Evaluation of two potential management strategies for garden lupin (*Lupinus polyphyllus*) in road verges

Utvärdering av två potentiella hanteringsmetoder för blomsterlupin (*Lupinus polyphyllis*) i vägkanter

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Sammanfattning

Invasiva växtarter är ett globalt problem som hotar inhemska ekosystem. Blomsterlupinen (*Lupinus polyphyllis*) är en invasiv flerårig växt som erhåller flera egenskaper som bidrar till dess framgångsrika etablering och spridning. Vägkanter agerar som landskapskorridorer för exotiska arter samtidigt som det är en viktig habitatrefug för många ängsväxter. För att effektivisera bevarandeåtgärder behövs en gedigen kunskap om målarten och dess egenskaper. Syftet med den här studien var att utvärdera två potentiella hanteringsmetoder av blomsterlupin i vägkanter. Slåtter är en redan etablerad strategi, men för att ta reda på om olika skördintensiteter påverkar återväxten av biomassa på olika sätt jämfördes en kontrollgrupp (ej skörd) och tre skördgrupper (1–3 skördar) med avseende på bladbiomassa, stambiomassa, blomställningsbiomassa och plantans totala biomassa. En annan potentiell hanteringsmetod utvärderades med fokus på fröbanken. Frön från blomsterlupin värmebehandlades i 40°C, 50°C och 70°C, och jämfördes mot frön utsatta för kontrolltemperatur (20°C). Varaktigheten för värmebehandlingar var 1, 5 och 15 minuter. Några frön utsattes för vattenbehandling innan värmebehandlingen för att bryta den fysiska vilan. Blomsterlupinfrön jämfördes mot tre andra arter i familjen ärtväxter (*Fabaceae*); blodklöver (*Trifolium incarnatum*), blålusern (*Medicago sativa*) och (*Louts corniculatus*). Det fanns en signifikant effekt beroende av skördintensitet på återväxt av stambiomassa, blomställningsbiomassa och plantans totala biomassa. Gällande bladbiomassa fanns ingen skillnad mellan grupperna. Faktumet att blomsterlupinen primärt investerar mer resurser på produktion av bladbiomassa kan anses positivt ur ett åtgärdsperspektiv. Återväxten av biomassa var emellertid beroende av plantans ursprungliga storlek, således skulle effekten av slåtter kunna skilja sig mellan individer i en population. Vattenbehandlingen bröt inte den fysiska vilan hos blomsterlupinfrön. Men en ökad temperatur under värmebehandlingen verkade ha en hämmande effekt för antalet grodda frön och MGT. Trots det återfanns ett relativt stort antal levande blomsterlupinfrön efter den sista observationsdagen. Det kan indikera att många frön skulle överleva en värmebehandling av fröbanken om behandlingen skulle implementeras som åtgärdsmetod. Metoden skulle förmodligen vara tidskrävande och dyr i förhållande till den önskade reducerande effekten på levande blomsterlupinfrön. För att utveckla resurseffektiva åtgärder för artens fröbank krävs mer kunskap. Slåtter verkar vara en bättre åtgärdsmetod om det genomförs vid rätt tidpunkt två gånger om året.

Abstract

Invasive plant species are a global problem that threaten native ecosystems. The garden lupin (*Lupinus polyphyllus*) is an invasive perennial herb and possesses several traits, which enables successful establishment and dispersal. Road verges serve as landscape corridors for exotic species, as well as being an important refuge habitat for many grassland species. To make conservation managements more efficient, an extensive knowledge of the target species and it's traits is required. The aim of this study was to evaluate two potential management strategies for garden lupin in road verges. Mowing is an established strategy already, but to find out if different cutting intensities will affect biomass regrowth differently, one control group (no cut) and three harvest groups (1-3 cuts) were compared regarding leaf biomass, stem biomass, inflorescence biomass and total plant biomass. Another potential management strategy was evaluated focusing on the seed bank. Seeds of garden lupin were heat treated at 40 °C, 50 °C, and 70 °C and compared to control seeds (20 °C). Duration of treatments was 1, 5, and 15 minutes. Some seeds were watered before the heat treatment to break physical dormancy. Seeds of garden lupin were compared to three other species in the Fabaceae family: *Trifolium incarnatum, Medicago sativa,* and *Lotus corniculatus.* I found a significant effect of cutting frequency on regrowth of stem biomass, inflorescence biomass, and total plant biomass. Regarding leaf biomass, there was no difference between harvest groups. The fact that garden lupin plants primarily invest more resources in leaf biomass regrowth can be regarded as positive from a management point of view. However, biomass regrowth was depended on initial plant size, and the mowing response might differ between individual plants in populations. The water treatment did not break the physical dormancy of garden lupin seeds. Increased temperatures during heat treatments seemed to have an inhibiting effect on both seed germination and mean germination time. However, a high number of vital garden lupin seeds were remaining after the last observation. This might indicate that many seeds would remain vital if seed bank heat treatments were implemented as a management strategy. The strategy would probably be time consuming and expensive in relation to the desired reducing effect on vital garden lupin seeds. To develop resource efficient management actions on the species' seed bank, more knowledge is needed. Mowing seems like a better management option if it is implemented at the right time twice a year.

Introduction

The dispersal of invasive vascular plants is a growing problem worldwide (Seebens et al., 2017). Invasive plant species affect the invaded habitats, causing diversity loss and change the species composition and the stability of ecosystems (Hejda et al., 2017). Preventing introductions of invasive species or limiting their impact is an expensive process and will require substantial resources (Vitousek et al., 1997). Therefore, it is important to use control methods adapted to the target species and its characteristics (Davis et al., 2006; Kettenring & Reinhardt Adams, 2011; Klinger et al., 2020).

