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Potential of the green dock beetle (*Gastrophysa viridula*) as a biocontrol agent of northern dock (*Rumex longifolius*) in Norwegian grassland

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Plant science

Preface

This thesis concludes my master's degree and five years of studies at the Norwegian University of Life Sciences (NMBU). I have been interested in biological control for a long time, and I am grateful for the opportunity to contribute along with researchers at NMBU and the Norwegian Institute of Bioeconomy Research (NIBIO) to the knowledge on the potential use of biological control in integrated pest management of *Rumex longifolius*.

The thesis is based on two experiments performed as part of this thesis as well as a field experiment conducted in 2009 at the NIBIO research station in Ås as part of "Control of docks (*Rumex* spp.) in organic fodder production – a true bottleneck in organic farmed branded dairy and meat products" (Project number 176812). Thank you to Uno Andersen, Lars Olav Brandsæter, Paul E. Hatcher, and Jan Netland for allowing me to use your data.

The execution of experiments and completion of this thesis would not have been feasible without the help of many people. First, I would like to give a large thank you to my main supervisor Lars Olav Brandsæter at NMBU/NIBIO and co-supervisor Nina Svae Johansen at NIBIO for excellent guidance, help and encouragement throughout this process. A thank you is also sent to my corresponding co-supervisor Paul E. Hatcher in the School of Biological Sciences at the University of Reading for his advice. For guidance and help with the statistics, I would like to thank Torfinn Torp at NIBIO. For their advice and insights, I would like to thank Therese With Berge, Kirsten Semb Tørresen, Uno Andersen, and Andrew Dobson at NIBIO. For their help with cultures, constructing, and removing cages, I would like to thank Henrik Antzée-Hyllseth, Elisa Gauslå, Marta Bosque Fajardo, Marit Helgheim and Andreas Beachell at NIBIO. For assistance regarding greenhouse rooms, I would like to thank Per Johan Henrik Haugs Jorde at Senter for klimaregulert planteforskning (SKP). For help with determining species, I would like to thank Torstein Kvamme at NIBIO and Kai Berggren. For help with translating articles, I would like to thank Haldor Fykse, Jens Dietrich, and Kristina Wathne Eftestøl. And for proofreading, I would like to thank Miriam Dybing Taksdal.

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Ida Dybing

Abstract

Docks (*Rumex* spp.) act as weeds in many parts of the world, and in Norwegian grasslands, the northern dock (*Rumex longifolius*) is the most widespread dock species. The ability of farmers to control docks without the use of chemical herbicides is thought to be a limiting factor in the conversion to organic farming. The purpose of this study, including three experiments, was to investigate the potential of the indigenous green dock beetle (*Gastrophysa viridula*) for biological control of *R. longifolius* via the inundative method in Norwegian grasslands. Firstly, the effect of different applied developmental stages and densities of *G. viridula* on *R. longifolius* seedling growth and survival, when grown under competition from perennial ryegrass (*Lolium perenne*), was examined in a field experiment in summer 2009 to summer 2010. Secondly, the effect of grazing by *G. viridula* on dock growth when *R. longifolius* seedlings were grown alone, under competition from Italian ryegrass (*Lolium multiflorum*), or a "ley mixture" containing forage species commonly used in Norwegian leys, was examined in a glasshouse experiment in autumn 2019. Thirdly, the dispersal of *G. viridula* in a perennial ley was examined in a field experiment in summer 2019. In the grazing field experiment, *G. viridula,* when applied as third instar larvae, significantly reduced the percentage leaf area remaining of the four oldest dock leaves, the survival of docks to the following year, and shoot weight the following year when compared to the control. While when applied as gravid females, *G. viridula* was not able to reduce dock growth or survival compared to the control. The most effective densities of third instar larvae in reducing dock growth and survival were 250 and 500 larvae per 16 dock plants. In the glasshouse experiment, *G. viridula* applied as gravid females and apparent males were able to significantly reduce the shoot and root growth of *R. longifolius* regardless of competition level. The most significant effect on root dry weight, when compared to an ungrazed, non-competing control, was found when dock plants were exposed to both grazing and competition, irrespective of with which species the docks were competing. The final root dry weight of grazed, competing docks were 71.9 – 72.9 % less than that of ungrazed, competing docks. The expected dispersal of *G. viridula* in a perennial ley within the completion of one generation was found to be between 5 and 15 meters. The potential of *G. viridula* as a biocontrol agent of *R. longifolius* in Norwegian grasslands was found to be high, with the highest efficacy when applying third instar larvae at a density of 250 larvae per 16 dock plants, but also a good effect of applying gravid females in one of the two experiments. The ultimate effect on dock root dry weight, when grazed by *G. viridula*, appears to be independent of whether it is grown in competition with Italian ryegrass or a ley mixture, indicating that *G. viridula* may be able to compensate to some extent for differences in competitive ability between grasses/ley mixtures. Short dispersal in the field indicates that releases of *G. viridula* in the field would have to be at close intervals (m) or within dock patches.

Samandrag

Høymole (*Rumex* spp.) opptrer som ugras i mange deler av verda, og i norsk grasmark er vanleg høymole (*R. longifolius*) den mest utbreidde arten. Bondens evne til å kontrollere høymole utan bruk av kjemiske herbicid er trudd å vere ein hemjande faktor i overgangen til økologisk landbruk. Føremålet med denne studien, som inkluderte tre forsøk, var å undersøkje den innfødde syrebladbillas (*Gastrophysa viridula*) potensial til biologisk kontroll av vanleg høymole ved overfløymingsmetoden i norsk grasmark. (1) I eit feltforsøk frå sommaren 2009 til sommaren 2010 vart effekten av syrebladbiller tilført ved ulike utviklingsstadium og tettleikar på frøplanter av vanleg høymole som vaks under konkurranse frå fleirårig raigras (*Lolium perenne*), undersøkt. (2) I eit veksthusforsøk hausten 2019 vart effekten av herbivori frå syrebladbiller på vekst av frøplanter av vanleg høymole, når plantene vaks aleine, med konkurranse frå Italiensk raigras (*Lolium multiflorum*) eller frå ei vanleg engfrøblanding, undersøkt. (3) I eit feltforsøk sommaren 2019 vart spreiinga av syrebladbiller i ei fleirårig eng undersøkt. I feltforsøket med herbivori reduserte syrebladbillene, når tilført i tredje larvestadium, signifikant prosenten av attverande bladareal av dei fire eldste høymoleblada, overlevinga til det fylgjande året, og skottørrvekta det fylgjande året når samanlikna med kontrollen. Når syrebladbillene var tilført som gravide hoer derimot, klarte dei ikkje å redusere verken veksten eller overlevinga av høymole når samanlikna med kontrollen. Tettleikane av tredje-stadiums larvar som mest effektivt reduserte høymolevekst og overleving var 250 og 500 larvar per 16 plantar. I veksthusforsøket, når syrebladbiller vart tilførde som gravide hoer og tilsynelatande hannar, klarte dei å signifikant redusere skot- og rottørrvekt av vanleg høymole, uavhengig av konkurransenivå. Den største effekten på rottørrvekt når samanlikna med ein kontroll utan verken herbivori eller konkurranse, vart funne når høymoleplantene vart utsett for både herbivori og konkurranse, uavhengig av kvifor nokre artar høymola konkurrerte med. Den avsluttande rottørrvekta var 71.9 – 72.9 % lågare hjå konkurrerande planter som var utsett for herbivori, enn konkurrerande planter som ikkje var utsett for herbivori. Den forventa spreiinga av syrebladbiller i ei fleirårig eng innføre fullførelsen av ein generasjon vart funne å vere mellom 5 og 15 meter. Syrebladbillas potensial som kandidat i biologisk kontroll av vanleg høymole i norsk grasmark vart funne å vere høgt. Den høgaste effekten vart funne ved tilføring av 250 tredje-stadiums larvar per 16 høymoleplanter, men ein god effekt vart også funne ved tilføring av gravide hoer og hannar i eit av to forsøk. Den endelege effekten på rottørrvekt, når høymoleplantene vart utsett for herbivori av syrebladbiller, var tilsynelatande uavhengig av om høymola konkurrerer med Italiensk raigras eller engfrøblandinga, som indikerer at syrebladbilla kan vere i stand til å kompensera for ulikheiter mellom konkurranseevna til i kvart fall nokre grasarter/engblandingar. Kort spreiing i enga indikerer at utslepp av syrebladbiller vil måtte vere med korte intervall (m) eller inni "flekker" av høymole.

