet present at the planting time of the main crop. This idea was put forward by Ilnicki and Enache (1992) regarding the use of subterranean clover as a cover crop under the natural conditions of the Mediterranean climate. Under these climate conditions with mild wet winters and dry hot summers, subterranean clover is sown in late summer, has a period of growth followed by a dormant period in winter, resumes growth in early spring and finally flowers and dies off in early summer. The main crop is planted in spring and grows strongly at the moment that subterranean clover has stopped growing and begins to die off. In such a system the cover crop is not competing for resources with the main crop. Such an approach, however, does not fit to the climatic conditions in the Netherlands which is, for instance, illustrated by the fact that in Dutch Flanders, naturally occurring subterranean clover has a summer annual life cycle.

It is important to note that the additional management of the companion crop is likely to be of a permanent nature. A farmer will need to monitor carefully, throughout the growing season, whether or not an extra effort is needed to control the growth of the companion crop, or to take an additional measure to achieve a sufficient weed control level. Also for the aspect of additional management of the companion crop it is important to realize that the choice of cover crop species has a direct relation to the type and intensity of the required management. Baumann et al. (2001a) proposed the use of a harvestable cash crop as companion crop for weed suppression. In this case, the competitive influence on the poorly competing main crop, would only result in an increased yield of the companion crop. The results of their study showed that a carefully chosen combination of crops and companion crop may enhance weed suppression without suffering yield loss (Baumann et al., 2002).

In the previous, it was discussed why clover is such an interesting group of species for use as companion crop. The ability to fix nitrogen from the air and the pest-suppressing effect of clover are very important assets as in the near future the developed world may not have the luxury of an ample supply of nutrients or the availability of effective chemical protection agents. The future situation will certainly increase the need for further knowledge on how to incorporate leguminous species such as clovers in our cropping systems. Although the ideal cover crop species was not identified, the current research revealed that white clover would certainly be a promising candidate for further investigations.

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Samenvatting

Achtergrond van het onderzoek

Vanaf de vroegste vormen van landbouw worden gewassen gehinderd door onkruiden die, voornamelijk via concurrentie om licht, water en voedingsstoffen, groei en opbrengst negatief beïnvloeden. In hoeverre een landbouwgewas zelf in staat is om onkruiden effectief te beconcurreren hangt af van het verschil in concurrentiekracht tussen gewas en onkruid. Concurrentiekracht hangt samen met een reeks eigenschappen, waaronder vroege groei, groeisnelheid, snelheid van gewassluiting, gewashoogte en de snelheid waarmee de maximale gewashoogte bereikt wordt. Omdat bij de veredeling van landbouwgewassen prioriteit gegeven wordt aan eigenschappen als opbrengst, resistentie tegen ziekten en plagen en geschiktheid voor bepaalde milieus, is er nauwelijks aandacht voor concurrentiekracht of onkruidonderdrukkend vermogen geweest. Onkruiden daarentegen zijn vaak pioniers; planten die zich op een vrijgekomen stuk grond, zoals een pas geploegde akker, als eerste een plek weten te bemachtigen. Het zijn meestal snelle groeiers die, samenhangend met hun pionierskarakter, sterk kunnen concurreren met andere planten. Het is dan ook niet verwonderlijk dat, zonder menselijk ingrijpen, de concurrentie tussen onkruiden en landbouwgewassen meestal in het voordeel van de onkruiden uitpakt. Het menselijk ingrijpen dat nodig is om onkruiden niet de overhand te laten krijgen, heeft zich vanaf het prille begin van de landbouw ontwikkeld. Waar het eerst een tijdrovende en arbeidsintensieve bezigheid betrof, gaat het nu om, door hoogwaardige technologie gedomineerde, werkzaamheden die, mede door de inzet van chemische middelen, een teler in staat stellen om met een geringe arbeidsinzet en lage kosten zijn gehele bedrijf vrijwel onkruid vrij te houden. Er zijn bovendien niet alleen steeds geavanceerdere herbiciden op de markt gekomen, maar tevens zijn er gewassen ontwikkeld die resistent zijn tegen bepaalde herbiciden, waardoor breed-werkende herbiciden ook na opkomst van het gewas ingezet kunnen worden.

Dat het gebruik van gewasbeschermingsmiddelen ook forse nadelen met zich meebrengt, is inmiddels duidelijk. De schade aan het milieu en de daarmee gepaard gaande publiciteit heeft inmiddels geleid tot een grotere bewustwording bij consumenten, beleidsmakers en overheid. De aangepaste regelgeving die hieruit is voortgekomen, uit zich onder meer in strengere toelatingseisen voor herbiciden en andere gewasbeschermingsmiddelen. Daarnaast is er een groeiende groep consumenten en telers die zich deels, of soms zelfs volledig, afgekeerd hebben van het gebruik van chemische middelen. Zo wordt er in de ecologische- of biologische landbouw niet alleen afgezien van het gebruik van synthetische gewasbeschermingsmiddelen, maar wordt voor de nutriëntenvoorziening geen gebruik gemaakt van kunstmest.

Telers die zonder gebruik te maken van herbiciden hun onkruidpopulaties moeten beheersen, worden geconfronteerd met een grote uitdaging. Handmatige verwijdering van onkruiden is immers amper een optie, omdat het in de Nederlandse situatie niet mogelijk is om op grote schaal goedkope arbeid in te zetten.

Gelukkig is er in de landbouwmechanisatie flinke voortgang geboekt, onder andere via de ontwikkeling van machines die in staat zijn om onkruiden in de gewasrij te verwijderen. Mechanische bestrijding heeft echter ook nadelen en een totaaloplossing is er met deze vorm van onkruidbestrijding dan ook niet gevonden. Er is daarom dringend behoefte aan nieuwe inzichten voor toepassing van geïntegreerde onkruidbeheerssystemen. Hierbij kan ook lering getrokken worden uit het functioneren van natuurlijke ecosystemen. Natuurlijke ecosystemen kennen een grotere soortenrijkdom dan agro-ecosystemen en zijn stabieler, in die zin dat er minder frequent een plotselinge, explosieve groei van bepaalde planten- of diersoorten optreedt.

