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Summary

Application of herbicides such as glyphosate has been the main weed control method on hard surfaces in most European cities. In recent years, however, several countries have implemented federal restrictions on the use of herbicides on hard surfaces due to the risk of leaching herbicides into ground or surface water. In Denmark, local authorities and state institutions have signed a voluntary agreement in 1998 about a total phase-out of herbicides on public areas. Therefore, many public authorities rely on the use of non-chemical weed control methods, primarily flame weeding. Whereas glyphosate provides an almost complete kill of the plant, all non-chemical methods mainly affect the above ground plant parts. Effective weed control with non-chemical methods therefore requires repeated treatments, but this also implies increased costs.

Most basic studies on the effect of thermal weed control methods cover the effect of a single treatment. However, weed species with protected growth meristems and/ or root-propagation will usually regrow after a single treatment even at high doses of energy. This thesis aims at a profound study of how weeds that are considered tolerant to flaming respond to repeated treatments. Knowledge of how repeated treatments influence the regrowth of weeds is essential to plan strategies for non-chemical weed control.

Three experimental designs were chosen to study how time period between treatments affected weed regrowth. Dose-response experiments conducted over two growth seasons showed that six treatments a year and a total dose estimated to around 631-674 kg propane ha^{-1}, depending on year, controlled *Lolium perenne* L. effectively (90% reduction in dry weight). The results emphasized the importance of applying a dose that is sufficient high in order to kill all above ground leaves. In this way, the number of treatments per season can be reduced, which is more economically feasible than carrying out more treatments with a lower dose.

The impact of time interval between flaming treatments on the regrowth and flower production of two grasses was studied in experiments on a constructed hard surface. Flaming treatments decreased plant biomass of *L. perenne* and *Poa annua* L. and also the ratio of flowering *P. annua* plants. However, only few plants were killed. The first flaming treatment affected aboveground biomass more than the second flaming treatment. A treatment interval of seven days provided the greatest reduction in regrowth of *L. perenne*, whereas the effect of

treatment interval varied between the first and second repetition of this experiment for *P. annua*.

Three years *in situ* experiments on traffic islands revealed the effect of different weed control methods on weed cover and estimated the number of required treatments. In 2004, weed cover could be kept on an acceptable level with eight flame treatments a year and a mean dose of 150 kg propane ha^{-1} per treatment. The doses were higher than planned due to the irregular shape of the traffic islands, overlap and impediments such as traffic signs. The weed flora was dominated by perennial grasses, especially the salt-tolerant species *Puccinellia distans* (Jacq.) Parl. In 2005 and 2006 weed cover was recorded every second week and treatments were carried out every time the weed cover exceeded 1.6% (1.8 % after mid July). In this way an estimation of the number of treatments needed to keep weed cover below a certain acceptance level could be obtained. On average during the two seasons, the following number of treatments was required: Glyphosate 2.5, hot water 3, flames 5, hot air/ flames or steam 5.5 treatments. It was expected that the number of required treatments with the non-chemical weed control methods would be higher, especially when the present weed flora of perennial weeds and grasses is taken into consideration. The results indicated that regular thermal treatments decreased the treatment frequency in the course of time, as fewer treatments were required to keep weed cover below the acceptance level in the third experimental year.

In conclusion, the results emphasise that it *is* possible to obtain acceptable control of larger plants and heat tolerant weeds such as grasses and perennial weeds with thermal weed control methods. However, one or two treatments may not kill them even at very high doses of energy. If it is assured that the dose is sufficiently high at each treatment, then the number of treatments per season can be reduced. Another way to reduce the number of treatments per year is to assess the need for weed control regularly by a simple method and adjust the weed control effort to the required visual street quality.

Sammendrag (Danish summary)

Ukrudt på faste belægninger har hidtil kunnet bekæmpes med herbicider, primært midler med aktivstoffet glyfosat, men herbicider kan være en trussel mod miljøet, især grundvandet. Derfor har mange lande indført restriktioner mod anvendelsen af herbicider. I Danmark indgik staten, amterne og kommunerne i 1998 en aftale om at udfase anvendelsen af herbicider på offentlige arealer. Mange offentlige myndigheder er derfor nødsaget til at bekæmpe ukrudt med pesticidfri metoder, især termisk bekæmpelse med gasbrændere. De termiske metoder virker primært på plantens overjordiske dele, hvorimod glyfosat-holdige midler virker systemisk på hele planten, også rødder og rhizomer. Effektiv bekæmpelse med de termiske metoder kræver derfor gentagne behandlinger for at undgå genvækst fra beskyttede vækstpunkter og/ eller underjordiske regenerative organer. Det øger udgifterne til ukrudtsbekæmpelsen.

De fleste kontrollerede undersøgelser af effekten af termisk ukrudtsbekæmpelse bygger på forsøg med kun en enkelt behandling. Men selv ved høje energidoseringer vil der være nogle ukrudtsarter, især græsser, som kan overleve og fortsætte deres vækst. Formålet med dette ph.d. projekt er at udføre en grundig undersøgelse af hvordan ukrudtsarter, som anses for at være tolerante over for termiske behandlinger, reagerer ved gentagne behandlinger. Viden om hvordan gentagne behandlinger påvirker ukrudtets genvækstevne er essentiel for at kunne planlægge langsigtede bekæmpelsesstrategier.

Tre forskellige forsøgsdesign var udvalgt for at kunne undersøge hvordan forskellige tidsintervaller mellem termiske behandlinger påvirkede ukrudtplanters genvækst. Dosis-respons markforsøg over to vækstsæsoner viste at seks flammebehandlinger med en total dosering på 631-674 kg gas ha^{-1}, afhængigt af forsøgsår, medførte et acceptabelt bekæmpelsesniveau for alm. rajgræs (*Lolium perenne* L., 90 % reduktion i tørvægt). Resultaterne understreger betydningen af at udføre behandlinger med tilstrækkelig høj dosering for at slå alle overjordiske plantedele ihjel. På den måde kan antallet af behandlinger per vækstsæson reduceres, og det er mere økonomisk rentabelt end at udføre flere behandlinger med en lavere dosering.

Betydningen af forskellige intervaller mellem flammebehandlinger for to græssers genvækst- og blomstringsevne blev undersøgt i forsøg som blev udført på et simuleret befæstet areal. Flammebehandlinger nedsatte biomassen af alm. rajgræs og enårig rapgræs

(*Poa annua* L.), og behandlingerne reducerede også enårig rapgræs' blomstring. Der var dog kun få planter som døde af behandlingerne. Den første behandling havde relativ større effekt på planternes overjordiske biomasse end en efterfølgende behandling. Et behandlingsinterval på 7 dage gav den største reduktion i overjordisk biomasse for alm. rajgræs, hvorimod effekten af de forskellige behandlingsintervaller varierede mellem de to gentagelser af forsøget for enårig rapgræs.

Tre års *in situ* forsøg på hellearealer var designet for at afdække effekten af forskellige bekæmpelsesmetoder på ukrudtsdækningsgrad og estimere antallet af nødvendige behandlinger per vækstsæson. I det første år kunne ukrudtets dækningsgrad holdes på et acceptabelt niveau med otte flammebehandlinger og en dosering på omkring 150 kg gas ha^{-1} per behandling. Den høje dosering skyldtes hellernes ujævne form, overlap og forskellige forhindringer på hellerne, for eksempel skilte. Ukrudtsfloraen var domineret af flerårige græsser, især den salttolerante art udspærret annelgræs (*Puccinellia distans* (Jacq.) Parl.). De efterfølgende to år var ukrudtets dækningsgrad bestemmende for antallet af behandlinger. Det blev målt hver anden uge, og hver gang dækningsgraden oversteg 1,6 % (1,8 % efter midten af juli), blev der udført en behandling. På den måde kunne det estimeres hvor mange behandlinger der var nødvendige for at opretholde det valgte tilstandskrav. Følgende antal behandlinger var nødvendige i gennemsnit per år: Glyfosat: 2,5; Hedvand: 3; Flammer: 5; hedluft/ flammer: 5,5; Damp: 5,5. Antallet af termiske behandlinger var overraskende lavt, især når man tager sammensætningen af ukrudtsfloraen i betragtning. Resultaterne indikerede at en jævnlig bekæmpelse med termiske behandlinger nedsatte behandlingshyppigheden med tiden, idet færre behandlinger var nødvendige for at overholde tilstandskravet i det 3. forsøgsår.

Afslutningsvis kan det konkluderes at det *er* muligt at opnå et tilfredsstillende bekæmpelsesniveau af større ukrudtsplanter og varmetolerante arter såsom græsser og flerårigt ukrudt med termiske metoder. Men det er nødvendigt at behandle planterne flere gange gennem vækstsæsonen. Antallet af behandlinger per vækstsæson kan reduceres ved at sikre at doseringen ved hver behandling er tilstrækkelig høj til at al overjordisk biomasse slås ihjel. En anden måde at reducere behandlingshyppigheden på er ved at vurdere ukrudtets dækningsgrad jævnligt med en simpel metode, og lade denne vurdering være bestemmende for bekæmpelsesbehovet.

Preface

Eight years ago I gained my first experiences with growing weeds and conducting experiments as a graduate student at Højbakkegård, the experimental research station of the Faculty of Life Sciences, University of Copenhagen. Plants of *Calystegia sepium* adorned growth houses, climate chambers and the outdoor paved experimental site. Since then, I have grown and tried to grow many other weeds. Mostly with success, but rarely without challenges. Growing plants may be a challenge but who would have thought that growing weeds would be as well? And then, after spending lots of time nursing the plants, most of them are cut, burned or killed in other ways…

However the work is far from meaningless. Environmental concern has lead to political decisions on reducing the use of pesticides. Much money is spend on weed control on hard surfaces and the municipalities, groundskeepers and private house owners face big challenges especially with the control of perennial weed species. The demand for knowledge on how to control the weeds has increased and further development of non-chemical weed control methods is required.

I have learned many things about growing weeds (!), experimental challenges, unpredictable weather conditions, breaking down the figures -and about myself! I am very grateful that LIFE, University of Copenhagen, supported this work with a scholarship. It has financed far the most of the work presented in this thesis. I am also thankful that the European Regional Development Fund (INTERREG IIIC, http:/www.interreg3c.net) partly funded the experiments presented in Paper IV and V through the project CleanRegion (http://www.cleanregion.dk).

Many people had an impact on this thesis in different ways and I am very thankful to all of them. Especially I would like to express my thankfulness to:

- My supervisors Palle Kristoffersen and Christian Andreasen for valuable discussions and for trying to help me to reduce the size of my experiments… Palle has the valuable gift of being able to see the essential part of data and Christian has supported me with precise and invaluable advice on experiments and publication of results.

- My colleague and “office-mate” Oliver Bühler, who is always willing to listen to everything from tricky statistics to complaints about sleepless nights.
- Jens Carl Streibig for his patience during very long and educative days on a NOVA PhD course in Latvia, where I learned how to use the statistical open-source program “R”.
- Christian Ritz for statistical support and always being able to get me back in the saddle when I felt being up against a brick wall.
- The technical staff at Højbakkegård, especially: Jens Bertelsen took very good care of my small grass plants in the growing houses and we spent many hours together lifting stones and washing roots! I could always count on Anders Nørgaard, who took care of my fields and spent many hours helping me with the biomass samples. Jens Erik Christensen was always careful and precise when assisting me with the flame treatments.
- …and of course to Ali Salanti for endless support, love and understanding, and my sweet children Eva-Luna and Linus for giving my thoughts a break from time to time.