Contents

1 Introduction

Dock (*Rumex* spp., Polygonaceae) species act as weeds in many parts of the world (Cavers & Harper, 1964; Holm & Korpelainen, 1999) and two of them, broad-leaved dock (*R. obtusifolius* L.) and curly dock (*R. crispus* L.) are counted among the world's worst weeds (Holm et al., 1977). Besides *R. obtusifolius* and *R. crispus*, the northern dock (*R. longifolius* DC.) is very common in Fennoscandia (Holm & Korpelainen, 1999), and the most widespread dock species (Fykse, 1986), and most problematic dicot weed in Norwegian grasslands (Haugland, 1993), where it is found throughout the country (Fykse, 1986). Docks have a lower palatability and digestibility for ruminants than grass (Timenes, 1986), and reduce quality (Hejduk & Dolezal, 2004) and quantity of grass yield in grassland and pasture (Oswald & Haggar, 1983). Of herbicides used in conventional grasslands in central Europe, 80 % are estimated to be used to control *Rumex* spp. [Galler, 1989 (not seen), cited by Ringselle et al., 2019]. In Norway, 80 % of organic agricultural land consists of grassland and meadow (Debio, 2019), and the ability of farmers to control docks without chemical herbicides is thought to be a limiting factor in conversion from conventional to organic farming (Hatcher et al., 2008). It is, therefore, important to find an effective non-chemical control method of docks. All farmers in both Norway and the EU are also required by law to practice integrated pest management (IPM) (Directive 2009/128/EC, 2009; *Forskrift om plantevernmidler*, 2015 § 26). The fourth principle of IPM states that "Sustainable biological, physical, and other non-chemical methods must be preferred to chemical methods if they provide satisfactory pest control" (ANNEX III of Directive 2009/128/EC, 2009).

Much research has been performed on non-chemical control of *Rumex* spp., and research on combining control methods has been called for (Zaller, 2004). In addition to research on, among others, mechanical control of *Rumex* spp. (Hujerová et al., 2016; Ringselle et al., 2019; van Evert et al., 2020) there has also been much research on using biological agents to control *Rumex* spp. (Davies & Turner, 2010; Grossrieder & Keary, 2004; Hatcher et al., 2008). Hatcher et al. (2008) claims that the most promising agents in Europe for inundative biological control are the indigenous green dock beetle (*Gastrophysa viridula* De Geer., Chrysomelidae) and a rust fungus [*Uromyces rumicis* (Schumach.) G. Winter].

Gastrophysa viridula is an oligophagous herbivore that prefers feeding on docks (Martinková & Honek, 2004). In Norway, *G. viridula* is found throughout the country (Artsdatabanken, n.d.) and have been observed feeding on *R. longifolius* in the field (Lars Olav Brandsæter, professor at the Faculty of Biosciences, NMBU. Personal communication). Their voltinism depends on their habitat and temperature, and in northwestern England, they routinely undergo three generations a year on managed grassland (Smith & Whittaker, 1980b). Their voltinism in Norway has not been studied, but as they have been observed far north in Norway (Artsdatabanken, n.d.), one can assume that they can complete at least one generation throughout the country. Calculations using air temperature 2 m above ground level (NIBIO, n.d.), the estimated lower developmental threshold for *G. viridula* (7.9 °C) and the required sum of degree-days to complete one generation (Kucherov & Kipyatkov, 2011) indicates that this species was able to complete three generations in southern and central Norway, and one generation in northern Norway in 2019. The average temperature in Norway was 1.2 °C above normal in 2019 (Grinde et al., 2020). Temperature would often be higher in the microclimate of crops, however (Robertson, 1953, not seen. Cited by Holmes & Dingle, 1965), and factors such as the timing of emergence from hibernation and habitat (Smith & Whittaker, 1980b) also affect their voltinism. Smith and Whittaker (1980b) found that *G. viridula* completed more generations per year when grown in regularly mown grassland than in uncut grassland or a dock monoculture. The fact that *G. viridula* is present in all of Norway, feeds on *R. longifolius* and is probably able to complete several generations in much of Norway makes it a promising potential biocontrol agent also in Norwegian grasslands.

Herbivory by *G. viridula* and infection of *U. rumicis*, both separately and combined, of *R. obtusifolius* and *R. crispus* has been found to reduce the growth of first-year docks (Hatcher et al., 1994; Hatcher, 1996). Herbivory of *R. crispus* by G. viridula was also found to reduce dock growth and survival on a shingle bank (Whittaker, 1982) and reduce the growth of *R. crispus* and *R. obtusifolius* when grown with interspecific competition from each other and alone at certain densities of applied gravid females and males (Bentley & Whittaker, 1979). In a ley, docks will grow with competition from the grass sward, and as competition from grass significantly affect dock seedling growth (Haugland, 1993; Jeangros & Nösberger, 1990), it is an important element to introduce in experiments on the efficacy of *G. viridula* as a biocontrol agent. However, few experiments have been performed on grazing by *G. viridula* on docks growing in competition with grass. Keary & Hatcher (2004) found that when *G. viridula* and *U. rumicis* on *R. obtusifolius* were combined with competition from *Lolium perenne* L., only herbivory by *G. viridula* was able to reduce seedling growth consistently. And Cottam et al. (1986) found that *G. viridula* was only able to reduce the growth of *R. obtusifolius* when grown in competition with grass.

The dispersal of green dock beetles in the field has been questioned as they have never been observed flying (Smith & Whittaker, 1980a). Smith and Whittaker (1980a), therefore, examined their dispersal eight meters out from a release point for one week, and found beetles at eight meters already after two days. In a different experiment, their movement was measured in a ten-meter radius for up to 22 days, where the average distance of recapture from the release point was three meters, and the maximum distance seven meters (Whittaker et al., 1979). In the last study, they had also observed a *G. viridula* migration of seemingly 35 meters to recolonize a shingle bank.

Despite much research performed on inundative biocontrol by *G. viridula* on other dock species, no studies have been published on *R. longifolius*, which is most common in Norway (Fykse, 1986). Also, no systematic studies have been performed on the expected dispersal of *G. viridula* in the field beyond ten meters, which is an important aspect if *G. viridula* is to be used as a biocontrol agent by the inundative method.

This study aimed to assess the potential of *G. viridula* as a biocontrol agent of *R. longifolius* in Norwegian grassland. Research questions posed were:

(I) Which applied developmental stage of *G. viridula* will most effectively reduce *R. longifolius* growth and survival?

(II) Which applied density (no. per plant) of a given developmental stage of *G. viridula* will produce the largest reduction in growth and survival of *R. longifolius*?

(III) Will the combined effect of competition from grasses and grazing by *G. viridula* give better control of *R. longifolius* than either factor by itself?

(IV) Is there a difference in the reduction of *R. longifolius* growth when docks under competition from either Italian ryegrass (*Lolium multiflorum* Lam.) or a ley mixture are grazed by *G. viridula*?

(V) How far from a release point in a perennial ley will imagoes and their offspring disperse over time?

These questions were examined in three experiments: (1) A field experiment performed in 2009 at Bioforsk (now NIBIO) as a part of an earlier running project (2007 – 2011) "Control of docks (*Rumex* spp.) in organic fodder production – a true bottleneck in organic farmed branded dairy and meat products" (Project number 176812), examined the degree of which *G. viridula* was able to reduce the growth and survival of *R. longifolius* seedlings at different applied densities and developmental stages of *G. viridula* when *R. longifolius* was under competition from perennial ryegrass (*Lolium perenne* L.). (2) A glasshouse experiment performed in 2019 as part of this thesis examined the effect of added competition from Italian ryegrass, or a “ley mixture” on the ultimate reduction in *R. longifolius* seedling growth when grazed by *G. viridula*. Italian ryegrass was chosen as it was found to suppress R. obtusifolius and R. crispus growth best when comparing four grass species by Niggli et al. (1993) along with a ley mixture containing three forage species commonly used in Norwegian leys (Molteberg, 2017). (3) And lastly, a field experiment performed in 2019 as part of this thesis where the distance of which *G. viridula* will disperse in a perennial ley was examined.

2 Materials and methods

2.1 Experiment 1: Grazing field trial

2.1.1 Experimental design

The first experiment took place in a field at Ås, in southeastern Norway (59º 40' N, 10º 46' E), 97 meters above sea level in summer 2009. The use of *Gastrophysa viridula* to reduce *Rumex longifolius* growth and survival was examined in a field experiment where the placement of two developmental stages (third instar larvae and gravid females) and three levels of individuals (100, 250 or 500 for third instar larvae, and 5, 15 or 25 for gravid females), as well as a control with no *G. viridula*, were compared on transplanted docks in a newly established ley. Three complete blocks were established; however, they were not randomized (Figure 2.1). Due to an insufficient number of gravid females, one plot of 15 gravid females was removed.