Meerdere onderzoekers hebben, naar aanleiding van waarnemingen in natuurlijke ecosystemen, gekeken naar de effecten van een toename in plantendiversiteit in een agro-ecosysteem. Dit type onderzoek richt zich in veel gevallen op het onderdrukken van schade veroorzaakt door herbivore insecten. Er zijn hiervoor meerdere mechanismen denkbaar, maar centraal staat dat in monocultures anders samengestelde insectenpopulaties worden waargenomen dan in agro-ecosystemen die bestaan uit meerdere plantensoorten, de zogenaamde polycultures. Ook voor het onderdrukken van onkruiden kan het laten toenemen van de plantendiversiteit een rol spelen. Het gaat dan niet noodzakelijk om onderdrukking van onkruiden door het introduceren van organismen op een ander trofisch niveau, maar ook om onderdrukking van onkruiden door concurrentie met planten die worden toegevoegd aan het hoofdgewas.

Het onderzoek dat gepresenteerd wordt in dit proefschrift maakte oorspronkelijk deel uit van een breed opgezet onderzoek naar de mogelijk gunstige effecten van het toevoegen van een tweede gewas aan het hoofdgewas. In dit onderzoek werden effecten van deze toevoeging op schadelijke insecten, plantenziekten en het voorkómen van onkruiden bestudeerd. In het eerste hoofdstuk van dit proefschrift wordt een pilot-experiment beschreven dat betrekking heeft op de effecten van het toevoegen van brouwgerst op onkruidonderdrukking in een spruitkoolgewas. De resultaten van de pilot-proef lieten het belang van de keuze van hoofdgewas en toegevoegd gewas zien. Een verkeerde combinatie van plantensoorten kan leiden tot grote schade aan de groei en opbrengst van het hoofdgewas, tot onvoldoende onkruidonderdrukking en/of het verhinderen en bemoeilijken van mechanische onkruidbestrijding. Omdat de combinatie brouwgerst – spruitkool wél geschikt bleek voor onderzoek naar effecten op insectenpopulaties en bodempathogenen maar minder geschikt voor onderzoek naar onkruidonderdrukking werd het deel van de onkruidbeheersing van het project voortgezet met andere plantensoorten.

De keuze voor klaversoorten en de focus van het onderzoek

In verschillende publicaties worden experimenten beschreven met klaversoorten als toegevoegd gewas aan het hoofdgewas. Het is aangetoond dat klaver een gunstige rol kan spelen als het gaat om de vermindering van schade door sommige herbivore insecten en het verminderen van aantasting door sommige plantpathogenen. Klaver kan daarnaast stikstof uit de lucht vastleggen, waardoor de bodemvruchtbaarheid toeneemt. Ook worden diverse klaversoorten geschikt geacht voor de onderdrukking van onkruiden. Desalniettemin is de ontwikkeling van mengteeltsystemen met klaver als toegevoegd gewas niet zo eenvoudig als misschien verwacht. Publicaties waarin het onkruidonderdrukkend vermogen van klaver wordt beschreven, maken meestal ook gewag van de schade die klaver, door middel van concurrentie, aan het hoofdgewas kan toebrengen. Het in dit proefschrift beschreven onderzoek spitst zich toe rond de vraag of klaver als toegevoegd gewas een gunstige rol kan spelen bij het onkruidbeheer, zonder dat de concurrentie tussen de klaver en het hoofdgewas tot onacceptabele opbrengstderving van dit laatste leidt.

Plantkarakteristieken in relatie tot concurrentiekracht

De eerste kwestie die zich aandiende bij het beantwoorden van deze vraag was of de keuze van de klaversoort invloed heeft op het functioneren van het systeem (de tripartite interactie van hoofdgewas, toegevoegd gewas (de klaversoort) en het onkruid). Is het empirisch vast te stellen dat er verschillen in planteigenschappen tussen de onderzochte klaversoorten zijn en hebben deze mogelijke verschillen ook een effect op karakteristieken zoals concurrentiekracht? Bij de beantwoording van deze vraag is eerst gekeken naar de verschillen in plantkarakteristieken (Hoofdstuk 2), terwijl in Hoofdstuk 3 vervolgens wordt ingegaan op de verschillen in concurrentiekracht tussen de klaversoorten. In Hoofdstuk 3 is onderscheid gemaakt tussen effecten op onkruiden, of planten die als model-onkruid fungeerden, en daarnaast de effecten op gewasplanten, waarbij prei is gebruikt als model-gewasplant. De resultaten van de veldexperimenten in 2001 en 2002 toonden aan dat de keuze van de klaversoort van groot belang is. Er waren meetbare verschillen in bijvoorbeeld groeisnelheid, relatieve groeisnelheid en hoogte-ontwikkeling tussen klaversoorten. Zo was Perzische klaver een zeer snelle groeier met een vlotte bodembedekking en deze laatste eigenschap hing samen met een hoge relatieve groeisnelheid (RGR) en een goede startpositie ten gevolge van een snelle kieming en een hoog percentage opgekomen kiemplanten.

Onderaardse klaver daarentegen ontwikkelde zich langzaam en deze soort vertoonde de minste hoogtegroei (maximale hoogte 12 cm). De hoogte-ontwikkeling van de verschillende soorten bleek niet gecorreleerd te zijn met de hoeveelheid biomassa die werd vastgelegd. Zo werd rode klaver 80 cm hoog, maar produceerde deze soort evenveel biomassa als onderaardse klaver, die, zoals gezegd, veel lager bleef. Witte klaver ontwikkelde zich in de beginfase minder snel in vergelijking met Perzische klaver maar herstelde zich snel en produceerde veel biomassa.

Ook qua concurrentiekracht werden grote verschillen tussen klaversoorten aangetoond. Een aantal soorten zoals Perzische klaver, rode klaver, incarnaatklaver, Alexandrijnse klaver en bastaardklaver bleek goed in staat om met onkruiden te concurreren, terwijl onderaardse klaver onkruiden veel minder sterk onderdrukte. Witte klaver bevond zich tussen de sterk- en de zwakconcurrerende soorten in.

De resultaten van Hoofdstuk 3, en van Hoofdstuk 5 in het bijzonder, tonen duidelijk aan dat soorten die in staat waren om de bodem vlot te bedekken ook sterk concurreerden met onkruiden. Perzische klaver is een goed voorbeeld van zo'n soort die, als gevolg van een snelle begingroei, gecombineerd met de productie van relatief dunne bladeren, een snelle toename in bladoppervlak kende.