Contents

Introduction

Regulation of pesticide use in urban amenity areas

Increasing concerns about leaching of pesticides into surface water or ground water have sparked public awareness and restrictions on herbicide use in urban amenity areas in many countries (Kristoffersen *et al*., 2008b). Water quality monitoring studies have demonstrated that there is a disproportionate contamination of waters by non-agricultural herbicide use in comparison with agricultural use (Saft & Staats, 2002; Augustin, 2003; Skark *et al.*, 2004; Kempenaar & Saft, 2006; Kempenaar *et al*., 2006; Kempenaar *et al.*, 2007). One of the main uses of herbicides in urban areas is to control weeds on hard surfaces. These areas are often constructed for rapid penetration of water or, more often, to encourage surface run-off to avoid flooding: this can result in contamination of nearby ditches, drains, sewage systems or ground water (e.g. Ramwell *et al.*, 2002; Skark *et al.*, 2004). Consequently, there is minimal opportunity for herbicide sorption and/ or degradation and the potential for removal of herbicides to surface waters is high.

In Denmark, the drinking water resource is based solely on groundwater (H.J. Albrectsen, pers. comm., DTU, Denmark). In recent decades, the political and public concern about pollution of our drinking water has lead to major restrictions on pesticide use in agriculture as well as in urban amenity areas. Recently, pesticides and their metabolites have been detected in 23% of the examined groundwater abstraction wells (Thorling *et al.*, 2010), causing renewed debate on pesticide use.

In order to give the public sector a leading position in efforts to minimise pesticide use, the Danish Ministry of Environment, municipalities and counties entered into a voluntary agreement on phasing out pesticide use in public amenity areas before 1 January 2003. As part of the voluntary agreement, the parties committed themselves to register pesticide consumption regularly.

Use of pesticides on public amenity areas in Denmark has decreased markedly since the first agreement was signed in 1998 (Figure 1). However, a total phase-out has not been possible to achieve. Glyphosate is the main active ingredient that has been used in urban areas, and the only active ingredient used on hard surfaces (Kristoffersen *et al*., 2008). The voluntary agreement was resigned in March 2007. It aims at continual reduction on pesticide use; however there is no specific goal of reduction as long as the parties work on a phase-out of pesticides. Exceptions are areas where phase-out of pesticides is not possible for safety or operational reasons (e.g. on railway tracks), as well as areas infested with *Heracleum mantegazzianum* Sommier & Levier.

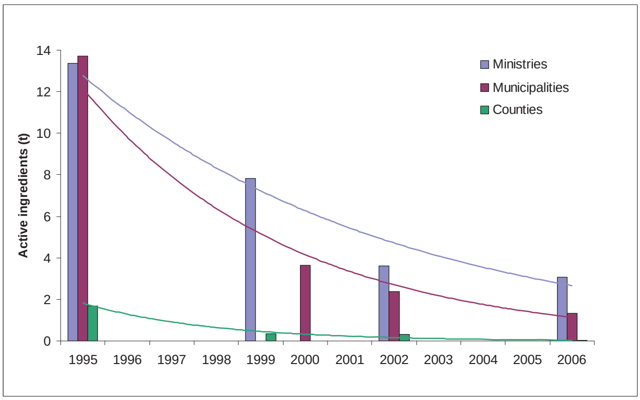

Figure 1. Annual use of pesticides in tonnes of active ingredients by Danish municipalities, counties and state institutions/ ministries. The first survey was carried out in 1995. The voluntary agreement on a total phase out of pesticide use was signed in 1998. The surveys built on questionnaires that were sent to the respective public authorities (Kristoffersen & Rask, 2007).

Non-chemical weed control on hard surfaces

As a result of the strong regulation of pesticide use in Denmark as well as in other European countries, there has been increasing interest in alternative ways to control weeds. Equipment for thermal treatments (e.g. gas burners and steamers), mechanical weed control (weed brushers) and equipment designed for semi-hard surfaces have been subject to continual research and development (Paper I). However, these alternative methods are often less cost-effective compared with spraying with glyphosate, which usually kills the weeds completely with few treatments per year (Augustin *et al*., 2001). Non-chemical weed control methods require more frequently repeated treatments primarily due to regrowth of tolerant weed species.

Within the last years, different strategies for weed control on paved areas have been developed and published as guidelines for the municipalities, and park and road managers (Tvedt *et al.,* 2000; Tvedt & Kristoffersen, 2002; Hansen *et al*., 2004; Kempenaar, 2004; Kristoffersen & Tvedt, 2005; Schroeder & Hansson, 2006).

Weeds on hard surfaces

Growth of weeds on hard surfaces is not desirable for several reasons:

- Weeds may cause damage to the surfaces by breaking up asphalt and the edge of road seals or enlarge cracks.
- Weeds can make asphalt footpaths slippery or impair pass ability.
- Accumulation of plant residues may clog water drains or make a substrate for new weed establishment. The presence of weeds may disturb sweeping operations.
- At road verges, traffic islands or roundabouts weeds can impair the visibility of traffic indicators, and thereby mislead road users or even cause accidents.
- Weeds make streets and pavement unsightly and the presence of weeds tend to indicate a city in decline.

The extent of the weed problem on a pavement highly depends on factors as design of the hard surface (e.g. joint width or thickness of asphalt), intensity of use, or weed pressure (e.g. neighbour upon a field or the edge of a ditch). Some weeds (e.g. bryophytes) cause less damage and are less unsightly than others (e.g. grasses and *Taraxacum officinale* F.H. Wigg), so the weed species composition affects the need of weed control. Additionally, the weed control effort can be graduated according to priority, e.g. differentiated among town centres, residential and industrial areas (Kortenhoff *et al.*, 2001; Tvedt & Kristoffersen, 2002; Melander *et al.*, 2009).

Controlling weeds with non-chemical weed control methods require more specific knowledge on the weed species composition in comparison with chemical control. The effectiveness of e.g. thermal weed control methods is strongly related to the present weed species and their growth stage at time of treatment. Ascard (1995a) divided weed species into four groups depending on their tolerance towards flaming. According to him, the most important factor distinguishing sensitive and tolerant weed species is not the heat tolerance of the leaves, but rather the ability of plants to regrow after the treatment. The most tolerant species were weeds with protected growth points located very near the soil surface, e.g. *Poa annua* L. and other grass species. *Poa annua* and other grass species were reported to be among the most frequent species in North-European countries (Melander *et al.*, 2009), increasing the need of knowledge on how to control grass weeds.

Pavements that are affected by trampling are frequently inhabited by species with their growth meristems located near the soil surface and are therefore protected against trampling damage, e.g. grasses, several Plantaginaceae or Asteraceae at the phenologic stage of the rosette (Benvenuti, 2004). Thus, non-chemical methods such as thermal treatments have lesser efficacy on these weed species as compared to weed species with more exposed growth meristems, such as *Chenopodium album* L., *Fumaria officinalis* L., *Urtica urens* L. and *Stellaria media* (L.) Vill. (Ascard, 1995b). Perennial weeds thrive especially well in urban environments due to vegetative propagation and/or having growth meristems located below the surface, e.g. *Elytrigia repens* (L.) Gould, *Cirsium arvense* (L.) Scop and *Equisitum arvense* L. (Ascard 1995b; Torstensson & Borjesson, 2004).

Problems to be investigated

Non-chemical weed control is generally regarded as less cost-effective compared with chemical weed management. This is mainly due to the fact that all non-chemical weed control methods require repeated treatments. Knowledge of the effects of repeated treatments on weed regrowth is essential to plan strategies for non-chemical weed control. The literature on thermal weed control covers several basic studies on the effect of a single treatment (e.g. Parish, 1989a; 1989b; 1990a; Ascard 1994; 1995a; 1995b; 1998; Hansson & Ascard, 2002; Ulloa et al. 2010a; 2010b). However, weed species which have protected growth meristems and/or propagate by regenerative roots or rhizomes will usually not be killed by a single treatment even at very high doses of energy (Ascard 1995a; Hansson & Ascard 2002; Ulloa 2010a; 2010b). There is a need to design and carry out long-term experiments under controlled or semi-controlled conditions in order to investigate how weeds, that are considered tolerant to thermal treatments, react on repeated treatments. The efficacy of the treatments should be monitored over an entire season, or several seasons to resolve the following questions:

- How is the relation between the dose of energy and the necessary number of treatments per season to control different weed species?
- How does the duration of the time period between treatments affect regrowth?
- Does it make a difference which type of thermal treatment that is carried out?
- Can the number of treatments be reduced in the growing season in order to save energy and costs?

Long-term experiments require careful planning:

- What are the advantages and disadvantages of different experimental designs?

- How can regrowth be measured without damaging the treatment plots, and how will this choice affect the result?

Structure of the thesis

This thesis consists of a general discussion and the following six papers/ manuscripts in the appendix. The first part of the thesis will link the central findings from the individual papers and relate them to each other. However, main emphasis is on the papers and manuscripts, which includes a review of literature and research studies on non-chemical weed control. The papers in the appendix are listed for the sake of coherence and continuity, and therefore not in chronological order.

Objectives

The general aim of this work was to study the effect of repeated treatments on regrowth of weeds that are considered tolerant to flaming. Most studies on treatment effect cover the effect of one treatment. However, knowledge on the long-term effects of these methods is crucial to be able to reduce the treatment frequency and thereby lower the costs. By the use of different experimental designs and sampling methods it was investigated how grass weeds responded to repeated thermal treatments. The regrowth of the two grass species *Lolium perenne* L. and *Poa annua* L. was studied in semi-controlled experiments. Both species are very hard to control with non-chemical weed control methods on hard surfaces, and *P. annua* was the most frequent species on pavements in a study on pavements in five European towns (Melander *et. al.*, 2009). In *in situ* experiments on traffic islands, the weed flora was dominated by hard to control perennial grasses as the salt-tolerant species *Puccinellia distans* (Jacq.) Parl., *Poa* spp. and *Elytrigia repens* (L.) Desv. Ex Nevski as well as several dicotyledonous weeds (mainly *Chenopodium album* L., *Chenopodium glaucum* L., *Lepidium ruderale* L. and *Taraxacum* spp.).

More specifically, the objectives were:

- To provide a comprehensive review of the scientific state of the art regarding non-chemical weed control on hard surfaces (paper I).
- To describe the relationship between dose of propane and repeated flame treatments on the regrowth of *L. perenne* (paper II).

- To determine whether split applications at the same total doses would increase the control effect (paper II).
- To design and test a semi-controlled experimental set up on a simulated hard surface where the effects of weed control on above as well as below ground plant growth can be investigated (paper III).
- To study the effect of treatment intervals between flame treatments on weed species that are considered tolerant to flaming (paper III).
- To study differences in treatment effect between different weed control methods on naturally developed weeds *in situ* experiments (paper IV and V).
- To estimate the required treatment frequency to keep weed cover below a certain limit *in situ* experiments (paper V).
- To discuss the use of simple image analysis to assess the response of weeds to repeated flame treatments (paper VI, a “short communication”).