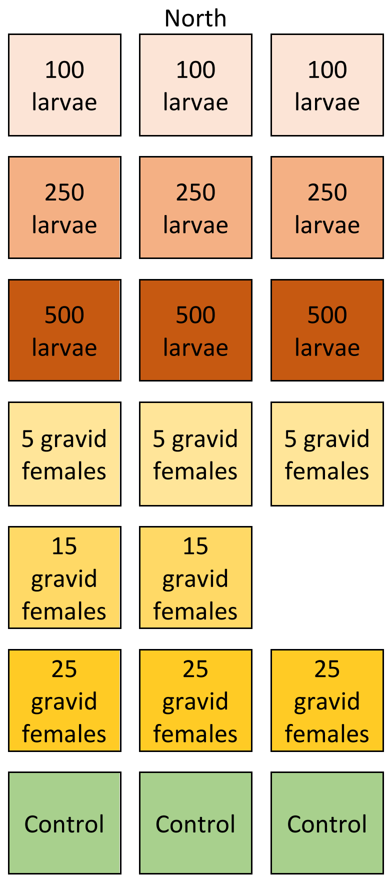


Figure 2.1. Setup of the experiment. Larvae used are at third instar. Numbers give the number of individuals in the plot. Control is ungrazed (without *G. viridula*).

2.1.2 Plant material

2.1.2.1 Grass

Grass seeds used in the ley were *Lolium perenne* L. (perennial ryegrass). Unknown cultivar and seed producer.

2.1.2.2 Docks

Rumex longifolium seeds used in the experiment were collected at Ås, Norway (59º 40' N, 10º 46' E) in autumn 2007, and dried in ambient room temperature (≈22 °C).

2.1.3 Beetles

The green dock beetles used in this experiment were collected in Øystre Sildre, Norway (61º 14' N, 8º 52' E), approximately 740 meters above sea level in 2008. The beetles were reared in glasshouses at Ås (59º 40' N, 10º 46' E) for a year, and underwent winter hibernation (diapause). Temperature and humidity were controlled, while there was no additional lighting in spring 2009. The exact temperature, humidity, and light is unknown. They were fed with *R. longifolius*.

2.1.4 Experimental site

Experimental plots were established in the middle of a newly sown ley on the grounds of the Norwegian university of life sciences. The site had a slope of approximately 7 % to the east and trees along the southern and western border that could cast some shadow in the evening. The soil consisted of sandy to silty clay loam [according to the definition used by NIBIO (NIBIO, 2017)] throughout the field (NIBIO, 1991). The experimental site was sown with perennial ryegrass in week 22 of 2009 and fertilized. Sowing rate of grass, amount and brand of fertilizer is unknown. Temperature and precipitation in Ås from the sowing of the ley and until the last assessment before winter can be found in Table 2.1.

Table 2.1. Total precipitation, average temperature, and deviation in temperature and precipitation in Ås from the normal-period of 1961 – 1990, from the sowing of grass and until the last assessment in 2009.

Month	Precipitation		Temperature		Source
	Total (mm)*	Percent of normal	Average (°C)**	Deviation in °C from normal	
May	56.0	75 - 100	10.9	+ 1.0 - 1.5	(Iden et al., 2009d)
June	29.6	25 - 50	14.5	+ 0.0 - 0.5	(Iden et al., 2009c)
July	59.4	200 - 250	16.2	+ 0.5 - 1.0	(Iden et al., 2009b)
August	60.2	125 - 150	15.3	+ 1.0 - 1.5	(Iden et al., 2009a)
September	28.2	50 - 75	12.0	+ 2.0 - 3.0	(Iden et al., 2009e)
Average≈	86.7	100 - 125	13.8	+ 1.0 - 1.5	

*(NIBIO, 2009a), **(NIBIO, 2009b)

2.1.5 Units and treatments

Plots of 1.2 x 1.2 m with docks planted in an area of 1 x 1 m were established in week 26. Between all plots, there was a minimum of 1 m distance, and the plots were placed where there was good establishment of the ryegrass. Blocks ran from north to south. The same day ryegrass was sown outdoors in week 22; dock seeds were sown by broadcasting in plastic trays and placed in a glasshouse. The plastic trays were moved outdoors after one week due to few germinated seeds, and germination increased. Approximately one week later, the seedlings were pricked out into plug trays and grew there for two weeks before being transplanted into the plots. When pricked into plug trays, plants were moved outside during the daytime, and inside at night for a period until they were acclimated, after which point, they stayed outside until transplantation into plots. Sixteen dock plants were transplanted into each plot and evenly spaced in a 4 x 4 grid pattern (Figure 2.2). Before transplanting, the dock plants had grown unevenly, and to ensure uniformity between plots, 2 larger and 14 smaller dock plants were planted in each plot. The field was irrigated well before planting, and to prevent desiccation in the dry and warm weather, the field was watered every second day in the first period (exact duration of period unknown).

To ensure that the beetles would remain in the plot where they were later placed, a cage was built over each plot, including control plots. The cage extended 10 cm out from the closest dock at all sides and was dimensioned to 1.2 x 1.2 x 0.5 m (length x width x height). Fly netting with 1 x 1 mm mesh was stapled to the framing and dug approximately 10 cm into the soil. Cages over plots with *G. viridula* had a lid. The lid was made so the lids netting would rest on top of the cage with wooden framing outside of the cage to weigh it down.

Third instar larvae and gravid females were transferred to the plots on June 25th and 26th. It is unknown whether the females had started ovipositing before initiation of the experiment. Beetles were placed on the center four plants in each plot.

After the second assessment July 21st the ryegrass was harvested (unknown stubble height) and removed from plots. There were taken no measures to minimize how many *G. viridula* were removed along with the grass.

Figure 2.2. Size of dock plants at transplantation into the ley (A) and placement of dock plants in plot (B). The placement of plants is marked by wooden labels. Photo (June 25th, 2009): Uno Andersen

2.1.6 Assessments

There were three assessments of dock plants in summer and fall of 2009, and one end assessment in summer 2010, respectfully July 7th, July 21st and September 15th to 17th in 2009 and July 9th, 2010. There were no systematic registrations of eggs, larvae, and imagoes of *G. viridula*. Still, there were some descriptions of what developmental stage most individuals were in at a given registration date.

At all three registration dates in 2009, up to four leaves per dock plant (four leaves were photographed if present), counting from the oldest true leaf discernible were photographed against

1 mm graph paper and later visually assessed to determine the percentage of the remaining area of live lamina per leaf relative to no leaf damage (100 %). Fourteen leaves were also analyzed with ImageJ (an open-source image processing program) to give the percentage of the remaining area of live lamina per leaf to determine the discrepancy in percentage between the two assessment methods. All plants in a plot were visually assessed. No absolute value (cm^2) of leaf area was determined for any leaf.

At the end assessment in 2010, the number of live plants per plot were counted (plants were considered living if they had any shoot growth), and the shoots of all plants were harvested and dried to give shoot dry weight.

2.1.7 Data analyses

For statistical analysis and visual representation, the percentage of leaf area remaining per leaf was averaged over each plant, and then all plants in a plot were averaged. Statistical analyses on the percentage of live lamina per four oldest leaves per plant were performed in SAS®. The percentage of live lamina per four oldest leaves per dock plant was first square root transformed and then modeled using a general linear mixed model with the treatments, the days after release, and their interactions as fixed factors and block as a random factor. The effect of repeated measurements on the area of live lamina per four oldest leaves per plant (%), which was observed in each plot within each block 11, 25, and 82 days after release, was modeled using an unstructured covariance structure for the random terms in the model. The random terms are assumed to be normally distributed. A Tukey-Kramer test was performed with a significance level of 0.05. The LS-means were retransformed for use in a graph, computed in Minitab®.

Statistical analyses on shoot dry weight per plot and percentage of surviving plants per plot were conducted in Minitab® version 19.2020.1, and contrasts were performed in SAS®. The response variables were transformed using the Box-Cox transformation $y^* = y^\lambda$ if all y's > 0 and $y^* = (y + 1)^\lambda$ if some y's < 0 before modeling, to better meet the assumptions of normality and homogeneous variance. The Box-Cox algorithm used to transform the data calculates a λ value, which determines the best mode of transformation (if any is required), and subsequently transforms it (Osborne, 2010). Transformations used are shown in results. A mixed-effects model was fitted to the transformed data, with treatment as a fixed factor and block as a random factor. Tukey pairwise comparisons were performed. Contrasts were performed in SAS® to determine differences in response between groups of treatments. Tukey pairwise comparisons and contrasts were performed with a significance level of 0.05. Graphs with means and corresponding standard error of the mean (S.E.) values were computed in Minitab®.

2.2 Experiment 2: Grazing glasshouse trial

2.2.1 Experimental design

The second experiment took place in a glasshouse at Ås, Norway (59º 40' N, 10º 46' E) in autumn 2019. Three levels of competition [no competition, competing with Italian ryegrass, or a ley mixture (Figure 2.3)] were combined with the presence or absence of grazing by *G. viridula* in an experiment with 'miniature leys' (Table 2.2). A 3 (factor: competition) x 2 (factor: *G. viridula*) randomized factorial block design was established with four complete blocks, where each block shared one worktable in a glasshouse room (Figure 2.4). There were two rooms with two worktables per room.

Table 2.2. Treatments in the experiment, with abbreviated names.