The Swedish Transport Administration, as the responsible authority for the country's road and railway infrastructure, plays a crucial role in limiting the dispersal of invasive plant species. On 1 January 2015, the EU Regulation on the prevention and management of the introduction and spread of invasive alien species came into force (European Commission, 2015), which requires the Swedish Transport Administration to prevent the spread of invasive species.

The garden lupin (*Lupinus polyphyllus*) is a perennial herb in the Fabaceae family. It is native to western North America and was introduced to Europe as an ornamental flower (Valtonen et al., 2006). Since then, the species has spread rapidly and is found in large parts of Sweden, mainly along road verges and railway embankments (Wissman et al., 2015). Road verges are typically highly disturbed and serve as landscape corridors for exotic species (Lázaro-Lobo & Ervin, 2019). Garden lupin populations along road verges reduce species diversity of both plants and lepidopteran fauna (Valtonen et al., 2006). Meanwhile, road verges act as important refuges for grassland species, since the area of semi-natural grassland has declined during the last century (Cousins, 2006; Öster m. fl., 2007; Auestad m. fl., 2011; Lázaro-Lobo & Ervin, 2019). Road verges function as important complementary habitats and are also the main habitats for many species, including some species on the Swedish Red List (Lennartsson & Gylje, 2009). The Swedish Transport Administration is currently working on control projects in several parts of Sweden to reduce dispersal of garden lupin by excavation of the upper soil layer where plants are present (The Swedish Transport Administration, 2020).

Another common method to reduce garden lupin in road verges is mowing, which is considered effective against tall plant species (Jantunen et al., 2007). Several studies have indicated that survival, regrowth, flowering, and dispersal are reduced by mowing and biomass removal (Jantunen et al., 2007; Fremstad, 2010; Klinger et al., 2020; Ramula, 2020). However, mowing the road verges after flowering when the seed pods are ripe can increase the dispersal of seeds

(Klinger m. fl., 2020). Mowing directly removes of biomass from the plant, and if management is implemented at the right time, the number of seed-producing individuals will decrease in the population in the following seasons (Ramula, 2020).

According to Jantunen et al. (2007) and Fremstad (2010), mowing should occur twice a year for 3-5 years, i.e., before flowering and two months later (Fremstad, 2010). To implement mowing as a management strategy but only cut the plants once a year involves the risk that individuals will recover later during the season and still produce viable seeds (Martínková et al., 2004). The garden lupin is a resprouter, and resprouters are known to allocate more biomass to roots than to shoots, with high starch concentrations in roots (Knox & Clarke, 2005). Removal of biomass in plant species able to regenerate asexually might stimulate a dormant bud bank at the root collar to produce new shoots (Klimešová & Klimeš, 2007). The natural population dynamics and age differences might affect the management outcome (Davis et al., 2006), since larger plants have a competitive advantage in resource uptake within the invaded range (van Kleunen et al., 2010).

The garden lupin possesses several traits associated tht facilitate successful invasion in alien plants. As mentioned earlier, the species can regenerate vegetatively, even though sexual reproduction seems to be the most common strategy (Ramula 2014). Every plant produces hundreds of seeds, which are ballistically dispersed (Aniszewski et al., 2001). The seeds can remain viable in the soil seed bank for several years or germinate the following spring (Timmins & Mackenzie, 1995), which is characteristic of species occurring in disturbed habitats (Matus et al., 2005).

Mowing and excavation are management strategies used in Sweden to control invasive plant species. However, since every garden lupin plant produces hundreds of seeds that can remain dormant in the soil, management strategies targeting the seed bank are worth investigating. Some studies have considered the use of microwave-induced heat to control invasive plant species (Ambrose et al., 2015; Sahin & Saglam, 2015). Elliot et al. (2011) conducted a study on garden lupin seeds and found that seed germination was not affected by heat shocks. However, their study was only performed on dry seeds. Germination in the family Fabaceae is often a two-stage process, starting with the breaking of physical dormancy, followed by actual germination (Elliot et al., 2011). Physical dormancy is facilitated by hard seed coats that do not allow the seed to absorb water and is especially common in the Fabaceae family (Van Assche et al., 2003; Elliot et al., 2011). Protective tissues also promote seed survival during fire events, and the duration of heat treatment can affect plant species differently (Gashaw & Michelsen,

2002). Germination of garden lupin seeds is highly asynchronous, and the morphology of seeds affect vitality and the time of germination (Klinger et al., 2020). According to Klinger et al. (2020), there are seasonal differences in seed morphology. Seeds produced early are green, soft, and tend to germinate in autumn, while seeds produced late were brown, hard, and tended to germinate in spring after winter stratification. According to Aniszewski et al. (2001), garden lupin has several different seed types based on morphological and genetical characteristics. This may mean that different seeds respond to a treatment in different ways. Finding out if seeds remain viable if seeds are "awakened" from physical dormancy before being subjected to heat might be a possible opening for treatment of the species' seed bank.