Implications of different experimental designs on hard surfaces

Three different experimental designs are presented in the thesis:

- Semi-controlled field experiments with one sown weed species (Paper II and VI, figure 2a).
- Semi-controlled outdoor experiments on a constructed hard surface where different weed species where planted separately (Paper III, figure 2b).
- *In situ* experiments on traffic islands and pavements with naturally occurring vegetation (Paper IV and V, figure 2c and 2d).

Several issues have to be taken into consideration when choosing an experimental design to investigate the effects of non-chemical weed control methods on hard surfaces. The semi-controlled field experiments presented in Paper II provide growth conditions that are quite different from the often harsh conditions in joints and cracks on hard surfaces (Paper I). The conditions may be more related to weed growth along road verges. On the other hand, the roots of the grass weeds may be more protected when the plant is growing in small cracks in a hard surface. This experimental design was chosen in order to assure good growth of the plants as well as uniformity of the treatment plots. *Lolium perenne* (L.) was chosen as a test weed as it is easy to establish on a field and forms a dense stand. It was decided to conduct this experiment with one species instead of a mixture of species to avoid difficulties in interpretation of the results. Thereby competition from invading weed species could be reduced. If a mixture of species is sown, some species may, by chance, be more abundant in some patches than in others. Additionally, when different treatments are being carried out some species may be favoured e.g. *Poa annua* L. at high doses of propane (Ascard, 1995a).

The constructed hard surface presented in paper III provided growth conditions that were more similar to hard surfaces. The weeds were not subjected to wear by trampling, but they were planted in very sandy soil and the surrounding pavement may have offered the roots some protection against the flame treatments. Additionally, the soil between the pavements was probably warmer than soil on a field resembling the warmer environment on paved areas in cities. A major disadvantage is that this kind of surface is expensive and labour demanding to construct. It would not have been possible to establish a constructed surface for the large-scale experiments as the dose-response experiment presented in Paper II.

In situ experiments have their 'pros' and 'cons' as well. On one hand experimental results with naturally occurring weeds on pavements may seem to be of highest practical value. On the other hand it is very difficult to find plots that have similar vegetation, growth conditions and weed pressure from surrounding fields or gardens (see figure 2d). Another problem with *in situ* experiments is that the experimental set up can be subjected to vandalism. Additionally, weeds may be controlled by mistake by citizens or road administrators who have not been properly informed about the experiments or disagree with the importance of non-treated plots.

In conclusion, the choice of experimental design depend on the objective of the study, the economy of the project and the possibilities of using pavements in cities as experimental areas.

Fig. 2. Photographs from semi-controlled field experiment (A, upper left), semi-controlled experiment on a constructed hard surface (B, upper right), *in situ* experiments on traffic islands (C, lower left) and pavements (D, lower right).

Measuring the efficacy of non-chemical weed control in long-term experiments

Measurements of plant regrowth after different treatments can be done in several ways. Examples are collecting biomass samples to measure changes in dry weight or fresh weight (e.g. Rask & Andreasen, 2007), counting plant numbers (e.g. Ascard, 1994), measuring light reflection, visual assessment of percentage weed control (e.g. Hansson & Ascard, 2002) or use of image analysis to count number of green pixels or area of leaves with a specific shape (e.g. Hansson, 2002). The choice of sampling methods may affect the results, especially when repeated treatments are carried out.

Dry weight of plants gives a useful and qualified estimate of the effect of the treatment. If the aim of the investigation is to measure the effect of repeated weed control treatments, the removal of the biomass makes it impossible to measure how regrowth would occur under natural conditions. Additionally it is very labor demanding. If there is a lot of withered grass in the samples that needs to be removed before weighing, this will increase the work load considerably. It may also be difficult to estimate when a plant is dead. The plant may still have some green parts or covered living shoots or buds even though most of the plant seems to be withered away (Paper III).

A B C

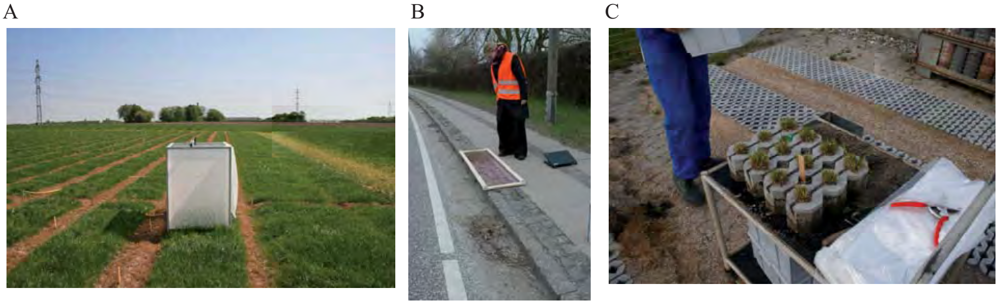

Fig. 3. Different ways to measure regrowth that were used in the experiments: A. Image of the tent used for image capturing. The frame that is seen on figure 4 and 5 is placed at the bottom of the tent: B. Image of a frame that was used to estimate percentage weed cover on pavements. The frame used on traffic islands was quadratic: C. Image of stone taken up in the grass reinforcement experiment in order to harvest above and below ground biomass.

In case the samples are small enough, it may be possible to count the number of surviving plants, either in the field or after harvest. Other methods are remote sensing, visual assessment with e.g. a frame to define the plot (Fig. 3b, Paper IV; Paper V; Melander *et al.*, 2009) or use of image analysis. However, visual rating of percentage weed control is a subjective assessment method and some scientific journals only accept visual assessment when data are partly supported by an objective measurement method (See e.g. Weed Technology, Editors Note, 2011).

Fig. 4. Images from October 2010 (14 days after the last treatment) showing different responses to flaming after A: 0 treatments/ control plot, B. 2 treatments, C: 4 treatments, D: 6 treatments, E: 8 treatments, or F: 10 treatments, all with the same dose (80 kg propane ha^{-1}).

The use of image analysis to measure vegetation changes during an entire growing season

Several attempts have been done to use image analysis in weed science. Andreasen *et al.* (1997) suggested a method to estimate weed densities by using image analysis and Gerhard *et al.* (2002) have used image analysis to identify weed seedlings. Hansson (2002) used image analysis to assess the weed control effect of a hot water treatment on *Sinapsis alba* L. The aim of using image analysis in the dose-response experiments presented in Paper II and VI was to measure vegetation changes in an objective and easy way during full season experiments without damaging the plots.

Dense stands of *Lolium perenne* were flamed with different doses of propane and different time intervals between treatments during two growing seasons. Images were taken every second week to measure changes in vegetation cover (Fig. 4). It was planned that the images should be analysed by a simple image analysis programme counting green pixels and/ or visual assessment. The digital image analyzer program "Imaging crop response analyzer" developed by Rasmussen *et al.* (2007) seemed promising on the first images from a pilot experiment in 2007 with mixed grass species. However, it turned out to be unusable in the long-term dose-response study, perhaps because the images were not bright enough or the algorithm was not appropriate for the leaf color of perennial ryegrass.

The only way to analyze the images from the field experiment was by developing an image analysis program where the algorithm could be defined. A macro was generated in the open source software ImageJ (http://rsbweb.nih.gov/ij/) with the plugin "Threshold color" as described in Paper VI. The same threshold was used on all images, as the aim was to be able to run the macro on all images automatically (Fig. 5).

It is important, when choosing image analysis as assessment method, that the colors of the leaves can be segmented easily from the colors of the soil and withered leaves. Sometimes the simple image analysis programme, that was used to count number of green pixels, could not discriminate completely between yellowish soil pixels, yellowish grass and green pixels. *Lolium perenne* had very light green leaves, almost yellowish, and it was particularly difficult to discriminate between the color of the soil and withered plant parts. The image analysis macro was developed during a PhD course in spring 2010 to analyze the images that were captured in 2008. Even though much effort was invested in improving light conditions when taking the images in 2010, it was not possible to obtain reliable estimations of percentage green cover by image analysis on the images captured in 2010 with the threshold procedure that was used for the 2008 images. The problem was that the green leaves could not be separated from the soil, which was more yellowish this year. It may be possible by using another color space, filters or improvements of the generated macro. Further work is needed to make these changes.

333 images from one sample date in 2008 are analyzed in Paper VI and effective doses estimated by dose-response curves are compared with those obtained by visual assessment or dry weight. Image analysis was an easy measurement method of vegetation cover but did not give precisely the same results as biomass measurements. All assessment methods showed a relation between dose and treatment interval on the reduction of plant weight or decrease in vegetation cover. However, there were significant differences in the estimated effective doses (ED_{90}) depending on assessment method and length of treatment interval.

Color corrections of images acquired under different illumination

In order to measure vegetation changes during an entire season it is necessary to carry out the image analysis with color corrections to adjust for different illumination. This can be done in ImageJ by the use of the color correction card that was placed within the frame (Fig. 4). In 2008, a white balancing card was used which contains white, black and gray areas (Fig. 5). The gray scale colors can be used as absolute reference colors to adjust the RGB channels individually to match a standard light source as gray scale colors reflect all colors equally. Ideally, the reference card should contain several evenly spaced values from pure black to pure white to get as many points on the correction curve as possible. An example of a correction curve with three points is given in Russ (2006). It is possible to make adjustments with only one or few points if it is assumed that the curve is linear. If one color is used, light gray is preferred.

I have developed a macro that can use this white balancing card for color adjustments; however, it can not be used without manually checking all the images. The reason is that the white balancing card was sometimes covered with grass, or the card was sometimes blackened from soot that was on the grass or soil after the flame treatments. In 2010, a full color reference card was used. Before taking an image the card was cleaned with a brush and all leaves were carefully removed from the card. Analysis of these images may shed light on the response of the weeds during an entire season after many different combinations of doses of propane and treatment intervals. For example, it could reveal whether the treatments in spring had higher effect than the treatments in summer. It could also show after which exact treatment and at which dose there was no further reductions in weed cover during the rest of the season. Further work is needed to be able to analyze these images. They could be analyzed by visual assessment of all images, as this method seemed to give qualified estimations of weed cover in Paper VI. However, it will take a long time, and different people may assess the weed cover differently. If the right macro could be developed, image analysis would be preferred to analyze the images as it is an objective method and possibly could be run automatically.

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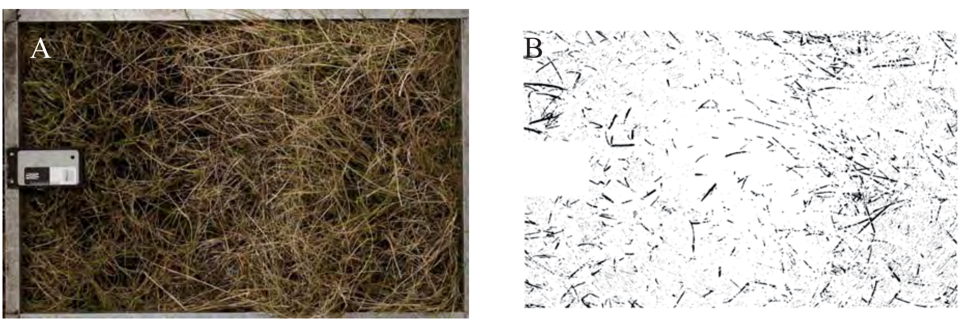


Fig. 5. Thresholded binary image (B) of an image taken in October 2008 (A). The frame and white balancing card was removed before number of green pixels was counted as seen on image B.