Het was niet verassend dat verschillende klaversoorten, naast een onkruid-onderdrukkend effect, ook een groeionderdrukkend effect op gewasplanten hadden. Alle klaversoorten hinderden het modelgewas maar ook hier werden forse verschillen tussen klaversoorten gevonden. Rode klaver en bastaardklaver brachten zeer veel schade toe, terwijl witte- en onderaardse klaver daarentegen duidelijk minder schadelijk waren voor het modelgewas.

Het resultaat van een correlatie-analyse toonde aan dat maximale planthoogte het sterkst gecorreleerd was met de aangerichte schade aan het gewas. Omdat onderaardse klaver substantieel minder hoogte-ontwikkeling liet zien dan witte klaver, was het opvallend en onverwacht om te constateren dat beide klaversoorten evenveel schade toebrachten aan het preigewas. Omdat onderaardse klaver regelmatig wordt genoemd als een potentieel geschikt gewas om toe te voegen aan het hoofdgewas, werd dit aspect met een beperkt aantal klaversoorten verder onderzocht. Naast onderaardse klaver en witte klaver werd ook Perzische klaver als sterk contrasterende soort gebruikt.

De concurrentiekracht van onderaardse klaver

De in Hoofdstuk 4 gepresenteerde potproeven hadden tot doel een verklaring te vinden voor de relatief grote schade die onderaardse klaver aan het modelgewas had toegebracht in de eerder gepresenteerde proeven. De hypothese die getoetst werd, was of onderaardse klaver wellicht een sterkere ondergrondse concurrent is dan witte klaver. In deze proeven werd de klavergroei in potten waarin zowel boven- als ondergrondse concurrentie mogelijk was, vergeleken met de groei in potten waarin ondergrondse concurrentie werd uitgesloten. Naast onderaardse en witte klaver werd ook de ondergrondse concurrentiekracht van Perzische klaver en Italiaans raaigras getoetst. Daarnaast werd het effect van bemesting, door het toevoegen van stikstof aan de potten, op concurrentiekracht onderzocht. De resultaten van de potproeven toonden aan dat onderaardse klaver inderdaad een sterke wortelconcurrent was en sterker om ondergrondse hulpbronnen concurreerde dan witte klaver. De ondergrondse concurrentie van onderaardse klaver was ook iets sterker dan die van Perzische klaver, terwijl Italiaans raaigras van de vier geteste soorten duidelijk over de sterkste ondergrondse concurrentiekracht beschikte. De stikstofbemesting had alleen op de concurrentiekracht van de laatstgenoemde soort een effect; deze soort werd een nog sterkere wortelconcurrent bij toenemende hoeveelheden beschikbare stikstof. Voor de concurrentiekracht van de klaversoorten geldt dat geen enkele respons op het toevoegen van stikstof werd waargenomen.

Hoe goed zijn de klavers in staat om onkruiden te verstikken?

In Hoofdstuk 5 wordt vervolgens het laatste deel van het experimentele werk gepresenteerd. In deze proef wordt de onkruidonderdrukkende capaciteit van Perzische-, onderaardse- en witte klaver ten opzichte van een monocotyl model-onkruid (Italiaans raaigras) en een dicotyl model-onkruid (quinoa) onderzocht. In grote plastic bakken werden Perzische-, onderaardse- en witte klaver op verschillende tijdstippen gezaaid, met een interval van twee en een halve week, zodat bakken met een klaverbedekking van verschillende leeftijd gecreëerd werden. In deze klaverbakken werden vervolgens éénmalig onkruidzaden of onkruidkiemplanten ingebracht. De resultaten van dit experiment toonden aan dat onderaardse klaver veel minder goed in staat is om onkruiden te verstikken dan witte klaver en Perzische klaver. Bij de laatste twee soorten was bovendien een veel snellere ontwikkeling van de capaciteit om onkruiden te verstikken waar te nemen dan bij onderaardse klaver. Perzische klaver was het best in staat om de onkruidplanten, afkomstig van zowel de zaden als van de kiemplanten, te verstikken. Het experiment toonde bovendien opnieuw aan dat de capaciteit van klaversoorten om onkruiden te verstikken gerelateerd was aan de, binnen een relatief korte periode, geproduceerde hoeveelheid biomassa. Deze resultaten bevestigen dat de snelle begingroei van Perzische klaver een plausibele verklaring is voor de goede onkruidonderdrukkende eigenschappen van deze soort.

De geschiktheid van klaver als onkruidonderdrukker

In de algemene discussie, Hoofdstuk 6 van dit proefschrift, wordt teruggekeerd naar de centrale onderzoeksvraag en wordt het onderzoek in een breder kader geplaatst. Ook wordt ingegaan op de dilemma's die zich bij het opzetten en uitvoeren van het experimentele werk voordoen. Het gaat dan bijvoorbeeld om de vraag hoe op een zo goed mogelijke wijze gelijke startcondities voor de verschillende soorten gecreëerd kunnen worden. Een andere vraag is hoe een goede vergelijking tussen soorten gemaakt kan worden als het niet gelukt is om gelijke startcondities te creëren. Ook wordt besproken wat de verschillen in plantkarakteristieken betekenen voor de concurrentiekracht van de verschillende klaversoorten, hoe de ideale plantensoort die toegevoegd kan worden, eruit zou kunnen zien en hoe Perzische-, witte- en onderaardse klaver zich verhouden tot zo'n ideale soort.