The aim of this thesis is to evaluate two potential management strategies for dealing with garden lupin along road verges. Mowing is an already established method with a known inhibitory effect on garden lupin stands. However, mowing is often implemented at the wrong time, for example, when seeds already are produced or if mowing is done too seldom. Thus, different cutting intensities will be evaluated with respect to their effects on biomass regrowth. The plant biomass will be divided into stem biomass, leaf biomass, and inflorescence biomass as well at the total biomass of the plant. In addition, heat treatment of seeds will be evaluated as a potential management strategy, focusing on the seed bank. Dry and wet seeds will be exposed to the same heat treatments to study if the seeds are more sensitive to heat treatments after physical dormancy has been broken. To evaluate how seeds of garden lupin respond to treatments in relation to other species in the same family, the Fabaceae family, *Trifolium incarnatum, Medicago sativa,* and *Lotus corniculatus* will be exposed to the same treatments. The research hypotheses were:

1) Biomass per plant will decrease if plants are cut several times. However, the original plant size might affect biomass regrowth since larger plants potentially have greater starch reservoirs.
2) Seed germination will be reduced if the physical dormancy has been broken, i.e., seeds exposed to water before heat treatment will be affected to a greater extent than dry seeds by heat treatments.
3) The highest number of germinated seeds will occur in the control treatment for each species. With increased temperature, the number of germinated seeds will decrease.
4) Higher temperatures during heat treatment will slow down the germination process.
5) Since all the plant species in this study belong to the Fabaceae family, the main responses to treatments should not differ.

Methods

Field experiment

The experiment was carried out in Karlstad municipality, Sweden, close to Highway E18 (59°24'09.9"N 13°37'17.4"E), during summer 2020. Along a 50 m stretch, 40 garden lupin plants (as well as eight reserve plants) of similar size were selected in May 2020. Each plant was marked with flower sticks provided with duct tape, and the tape was numbered with plant-ID (1-48). Plants were randomly divided into groups. The study objects in the field consisted of four groups of 10 plants each. One group served as a control and was only cut at the final harvest on 12 August when all plant biomass was collected. The other groups were harvested once, twice, and three times respectively (harvest groups). The plants were measured (canopy height and diameter) and cut with scissors 2-3 centimeters above ground level. Each plant was photographed during the field visits to follow each individual plant's development (fig. 1). Plants were placed in paper bags and brought to the laboratory at Karlstad University. Here, the plants were dried in a drying cabinet (Termaks TS 8000) at 75 °C for at least three days. When the plants were dry, the stem, leaves, and inflorescences of each plant were sorted separately and weighed. The process was repeated after each harvest. The first harvest was done on 28 May, when all the plants except the control group plants were cut. At that time, all plants had a well-developed inflorescence. The second harvest was carried out on 16 June, and the third harvest was carried out on 10 July.

Figure 1. Development of a plant in the control group. Upper left: May; upper right: June; lower left: July; and lower right: last visit in August.

Laboratory experiment

The experiment to evaluate potential management strategies was performed in a laboratory at Karlstad University. 12,000 seeds from garden lupin were collected from the field study population. The seeds were collected in July, whereas seeds from *Trifolium incarnatum, Medicago sativa,* and *Lotus corniculatus* seeds (12,000 per species) were bought from Impecta (www.impecta.se/, Julita). In each Petri dish, two filter papers and 50 seeds were placed. Ten replicates for each treatment were prepared, and this was repeated for all study species. Temperature treatments were set to 20 °C (control temperature), 40 °C, 50 °C, and 70 °C, and the duration for the heat treatments were 1 minute, 5 minutes, and 15 minutes. Half of the seeds were dry during the heat treatment, while the other half had 4 ml of distilled water added 24 hours before the heat treatment. Seeds were always heat treated on Thursdays to be able to follow a consistent schedule observing the Petri dishes. After the heat treatment, 5 ml of water

were added to the dry seeds, and 1 ml of water was added to the wet seeds. Thereafter, all seeds were incubated in a plant growth chamber (Panasonic MLR-352) at a constant temperature of 20 °C. The lighting in the chamber varied from dark to light (12,200 LUX). Between 08:00 and 22:00, the fluorescent lamps in the chamber were lit, and between 22:01 and 07:59 lights were off to simulate the rising and setting sun during summer. The day after heat treatment, lupin seeds were scarified (a thin cut in the seed coat) with a scalpel to speed up the germination process. Scarification was considered necessary due to the thick seed coat making it difficult for seeds to absorb water (Brain, 1952). The seeds of *Trifolium incarnatum, Medicago sativa,* and *Lotus corniculatus* had thinner seed coats, thus no scarification was needed for these species. The Petri dishes were examined every Monday, Wednesday, and Friday for 20 days. Germinated seeds were counted and removed from the Petri dishes, and if necessary, more distilled water was added.

Statistical analyses

Data were sorted in Excel Office 365 and statistical analyses were performed in IBM SPSS Statistics 24. To examine whether the individuals randomly assigned to the four harvest groups differed in size, a one-way ANOVA was performed for both canopy height and diameter as a dependent factor. Number of harvests (control, 1 harvest, 2 harvests, 3 harvests) was used as a fixed factor.

To analyse how regrowth of biomass was affected by the number of times the plant was cut, biomass from all plant harvests was summed and expressed as per individual and also separated into leaf biomass, stem biomass, inflorescence biomass and total plant biomass. To account for initial size differences among individuals, canopy height×diameter was used as a covariate. A one-way ANCOVA was performed for each dependent factor (leaf biomass, stem biomass, inflorescence biomass, and total plant biomass) with the number of harvests as a fixed factor. The significance level was set at 95%. To test which of the harvest groups were significantly different from each other, TukeyHSD tests were performed.

To analyze if the plant size in May was related to regrowth of biomass, a regression analysis was performed. For the analysis, I used the total biomass of all harvest groups except for the control. The size was estimated as canopy height×diameter from the measurement in May and was set as an independent factor. The total biomass was set as a dependent factor.