Effective non-chemical weed control requires repeated treatments

How many treatments?

There are several things that are essential to obtain effective control of perennial weeds on hard surfaces with non-chemical weed control methods: The dose need to be high enough to kill all above-ground leaves and the treatments need to be repeated in order to starve and eventually kill below ground plant parts, such as rhizomes or regenerative roots. There is a potential for adjusting the energy dose and the number of treatments to the weed flora, according to the plants' morphology (e.g. position of growth meristems), flowering period (Hartin, 1989; Benvenuti, 2004) and to the stage of development (Hansson & Ascard, 2002).

According to Ellwanger *et al.* (1973a, 1973b), cellular death after a single thermal treatment is primarily due to the initial thermal disruption of cellular membranes rapidly followed by dehydration of affected tissue. Daniell *et al.* (1969) found that the structural changes were more pronounced when the temperature of the cell changes rapidly as in a flame treatment than when the temperature changes were more gradual as in a hot-water-bath treatment. Lethal leaf temperatures in the range from 55 to 94 ºC have been reported (Anderson *et al.,* 1967; Daniell *et al.*, 1969; Hoffmann, 1989). Exposure times to the flame in the range from 0.065 and 0.13 s has been assumed adequate to kill all weeds encountered in a crop although an extremely wide range of weeds were considered (Thomas 1964; Daniell *et al.* 1969).

Plant survival of high propane doses is largely dependent on their ability to regrow after thermal treatment (Vester 1985; 1990; Ascard 1995a). Therefore, effective non-chemical weed control requires more frequent repeated treatments than chemical weed management (Popay *et al.*, 1992; Elmore 1993; Augustin *et al.*, 2001; Reichel, 2003; Kristoffersen *et al.*, 2004, Paper I-V).

Usually, treatment intervals during the growing season of 2 to 5 wk have been suggested as necessary (e.g. Kreeb & Warnke 1994; Hansson 2002; Paper I; Paper IV). Hansen *et al.* (2004) found that 11-12 treatments per growing season were necessary to achieve acceptable weed control on areas heavily infested with perennial weeds, resulting in treatment intervals of about 1 to 2 wk. In Paper V, 2-7 yearly treatments with the thermal methods were necessary to keep the weed cover below 2%. However, it was expected that the number of required treatments would be higher. The results from Paper V are closer to results by

Vermeulen *et al.* 2006. They reported a lower treatment frequency to be necessary: 4-6 brushings, 3-5 flamings, and 3-5 hot water applications per growing season. Because a high treatment frequency increases the costs of weed control, knowledge of treatment efficiency can provide practical advice on how to reduce the number of treatments and lower the costs of control.

If all aboveground plant parts are killed at each treatment, there should be no difference in the number of required treatments per season regardless of which thermal weed control method that is used (Hansson, 2002). However, the results from Paper IV and V indicates, that the hot water method with foam (Waipuna) may have a longer lasting effect on the weeds. Whether it is because the other methods that were tested did not have the ability to kill the growth meristems of e.g. *Puccinellia distans,* which was a species that was highly represented on the trial sites, or because the insulating effect of the water and foam had the ability to kill superficial roots, remains to be investigated.

Relationship between dose and treatment intervals

The effect of repeated treatments with different doses of propane on the regrowth of well-established *Lolium perenne* plants was investigated in field experiments (Paper II). The first experiment was carried out from May to October 2008 and the entire experiment was repeated in 2010. Biomass samples were collected three times during each season, in week 26, 34 and 40 (two weeks later in 2010). The procedure is explained in Paper II; however, only results from the last sample date in 2008 and 2010 are included in the manuscript. The results from the first and second sample date are summarized in the next two sections. Images were taken every other week throughout the season to measure vegetation changes by image analysis (see section 3 and Paper VI).

Mean dry weight of *L. perenne* was highly dependent on the dose of propane that was applied as well as treatment intervals (Fig. 6). The treatments were more effective in 2010 in comparison with 2008. There are several possible explanations for this: Mean winter temperatures were considerably lower in 2009/ 2010 in comparison with 2007/2008 (5.0 ˚C in October to March 2007-2008 and 2.8 ˚C in 2010). Even though the experiment was started two weeks later in 2010, the grass height was about 5 cm lower and more uniform in 2010. That meant that the first treatments had higher effect in 2010, because the flames could easier penetrate the dense leaf cover. Another reason may be that the field surface was more even in 2010 in comparison with 2008, which facilitated the operation of the gas burner.

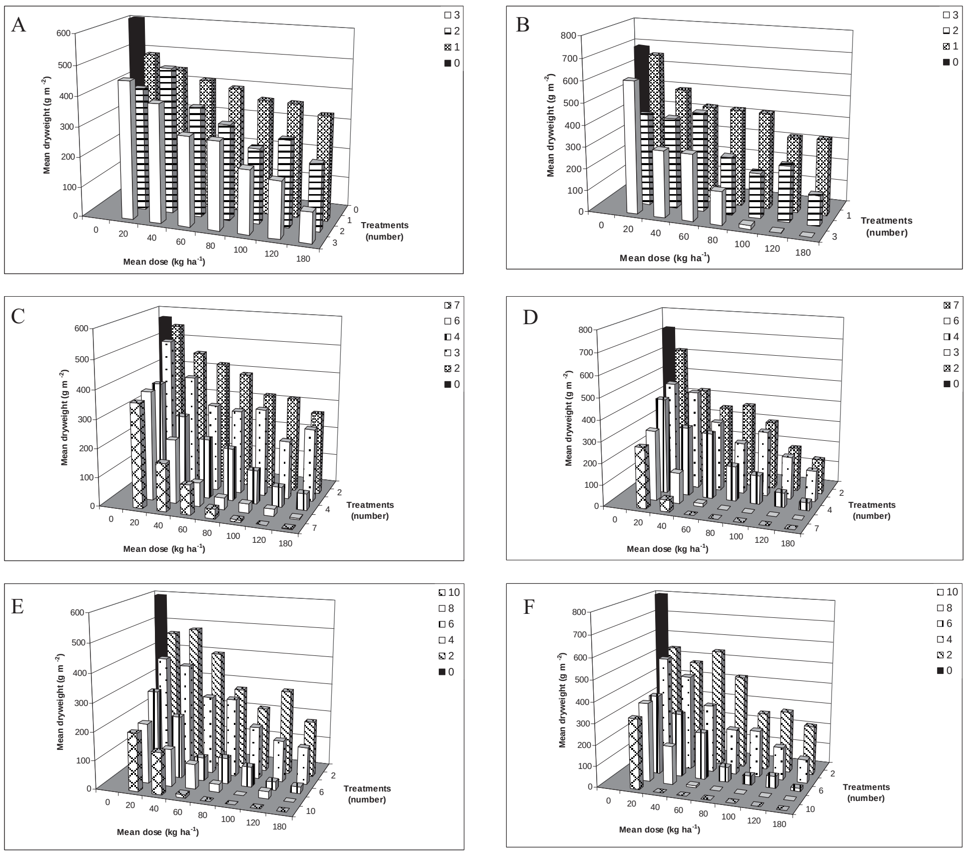


Fig. 6: 3D figures on the relationship between dose of propane, treatment interval and mean dry weight (g m^{-2}). The doses are mean pre-planned doses of propane in kg ha^{-1}. Each bar corresponds to the mean of nine replicates (three per block, 18 replicates for control plants). Biomass samples were collected after 0-3 treatments in 2008 (A) or 2010 (B), 0-7 treatments in 2008 (C) or 2010 (D) or 0-10 treatments in 2008 (E) or 2010 (F).

The experiment was designed as a long term dose-response study. The effect of different doses of propane was tested in a pilot study on a grass lawn in autumn 2007. Two treatments two weeks apart were carried out in order to define which doses that should be applied to obtain responses in both ends of the dose-response curve. It would have been an advantage to have different dose levels depending on the treatment interval. That means, that the dose-response curves for each treatment interval would have been more precise if high dose levels were chosen for long treatment intervals and low dose levels were chosen for short treatment intervals. However, it would not have been possible to obtain neither higher nor lower doses with the gas burner that was used in these experiments. The dose was mainly regulated by the driving speed, and at the lowest doses the speed was so high that the machine operator had to run behind the machine. The highest dose that was selected was the lowest possible driving speed of the machine.

Fig. 7: The gas burner HOAF thermHIT® 75M that was used in the experiments presented in Paper II and III.

Ascard (1995) has shown that the effect of flaming can be described by dose-response curves similar to those described by Streibig *et al.* (1993) for herbicides:

$$y = c + \frac{(D-C)}{1+\exp\{b(\log(x)-\log(e))\}} \qquad (1)$$

where Y is the response variable (biomass in g dry weight in this example) and x is the energy dose in kg propane ha^{-1}. *D* is the upper limit of the curve and *C* is the lower limit. The parameter *e* is equivalent to ED_{50}, which is the dose to achieve a 50% reduction in dry weight of the weeds. Parameter *b* describes the slope of the curve around *e* (inflexion point).

The upper asymptote corresponds to the mean dry weight of the control plants and the lower asymptote corresponds to no biomass production. At first the above four parameter model was chosen but it was not possible to fit the curves with this model. Lack of data points around the lower limit for some of the curves resulted in one case of negative *C* values. Knezevic *et al.* (2007) recommends the use of the three parameter log logistic model in this case. Moreover, this model was found to be more closely related to data and it is expected that the response will go towards 0 with increasing dose:

$$y = \frac{D}{1+\exp\{b(\log(x)-\log(e))\}} \qquad (2)$$

The quantity effective dosage (ED) is commonly used to compare different dose-response curves. ED is a function of the parameters: ED_y is defined as the dose that yields a response which is (100-y)% of the maximum response D (a reduction of y%). ED_y can be expressed by means of the parameters *b* and *e* in the three parameter logistic model:

$$ED_y = e(y(100-y))^{1/b} \qquad (3)$$

The package drc in R provides functions to compute ED_y values. The values are derived from the regression model utilizing the delta method (Knezivic *et al.*, 2007)

Estimates of ED_{50}, ED_{80}, ED_{90}, ED_{95} and their standard errors for all curves were calculated. ED_{90} and ED_{95} were outside the observed dose range for several curves, so most emphasis is on ED_{50} and ED_{80} estimates. ED_{95} estimates are not shown in the tables with parameter estimates.

Depending on the control situation, different control levels may be chosen. An 80% reduction in dry weight of weeds (assessed two weeks after last treatment) may not be sufficient on hard surfaces. However, in these experiments mean dry weight of control plants were around 600-800 g dry weight m^{-2}. Grass cover was dense and the doses of propane that were required were probably higher in comparison with the doses that would be necessary for effective weed control on hard surfaces.

However, even in these experiments with very high plant densities, it was possible to kill *Lolium perenne* completely with a combination of relatively high doses of propane and short treatment intervals, especially in 2010 (Fig. 6f). The field that had been treated in 2008 was left untouched in 2009-2010. In June 2009, i.e. nine months after the last treatment, no weed growth was observed on treatment plots that had received high doses (mean doses above 120 kg propane ha^{-1}) and eight or ten treatments from May-September 2008 (not shown).

The appropriateness of the dose-response models was tested by a graphical check of the data. A residual plot showed a weak tendency towards increasing variation with increasing values, i.e. the variance was not constant. It was not possible to use Box-Cox transformation (Streibig *et al.,* 1993) as many values in the dataset were zero or close to the lower limit.