Treatment name	Explanation
Control	Dock only
Grazing	Dock + green dock beetle
It. ryegrass	Dock + Italian ryegrass
It. ryegrass-Grazing	Dock + Italian ryegrass + green dock beetle
Ley mixture	Dock + ley mixture
Ley mixture-Grazing	Dock + ley mixture + green dock beetle

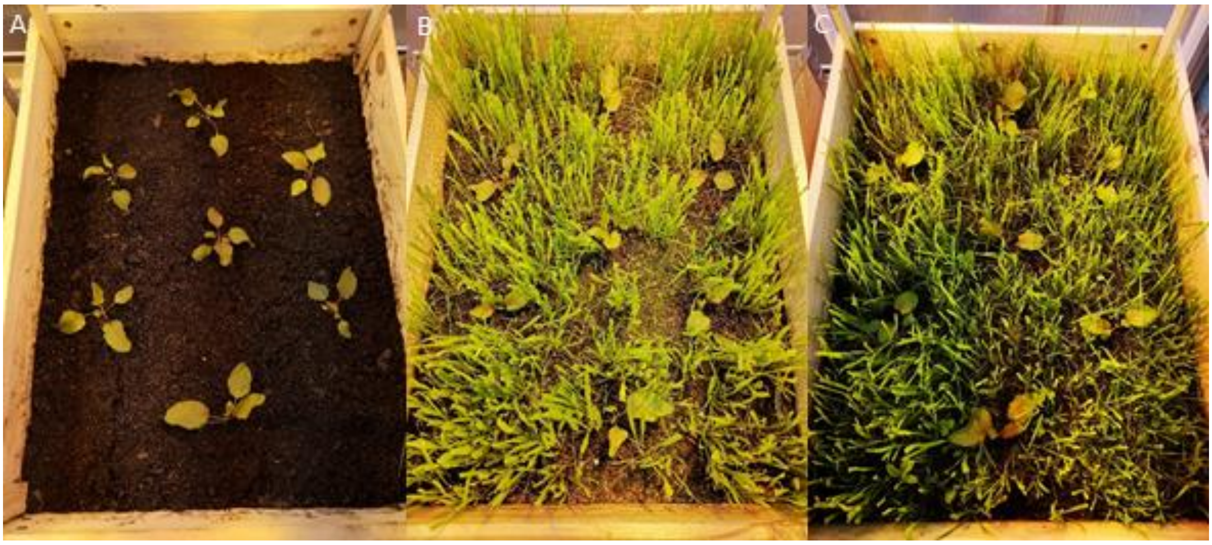


Figure 2.3. Competition levels for *R. longifolius*. (A) no competition, (B) competition with Italian ryegrass, and (C) competition with a ley mixture. Photo (October 7th, 2019): Ida Dybing

Entrance from hallway			
Room 1		Room 2	
Worktable I	Worktable II	Worktable III	Worktable IV
Ley mixture	It. ryegrass-Grazing	Grazing	It. ryegrass-Grazing
Control	It. ryegrass	Control	Ley mixture-Grazing
It. ryegrass-Grazing	Ley mixture	Ley mixture	Grazing
It. ryegrass	Control	Ley mixture-Grazing	It. ryegrass
Ley mixture-Grazing	Grazing	It. ryegrass	Control
Grazing	Ley mixture-Grazing	It. ryegrass-Grazing	Ley mixture
Outer wall			

Figure 2.4. Setup of the experiment. Light colors are treatments without beetles, dark colors with beetles. Green, yellow and red are treatments with respectively no competition, competition with Italian ryegrass, and competition with a ley mixture.

2.2.2 Plant material

2.2.2.1 Forage species

Forage species seeds used in the experiment were *Lolium multiflorum* (Italian ryegrass) 'Meroa' (Jorion Philip-Seeds, Belgium), and a ley mixture of 70 % *Phleum pratense* L. (timothy) 'Grindstad', 20 % *Schedonorus pratensis* (Huds.) P.Beauv. (meadow fescue) 'Minto' and 10 % *Trifolium pratense* L. (red clover) 'Lea' ('Spire surfôr normal', Felleskjøpet, Norway)

2.2.2.2 Docks

Rumex longifolium seeds were collected at Ås, Norway (59º 39' N, 10º 44' E) in August 2019, and dried in ambient indoor temperature (≈20 °C).

2.2.3 Beetles

Green dock beetles used in the experiment were collected in May 2019 from Rhubarb (*Rheum rhabarbarum*) at Ringsaker, Norway (61º 4' N, 10º 34' E), 505 meters above sea level. They were reared in growth chambers (NIBIO, Ås) with a constant temperature of 23 °C, 80 % relative humidity, and a photoperiod of 16/8 h (day/night). Light sources were fluorescent daylight lamps (Philips MASTER TL-D 90 Graphica 36W/950 SLV/10). They were fed with *Rumex obtusifolius*. To check for adverse effects when changing host plant to *R. longifolius*, 30 imagoes were placed in a cage with *R. longifolius* plants for 12 days, beginning August 28th. The mortality rate was no higher than on the original host plant, egg-laying and larval development appeared normal.

2.2.4 Units and treatments

There were created 'miniature leys' within a pallet collar of 55*75 cm on top of a plastic tray. In every pallet collar, there was used 80 liters of limed peat enriched with nutrients [Tjerbo Torvfabrikk' P-jord', containing 80 % (volume percent) sphagnum peat, 10 % composted bark and 10 % fine sand. The soil was enriched with 6 kg limestone flour and 2 kg fertilizer (NPK 12–4–18) per m^3 soil mixture, pH 5.5 – 6.5, and density 360 kg m^{-3} (applied volume)]. *Rumex longifolius* seeds were sown by broadcasting in plastic trays in room one for later transplantation of small plants into the 'miniature leys', and grass (+ clover) seeds were sown by broadcasting directly in the pallet collars (miniature leys) September 3rd. The sowing rate was similar to 25 kg ha^{-1} for the ley mixture and 35 kg ha^{-1} of Italian ryegrass. Throughout the experiment, all plants were watered as needed. The experiment was performed with a set room temperature of 18 °C/12 °C (day/night), 70 % relative humidity, and a photoperiod of 16/8 h (day/night). In addition to natural sunlight, the light source was warm white high-pressure sodium lamps [Lucalox™ PSL LU400W/PSL/T/E40, giving minimum 180 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) in room one and 210 µmol m^{-2} s^{-1} PPFD in room two at plant height (measured without netting cover)].

Fourteen days after sowing, *R. longifolius* were pricked out into plug trays. Plants at approximately the same stage were chosen (with emerging first true leaf). A month after sowing, the ley was cut to a stubble height of 5 cm (4th - 6th of October). The ley was cut to imitate a first harvest as the sward had grown to approximately 70 cm. This harvest also gave the dock plants good growth conditions. Docks were transplanted to the 'miniature leys' on October 7th. Similar sized plants with 4 - 6 true leaves were chosen. In Experiment 1, 16 dock plants were used per m^2, and to keep the experiments comparable, it was decided to use the equivalent in this experiment. Since these leys were 0.41 m^2, seven plants were established in each unit. After planting, it was irrigated equally in all plots with a nutrient solution (2 mS cm^{-1}, 57 % YaraLiva™ Calcinit™ and 43 % Kristalon™ Indigo, Yara, Norway).

To ensure that the beetles remained in the unit they were placed, cages were built over each pallet collar (Figure 2.5). To produce the same climate conditions in each 'miniature ley', cages with lids were also built over treatments without beetles. The cage roof was 45 - 50 cm above the soil surface. Insect proof netting (soft tulle) of 0.5 x 0.5mm mesh was stapled tightly around the framing, and duct tape was used to close gaps where imagoes/larvae could escape. The lid was made so that the lids netting would rest on top of the cage. A sealing strip was placed on top of the frame of cages that would contain beetles, and screws were placed in corners of lid and framing, with rubber bands to pull the lid further down (Figure 2.5).