To assess different aspects of germination, the number of germinated seeds was calculated for each Petri dish, and mean germination time (MGT) was calculated as the average time (days)

for germination. MGT is a weighted average of the time it takes for seeds to germinate, where the number of germinated seeds at different times is used as a proxy?? for weight. MGT is calculated as:

$$MGT = \sum(n \times d) / N,$$

where n = the number of germinated seeds at each observation, d = the number of days from the start of the experiment, and N = the total number of germinated seeds at the end of the experiment (Ellis & Roberts, 1981).

Analyses of the number of germinated seeds and MGT were performed with a significance level of 5%. The analyses focused on main effects of the variables. To find out whether the number of germinated seeds depends on temperature, treatment time, or water treatment, a three-way ANOVA was performed, where the logarithmic values for the number of germinated seeds and MGT were set as dependent factors. Analyses were performed for each species separately where temperature, treatment time, and water treatment were used as fixed factors. As there was only a significant effect of water treatment on germination for one species, wet seeds were excluded from all further analysis. Instead, a three-way ANOVA was performed, in which the logarithmic values for the number of germinated seeds and MGT were set as dependent factors, and species, temperature, and treatment time as fixed factors (significance level 5%). A TukeyHSD test was performed to test for differences in the number of germinated seeds and MGT between factor levels for the factors temperature, species and treatment time. Even with ln-transformed data, the variances were not homogenous. This might be due intraspecific differences. However, the results are considered robust since the study design is well balanced (Blanca et al., 2017).

To quantify the effect sizes of temperature treatments on seed germination, the log response ratio (RR) was calculated for germinated seeds heat-treated at 40 °C, 50 °C, and 70 °C. To calculate the RR for germinated seeds, the number of germinated seeds for each Petri dish was divided by the mean value of the number of germinated seeds in the controls for each species. RR values were then logged to achieve normality. Values for lnRR germinated seeds were used as a dependent variable in a two-way ANOVA (significance level 95%), where the purpose was to see which effect the temperature treatments had compared to the control treatment. Temperature and species were set as fixed factors. In the same analysis, a TukeyHSD test was

performed to see if there were any interspecific differences, and a TukeyHSD test for heat treatments was performed to find out if there were differences between the heat treatments on seed germination.

Results

Field results

For this study, 40 flower lupin plants were selected and randomly sorted into four harvest groups. There was no difference in canopy height between the groups at the start in May ($F_{3,36}$ = 0.039, P = 0.989), nor was there any difference in diameter ($F_{3,36}$ = 1.411, P = 0.255). As there was no significant size difference between the groups, the plants were selected for the study.

There was a significant effect of cutting on biomass regrowth for stem biomass ($F_{3,35}$ = 9.237, P <0.001), inflorescence biomass ($F_{3,35}$ = 15.741, P <0.001), and total plant biomass ($F_{3,35}$ = 8.400, P <0.001) after controlling for the original size of the plant ($F_{1,35}$ = 22.129, P <0.001). There was no significant effect between the different groups regarding leaf biomass ($F_{3,35}$ = 0.529, P = 0.665). TukeyHSD tests showed for both stem biomass and total plant biomass that the control group and the group that was cut twice were not significantly different from each other (Fig. 2).
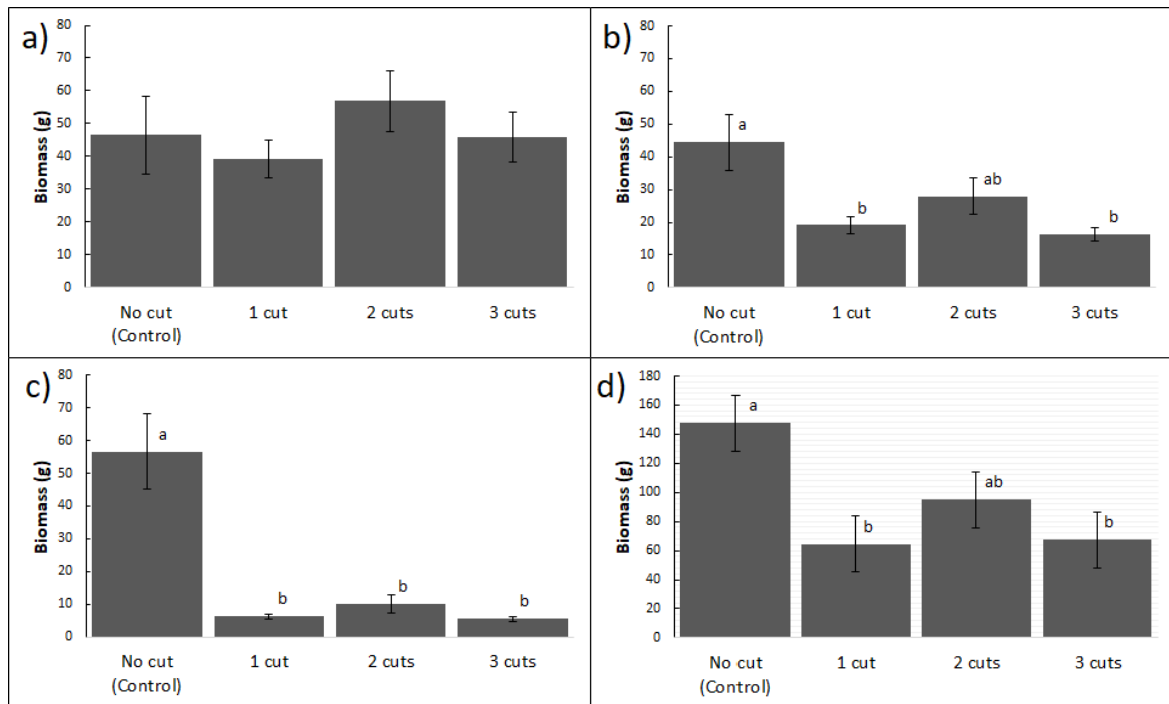
*Figure 2. Effects of cutting frequency on **a)** leaf biomass, **b)** stem biomass, **c)** inflorescence biomass, and **d)** total plant biomass. Bars show means ±SE. Different letters indicate significant differences (Tukey-test, P <0.05).*