Geconcludeerd wordt dat de soortskeuze van de onkruidonderdrukker een van de meest bepalende factoren is voor het functioneren van het systeem. De klaversoorten zijn dermate verschillend van elkaar dat, bij gebruik van een andere soort, ook grote verschillen in effecten op onkruiden en op het hoofdgewas te verwachten zijn. Eén van de meest opvallende uitkomsten van het onderzoek was dat onderaardse klaver, de soort die vanwege z'n relatief geringe planthoogte veelvuldig aangeraden wordt als bodembedekker, op geen enkele wijze geschikt lijkt te zijn als onkruidonderdrukker. Door de trage begingroei worden onkruiden onvoldoende verstikt en mede door een sterke ondergrondse concurrentie, loopt het hoofdgewas meer risico op opbrengst-verlies dan ingeschat op basis van planthoogte. Perzische klaver is door zijn snelle groei een prima onkruidonderdrukker, maar de soort veroorzaakt een veel te hoge concurrentiedruk, en zorgt daarmee potentieel voor teveel schade aan een hoofdgewas. Zo kan worden geconcludeerd dat het introduceren van klaver in een hoofdgewas voor een verbeterde onkruidonderdrukking niet mogelijk is zonder aanvullend beheer. Is er geen aanvullend beheer nodig om de schade aan het hoofdgewas te beperken, dan is er wel aanvullende onkruidbestrijding nodig om een voldoende mate van onkruidbeheer te realiseren. Van alle onderzochte klaversoorten lijkt witte klaver als toegevoegd gewas voor onkruidonderdrukking nog het meest veelbelovend, al geldt ook voor deze soort dat aanvullend management, door bijvoorbeeld maaien van de klaver, noodzakelijk is om de schade aan het hoofdgewas te beperken.

Summary

Since the early days of agriculture, weeds have been a nuisance to crop production, mainly through competition for light, water and nutrients. Whether a crop is able to effectively suppress weeds, largely depends on the difference in competitive ability between crop and weed. Competitive ability is a result of a range of characteristics of plants and populations of plants such as early growth, growth rate, earliness of canopy closure, maximum plant height and plant height development. As crop breeding is primarily focused on yield, resistance against pest and diseases and suitability for specific environmental conditions, there is very little attention given to the ability to suppress weed growth. Weeds, on the other hand, are often pioneer plants which are perfectly suited to colonize bare land, such as recently tilled farmland. Even more, weeds are usually growing profusely and are, in accordance with their pioneer character, strong competitors. It is, therefore, not surprising that, without human intervention, weeds often outcompete crops.

Ever since the early stages of agriculture, weed control methods have been developed. In the past, weed management was labour intensive and time consuming, whereas nowadays weed management is dominated by 'high tech' approaches that, mainly because of the availability of synthetic herbicides, enable a farmer to keep large areas of arable land nearly weed-free. On top of that, herbicide tolerant crops have been developed that allow for the application of broad-spectrum herbicides after crop emergence. At the same time, the drawbacks of an over-use of pesticides have become evident. The damage to the environment and the negative publicity around it has led to an increased awareness within the general public, policymakers and governments. This has resulted in stricter regulations regarding the use and admission of crop protection agents, such as herbicides, pesticides and fungicides. In addition, there are a growing number of consumers and farmers who favour agricultural production systems that are less reliant on, or completely exclude, the use of synthetic products such as crop protection compounds and synthetic fertilizers. Farmers that strive to control their weeds without the use of herbicides are faced with a significant challenge. In countries like the Netherlands manual weeding is difficult because of lack of labour and the enormous costs that are involved. Fortunately, mechanization has made a significant progress through, for example, the development of in-row weeding devices that are able to remove weeds in the crop row. Mechanical weeding on the other hand does not offer a complete solution and has its own drawbacks. This is why there is an urgent need for new insights in how to strengthen integrated weed management systems. In this regard, lessons might be learned from the functioning of

natural ecosystems, where species richness creates more stable circumstances and sudden dramatic increases in population density of certain species seem to occur less frequently.

Based on observations in natural ecosystems, a number of researchers have investigated the influence of plant species diversity on agro-ecosystem functioning. This type of research has often been focused on how to decrease damage caused by herbivorous insects. A number of underlying mechanisms have been proposed. Most of these have in common that the insect populations found in polycultures have a different composition compared to those in monocultures. In relation to the management of weed populations, enhanced plant species diversity was found to be a significant factor in weed control. Not necessarily through weed suppression by insects, but also through increased competition between weeds and the plant species that are added as a companion cover crop, to the main crop.

The research that is presented in this thesis was originally part of an integrated project on the potential benefits of adding a second plant species to the main crop. In this integrated research project, effects of the companion crop on herbivorous insects, plant pathogens and the suppression of weeds were investigated. In the first chapter of this thesis a pilot-study is presented on the effects of the addition of barley to Brussels sprouts. The results of the pilot-experiment showed the importance of choosing a suitable combination of main and companion crop, as the growth and yield formation of Brussels sprouts was severely reduced and barley provided a very poor suppression of weeds, whereas it also hindered the application of mechanical weeding. As the combination of barley – Brussels sprouts appeared to be suitable for research on insect populations and soil-borne pathogens the research on weed suppression was separated and continued by using a different set of plant species.

The aim of the research project and reasons for using clover species

Various publications describe experiments with clover as a companion crop. It has been shown that adding clover to the main crop can in some instances reduce damage by herbivorous insects and plant pathogens. Furthermore, clover has the ability to fix nitrogen from the air which enhances soil fertility. In addition, various clover species are considered to be suitable as a weed suppressor. There are many questions remaining regarding the use of clover species as a companion crop in mixed cropping systems. Publications that address the introduction of clover as a companion cover crop often mention the detrimental effects clover can have on the main crop through competition. The research project described in this thesis focused on the question whether clover can be added as a companion cover crop for weed suppression without substantially harming the main crop.

Plant characteristics in relation to competitiveness

The first issue that was addressed in an attempt to answer this question was whether the choice of clover species matters. Is there empirical evidence for differences in traits between clover species and do these differences have an effect on species characteristics such as competitive ability? In addressing these questions, differences in species traits were first assessed in Chapter 2, while in Chapter 3, the competitive strength of a range of clover species was investigated. Also in Chapter 3, a distinction was made between effects of clover on weeds or plant species that were used as model weeds and the effects on crop plants, in this case transplanted leek. The results of the field trials conducted in 2001 and 2002 clearly showed the importance of the choice of clover species. There were measurable differences in a range of parameters such as growth rate, relative growth rate (RGR) and height development of the clover species. Persian clover grew fast and covered the soil rapidly. Its ability to reach full canopy closure so quickly was related to a high RGR and a good starting position as a result of rapid germination and a high fraction emergence. Subterranean clover on the other hand developed slowly and this species only reached a maximum plant height of 12 cm. Height development of the various clover species did not appear to be correlated to the amount of accumulated shoot dry matter. Red clover, for example, reached a plant height of 80 cm but this species accumulated the same amount of shoot dry matter as subterranean clover. White clover initially developed relatively slowly compared to Persian clover, but this species recovered and ultimately accumulated a similar amount of biomass.