In general, the three parameter logistic model gave good descriptions of the dose –response relationships. However, in one case it was not possible to fit a curve due to a very low response on the treatment.

In many cases the curves were not parallel. When slopes of dose-response curves are parallel, then differences between treatment intervals will be less when doses are chosen close to the upper or lower limit in comparison with the middle region of the curves. Dose-ranges in the middle part of the curve will be almost independent of dose levels. In these experiments, where slopes in some cases are non-parallel, the results of vertical, as well as horizontal, assessment is highly dependent on the chosen dose level (Ritz *et al.*, 2006). Therefore, the curves generally have to be evaluated at a certain control level.

The effect of one to three treatments from May to June

The first biomass samples were collected in end of June 2008 and beginning of July 2010, respectively. The samples were collected two weeks after the last treatment in order to assess the regrowth of the weeds after flaming, and not the immediate response.

It was not possible to achieve 80% reduction in dry weight of *Lolium perenne* within the chosen dose intervals with neither one nor two treatments (both years, Table 1). In 2010, three treatments carried out every other week from May to June with a total dose of 225 kg propane ha^{-1} could reduce dry weight of *Lolium perenne* with 80% (mean dose 75 kg propane ha^{-1} per treatment).

Differences between the effects of different treatment intervals on the required dose can be investigated by looking on differences in ED_{50}, which is within the observed dose range for all treatment intervals. In both years, there were no difference in total dose requirement when two or three treatments were carried out. From an economical and practical point of view, it may therefore be an advantage for the road manager to carry out two treatments with higher doses, instead of three treatments with lower doses. In these experiments, to obtain the same effect on biomass reduction (50%) a 32 kg ha^{-1} higher dose per treatment was required in 2008 and 15 kg ha^{-1} per treatment in 2010 (Table 1) if the number of treatments should be reduced from three to two. The response in g dry weight after one flame treatment in May 2008 was so low that a dose-response curve could not be drawn. In 2010, one treatment with a dose of around 151 kg propane ha^{-1} in May could reduce weed dry weight with 50% when biomass samples were taken in beginning of July.

Table 1: Parameter estimates of regression (model 2) after flame treatment of *Lolium perenne* with different time intervals. Dose is calculated as total dose in kg propane ha^{-1} after 1-3 treatments during the entire growing season. Standard errors (SE) are given in parenthesis. The response after one flame treatment in May 2008 was so low that a dose-response curve could not be drawn.

		Slope of decrease	Total dose			Mean dose		
Year	**Treatments**	***b* (SE)**	**ED50 (SE)**	**ED80 (SE)**	**ED90 (SE)**	**ED50 (SE)**	**ED80 (SE)**	**ED90 (SE)**
	Number		kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
2008	**1**	.		.	.	.	.	.
	2	0.76 (0.191)	194 (37.1)	1193 (598.4)*	3447 (2603.1)*	97 (5.3)	569 (280.0)*	1606 (1191.6)*
	3	1.18 (0.161)	196 (22.6)	632 (87.4)*	1253 (268.5)*	65 (7.5)	205 (28.0)*	402 (85.2)*
2010	**1**	1.04 (0.171)	151 (20.1)	571 (164.9)*	1245 (507.8)*	151 (20.1)	571 (164.8)*	1245 (507.2)*
	2	0.92 (0.190)	116 (18.1)	526 (149.0)*	1273 (573.7)*	58 (9.1)	265 (75.8)*	647 (294.7)*
	3	2.48 (0.280)	128 (8.9)	225 (13.6)	312 (25.5)	43 (3.0)	75 (4.5)	104 (8.5)

* Outside observed dose range

The data from 2010 contained non-parallel curves, resulting in non-constant horizontal distance between the curves. The slope, parameter *b*, was steeper for plants that have received three treatments (Fig. 8, Table 1). The slope of the dose-response curve may reflect variation in heat tolerance between plants within the same stand. A flat slope may be attributed to variations in control effect, because of uneven soil conditions, or irregular performance of the flame weeder (Ascard, 1995a).

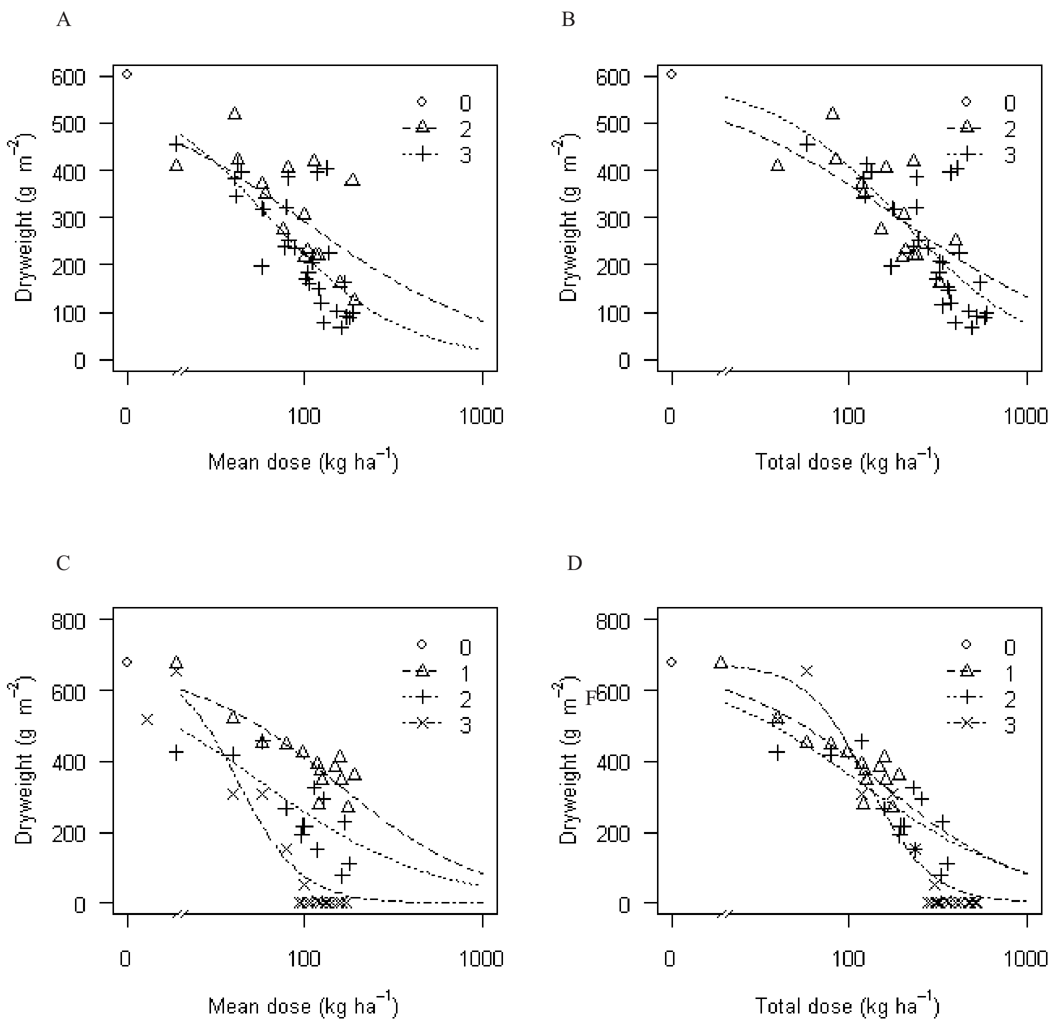


Fig. 8: Dose response curves for the mean and total propane dose effect on mean plant dry weight in 2008 (a, b) and 2010 (c,d) after flaming *Lolium perenne* with different time intervals. Each data point corresponds to the mean of three replicates. The upper asymptote (control plants) is based on the mean from 18 biomass samples. Parameter estimates are given in Table 1.

The effect of two to seven treatments from May to August

The second biomass samples were collected in end of August 2008 and beginning of September 2010, respectively, two weeks after the last treatment.

The slope, parameter *b*, was significantly steeper for plants that have received six or seven treatments in comparison with two to four treatments (both years, Table 2, Fig. 9). Two to three treatments carried out from May to August could not reduce dry weight of *L. perenne* with 80% within the chosen dose intervals (Table 2, both years). In 2008, there was no significant difference between the total dose requirement to obtain 80% reduction in biomass after four, six or seven treatments (six and seven in 2010). Four treatments with a mean dose per treatment of around 103 kg propane ha^{-1} could reduce weed dry weight with 80%. A 90% reduction was not obtained within the observed dose interval. When the number of treatments was six or seven, the required mean dose per treatment was one half or one third of the dose that was required when four treatments were carried out.

As in the previous section, the experiments show that there is a trade-off between carrying out more treatments or increasing the dose per treatment. However, when the treatment intervals become too long, even very high doses are not sufficient to control grass weeds as *L. perenne*. On the other hand, when treatment intervals are short, increasing the number of treatments with lower doses has limited effect. The results from the present biomass samples show that the dose requirement (total dose as well as mean dose per treatment) to obtain a 90% reduction in dry weight is the same with six or seven treatments. In other words, six treatments with a mean dose around 42 to 81 kg ha^{-1} would have been sufficient to obtain 90% control of *L. perenne*.