The regression analysis showed that plant individuals that were larger in May had a better recovery despite cutting ($F_{1,28} = 49.538$, P <0.001, $R^2 = 0.639$), thus had the plant's original size a positive effect on regrowth of total biomass (Fig. 3).
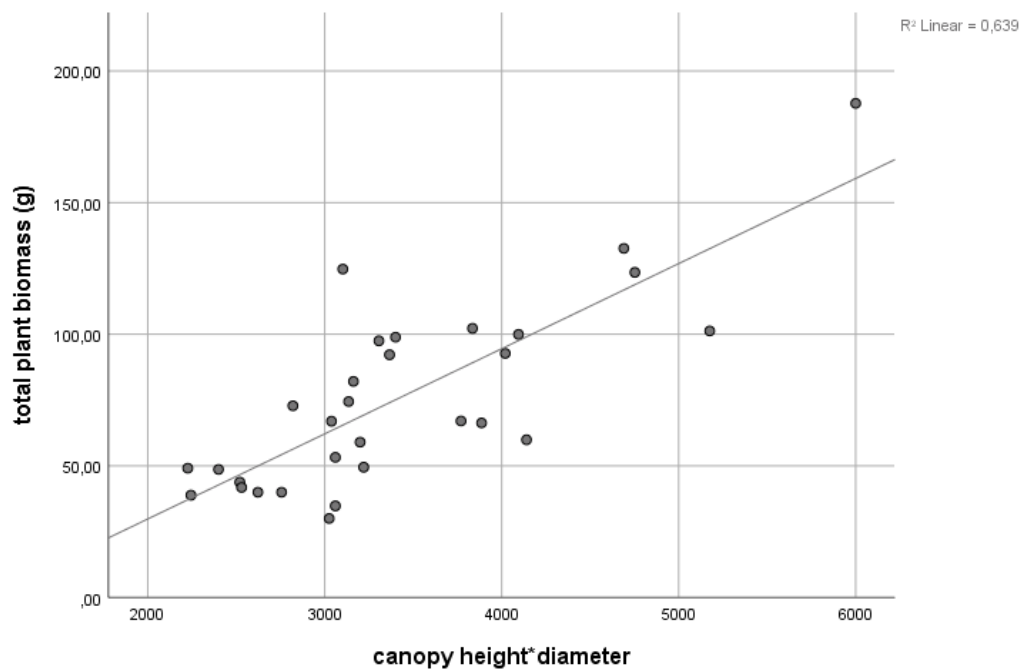

Figure 3. Relationship between plant size in May and the total biomass produced by each plant after cutting. Control group not included.

Laboratory results

A total of 48,000 seeds were used during this study, of which 37,712 had germinated at the last observation. The species were significantly different in terms of germinated seeds ($F_{3,432}$ = 1016.4 P <0.001). The number of germinated seeds per sample in each species was (mean ± SE): *Lupinus polyphyllus* (43.92 ± 0.31), *Medicago sativa* (40.61 ± 0.28), *Trifolium incarnatum* (46.72 ± 0.35), and *Lotus corniculatus* (28.18 ± 0.32). During the last observation day, remaining vital and dead seeds in every Petri dish were counted (table 1).

Table 1 Total number of vital and dead seeds from each species in all heat treatments and replicates during the last observation day.

Species, vital seeds	20 °C	40 °C	50 °C	70 °C	Total
Lupinus polyphyllus	100	170	166	245	**681**
Trifolium incarnatum	11	14	12	21	**58**
Medicago sativa	46	55	79	75	**255**
Lotus corniculatus	290	280	181	171	**922**
Species, dead seeds	20 °C	40 °C	50 °C	70 °C	Total
Lupinus polyphyllus	1	12	22	23	**58**
Trifolium incarnatum	8	22	96	210	**336**
Medicago sativa	179	206	240	247	**872**
Lotus corniculatus	305	356	538	498	**1,697**

There was a significant effect of the water treatment on the number of germinated seeds for *Trifolium incarnatum* ($F_{1,214}$ = 137.692, P <0.001). For *Lupinus polyphyllus* ($F_{1,216}$ = 0.147, P = 0.702), *Lotus corniculatus* ($F_{1,216}$ = 2.780, P = 0.097) and *Medicago sativa* ($F_{1,216}$ = 3.732, P = 0.055), water treatment did not affect the number of germinated seeds. Therefore, only dry seeds were kept for further analysis of the number of germinated seeds and MGT.