Large differences were also observed regarding the competitive effect of clover species. Weed suppression of a number of species, particularly Persian, red, crimson, berseem and alsike clover was superior, whereas subterranean clover exhibited poor weed suppression. White clover held an intermediate position. The results of Chapter 3, and Chapter 5 in particular, clearly show that species that covered the soil rapidly were also able to compete strongly with weeds. Persian clover is a good example of such a species; its ability to accumulate biomass rapidly, combined with the production of thin leaves resulted in a quick leaf area development. Not surprisingly, the addition of the clover species did not only result in weed suppression, but also affected the growth of the main crop. All species hindered the growth of transplanted leek severely, but also for this feature clear differences between clover species were observed. Red clover and berseem clover caused the highest reductions, whereas white clover and subterranean clover caused less damage than the other clover species. Correlation analysis showed that maximum plant height was the clover trait that correlated most strongly with the damage to the main crop. As subterranean clover was significantly shorter than white clover, it was quite remarkable that subterranean and white clover caused similar amounts of damage to the leek crop. Particularly since subterranean clover is often considered a suitable companion crop, this aspect was further investigated with a subset of clover species. Apart from subterranean clover and white clover, Persian clover was included as a contrasting third species.

The competitive ability of subterranean clover

The purpose of the pot experiments, presented in Chapter 4, were to find a possible explanation for the relatively high level of damage that subterranean clover imposed on the model crop. The hypothesis tested was that subterranean clover was a stronger competitor for below-ground resources than white clover. In the experiments, growth of clover in pots that allowed above- as well as below-ground competition were compared with the growth of clover in pots in which a PVC tube prevented below-ground competition. Apart from subterranean and white clover, the below-ground competitive ability of Persian clover and Italian rye grass were tested. Additionally, the effect of nitrogen availability on the competitiveness of these species was investigated. The results of the pot experiments showed that subterranean clover was indeed a strong root competitor that out-competed white clover for below-ground resources. The ability of subterranean clover to compete for below-ground resources was even slightly stronger than that of Persian clover while Italian rye grass was clearly the species with the strongest ability for below-ground competition. Nitrogen availability only had an effect on the competitive strength of Italian rye grass, as this species became an even stronger root competitor with increasing amounts of available nitrogen. No effect of nitrogen availability on the competitive strength of any clover species tested was observed.

The smothering ability of clover species

Finally, in Chapter 5, the remaining part of the experimental work was presented. In this experiment, the weed-suppressive ability of Persian, subterranean and white clover was further compared using a monocot (Italian rye grass) and a dicot (quinoa) model weed species. Three different sowing dates, with intervals of two to three weeks, were used to create clover canopies in different stages of development for each of the three clover species. In these clover plots, both weed seeds and weed seedlings were planted at one moment in time, when clover canopies were between 3.5 – 8.0 weeks old. The results of this experiment showed that subterranean clover was unable to smother weeds to the same extent as white and Persian clover. Furthermore, the latter two species showed a much quicker development in their weed-smothering ability than subterranean clover. Persian clover was clearly the species with the strongest ability to smother weed plants. The results of the experiment re-inforced a strong correlation between smothering ability and the amount of shoot dry matter that was accumulated. These results confirm that the rapid early growth of Persian clover is the most likely explanation for its strong weed-suppressing ability.

Suitability of clover species for weed suppression

In the general discussion (Chapter 6) attention is focused on the main research question of this thesis and the research project is placed in a broader context. Various dilemmas that were encountered throughout the experimental phase of the research project are also mentioned and discussed. For example, how to create equal starting conditions for species differing in seed size and how to handle such comparisons if equal starting conditions cannot be realized. Also, the differences in plant characteristics between clover species and how these relate to differences in competitive ability is discussed. Furthermore, the characteristics of the ideal companion crop for weed suppression are described and it is discussed how Persian, white and subterranean clover compare to such an ideal species.

It was concluded that the choice of clover species is one of the most determining factors for functioning of the mixed crop system. The characteristics of the various clover species differ to such a degree that the choice of species creates large differences in the suppression of weeds and the competitive damage to the main crop. One of the most striking results of this thesis research is that subterranean clover, the species that, because of its low plant height, is often referred to as a suitable cover crop, does not seem to meet its expectations. On the one hand, its slow early development results in an insufficient smothering of weeds, whereas on the other hand, its strong ability to compete for below-ground resources contributes to a greater risk on yield reduction of the main crop than would be expected based on its maximum plant height. The weed-suppressive ability of Persian clover is exceptional, but the strong competitive pressure it puts on the main crop will eventually cause too much yield loss. In conclusion, it can be stated that the introduction of a companion clover crop for enhanced weed suppression is not possible without additional management. This management will be required to either reduce the competitive damage to the main crop, or to attain an adequate level of weed management. Of all clover species that were investigated, white clover appears to be the most promising species for use as a weed suppresser in a mixed cropping design. However, additional management will still be necessary to minimize the competitive damage of this species to the main crop.

Résumé

Depuis les débuts de l'agriculture, les mauvaises herbes ont été une nuisance pour la production de cultures, principalement du fait de la compétition pour la lumière, l'eau et les nutriments. La capacité d'une culture à lutter contre les adventices dépend en grande partie de la différence de compétitivité entre la culture et la mauvaise herbe en question. La compétitivité est le résultat d'un ensemble de caractéristiques des plantes et des populations de plantes tels que la croissance précoce, le taux de croissance, la précocité de la fermeture de la couverture végétale, la hauteur maximale et l'évolution de la hauteur des plantes. La production agricole étant principalement ciblée sur le rendement, sur la résistance aux ravageurs et aux maladies et sur l'adaptabilité à des conditions environnementales spécifiques, très peu d'attention est portée sur la capacité d'une plante à lutter contre les mauvaises herbes. Par ailleurs, les adventices sont souvent des plantes pionnières qui sont parfaitement adaptées à la colonisation de terres nues, comme les terres agricoles récemment labourées. De plus, les mauvaises herbes ont généralement une croissance profuse et ont, conformément à leur caractère pionnier, une forte compétitivité. Il n'est donc pas surprenant de constater que, sans l'intervention de l'homme, les adventices gagnent sur les cultures.