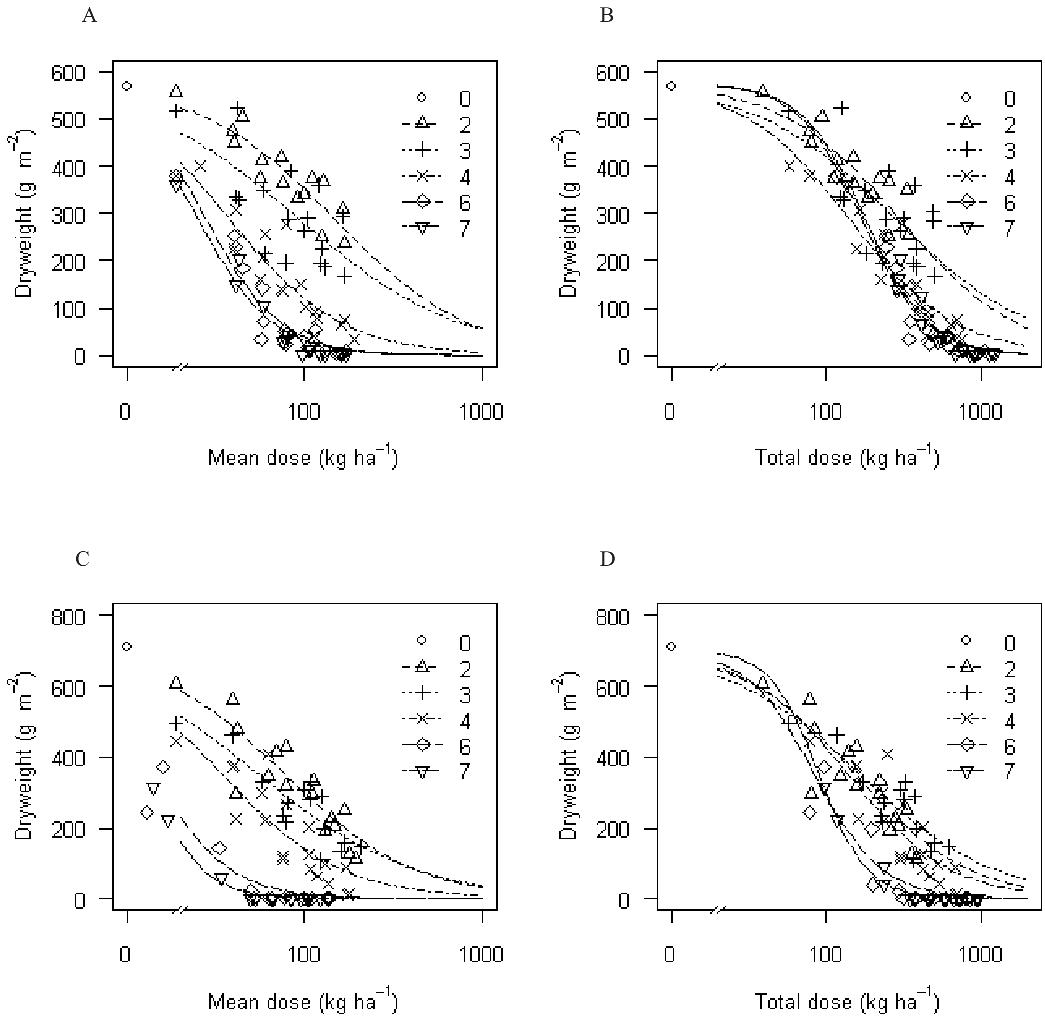


Fig. 9: Dose response curves for the mean and total propane dose effect on mean plant dry weight in 2008 (a, b) and 2010 (c,d) after flaming *Lolium perenne* with different time intervals. Each data point corresponds to the mean of three replicates. The upper asymptote (control plants) is based on the mean from 18 biomass samples. Parameter estimates are given in Table 2.

Table 2: Parameter estimates of regression (model 2) after flame treatment of *Lolium perenne* with different time intervals. Dose is calculated as total dose in kg propane ha^{-1} after 2-7 treatments during the entire growing season. Standard errors (SE) are given in parenthesis.

Year	Treatments	Slope of decrease	Total dose per season			Mean dose per treatment		
		b (SE)	**ED50 (SE)**	**ED80 (SE)**	**ED90 (SE)**	**ED50 (SE)**	**ED80 (SE)**	**ED90 (SE)**
	Number		Kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
2008	**2**	1.15 (0.201)	299 (38.1)	1002 (287.4)*	2033 (819.7)*	147 (18.4)	488 (138.9)*	983 (394.4)*
	3	0.96 (0.148)	282 (33.5)	1195 (306.4)*	2785 (1048.8)*	93 (10.9)	390 (98.8)*	903 (336.5)*
	4	1.27 (0.167)	142 (16.6)	423 (49.6)	803 (145.8)*	38 (4.0)	103 (11.3)	185 (31)*
	6	2.10 (0.263)	172 (15.2)	332 (27.9)	489 (55.8)	28 (2.5)	55 (4.6)	81 (9.3)
	7	2.11 (0.270)	180 (16.0)	348 (31.0)	511 (61.6)	25 (2.3)	49 (4.4)	73 (8.8)
2010	**2**	1.19 (0.154)	155 (14.8)	494 (73.3)*	973 (218.1)*	77 (7.4)	247 (36.9)*	488 (110.0)*
	3	1.00 (0.137)	164 (18.9)	655 (113.4)*	1467 (396.1)*	55 (6.3)	219 (38.2)*	494 (134.3)*
	4	1.32 (0.156)	139 (15.1)	396 (41.4)	733 (115.2)*	34 (3.8)	99 (10.4)	185 (29.3)*
	6	2.01 (0.306)	85 (9.2)	169 (16.5)	253 (34.4)	14 (1.5)	28 (2.75)	42 (5.8)
	7	2.77 (0.738)	90 (8.0)	149 (16.3)	199 (35.1)	13 (1.2)	21 (2.3)	29 (4.9)

* Outside observed dose range

The effect of two to ten treatments from May to September

The third biomass samples were collected in the end of August 2008 and the beginning of September 2010, respectively, two weeks after the last treatment. The data from these samples are analysed in Paper II, which is accepted for publication in Weed Research. Dose-response models on mean doses are not shown in Paper II, and are therefore included in this section (Figure 10, A, C).

It was not possible to achieve an 80% reduction in dry biomass with two (both years) or four treatments (in 2008) carried out from May to September (Table 3). To achieve a control level of 90% with six treatments a year, mean doses per treatment where estimated to around 106-113 kg ha^{-1}, depending on year. With shorter treatment intervals the dose requirement per treatment was decreased, however not significantly when the number of treatments were increased from eight to ten treatments (both years). That means that the same control level could be obtained with either eight or ten treatments with the same dose per treatment.

Regarding the total dose requirement per season, there was no significant difference between carrying out 6 to 10 treatments in 2008 to obtain a 90% or 80% reduction in dry biomass of weeds (8 to 10 in 2010). In both years, increasing the number of treatments from two to four treatments per growing season did not increase the required total dose significantly at the ED_{50} or ED_{80} control level. That means that in 2008, increasing the number of treatments from four to six required the highest increase in total dose to achieve 50% or 80% reduction in dry biomass (Fig. 10). In 2010, nonparallel (and crossing) curves made comparisons at the ED_{50} level more complicated. Only ED_{50} after eight treatments was significantly lower in comparison with two and four treatments. As in 2008, the highest significant difference in dose requirement at ED_{80} was when the number of treatments was increased from four to six yearly treatments. However, the dose requirement, when the number of treatments was increased from six to eight, was significant as well.

Especially the data from 2010 contained non-parallel curves. The slopes were steeper for plants that have received eight or ten treatments a year (Table 3). Two treatments a year resulted in a significantly flatter curve in comparison with the other treatment intervals. In 2008, all slopes were more similar; however, the slope for plants that had received four treatments a year was significantly flatter than the other slopes.

Table 3: Parameter estimates of regression (model 2) after flame treatment of *Lolium perenne* with different time intervals. Dose is calculated as total dose in kg propane ha^{-1} after 2-10 treatments during the entire growing season. Standard errors (SE) are given in parenthesis.

		Slope of decrease	Total dose per season			Mean dose per treatment		
Year	**Treatments**	***b* (SE)**	**ED50 (SE)**	**ED80 (SE)**	**ED90 (SE)**	**ED50 (SE)**	**ED80 (SE)**	**ED90 (SE)**
	Number		kg ha^{-1}	kg ha^{-1}	Kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
2008	**2**	1.23 (0.168)	189 (16.0)	585 (99.8))*	1132 (286.0)*	94 (7.9)	287 (49.1)*	553 (140.5)*
	4	1.03 (0.131)	227 (24.0)	875 (133.5)*	1929 (464.7)*	56 (6.0)	218 (33.7)*	483 (117)*
	6	1.40 (0.178)	131 (15.9)	353 (34.1)	631 (91.3)	21 (2.6)	59 (5.7)	106 (15.4)
	8	1.43 (0.224)	112 (18.5)	296 (31.8)	523 (80.8)	14 (2.3)	36 (4.0)	65 (10.2)
	10	1.76 (0.288)	145 (19.8)	319 (31.6)	505 (71.0)	14 (2.0)	32 (3.2)	50 (7.17)
2010	**2**	0.91 (0.12)	170 (17.1)	774 (158.0)*	1881 (593.3)*	85 (8.6)	392 (81.1)*	959 (307.6)*
	4	1.20 (0.13)	169 (15.3)	537 (55.6)	1055 (169.0)*	42 (3.9)	135 (14.1)	267 (43.4)*
	6	1.34 (0.14)	131 (14.4)	368 (33.0)	674 (87.7)	21 (2.4)	61 (5.5)	113 (14.9)
	8	2.21 (0.28)	129 (9.8)	240 (19.0)	347 (39.0)	16 (1.2)	30 (2.4)	43 (4.8)
	10	4.33 (1.35)	151 (7.1)	208 (17.4)	250 (34.5)	15 (0.7)	21 (1.7)	25 (3.3)

* Outside observed dose range

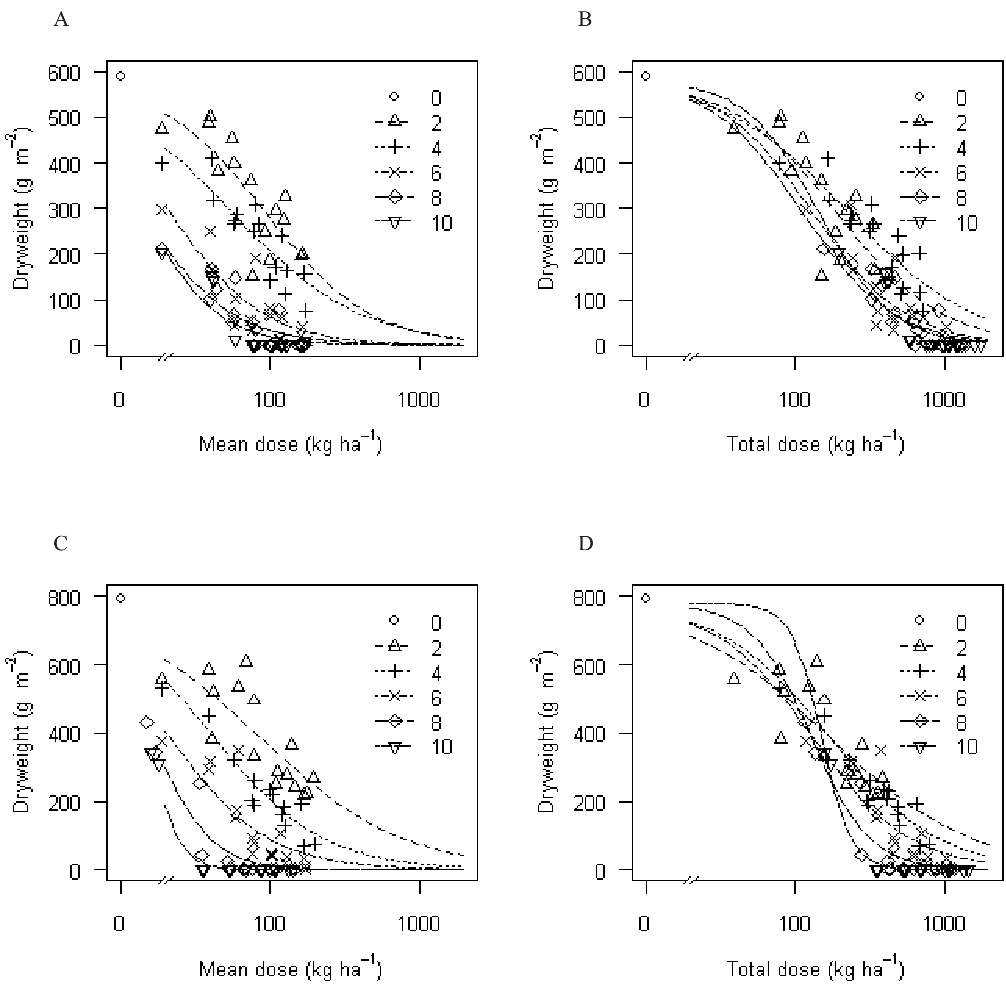


Fig. 10: Dose response curves for the mean and total propane dose effect on mean plant dry weight in 2008 (a, b) and 2010 (c,d) after flaming *Lolium perenne* with different time intervals. Each data point corresponds to the mean of three replicates. The upper asymptote (control plants) is based on the mean from 18 biomass samples. Parameter estimates are given in Table 3.