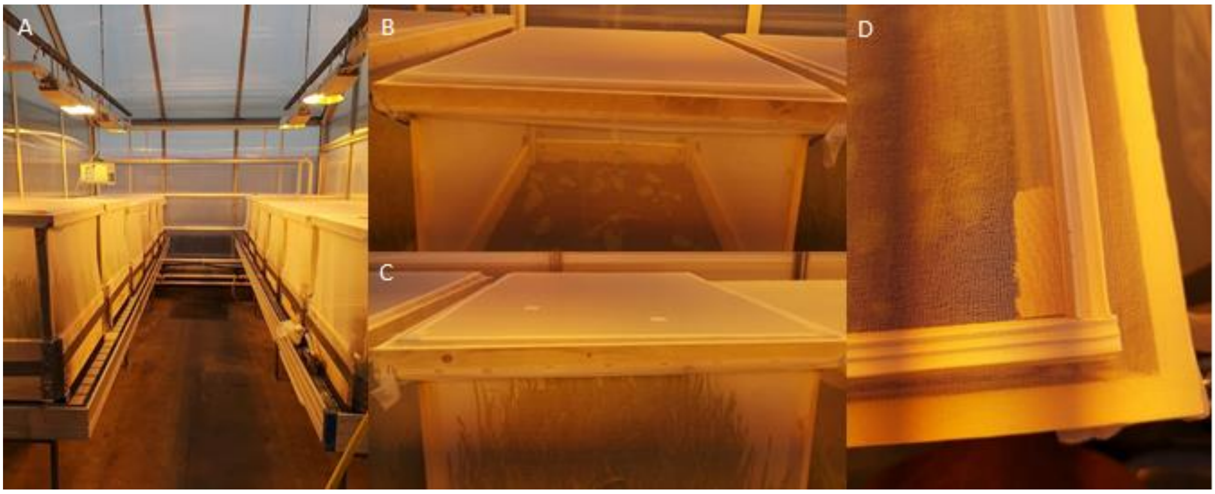


Figure 2.5. Cages (A). Lids in cages with beetles secured with rubber bands (B) and a sealing strip (D). Lids on cages without beetles simpler (C). Photo: Ida Dybing

Beetles were transferred to the cages on October 11th. Ten gravid females and ten non-gravid (apparently male) imagoes were placed in all 'miniature leys' of treatments with beetles. This density equates to 25 gravid beetles per 16 plants (which was one of the treatments in Experiment 1). In the treatments of worktable four, nine gravid females and one assumed gravid female were used due to lack of enough clearly gravid females. Most beetles had started ovipositing before being transferred to the cages. The first beetles started ovipositing October 7th.

A second harvest of grass (+ clover) and a first harvest of docks was simulated on November 5th and 6th, four weeks after the start of the experiment (approximately 500 degree-days had accumulated since the first harvest). Grass (+ clover) was harvested with a stubble height of 5 cm. Two days later (November 7th and 8th), all docks (including control treatment) were harvested at 9 cm (the current height of the sward) as not to give them an unfair advantage or disadvantage over the sward. All dock leaves were manually stretched up before cutting, as they had laid down after the harvest of grass (+ clover). Larvae and imagoes found on grass (+ clover) and dock plants were placed back into the cage after the plants were harvested, as they, in a real-life scenario, would have time to move over to live plants. Egg clusters were taken out with the plants.

2.2.5 Assessments

The experiment was run for eight weeks. When the beetles were released, the number of unfolded leaves was counted on two random dock plants in each 'miniature ley'. Every second week (i) number of unfolded leaves per dock plant [including dead leaves (data not shown)] and (ii) number of leaves with a living center nerve of over five centimeters (arbitrarily chosen limit to show the number of leaves of a notable length) per dock plant was assessed for all dock plants. The BBCH value of ten random grass plants per 'miniature ley' was also determined. In treatments with the ley mixture, the

BBCH value was determined for five timothy plants, five meadow fescue plants and five red clover plants. After two and six weeks, the leaf area of the two longest unfolded leaves of each dock plant was recorded non-destructively. A picture was taken that was later analyzed with WinFOLIA™ to give the living leaf area in cm^2. When analyzing pictures of dock leaves with WinFOLIA™, the petiole was excluded. This measurement method was imperfect, as dock leaves are undulating to a varying degree so that not all lamina was recorded, but it gave a satisfying estimate. Both when counting and analyzing leaves, the leaf/part of the leaf was counted as dead when completely yellow or necrotic and without turgor. After four weeks, grass (+ clover) was harvested, and fresh weight recorded. All dock leaves protruding more than nine centimeters from the soil were harvested, scanned, and later analyzed with WinFOLIA™. The dry weight of harvested dock leaves (excluding petioles) was determined. After eight weeks, the experiment was concluded. The above-soil plant material was harvested, fresh and dry weight of the forage species was determined, all dock leaves were scanned to be later analyzed by WinFOLIA™, and dry weight of the shoot was determined. Taproots were dug up, carefully cleaned and dried to determine dry weight.

There were six beetle registrations, the first registration was performed three weeks after the start of the experiment, and then one registration per week. The number of egg clusters, live imagoes, and larvae on/under dock plants and on cage walls and roof were counted each time. When the grass (+ clover) was harvested after four weeks, egg clusters, live larvae, and imagoes on grass straws and clover leaves were counted as the plants were harvested. At the registration after five weeks, the sward was so low that the imagoes were visible on the entire soil surface and were therefore counted. At the registrations after six and seven weeks, all imagoes (living and dead) in the grazing treatment were counted, as there had emerged many first-generation imagoes which had died of starvation by the time I registered them. At the end of the experiment, living and dead beetles in the whole cage of every treatment were counted.

2.2.6 Data analyses

Statistical analyses were performed in Minitab®, except for contrasts, which were performed in R commander version 3.3.1. For initial leaf data, a mixed-effects model with room and worktable as random factors and competition and *G. viridula* as fixed factors was used. For the rest of the data, a mixed-effects model ANOVA with room and worktable as random factors and competition, *G. viridula*, and their interaction (C*$G.\ v$) was first performed (full model). The response variables were transformed using the Box-Cox transformation $y^* = y^\lambda$ if all y's > 1 and $y^* = (y + 1)^\lambda$ if some y's < 1 before modeling, to better meet the assumptions of normality and homogeneous variance. ANOVA table of the responses with their respective λ and transformations can be found in Table S2. Due to a large difference between the control treatment and all other treatments, the control was excluded from the rest of the statistical analyses to be better able to detect differences between the remaining treatments. A mixed-effects model ANOVA with treatment as a fixed factor, and room and worktable as random factors, was then used for all dock data. All response variables were examined to see if they would better meet the ANOVA assumptions after Box-Cox transformation, and responses that showed improved residual plots and R^2 were used in their transformed state. Transformations used are shown in results. Means of transformed data from the model were retransformed before use in tables. Tukey pairwise comparisons were performed to determine significant differences between treatments. Contrasts were performed in R commander to determine differences in response between groups of treatments after 14 days. Room was removed from the model for the contrast, as it had zero variance. The Tukey pairwise comparisons and contrasts used significance level 0.05. Standard error of the mean of all forage species data was computed in Minitab®. All graphs with means and their corresponding S.E. and confidence intervals were computed in Minitab®.

2.3 Experiment 3: Dispersal field trial

A field trial was established in the summer of 2019 at two different sites close to Vestby, in southeastern Norway. The dispersal over time of *G. viridula* in a perennial ley from a center release point was examined. Three plots were established at each site.

2.3.1 Experimental sites

The first experimental site (59º 37' N, 10º 43' E) was 101 meters above sea level and had a flat terrain with minimal shadowing of the field by trees or buildings (Figure 2.6). It was a field of perennial ley established with a ley mixture of 50 % *Phleum pretense* L. (timothy) 'Grindstad', 20 % *Schedonorus pratensis* (Huds.) P.Beauv. (meadow fescue) 'Fure', 15 % *Poa pratensis* L. (smooth meadow-grass) 'Knut', 10 % *Trifolium pratense* L. (red clover) 'Lea' and 5 % *Trifolium repens* L. (white clover) 'Litago'/' Hebe' ('Strand nr 13', Norgesfor, Norway) and a companion crop of *Hordeum vulgare* L. (barley), *Pisum sativum* L. (field pea) and *Vicia sativa* L. (common vetch) (Unknown percentage and product name) in 2015. Two edges of the field were sown in 2019 with the same seed mixtures. The field was approximately 1.29 ha in size and was heavily infested with *Rumex* spp. when first visited in May 2019. The dock distribution was patchy, and most plants observed were *R. longifolius.*

The second experimental site (59º 34' N, 10º 46' E) was 33 meters above sea level and had a sloping terrain towards the east with a forest to the west that made the sun set early on the field (Figure 2.7). It was a field of perennial ley established with the same seed mixtures as the first site in 2018, but due to a warm and dry summer, there was low germination, and much of the fields flora most likely germinated from an existing seed and bud bank. The field was approximately 4.2 ha in size and heavily infested with *Rumex* spp. and various other weed species such as *Cirsium arvense, Urtica* sp., *Ranunculus repens,* and *Stachys palustris*. The dock distribution was approximately uniform, and most plants observed were *R. longifolius.* Both fields had symptoms of herbivory. Some of the most abundant insects on docks were collected, and the most abundant beetles were determined to be *Apion* sp.

Before starting the experiment, both sites were examined systematically for existing *G. viridula* populations. The field was examined northwards from the south end. For every 30 meters, the field was traversed from west to east/east to west, where every ten meters up to five plants (five plants were examined if present) were examined within a 0.5-meter radius. The plant was first scrutinized from all angles without touching it to avoid *G. viridula* dropping to the ground, and then all leaves were turned. The screening of the fields resulted in respectively 47 and 147 points of registration at the first and second site. *Gastrophysa viridula* was also continually looked for while moving, without examining individual plants. No *G. viridula* were found.

Figure 2.6. First experimental site with the approximate placement of the center beetle release point of the three plots. Source: norgeibilder.no (accessed 06.07.19).

Figure 2.7. Second experimental site with the approximate placement of the center beetle release point of the three plots. Source: norgeibilder.no (accessed 29.06.19).