Des méthodes de lutte contre les mauvaises herbes ont été développées depuis les premiers pas de l'activité agricole. Par le passé, la lutte contre les adventices demandait beaucoup de main-d'œuvre et de temps, alors que de nos jours cette lutte est dominées par les approches 'haute technologie' qui, grâce à la mise à disposition d'herbicides synthétiques, permettent à l'agriculteur de conserver de larges parcelles de terre arable sans mauvaises herbes. En plus de cela, des cultures tolérantes aux herbicides ont été développées, permettant ainsi l'application d'herbicides à large spectre après levée de la culture. Dans le même temps, les désavantages de la sur-utilisation de pesticides sont devenus manifestes. Les dégâts sur l'environnement et la publicité négative à ce sujet ont conduit à une augmentation de la prise de conscience du public, des décideurs politiques et des gouvernements, avec pour conséquence une réglementation plus stricte en matière d'utilisation et d'accès autorisé à des agents de protection des cultures tels que les herbicides, pesticides et fongicides. Par ailleurs, un nombre croissant de consommateurs et d'agriculteurs sont en faveur d'un système de production agricole moins dépendant de, ou excluant complètement, l'utilisation de produits synthétiques. Les agriculteurs qui s'efforcent à lutter contre les mauvaises herbes sans avoir recours à des herbicides font face à un défi majeur. Dans des pays comme les Pays-Bas, le désherbage manuel est difficile du fait du manque de main d'œuvre et de l'importance des coûts associés. Heureusement, la mécanisation a fait des progrès importants à travers, par exemple, le développement de dispositif de désherbage capable d'enlever les adventices dans les rangs de cultures. Le désherbage mécanique néanmoins n'offre pas une solution complète et a ses propres désavantages. C'est pourquoi il y a un besoin urgent d'idées nouvelles sur la manière de renforcer les systèmes intégrés de lutte contre les adventices. A cet égard, des leçons peuvent être tirées du fonctionnement des écosystèmes naturels, où la richesse des espèces crée un terrain plus stable et où les augmentations soudaines et notables de densité de population de certaines espèces semblent survenir moins fréquemment.

Sur la base de l'observation des écosystèmes naturels, des chercheurs ont enquêté sur l'influence de la diversité des espèces sur le fonctionnement des agro-écosystèmes. Ce type de recherche a souvent porté son attention sur la façon de réduire les dégâts causés par les insectes herbivores. Un certain nombre de mécanismes sous-jacents ont été proposés. La plupart de ces derniers ont en commun le fait que les populations d'insectes rencontrées en polycultures ont une composition différente de celles rencontrées en monocultures. Par rapport à la gestion des mauvaises herbes, la diversité accrue des espèces de plantes apparaît comme un facteur majeur de contrôle des adventices. Pas forcément du fait de la suppression des mauvaises herbes par les insectes, mais aussi grâce à la compétition accrue existante entre les adventices et les espèces de plantes ajoutées comme plantes compagnes couvre-sol à la culture principale.

Les travaux de recherche présentés dans cette thèse faisaient à l'origine partie d'un projet intégré sur les avantages potentiels de l'ajout d'une deuxième espèce de plante à une culture principale. Dans ce projet de recherche intégrée les effets de la plante compagne sur les insectes herbivores, les pathogènes des plantes et la suppression des mauvaises herbes ont été étudiés. Dans le premier chapitre de cette thèse, une étude pilote présente les effets de l'addition de l'avoine sur les choux de Bruxelles. Les résultats de cette expérience pilote ont montré l'importance du choix d'une combinaison entre culture principale et plante compagne: la croissance et le rendement des choux de Bruxelles ont été sévèrement réduits et l'avoine n'a conduit qu'à une très faible suppression des adventices, tout en empêchant par ailleurs la mise en œuvre de désherbage mécanique. Alors que la combinaison avoine – choux de Bruxelles est apparu comme adaptée pour la recherche sur les populations d'insectes et les pathogènes du sol, la recherche sur la suppression des adventices a été rompue et poursuivie avec l'utilisation d'autres espèces de plantes.

L'objectif de ce projet de recherche et les motivations quant à l'utilisation d'espèces de trèfle

De nombreuses publications décrivent des expériences où le trèfle est utilisé comme

plante compagne. Il a été démontré que l'addition de trèfle à la culture principale peut, dans certaines situations, réduire les dégâts occasionnés par les insectes herbivores et les pathogènes des plantes. Par ailleurs, le trèfle a l'habilité de fixer l'azote de l'air ce qui renforce la fertilité du sol. De plus, plusieurs espèces de trèfle sont considérées comme adaptées à la lutte des adventices. De nombreuses questions demeurent néanmoins quant à l'utilisation d'espèces de trèfle comme plantes compagnes dans des systèmes de culture mixte. Les publications adressant l'introduction du trèfle comme plante compagne mentionnent souvent les effets négatifs que peut avoir le trèfle sur la culture principale du fait de la compétition. Le projet de recherche décrit dans la présente thèse se concentre sur la question de savoir si le trèfle peut être ajouté entant que plante compagne pour la lutte contre les mauvaises herbes sans nuire considérablement à la culture principale.

Les caractéristiques des plantes par rapport à leur compétitivité

Afin de répondre à cette question, la première étape a été d'évaluer si le choix de l'espèce de trèfle avait de l'importance. Y a-t-il des preuves empiriques de différences de traits entre les espèces de trèfle et est-ce que ces différences ont un effet sur les caractéristiques de l'espèce telles que sa compétitivité? Afin de répondre à ces questions, une évaluation a tout d'abord été faite dans le chapitre 2 des différences de traits des espèces, alors que dans le chapitre 3 la compétitivité de diverses espèces de trèfle a été étudiée. Par ailleurs, dans le chapitre 3, une distinction a été faite entre les effets du trèfle sur des mauvaises herbes ou des espèces de plante utilisées comme adventice modèle, et les effets sur des plantes de culture, dans le cas présent du poireau transplanté. Les résultats des expériences de terrain effectuées en 2001 et 2002 ont nettement montré l'importance du choix de l'espèce de trèfle. Des différences ont été mesurées sur une série de paramètres tels que le taux de croissance, le taux de croissance relative (TCR) et l'évolution de la hauteur des espèces de trèfle. Le trèfle persan a poussé rapidement et couvert le sol très vite. Sa capacité à atteindre la fermeture du couvert végétal était liée à son fort TCR et à sa bonne position de départ du fait d'une germination rapide et de la levée d'une grande fraction. Le trèfle souterrain pour sa part s'est développé lentement et cette espèce a atteint une hauteur de plante maximale de 12 cm. L'évolution de la hauteur des différentes espèces de trèfle n'est pas apparue comme corrélée à la quantité amassée de matière sèche au niveau de ses pousses. Le trèfle rouge par exemple a atteint une hauteur de plante maximale de 80 cm mais cette espèce a amassé la même quantité de matière sèche au niveau de ses pousses que le trèfle souterrain. Le trèfle blanc s'est initialement développé relativement lentement par rapport au trèfle persan, mais cette espèce a récupéré et finalement amassé une quantité de biomasse similaire.