Dose-response models comparing different treatment intervals can be used to shed light on the effect of split applications (Paper II). In experiments by Ascard (1995a), split application with two half dose treatments one week apart did not give a higher plant number reduction than a single flame treatment at the same total dose, when naturally emerged weeds were flamed at early stages. However the weed flora consisted predominantly of susceptible weed species. In these experiments, split applications generally increased the effect of the treatments. This is in accordance with the assumption that repeated treatments are necessary to starve larger plants and heat tolerant weeds such as grasses and perennial weeds, that will regrow after a single treatment. Experiments by e.g. Håkansson (1982; 2003) and Rask & Andreasen (2007) show that, in order to starve perennial plants sufficiently, the second and subsequent treatment should be carried out after an initial regrowth but before regrowing shoots have become too large.

In summary, six treatments carried out from May to September and a total dose estimated to around 631-674 kg propane ha^{-1}, depending on year, controlled *L. perenne* effectively (90% reduction in dry weight). All weeds were killed with doses above 80 kg propane ha^{-1} when treatments were carried out every other week (10 treatments per growing season and a total dose of 800 kg ha^{-1}) and no regrowth was seen the following two weeks.

The effect of time intervals.

The impact of time different intervals between flame treatments was studied in experiments on a specially designed hard surface (Paper III). The aim of the experiments was to investigate how *Poa annua* L. (Annual bluegrass), *Lolium perenne* L. (Perennial ryegrass), *Taraxacum officinale* F.H. Wigg (Common dandelion) and *Plantago major* L. (Common plantain) responded to flaming with different time intervals, and to investigate the relation between regrowth of the plants and below- and aboveground biomass at the date of second treatment. Unfortunately the two broadleaf species did not germinate, and when it was discovered it was too late to include them in the study.

P. annua and *L. perenne* were successfully established and used in the experiments in 2008. Both species are hard to control on hard surfaces, and *P. annua* was the most frequent species on pavements in a study on pavements in five European towns (Melander *et. al.*, 2009). *Lolium perenne* was chosen as it was used in the field experiment as well.

The results of the present study are partly covered in Paper III.

The hypotheses tested in the experiments were that:

a) Flaming would reduce plant dry weight and number of flowering plants substantially in comparison with untreated plants.
b) Increasing time between treatments would increase regrowth.
c) Regrowth after two treatments would depend on root weight at the time of second treatment. It was hypothesized that the root weights would decrease substantially after the first treatment when new leaves were formed. It was assumed that the plants would be most susceptible to the second treatment at the time of minimum root weights (the compensation point). After this time the new leaves would become so large that the production of photosynthates would exceed losses through respiration. Regrowth would increase as the plants had rebuilt their root resources.

Two identical experiments repeated in time were carried out (one week between experiments). Above- and below ground biomass from 72 plants per treatment was harvested and dry weights were recorded at regular intervals to investigate how the plants responded to flaming. Regrowth of the grasses was measured by harvesting aboveground biomass two weeks after the second flaming treatments that were implemented at different time intervals. Data on below ground biomass is not presented in Paper III. After the first treatment roots were collected at regular intervals by lifting the stones, digging out all the roots, and place them in numbered bags. The roots were washed and dried for 24 hours at 80 C.

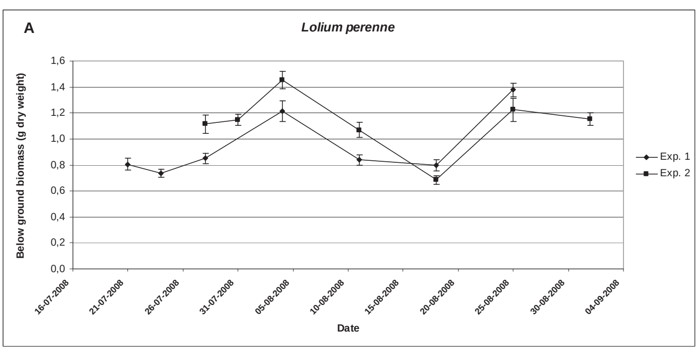
There were several issues related to the harvest of roots:

- Roots of especially control plants had grown into the mat that was placed below the grass reinforcement flag stones. It was difficult to remove all roots from the mat.
- The soil mixture (described in Paper III) consisted mainly of sand; however, there was a very small amount of humus (2%). The humus contained small wood pieces which were very difficult, and sometimes impossible, to remove from the roots (Fig. 11). In that case they were included in the dry weight. It is difficult to say how this issue influenced the results; however it has most likely increased variation in root weight and uncertainty of the results.
- It seemed that differences in root weights where more dependent on fluctuations in water supply and perhaps soil temperature, than the treatments (Figure 12). Root weights followed mainly the same seasonal pattern in the two experiments, even though the plants were watered daily, whereas the effect of flaming was not consistently related to regrowth after different time intervals.

However, even though the hard work collecting below ground biomass was not as fruitful as expected; the experiment did provide valuable results.

As presented in Paper III, flaming reduced above ground biomass substantially, but only few plants were killed. There was relatively more effect of the first flaming treatment than of the second. A flaming interval of 7 days reduced regrowth of *L. perenne* the most (Fig. 13), whereas the effect of treatment intervals on *P. annua* varied between the two runs of this experiment (Fig. 14). Very short treatment intervals (3 days) should be avoided, as these did not reduce weed biomass in comparison with 7 days treatment intervals.

Fig. 11: Roots have been dug up from the flag stones, and are being washed. Small pieces of wood from the soil were very difficult to remove



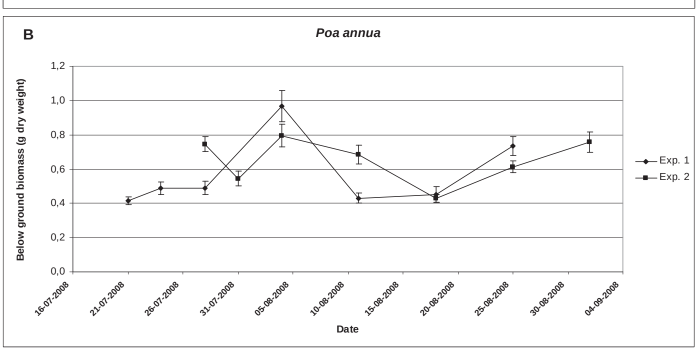


Fig. 12: Root weight of *Lolium perenne* (A) or *Poa annua* (B) following a flame treatment on August 21 (Exp. 1) or August 28 (Exp. 2).

It was expected that regrowth of the plants would increase more than it actually did with increasing time between treatments. Generally, however, this was not observed in the experiments, perhaps because of the time of the year. The experiments were performed late in the season (end of July to mid September), and whether the results would be the same in spring remains to be investigated. How treatment intervals longer than 21 days affect biomass production both in spring and late in the season should be explored. Treatment intervals of 28 and 35 days were included in the study, but it turned out to be too late in the season to provide reliable results. It could have been an advantage to harvest plants after different day degrees instead of number of days. It was not possible in practice due to the high work load of this study but may be recommended if similar studies are carried out.

Differences in effect between time intervals were relatively small in this study. Therefore, it should be investigated further whether a 7 days interval between the first two treatments actually would reduce the number of required treatments to control *L. perenne* during the rest of the season. Additional research is required to address this issue.

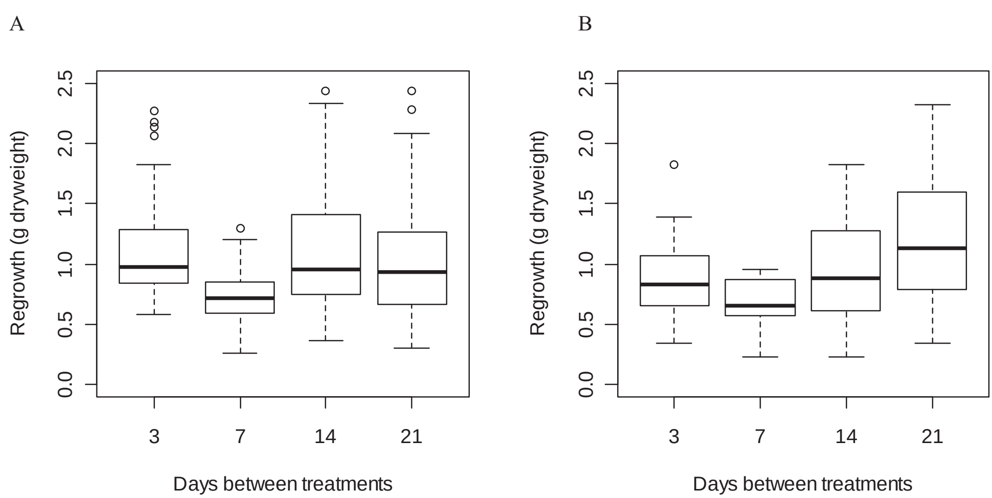


Fig. 13: Effect of time intervals. Box-and-whiskers plot of regrowth data (g dry weight of above ground biomass after two treatments carried out with different time intervals). The box contains the middle 50% of the response values with whiskers extending to the most extreme value which is no more than 1.5 times the interquartile range from the box. The thick solid lines inside the boxes are the medians. A) *Lolium perenne*, experiment 1, B) *Lolium perenne*, experiment 2.

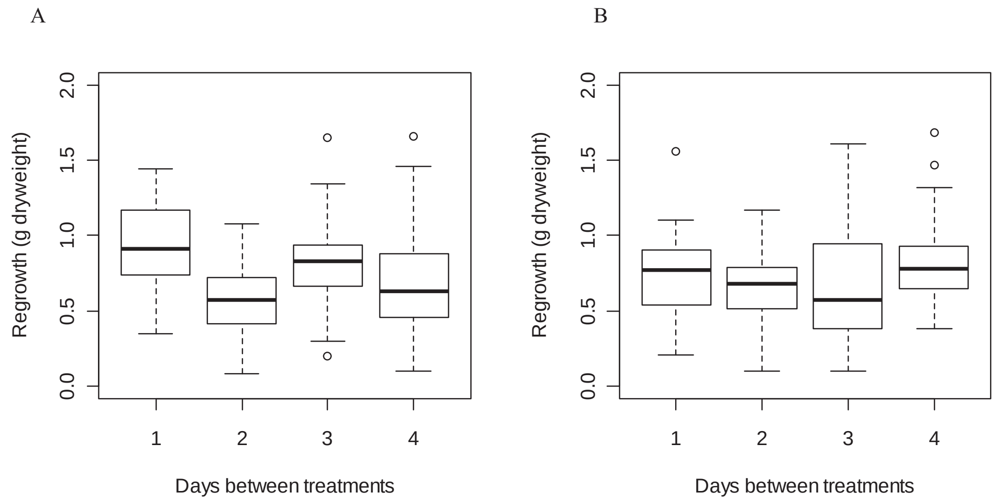


Fig. 14: Effect of time intervals. Box-and-whiskers plot of regrowth data (g dry weight of above ground biomass after two treatments carried out with different time intervals). The box contains the middle 50% of the response values with whiskers extending to the most extreme value which is no more than 1.5 times the interquartile range from the box. The thick solid lines inside the boxes are the medians. A) *Poa annua*, experiment 1, B) *Poa annua*, experiment 2.