2.3.2 Beetles

Green dock beetles used in this experiment were collected in June 2019 from *R. obtusifolius* at Karmøy, Norway (59º 16' N, 5º 12' E), 31.5 meters above sea level. They were reared in ambient room temperature (approximately 21±3 °C) in Stavanger, Norway, and fed with a mix of *Rumex* spp., *mostly R. obtusifolius.* They were placed in a room lit with mostly natural sunlight, and daylength was decided by the natural daylength at the time. They were shaded from direct sunlight.

2.3.3 Experimental design and units

The experimental plots consisted of circles of 29 m radius. Due to a small size of the first experimental site, approximately 4 – 11 % of each plot was in a newly established part of the ley with different species composition and, in two cases, an area with lower sward-height than most of the field. Fifty imagoes were placed evenly on the three dock plants closest to the center of the circle. The plant furthest from the center was 31.7±5.2 [Standard deviation (S.D.)] centimeters away. Due to a limitation of gravid imagoes, there were only 12 gravid females in each plot. Most of them were ovipositing at the initiation of the experiment. The first beetles started ovipositing approximately a week before starting the experiment. The beetles were released on July 7th at the first experimental site and July 26th at the second experimental site, approximately two weeks after the first harvest of the respective sites. Delay between the sites was due to wet soil conditions forestalling the harvest at the second site. At the start of the experiment, dock and forage plants were approximately 10 - 25 cm tall.

2.3.4 Assessments

The first registration was performed 5 - 6 and 12 - 13 days after the release of beetles in the first and second site, respectively. The two sites were not registered at the same interval of days because the registration dates had to be adjusted to the weather (as the beetles drop to the ground in rain, windy conditions and low temperatures), as well as other necessary activities such as tending to beetles to be used in the glasshouse experiment. From there on, they were registered approximately every two weeks until the end of the experiment, 39 and 53 days, respectively, after the experiment was initiated. The shorter registration period of the first site was due to a lack of sward harvesting that had caused the sward to lay down, and a noctuid moth that consumed most dock plants, making it hard to find any *G. viridula* that might be present.

At each registration, dock plants were examined for number of *G. viridula* imagoes, larvae and egg clusters. All dock plants were examined in a central circle of two-meter radius, and beyond there were six concentric annuli respectively 4 - 6, 9 - 11, 14 - 16, 19 - 21, 24 - 26, and 27 - 29 meters from the center, where up to 40 plants were examined in each annulus (Figure 8). There were eight registration points in each annulus, where up to five plants, if present, were examined for *G. viridula* in a one-meter radius. These registration points were placed in the eight cardinal directions from the center (Figure 2.8). For each plant examined, it was first scrutinized from all angles accessible without touching it, and then all leaves were turned to be examined. At the last registration of site one after 37 - 39 days, there were only four registration points per annulus (north, east, south, and west) due to a long sward and few dock plants that made registration time-consuming. Registration points that no dock plants had ever been found in were also omitted.

Distribution of dock plants in the plots were mapped in a similar manner in August over two registration days at the first site (9th and 12th) and three registration days at the second site (13th to 27th). Number of plants were counted in seven concentric annuli, respectively 3.5 - 4.5, 7.5 - 8.5, 11.5 - 12.5, 15.5 - 16.5, 19.5 - 20.5, 23.5 - 24.5 and 27.5 - 28.5 meters from the center. There were eight registration points in each annulus, one for each of the eight cardinal directions, where the number of dock plants (with at least one developed true leaf) within one square meter were counted. Many were without any lamina and were counted if they had a living center nerve on at least one leaf or a recently developed inflorescence.

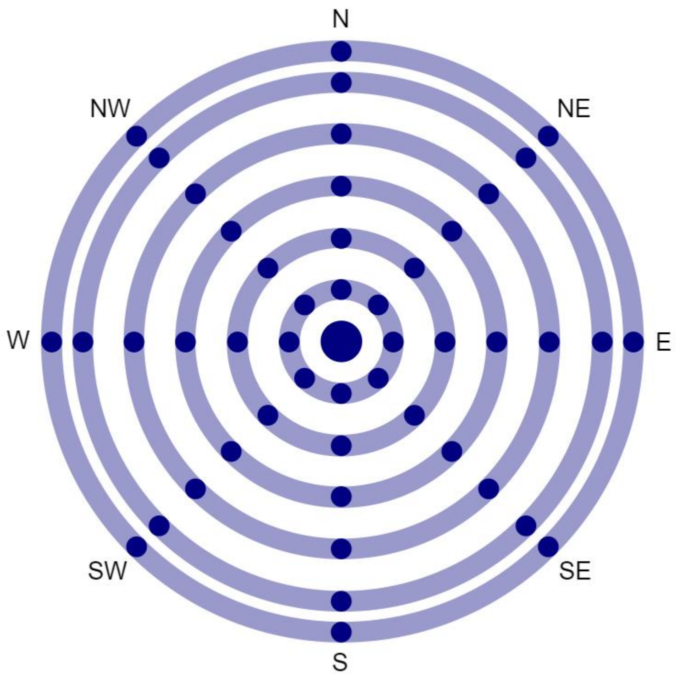


Figure 2.8. Registration pattern within each replication. A central circle and six concentric annuli, respectively 0 - 2, 4 - 6, 9 - 11, 14 - 16, 19 - 20, 24 - 26, and 27 - 29 meters from the center were examined for G. viridula. Dark circles represent the examined area.

2.3.5 Data analyses

Statistical analyses were performed in Minitab® 19. Data of detected imagoes, larvae, and egg clusters were summed together and converted to binary (found/not found) data. A binary logistic regression model was fitted for detection of *G. viridula* data, with days after start, meters from the release point, and their interaction as continuous predictors and site as a categorical predictor. The resulting model was used to predict the probabilities of detecting *G. viridula*. The mean number and S.D. of *G. viridula* found, and plants examined was calculated. The standard deviation of *G. viridula* found was calculated as sample standard deviation as it was desirable to be able to generalize the results to all *G. viridula*, while plant S.D. was calculated as population standard deviation as the ones found were the only ones of interest.

3 Results

3.1 Experiment 1

3.1.1 Applied developmental stages of *G. viridula*

Grazing by *G. viridula* significantly reduced the area of live lamina per four oldest leaves present per plant (%) from the release of the beetles and until the last assessment in 2009 when compared to the ungrazed control (Table 3.1; Figure 3.1). The visual assessment of the percentage of leaf area remaining was, on average, 7.4±11.7 (S.D.) % lower than the percentage calculated in ImageJ (data not shown). Grazing also reduced shoot dry weight of docks per plot ($P < 0.05$) and the percentage of docks that survived until July 2010 ($P < 0.05$) when compared to the control (Figure 3.2).

When comparing the introduction of gravid females and third instar larvae there was an interaction between treatment and time (Days after start) where gravid females close to linearly reduced the remaining leaf area of the four oldest leaves present to 6.8 – 21.4 of 100 % at the last assessment, while third instar larvae strongly reduced the leaf area remaining to 7.1 - 43.2 of 100 % within 11 days after release, and afterward slowly decreased the remaining percentage of leaf area to 1.4 – 13.9 of 100 % (Table 3.1; Figure 3.1). Only third instar larvae were able to reduce the area of live lamina per four oldest leaves present per plant (%) of first-year docks (Figure 3.1), the survival of docks to the following summer (gravid females, $P = 0.39$ and third instar larvae, $P < 0.001$; Figure 3.2) and the shoot dry weight of docks per plot the following summer (gravid females, $P = 0.50$ and third instar larvae, $P < 0.01$; Figure 3.2) when compared to the control.

Table 3.1. Repeated measures ANOVA, type III test for fixed factors of treatment (0, 5, 15 or 25 gravid females, 100, 250 or 500 third instar larvae), assessment date (11, 25 or 82 days after start), and their interaction on the percentage of leaf area remaining $leaf^{-1}$ $plant^{-1}$ of the four oldest leaves present [n = 3 (15 gravid females = 2)].