Temperature had a significant effect on seed germination ($F_{3,432}$ = 50.183, P <0.001; Fig 4a). Across all other factors, germination decreased significantly from 20 °C (42) to 40 °C (41) and was further reduced when seeds were exposed to 50 °C (39) and 70 °C (38); the latter two temperatures did not differ significantly from each other. The duration of heat treatment had a significant effect on the number of germinated seeds ($F_{2,432}$ = 4.491, P = 0.012; Fig 4b).
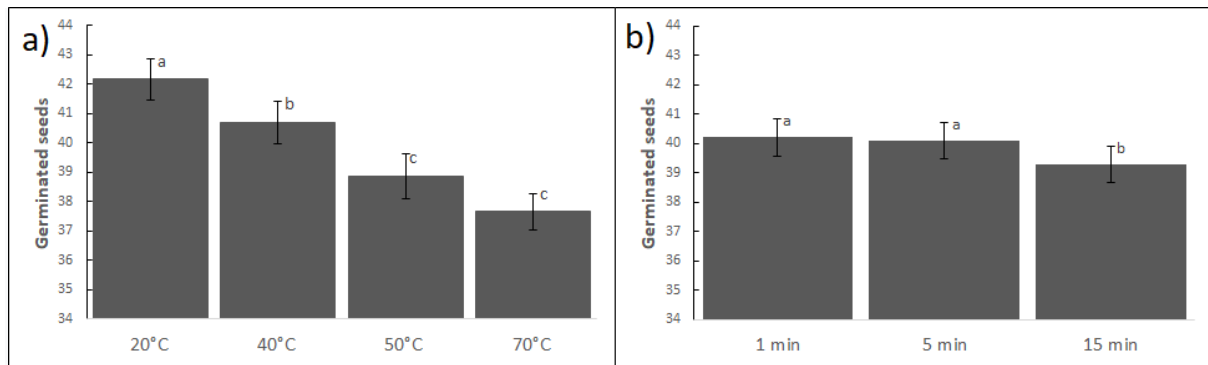
Figure 4. Number of germinated seeds in relation to **a)** temperature during heat treatment and **b)** duration of heat treatment. Bars show means ±SE. Different letters indicate significant differences (Tukey-test, P <0.05). Statistical analysis was done on log-transformed data.

The number of days required for seed germination varied between species ($F_{3,432} = 1519.4$, P <0.001). MGT was (mean days ± SE) *Lupinus polyphyllus* (6.56 ± 0.1), *Trifolium incarnatum* (2.41 ± 0.07), *Medicago sativa* (1.79 ± 0.05) and for *Lotus corniculatus* (4.5 ± 0.07). There was a significant effect on MGT depending on the temperature the seeds were exposed to during the heat treatment ($F_{3,432} = 34.225$, P <0.001). MGT for seeds at the control temperature (20 °C) was (3.81 ± 0.22), 40 °C (3.88 ± 0.2), 50 °C (3.54 ± 0.16) and 70 °C (4.03 ± 0.14; Fig 5a). The time seeds were exposed to heat treatment also had a significant effect on MGT ($F_{2,432} = 8.959$, P <0.001), where seeds exposed to heat treatment for 5 minutes had the shortest MGT (3.7 ± 0.17). Heat treatment for 1 minute (3.83 ± 0.16) and 15 minutes (3.91 ± 0.16) were sorted into the same homogeneous groups in TukeyHSD test (Fig. 5b).
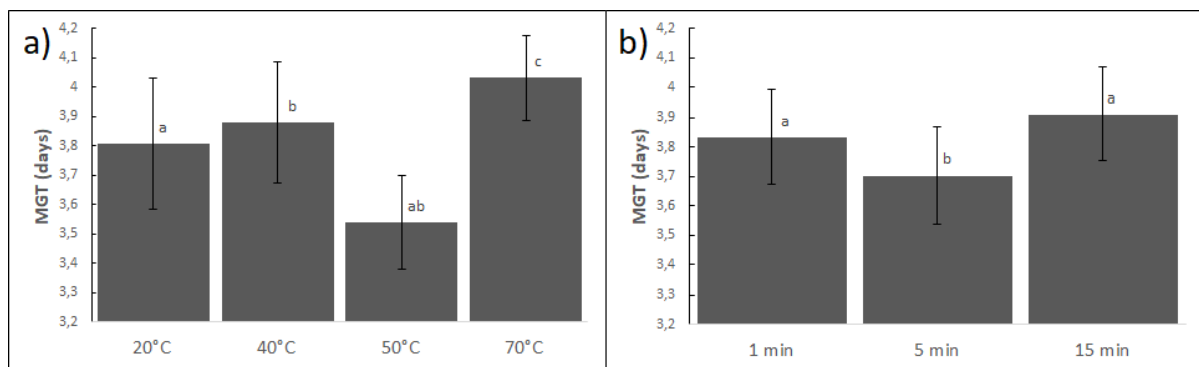

Figure 5. Number of days calculated on lnMGT depending on **a)** temperature during heat treatment and **b)** duration of heat treatment. Bars show means ±SE. Differnet letters indicate significant differences. (Tukey-test, P <0.05).

To find out how seeds exposed to higher temperatures during heat treatments germinated in relation to seeds in the control temperature (20 °C), logarithmic Response Ratio values were used in the analysis. In comparison with controls, the number of germinated seeds decreased at a higher temperature during the heat treatment ($F_{2,348} = 23.451$, P <0.001). There was a significant effect on germinated seeds in relation to control seeds depending on species ($F_{3,348}$