De larges différences ont aussi été observées quant à la compétitivité des différentes espèces de trèfle. Ainsi un nombre d'espèces, notamment le trèfle persan, le trèfle rouge, le trèfle incarnat, le trèfle d'Alexandrie et le trèfle hybride, ont montré une bonne suppression des mauvaises herbes, contrairement au trèfle souterrain. Le trèfle blanc a occupé une position intermédiaire. Les résultats des chapitres 3 et 5 en particulier, montrent clairement que les espèces couvrant le sol rapidement étaient aussi celles capable de faire concurrence avec les adventices. Le trèfle persan est un bon exemple d'une telle espèce; sa capacité à amasser de la biomasse, combiné à la production de fines feuilles a conduit à une évolution rapide de sa surface foliaire. Comme on pouvait s'y attendre, l'addition d'espèces de trèfle a non seulement conduit à une suppression des mauvaises herbes, mais a aussi affecté la croissance de la culture principale. Toutes les espèces de trèfle ont sérieusement entravé la croissance du poireau transplanté, mais là aussi des différences nettes ont été observées entre les différentes espèces. Le trèfle rouge et le trèfle d'Alexandrie ont causé les réductions les plus élevées, alors que le trèfle blanc et le trèfle souterrain ont causé des dégâts moins importants que les autres espèces de trèfle. L'analyse de corrélation a montré que la hauteur maximale de la plante était le trait du trèfle le plus fortement corrélé aux dégâts occasionnés sur la culture principale. Le trèfle souterrain étant sensiblement plus petit que le trèfle blanc, il a été surprenant de constaté que ces deux espèces de trèfle ont endommagé de façon similaire la culture de poireau. Le trèfle souterrain étant souvent considéré comme une plante compagne adaptée, cet aspect a été étudié de façon plus approfondie avec une plus petite sélection d'espèces de trèfle. Afin de contraster avec les trèfles souterrains et trèfles blancs, le trèfle persan a été inclus comme troisième espèce.

La compétitivité du trèfle souterrain

L'objectif des expériences en pots présentées dans le chapitre 4 a été de trouver une explication possible quant au niveau de dégâts relativement élevé causés par le trèfle souterrain sur la culture principale. L'hypothèse testée était que le trèfle souterrain est un concurrent plus fort pour les ressources sous-terraines que le trèfle blanc. Dans les expériences, la croissance du trèfle dans des pots permettant la compétition aussi bien aérienne que souterraine a été comparée avec la croissance du trèfle dans des pots où un tube de PVC empêchait la compétition sous-terraine. En dehors du trèfle souterrain et du trèfle blanc, la compétitivité souterraine du trèfle persan et le ray-grass d'Italie a elle aussi été testée. De plus, l'effet de la disponibilité en azote sur la compétitivité de ces espèces a été étudié. Les résultats de ces expériences en pots ont montré que le trèfle souterrain était en effet un fort concurrent au niveau des racines et qu'il gagnait de ce fait sur le trèfle blanc quant aux ressources souterraines. La capacité du trèfle souterrain à faire concurrence pour les ressources souterraines était même légèrement supérieure à celle du trèfle persan alors que le ray-grass d'Italie était clairement l'espèce avec la plus forte compétitivité souterraine. La disponibilité en azote a seulement eu un effet sur la compétitivité du ray-grass d'Italie: plus la quantité d'azote disponible était importante, plus forte était la compétitivité de cette espèce au niveau des racines. La disponibilité en azote n'a montré aucun effet sur la compétitivité des espèces de trèfle testées.

La capacité d'étouffement des espèces de trèfle
Finalement, le chapitre 5 présente la dernière partie des travaux d'expérimentation. Dans cette expérience, la capacité de lutte contre les mauvaises herbes des trèfles persans, souterrains et blancs a été comparée de façon plus approfondie en utilisant un adventice modèle monocotylédone (ray-grass d'Italie) et dicotylédone (quinoa). Trois dates d'ensemencement différentes, avec des intervalles de deux à trois semaines, ont été utilisées afin de créer des couverts végétaux à différentes étapes de développement pour chacune des trois espèces de trèfle. Dans ces parcelles de trèfle, les graines et plantules d'adventices ont été plantées ensemble à un moment donné, quand le couvert végétal avait entre 3.5 et 8.0 semaines. Les résultats de cette expérience ont montré que le trèfle souterrain a été incapable d'étouffer les mauvaises herbes dans la même mesure que l'ont fait le trèfle blanc et le trèfle persan. Par ailleurs, ces deux dernières espèces ont montré un développement beaucoup plus rapide de leur capacité d'étouffement que le trèfle souterrain. Le trèfle persan a été nettement l'espèce avec la plus forte capacité à étouffer les adventices. Les résultats de cette expérience renforcent la forte corrélation entre la capacité d'étouffement et la quantité de matière sèche de pousses amassée. Ces résultats confirment que la croissance précoce rapide du trèfle persan est l'explication la plus plausible à sa forte capacité de suppression des mauvaises herbes.

Aptitude des espèces de trèfle à la lutte contre les mauvaises herbes
La discussion générale (chapitre 6) porte son attention sur la principale question de recherche de la présente thèse et place le projet de recherche dans un contexte plus large. Les dilemmes variés rencontrés au cours de la phase expérimentales du projet de recherche sont mentionnés et discutés. Par exemple, comment créer des conditions de départ égales pour des espèces ayant des tailles de graine différentes, et comment traiter de telles comparaisons si des conditions de départ égales ne peuvent être réalisées. Sont discutées aussi les différences de traits entre espèces de trèfle et comment celles-ci sont liées aux différences de compétitivité. Par ailleurs, les caractéristiques de la plante compagne idéale pour la lutte contre les adventices sont

décrites et les trèfles persans, blancs et souterrains sont comparés à une telle espèce idéale.