Concluding remarks

The risk of pollution of groundwater and surface waters has led to restrictions on the use of pesticides in urban areas in many European countries. As a result of the strong regulation of pesticide use in Denmark as well as other European countries, there has been increasing interest in alternative ways to control weeds. Equipment for thermal treatments (e.g. gas burners and steamers), mechanical weed control (weed brushers) and equipment designed for semi-hard surfaces have been subject to continual research and development (Paper I). However, these alternative methods are often less cost-effective compared with spraying with glyphosate, which usually kills the weeds completely with few treatments per year. Non-chemical weed control methods require more frequently repeated treatments primarily due to regrowth of tolerant weed species.

The results presented in this thesis are in accordance with this assumption. Non-chemical treatments need to be repeated to kill larger plants and heat tolerant weeds such as grasses and perennial weeds that will regrow after a single treatment. The treatment frequency depend on factors, such as weed species composition, weed cover, weed acceptance level, weed control method, climate and type of hard surface. Because a high treatment frequency increases the costs of weed control, knowledge of treatment efficiency can provide practical advice on how to reduce the number of treatments and lower the costs of control.

In the semi-controlled experiments (Paper II and III) we investigated the regrowth of the two weed species: *Lolium perenne* L. and *Poa annua* L. Both species are very hard to control with non-chemical weed control methods on hard surfaces, and *P. annua* was the most frequent species recorded on pavements in five European towns (Melander *et. al.*, 2009). In the *in situ* experiments presented in Paper IV and V, the weed flora was dominated by perennial grasses as the salt-tolerant species *Puccinellia distans* (Jacq.) Parl., *Poa* spp. and *Elytrigia repens* (L.) Desv. Ex Nevski as well as several dicotyledonous weeds (mainly *Chenopodium album* L., *Chenopodium glaucum* L., *Lepidium ruderale* L. and *Taraxacum* spp.).

In Paper II, six treatments a year and a total dose estimated to around 631-674 kg propane ha^{-1}, depending on year, controlled *L. perenne* effectively (90% reduction in dry weight). All weeds were killed with doses above 80 kg propane ha^{-1} when treatments were carried out every other week (10 treatments per growing season and a total dose of 800 kg ha^{-1}) and no regrowth was seen the following two weeks. The experiments were carried out on a field with

L. perenne in its second year of growth. On hard surfaces also other grasses (e.g. *Poa* spp., *Festuca* spp., *E. repens*) are difficult to control and dicotyledonous weeds as e.g. *Taraxacum* spp. may be present in quite high densities and differing developmental stages. The results from Paper II show an important relation between applied dose and number of treatments, but it is not possible to give specific recommendations on which dose and which treatment interval that is optimal on hard surfaces. However, the results show the importance of applying a dose that is sufficient high in order to kill all above ground leaves. Thereby, the number of treatments per season can be reduced, which is more economically feasible than carrying out more treatments with a lower dose.

The constructed hard surface presented in paper III provided growth conditions that were more similar to pavements. It was specially designed to study the impact of time interval between flaming treatments on the regrowth and flower production of hard to control weeds. Flaming treatments decreased plant biomass of *L. perenne* and *P. annua* and also the ratio of flowering *P. annua* plants. However, only few plants were killed. The first flaming treatment affected aboveground biomass more than the second flaming treatment. A treatment interval of seven days provided the greatest reduction in regrowth of *L. perenne*, whereas the effect of treatment interval varied between the first and second repetition of this experiment for *P. annua*. In general, short treatment intervals (three days) should be avoided, as they did not increase the reduction of aboveground biomass compared with the seven day treatment interval.

In the *in situ* experiments on traffic islands that are presented in Paper IV, weed cover could be kept on an acceptable level with eight flame treatments a year and a mean dose of 150 kg propane ha^{-1} per treatment. The doses were higher than planned due to the irregular shape of the traffic islands, overlap and impediments such as traffic signs. In 2005 and 2006 a treatment was carried out every time the weed cover exceeded 1.6% (1.8% after mid July). In this way an estimation of the number of treatments needed to keep weed cover below a certain acceptance level could be obtained (Paper V). On the control areas a rapid increase in weed cover was observed, whereas 2-7 treatments per year could keep weed cover below 2%. On average during the two seasons, following number of treatments were required: Glyphosate 2.5, hot water 3, flames 5, hot air/ flames or steam 5.5 treatments. It was expected that the number of required treatments with the non-chemical weed control methods would have been higher, especially when the present weed flora of perennial weeds and grasses is taken into consideration. The results indicated that regular thermal treatments decreased the treatment frequency in the course of time, as fewer treatments were required to keep weed cover below the acceptance level in the third experimental year. It may also be possible that the hot water method with foam (Waipuna) has a longer lasting effect on the weeds. Whether it is because the other methods that were tested did not have the ability to kill the growth meristems of e.g. *Puccinellia distans,* which was a species that was highly represented on the trial sites, or because the insulating effect of the water and foam had the ability to kill superficial roots, remains to be investigated.

Measurements of plant regrowth after different treatments can be done in several ways. The choice of sampling methods may affect the results, especially when repeated treatments are carried out. In the study presented in Paper VI, the reductions in weed weight or weed cover after repeated flaming was measured in three ways: 1) plant dry weight, 2) percentage weed control by visual assessment of randomized images and 3) percentage green pixels by image analysis. Dry weight of plants gives a useful and qualified estimate of the effect of the treatment. However, if the aim of the investigation is to measure the effect of repeated weed control treatments, the removal of the biomass makes it impossible to measure how regrowth would occur under natural conditions. Additionally, collecting biomass samples is very labor demanding in large-scale experiments and if the samples contain a lot of withered grass that needs to be removed before weighing, this will increase the work load considerably. Another problem can be that it may be difficult to estimate when a plant is dead. The plant may still have some green parts or covered living shoots or buds even though most of the plant seems to be withered away (Paper III). Visual assessment with a frame to define the plot was used in the experiments on traffic islands (Paper IV and V) and in paper VI, visual assessment was carried out on randomized images from the experiments. Estimation of weed cover by visual assessment is a method which is very easy and quick to use in hard surface experiments. However, it is also a subjective method and may not always be accepted by scientific journals unless data are partly supported by objective measurements. The biased nature of visual assessment is not vital in experiments where the main objective is to compare different treatments within the same experiment. However, when results from different experiments are of interest, different people will often assess the control level differently, which make comparisons difficult. Therefore we tried a third method to measure weed cover, which should be easy and objective: image analysis. Once a well-working program is developed, many images can be analyzed objectively in very short time. Image analysis counting the number of green pixels could have been valuable to measure the amount of living above ground plant material in the experiment that was carried out on a constructed hard surface (Paper III). In this thesis, image analysis was used to measure changes in weed cover after repeated flaming (Paper VI). There was generally a clear relation between dose and treatment interval on the reduction of plant weight or decrease in vegetation cover. However, there were significant differences in the estimated effective doses (ED_{90}) depending on assessment method and length of treatment interval. The estimated effective doses (ED_{90}) were generally lower when the weed control effect was measured in dry weights in comparison with weed cover when treatment intervals were short (six to ten treatments a year). At long treatment intervals (four treatments a year) 90% control was only obtained when the control effect was assessed by weed cover probably because of relatively higher amounts of withered grass in the biomass samples. Two treatments a year showed almost no response on weed cover as these treatments only caused differences in vegetation heights. It is concluded that assessment of control effect after repeated treatments by image analysis is most useful when weed cover is low and when it is expected that the plots will contain relatively much withered plant material. However, when weed cover is close to 100%, or vegetation is mainly differing in heights, dry weights are preferred.

In conclusion, the results emphasise that it *is* possible to obtain acceptable control of larger plants and heat tolerant weeds such as grasses and perennial weeds with thermal weed control methods. However, it is necessary to assure that the dose is sufficiently high at each treatment in order to reduce the number of treatments per season. Another way to reduce the number of treatments per year is to assess the need for weed control regularly by a simple method and adjust the weed control effort to the required visual street quality.

Suggested future research

The results indicated that regular thermal treatments decreased the treatment frequency in the course of time, as fewer treatments were required to keep weed cover below the acceptance level in the third experimental year (Paper V). When treatments are carried out every second to fourth week, it will not be possible for new seedlings to establish. The effect of the non-chemical methods, which have the highest impact on small weeds, will therefore increase in the course of time. This long-term effect of regular treatments with non-chemical methods should be investigated further.

There is a potential for development of some of the weed control methods that are still at an experimental stage, such as weed control by laser radiation or UV-light (Paper I). It is also necessary to develop the existing equipment further and to make controlled tests or dose-response studies on the effect of some of the newer weed control methods, such as the hot water method with foam (Waipuna) or steam. The development of automated weed detection systems of weed cover would help rationalise a graduated weed control program, such as the "Wave" hot water equipment (http:// www.front2front.nl). However, the cost of sophisticated equipment would need to be balanced against faster operation speeds, reductions in water and energy consumption.

Non-chemical weed control requires more knowledge on the present weed flora, as especially perennial weeds are hard to control. This thesis concentrates on grass weeds, however, notably *Taraxacum officinale* F.H. Wigg, should receive particular attention as it is consistently recorded on hard surfaces (Melander *et al.*, 2009), and difficult to control with thermal methods (Ascard 1995; Hansson 2002).

Repeated use of any weeding method is likely to cause a shift in the weed flora to resistant or tolerant species. Such changes would limit the effectiveness of that particular weeding strategy. Therefore, an integration of combinations or sequences of different weed control techniques could reduce the risk of a selective pressure leading to the predominance of certain species (Paper I). For example, a combination of weed control methods, e.g. by brushing or sweeping at the beginning of the growing season to remove dirt and destroy the aboveground plant parts followed by thermal treatments at regular intervals throughout the season, may be advantageous. It was regrettable that there were no data on weed species composition before beginning of the trial on traffic islands (Paper IV-V). This information could have revealed how three years treatments with the same weed control method may favour certain weed species. Further studies are needed to reveal this important issue.

The development of simple and objective assessment methods like the “Imaging crop response analyzer” provide the possibility to standardise the assessment of the response of plants to repeated weed control. However, it would be an advantage if a program with the possibility of manual adjustment could be developed, in order to adjust for different colour thresholds and different light conditions, which can be measured with a calibration object in the image.

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Appendix

- **Paper I**

A M Rask & P Kristoffersen (2007). A review of non-chemical weed control on hard surfaces. *Weed Research* 47 (5), 370-380. (free access)

- **Paper II**

A M Rask; C Andreasen & P Kristoffersen (2012) Response of *Lolium perenne* (L.) to repeated flame treatments with various doses of propane. *Weed Research* 52 (2), 131-139

- **Paper III**

AM Rask, P Kristoffersen & C Andreasen (2011) Controlling grass weeds on hard surfaces: The effect of time intervals between flame treatments *Weed technology* 26, (1), pp. 83-88.

- **Paper IV**

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- **Paper V**

AM Rask, SU Larsen, C Andreasen & P Kristoffersen (2012) Repeated weed control on traffic islands using glyphosate and non-chemical weed control methods. (currently under review in *Weed Research*)

- **Paper VI**

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