Source of variation	Num DF	Den DF	F Value	Pr > F
Treatment	6	13	28.53	< .0001
Days after start	2	12	232.13	< .0001
Treatment*Days after start	12	13.94	6.44	0.0008

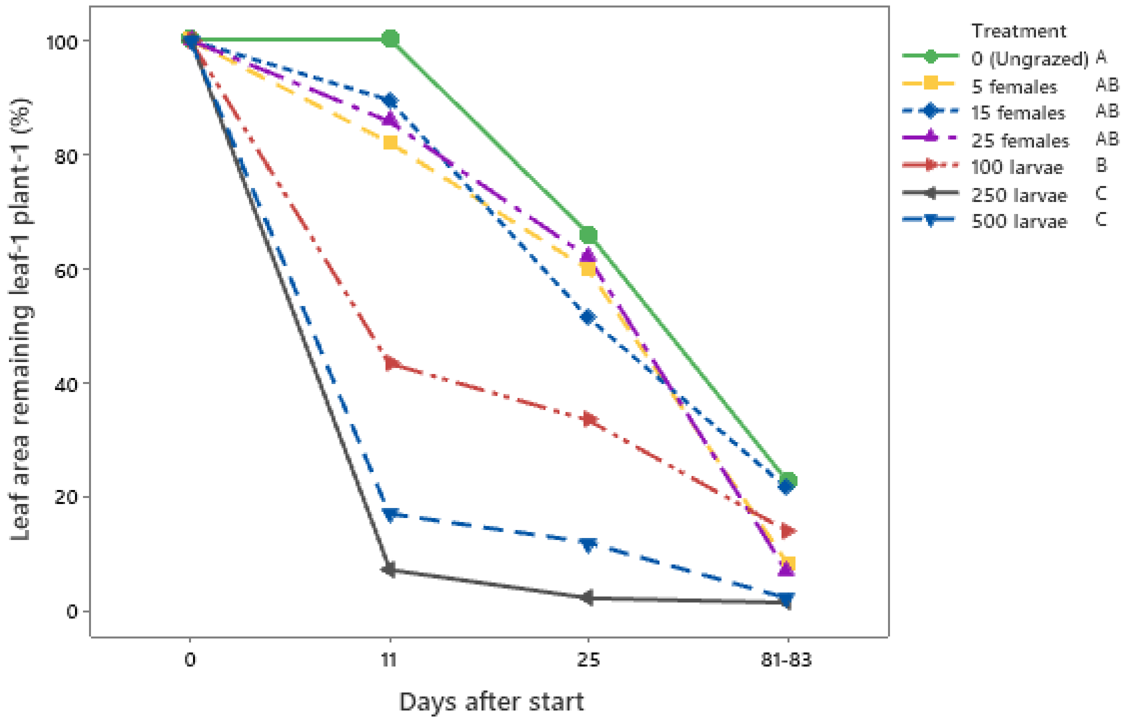


Figure 3.1. Retransformed LS-mean of percentage of area of live lamina leaf^{-1} dock plant^{-1} of the four oldest leaves present under different numbers of gravid females or third instar larvae over time [n = 3 (15 gravid females = 2)]. Treatments that do not share a common letter in the legend are significantly different according to a Tukey-Kramer test (P < 0.05). It was assumed that all plants had undamaged leaves at the initiation of the experiment.

The control treatment was unfortunately affected by *G. viridula* as well. Between 11 and 25 days after the release of the beetles, imagoes of *G. viridula* had entered the control plots, and by 25 days, they had laid eggs on the dock plants (Table 3.2). By the end assessment, herbivory had removed the same percentage of the lamina from the control plots as the plots with 15 gravid females (Figure 3.1). In treatments with gravid females, no eggs were found on dock plants before 25 days after the release of the beetles (Table 3.2).

3.1.2 Applied densities of *G. viridula*

3.1.2.1 Gravid females

There was no difference between the applied densities of gravid females on either remaining leaf area of the four oldest leaves present throughout the assessment period in 2009, the survival of docks per plot in 2010, or the shoot dry weight per plot in 2010 (Figure 3.1; Figure 3.2). There was, however, a tendency towards lower survival of docks at higher densities, and an opposite tendency towards higher shoot dry weight per plot at higher densities (Figure 3.2).

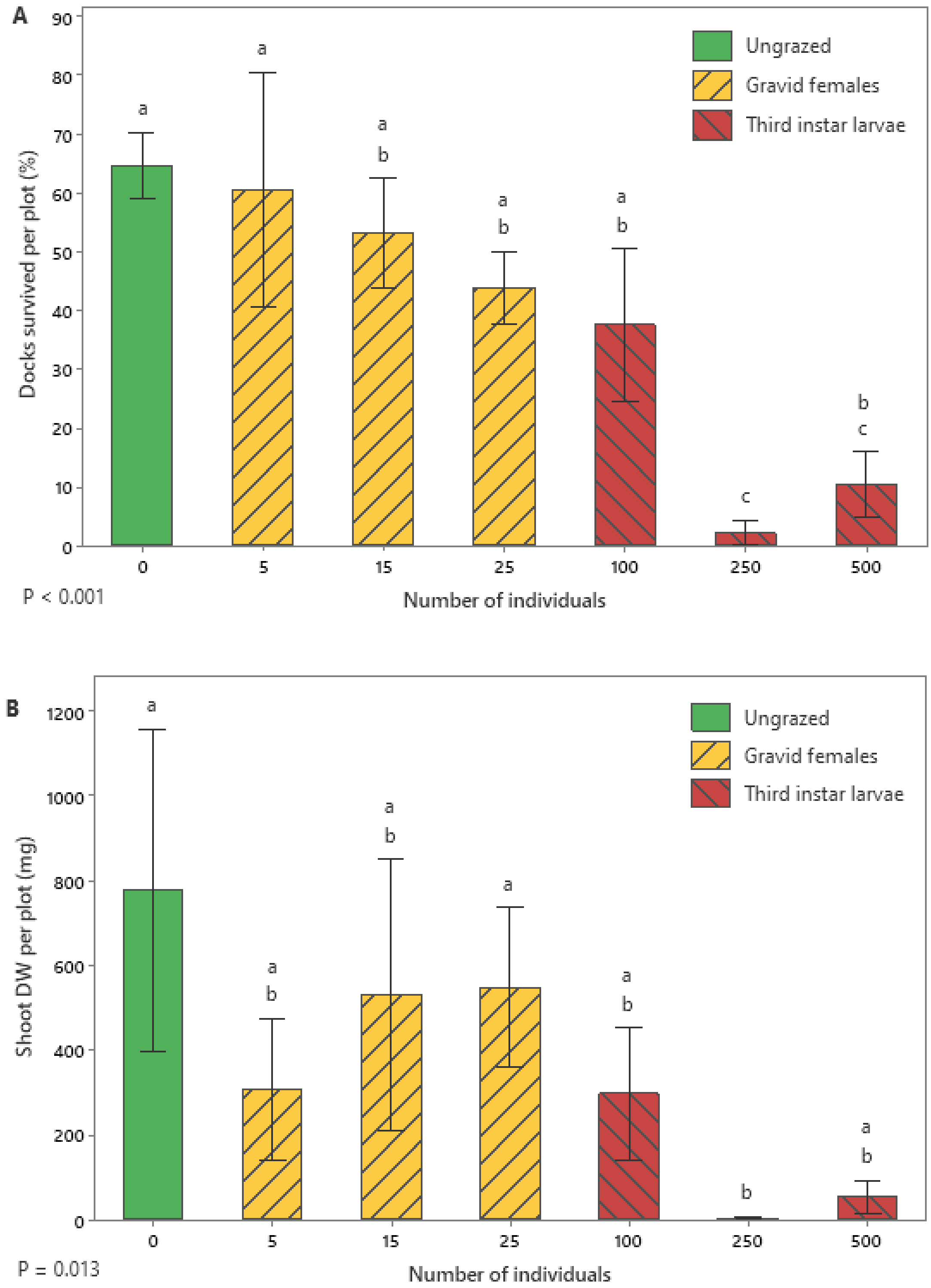


Figure 3.2. Mean (± S.E.) percentage of docks per plot that were alive as per 9th of July 2010, almost a year after the application of beetles (A) and shoot dry weight of docks per plot (B) under different treatments [n = 3 (15 gravid females = 2)]. Standard error of the mean is calculated by individual standard deviations. Bars that do not share a common letter are significantly different according to Tukey pairwise comparisons ($P < 0.05$). Data used in the model to perform Tukey pairwise comparisons were (A) $\sqrt{Y+1}$ transformed ($\lambda = 0.5$) and (B) $\sqrt[5.043906]{Y+1}$ transformed ($\lambda = 0.2$).

3.1.2.2 Third instar larvae

When comparing different densities of third instar larvae in a plot, 250 and 500 larvae had by the last assessment reduced the percentage of the area of live lamina per four oldest leaves present per plant (< 2.1 %) more than 100 larvae (13.9 %; Figure 3.1). However, only 250 larvae produced a significantly lower percentage of dock survival than 100 larvae (Figure 3.2), with a fitted survival mean of 1.4 % (data not shown). The shoot dry weight per plot was not affected by the density of larvae, but there was a tendency towards lower shoot dry weight in plots with 250 and 500 larvae compared to 100 larvae (Figure 3.2).

Table 3.2. Developmental stages of *G. viridula* described to be found on dock plants of different applied developmental stages of *G. viridula* at the three assessments. Accumulated degree-days are measured from two meters above soil level at Ås (NIBIO, 2009c) with the lower developmental threshold of *G. viridula*, 7.9 °C (Kucherov & Kipyatkov, 2011) as base temperature.