Il a été conclu que le choix de l'espèce de trèfle est le facteur le plus déterminant au fonctionnement d'un système de culture mixte. Les caractéristiques des différentes espèces de trèfle diffèrent d'un tel degré que le choix de l'espèce créé de larges différences en termes de suppression des mauvaises herbes et de dégâts occasionnés à la culture principale. Un des résultats les plus remarquable de cette thèse de recherche est que le trèfle souterrain, espèces qui du fait de sa faible hauteur de plante est souvent considéré comme une plante compagne adaptée, ne semble pas répondre à ce qu'on attend de lui. D'une part la lenteur de son développement précoce donne lieu à un étouffement insuffisant des mauvaises herbes, d'autre part sa capacité à faire concurrence pour les ressources souterraines contribue à un plus grand risque de réduction de rendement de la culture principale qu'il ne serait attendu en se basant sur la hauteur de plante maximale. La capacité de suppression des adventices du trèfle persan est exceptionnelle, mais la pression compétitive exercée sur la culture principale finira par engendrer une perte de rendement trop importante. En conclusion, on peut déclarer que l'introduction d'une culture de trèfle compagne pour l'amélioration de la suppression des mauvaises herbes n'est pas possible sans d'autres mesures de gestion. Ces mesures de gestion seront nécessaires soit pour réduire les dégâts occasionnés par la pression compétitive sur la culture principale, soit pour atteindre un niveau suffisant de lutte contre les adventices. De toutes les espèces étudiées, le trèfle blanc apparaît comme l'espère la plus prometteuse pour être utilisée comme suppresseur de mauvaises herbes dans un système de culture mixte. Cependant, des mesures de gestions complémentaires seront tout de même nécessaires pour minimiser les dégâts occasionnés par la pression compétitive sur la culture principale.

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PE&RC PhD Education Certificate

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

Review of literature (4.5 ECTS)
- Clover as a cover for weed suppression

Writing of project proposal (3 ECTS)
- Clover as a cover crop for weed suppression

Post-graduate courses (5 ECTS)
- Advanced statistics; WUR (2002)
- PE&RC Winterschool 2002; organizing committee (2002)
- Multivariate analysis; WUR (2004)

Invited review of (unpublished) journal (1 ECTS)
- Weed research; Crop weed interactions (2003)

Deficiency, refresh, brush-up courses (4.5 ECTS)
- Simulation of ecological processes (2001)
- Basic statistics (2001)

Competence strengthening / skills courses (1.5 ECTS)
- Scientific writing; WUR (2001)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)
- PE&RC Annual meeting (2000/2004)

Discussion groups / local seminars / other scientific meetings (6.6 ECTS)
- Plant and crop ecology (2000/2005)
- Workshop agrobiodiversiteit (2004)

International symposia, workshops and conferences (8.3 ECTS)
- 12th EWRS Symposium; Papendal (2001)
- EWRS Working group Crop-weed interactions; Wageningen (2001)
- EWRS Working group Crop-weed competitive interactions; oral presentation; Viterbo, Italy (2003)
- EWRS Working group Weeds and biodiversity; oral presentation, abstract on internet; Bristol, UK (2003)
- INRA-EWRS 12th International conference on Weed Biology; oral presentation; Dijon, France (2004)

Lecturing / supervision of practical's / tutorials (5.5 ECTS)
- Computerpracticum Populatiedynamiek; 6 days (2002)
- PGO Populatie ecologie; 5 days (2003)
- Computerpracticum Populatiedynamiek; 6 days (2003)

Supervision of 1 MSc student (3 ECTS)
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After having been employed by the Royal Tropical Institute, I applied for a PhD position within the 'Enhanced biodiversity for sustainable crop protection' project of which this thesis is the result. Martin, I would like to thank you for giving me a chance, I appreciate your enthusiasm, problem solving attitude, openness and friendliness.

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Curriculum vitae

Nicolaas Gerard den Hollander was born on 6 July 1970 in Heijningen. He graduated from the Gertrudislyceum (Atheneum) in 1989. He is a graduate from the Hogeschool West-Brabant (1993; environmental technology) and the University of Amsterdam (1996; biology).

He is currently employed by the Netherlands Organisation for Scientific Research (NWO, The Hague). He is the overall coordinator of the Innovational Research Incentives Scheme (the Veni, Vidi and Vici grant scheme).

Between June 2010 and September 2011 he was invited by the Netherlands Association of Universities (VSNU, The Hague) to take up the position of policy officer. He was leading discussion groups (university policy officers and liaison officers) and was the secretary of the steering group Research and Valorisation which was chaired by the Rector Magnificus and President of Leiden University. He worked on university profiles, thematic research priorities, research funding (national and EU) 'Topsectoren' policy and quality management. He has an extensive network and has been representing NWO and VSNU in various settings (OCW, EL&I, KNAW, universities, companies, VNO-NCW, KIA, ERC, DG for Research and Innovation).

He has been employed by NWO since 2005 and has gained extensive experience in management processes, selection procedures, finances, and policy making. He has been responsible for the development and implementation of various subsidy programmes such as Casimir, Toptalent and the Graduate Programme.

He held a PhD student position between 2000 and 2005 at Wageningen University and this thesis is the final result. He worked as a junior researcher between 1999 and 2000 at the Research Institute for Agro-Biology and Soil Fertility (Wageningen), and was employed by the Royal Tropical Institute, Department of Agriculture and Enterprise Development (Amsterdam) between 1996 and 1999. He was working on the project 'Management of aquatic weeds in the Senegal River Basin'. As a student he carried out research in 1995 for the International Maize and Wheat Improvement Center (CIMMYT) at the Kibos Sugar Research Station (Kisumu, Kenya). In 1993, he was working as a student at the Dutch Sea Research Institute (NIOZ, Texel) where he studied the effect of shellfish fisheries on the shellfish population (*Spisula subtruncata*) in the Dutch Wadden Sea.

He is married to Martine Leman, Director Advisory Group Oil & Gas of Royal Haskoning, and they have two children, Annabel and Luca.