= 2.690, P = 0.046). *Lotus corniculatus* showed the greatest difference between heat treatment and control temperature and was distinguished from other species in the TukeyHSD test (Fig 6a). There was a significant difference in how the species reacted to different temperatures during the heat treatment in relation to controls ($F_{6,348} = 5.757$, P <0.001; Fig. 6b).
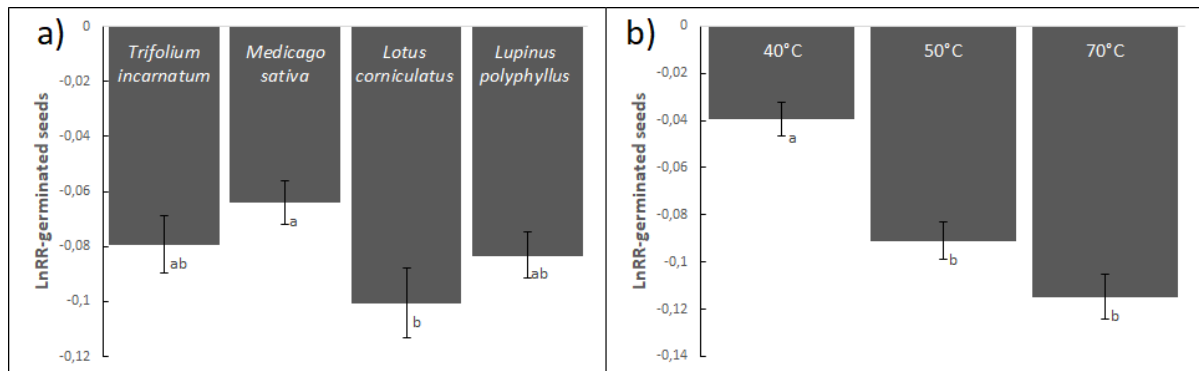

*Figure 6. Response ratio calculated on lnRR depending on **a)** species and **b)** temperature during heat treatment. Bars show means ±SE. Differnet letters indicate significant differences. (Tukey-test, P <0.05).*

Discussion

To evaluate two potential management strategies for garden lupin in road verges, two experiments were performed. In a field experiment, garden lupin plants were randomly assigned to four groups that were cut with different frequencies. Biomass per plant was expected to decrease if plants are cut several times, and cutting seemed have a reducing effect on biomass regrowth. Although, the original plant size affected biomass regrowth as larger plants in May had a better recovery rate while cut than smaller plants. To investigate how garden lupin seeds react to heat treatment, seeds were exposed to different temperatures for different durations, with or without water treatment. Seed germination was expected to be reduced if the physical dormancy has been broken, i.e., seeds exposed to water before heat treatment should be more affected than dry seeds by heat treatment. However, the water treatment did not have any effect on garden lupin seeds. The highest number of germinated seeds occurred in the control treatments, and increased temperatures had an inhibiting effect on seed germination. Higher temperatures during heat treatments slowed down the germination process to some extent, and the main effects in the study species' response to heat treatments and duration of heat treatment were the same.

Regrowth regarding stem biomass and inflorescence biomass was significantly lower in harvest groups than in the control group, with one exception, Here the group cut twice could not be separated from the control group and the other harvest groups. While not a clean result, the

result in general is consistent with several previous studies, mowing has an inhibitory effect on biomass regrowth (Jantunen et al., 2007; Fremstad, 2010; Klinger et al., 2020; Ramula, 2020). This might be described by one or a few individuals in harvest group 2 regenerating particularly well. Although the groups were not significantly different in average size when the plants were selected for the experiment, individual regrowth was positively related to initial size, regardless of how many times they were cut. The original size of plants had a positive effect on the total plant biomass, which means that larger plants in May recovered better than small plants. This could be explained by how a large plant size provides a competitive advantage in resources uptake within the invaded range (van Kleunen et al., 2010), depending on a large and well-developed root system with greater energy resources that facilitates rapid regrowth (Knox & Clarke, 2005; Klimešová & Klimeš, 2007). Therefore, population age/size differences might be important for how well control methods work (Davis et al. 2006). The study conducted by Ramula (2020) was performed on contemporary garden lupin plants under controlled conditions, and her results might not be applicable to the results of this study. This study was conducted for a limited time (May-December) while Ramula determined that if cutting is implemented annually, the plant's chance of survival is reduced in the coming year. As it was not possible to follow up the cut plants the next season, this study cannot conclude any result in unevenly aged stands for the following year.

Leaf biomass was not significantly different from the control group for any of the harvest groups, indicating that the plant invests most resources into leaf regrowth after cutting, while stem and inflorescence regrowth are secondary. If populations are mowed twice a year (Jantunen et al., 2007; Fremstad, 2010), the first time before flowering and the second time two months later with follow-up managements according to Fremstad's (2010) recommendations, it should be possible to eradicate populations in the long run. Cutting another 1-2 times per season cannot be motivated from my study, as there were no direct differences between the harvest groups regarding biomass regrowth. The fact that garden lupin plants invest most resources in leaf biomass, regrowth can be regarded as positive from a management point of view as the seed production should be reduced if mowing is performed as recommended.

In the laboratory experiment, I expected that the vitality of seeds would be affected more if the physical dormancy had been broken. Thus, seeds that absorbed water before the heat treatment were expected to be affected more by higher temperatures than seeds that were dry during the heat treatment. Temperatures and the duration for heat treatments were limited to a maximum of 70 °C and 15 minutes to be applicable in field. However, the prediction was true only for

Trifolium incarnatum, while there was no effect of the water treatment for garden lupin, *Medicago sativa* and *Lotus corniculatus*. Without water absorption before heat treatment, *Trifolium incarnatum* had the highest number of germinated seeds. Overall, the main effects in the study species' response to heat treatments and duration of heat treatment were the same. The water treatment did not break the physical dormancy in garden lupin seeds, probably due to the thick seed coat preventing water absorption (Van Assche et al., 2003). Scarification of garden lupin seeds occurred one day after heat treatment, but to get any effect from water treatments regarding the seed germination, scarification probably had to be done before heat treatments. The results of the current study are corroborated by Elliot et al. (2011), who showed that the vitality of dry garden lupin seeds was not affected by sudden heat shocks. However, morphological traits in garden lupin seeds seem to be important for vitality and when seeds tend to germinate (Klinger et al., 2020). Seeds used for this study were collected late during the season (July), which may have affected the outcome of the results. According to Klinger et al. (2020), seeds produced late tend to be harder and germinate the following season. Seeds from garden lupin in this study had the longest MGT of the studied species, while the number of living seeds that remained after last observation was relatively high. This could be an indication that the seeds would have germinated the following season under natural conditions. The results might have been different if seeds had been collected earlier in the season. Aniszewski et al. (2001) found that there are four different types of garden lupin seeds based on morphological characterization of seed coat ornamentation and genetical traits. They suggest that garden lupin contains different subspecies which may mean that different garden lupin seeds respond to treatments in different ways. Since there seems to be morphological, genetical and seasonal differences in garden lupin seeds, different responses to treatments may be expected. If this study had been conducted on seeds developed early in the season with thinner seed coats, the outcome would probably have been different.

High temperatures inhibited seed germination to a greater extent than low temperatures, which was an expected result. The highest number of germinated seeds were found in control treatments (20 °C) for all species, and in general the number of germinated seeds was reduced most in the 50 °C, and 70 °C heat treatments. Although hard seed coats are common in species within the Fabaceae family, leading to physical dormancy (Van Assche et al., 2003) and providing protection against external stress, the number of germinated seeds was affected by higher temperatures. Despite this, a high number of vital garden lupin seeds remained after the last observation. This might indicate that if seed bank heat treatments were implemented, many

seeds would still be viable. Furthermore, the duration of heat treatment had a general effect on the number of germinated seeds. This can be explained by higher stress levels if seeds are exposed to a heat treatment for a longer period. The variation between species may depend on the thickness of the seed coat (Gashaw & Michelsen, 2002), but also on how long the germination process generally takes for each species after water absorption. One prediction was that it would take a longer time for seeds to germinate at higher temperatures, and this corresponded with the experimental results. However, no major differences were observed in MGT for the temperature treatments below 70 °C, which may be an indication that the germination process is generally slower only when a certain temperature has been reached. Garden lupin seeds were significantly different from the other species with respect to MGT and required the longest time to germinate, which may be due to the seeds being collected late in the season (Klinger et al. 2020) or the thicker seed coat (Brain, 1952).

Limiting the impact of established invasive species is an expensive process and will require substantial resources (Vitousek et al., 1997). Therefore, it is important to use management strategies adapted for the target species and its characteristics (Davis et al., 2006; Kettenring & Reinhardt Adams, 2011; Klinger et al., 2020). The garden lupin has several traits that contribute to its successful establishment and spread. It thrives in highly disturbed habitats, such as road verges (Wissman et al., 2015), it reproduces both vegetatively and sexually (Ramula 2014) and produces many seeds (Aniszewski et al., 2001), which can be preserved in the seed bank for a long time (Timmins & Mackenzie, 1995). The seed germination is asynchronous, and the morphology and genetics in seeds are important for their vitality and when they tend to germinate (Aniszewski et al. 2001; Klinger et al., 2020). The Swedish Transport Administration, as the authority responsible for the country's state road and rail infrastructure, is regulated by the EU regulation on the prevention and management of the introduction and spread of invasive alien species. Therefore, it is obliged to prevent the spread of invasive species (European Commission, 2015). Dealing with a species like the garden lupin is a challenging task. The Swedish Transport Administration is currently conducting a control project, which involves removing the upper soil layer where flower lupin occurs (Swedish Transport Administration, 2020), but further management is needed to reduce the occurrence of garden lupin.

Conclusions

In this study, two potential management strategies for garden lupin were evaluated. Mowing is a previously known effective method for reducing the spread of garden lupin (Jantunen et al.,

2007; Fremstad, 2010; Klinger et al., 2020; Ramula, 2020), but to streamline resource usage, this study evaluated if several mowings reduced the regrowth of biomass to a greater extent. Regrowth of biomass seemed to depend on plant size, which indicates that population age differences might be important for how well control methods work (Davis et al., 2006). Cutting plants reduced stem biomass and inflorescence biomass, which is important for reducing seed dispersal. According to this study, mowing once during a season significantly reduces plant biomass with no difference between one, two, or three mowing occasions. However, cutting plants once a year involves the risk that individuals will recover later during the season and still produce viable seeds (Martínková et al., 2004). Mowing three times during a season does not result in a greater reduction in garden lupin stands. Mowing populations once before inflorescences have fully developed, and one more time two months later with follow-up managements following years according to Fremstad's (2010) recommendations seems to be the most reliable method for reducing garden lupin populations in road verges.

The second potential management strategy targets the soil seed reservoir of garden lupin. Although high temperatures had an inhibiting effect on seed germination, biological differences between temperature treatments are rather small. The hard seed coat is assumed to protect against external stresses to a high degree, which makes it difficult to apply heat treatment of the seed bank as an effective management strategy. It would probably be time consuming and expensive in relation to the desired reducing effect on vital seeds. However, there are studies that indicate that the garden lupin seeds are genetically and morphologically different (Aniszewski et al. 2001; Klinger et al., 2020), which may mean that different seeds respond to heat treatment in different ways. For further evaluation of the effect of heat treatments on garden lupin seeds, seeds collected at different times during the season should be exposed to heat treatment, as well as how the seed coat can be less resistant to external stresses.

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