Days after start	Degree-days (°C)	Applied *G. viridula*		
		None	Gravid females	Third instar larvae
11 (July 7th)	133.3	No *G. viridula*	Imagoes	Larvae
25 (July 21st)	240.9	Eggs	Imagoes and eggs	Imagoes
81 - 83 (September 15th to 17th)	621.0 - 624.9	No/few *G. viridula*	No/few *G. viridula*	No/few *G. viridula*

3.2 Experiment 2

3.2.1 Effect on dock growth of competition

Throughout the experiment in ungrazed docks, interspecific competition from grass (and clover) caused a clear reduction in dock growth compared to dock plants without competition. The dock plants were roughly the same size, and there was no difference in leaf number between any of the treatments at the initiation of the experiment (Table S1). The non-competing, ungrazed docks (control treatment) quickly grew significantly larger than the docks of all other treatments, with more leaves of a notable size (Figure 3.3) and larger leaf area of the longest leaves within 14 days (Table 3.3). The difference in leaf number and leaf area between the control, and all other treatments became larger as the experiment progressed (Figure 3.3; Table 3.3).

The number of leaves of a notable size, 27 – 28 days after the start of the experiment, was lower in the ungrazed treatments with the ley mixture than in the ungrazed treatment with Italian ryegrass (Figure 3.3). After the first dock harvest, however, the docks competing with the ley mixture recovered fastest, and docks competing with Italian ryegrass produced fewer leaves of notable size for the remainder of the experiment (Figure 3.3). This difference was not reflected in any leaf area measurements or dry weight of leaves, which showed no difference between ungrazed treatments (Table 3.3). The root dry weights of docks competing with Italian ryegrass were, however, clearly higher than that of docks competing with the ley mixture (Table 3.3).

After the second grass (+ clover) harvest, there was much less grass growth until the final harvest (Table 3.4). The growth appeared to be less reduced in the clover plants (personal observation). However, the competition pressure on dock plants appears to have been the same on both grazed and ungrazed treatments, as there was no difference between them in the developmental stage, or weight of harvested grass (+ clover) (Table 3.4).

3.2.2 Effect on dock growth of grazing

Grazing by itself reduced the dock growth significantly throughout the experiment compared to the non-competing, ungrazed control (Figure 3.3; Table 3.3). The leaf area, weight, and number of notable leaves was reduced by more than 99.9 % by the end of the experiment compared to the control (Figure 3.3; Table 3.3). The root weight was less affected, but still showed 99.6 % reduction in root dry weight when compared to the control (Table 3.3). However, the root weight of the non-competing grazing treatment showed no difference from the ungrazed treatments with competition (Table 3.3).

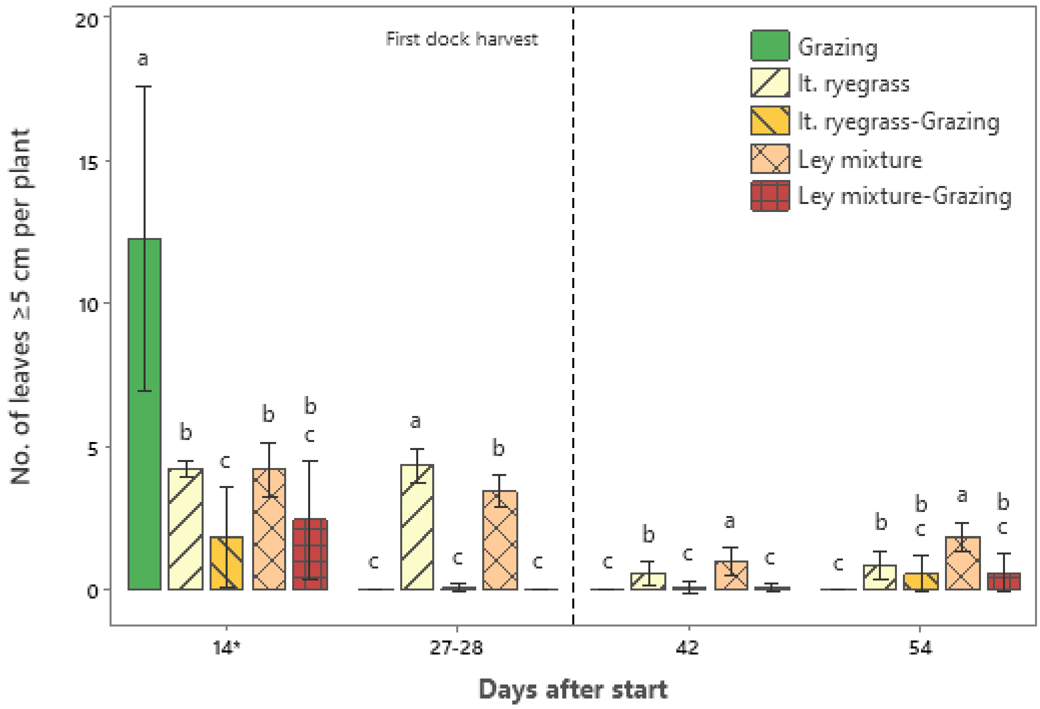


Figure 3.3. The mean number of dock leaves longer than 5 cm per plant under different treatments over time from the release of beetles (n = 4). Interval bars give the 95 % confidence interval, calculated by the standard deviation of each treatment x time. Bars that do not share a common letter are significantly different according to Tukey pairwise comparisons ($P < 0.05$). Pairwise comparisons are calculated per date. The ungrazed, non-competing (control) treatment was excluded from the figure due to the large difference between the control and all other treatments. Corresponding means and confidence intervals for the control are 20.14±3.47, 38.86±3.30, 22.32±3.20 and 23.47±5.74 for respectively 14, 27 - 28, 42, and 54 days after start. *Transformed data (square root, $\lambda = 0.5$) used in the model to perform the Tukey pairwise comparison.

3.2.3 Effect on dock growth of combined competition and grazing

Grazing treatments with competition also showed a reduction in dock growth throughout the experiment when compared to their respective ungrazed competing treatments (Figure 3.3; Table 3.3). Fourteen days after the release of beetles, there had been a larger effect of only competition than only grazing on the number of leaves of a notable size (Figure 3.3), and on leaf area of the two longest leaves ($P < 0.01$; Table 3.3). Treatments with both competition and grazing had fewer leaves of a notable size ($P < 0.01$) and smaller leaf area ($P < 0.001$) than treatments with only competition (Figure 3.3; Table 3.3). Nineteen days after the release of beetles, many more egg clusters had been laid and larvae hatched in the non-competing, grazing treatment than in treatments with competition and grazing (Figure 3.4). Many more third instar larvae were observed in the non-competing grazing treatments than the other grazing treatments after 19 and 25 – 26 days (personal observation). The higher beetle population in the non-competing, grazing treatment than in treatments with competition and grazing persisted until 47 days into the experiment (Figure 3.4).

Table 3.3. Effect of competition and grazing by *G.* viridula on leaf area and weight assessments of dock plants. Fitted means and S.E. given from mixed-effects model ANOVA (n = 4). Each observation is an average of the seven plants in each 'miniature ley'. Means that do not share a common letter in the superscript are significantly different according to a Tukey pairwise comparison (P < 0.05). The ungrazed, non-competing (control) treatment is left out of the model due to large differences from all other treatments.

Days after release of beetles	No competition		Competition with a ley mixture		Competition with Italian ryegrass		S.E.
	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	
13 days:							
Summed leaf area of the two longest leaves plant^{-1}, cm^2	269.08	81.57^{A}	58.85^{AB}	22.15^{CD}	43.17^{BC}	13.78^{D}	6.99
27-28 days, first dock harvest:							
*Leaf area of harvested leaves plant^{-1}, cm^2	4 208.21	0.00^{B}	100.75^{A}	0.04^{B}	82.33^{A}	0.27^{B}	-
Dock leaf dry weight plant^{-1}, mg	6 616.79	0.00^{B}	152.14^{A}	0.00^{B}	144.29^{A}	0.36^{B}	18.38
41 days:							
*Summed leaf area of the two longest leaves plant^{-1}, cm^2	324.93	0.00^{C}	17.51^{A}	1.87^{B}	13.09^{A}	1.72^{B}	-
55-58 days, final harvest:							
**Total leaf area plant^{-1}, cm^2	1 517.58	0.61^{B}	28.33^{A}	12.94^{A}	18.16^{A}	12.83^{A}	-
Dock shoot dry weight plant^{-1}, mg	4 382.14	1.43^{B}	71.07^{A}	23.57^{B}	58.93^{A}	24.64^{B}	7.77
Dock root dry weight plant^{-1}, mg	9 255.71	314.64^{B}	374.64^{B}	105.36^{C}	471.07^{A}	127.50^{C}	39.52

*Log$_e$ (λ = 0) transformed and **square root (λ = 0.5) transformed data used in model, mean retransformed for table. – S.E. of retransformed data removed as a retransformed S.E. would be meaningless.

By 25 – 26 days, the dock plants only exposed to grazing had collapsed (all leaves, including leaves with remaining lamina wilted), and most did not produce any more leaves (data not shown). Plants that recovered produced no more leaves until after 33 days when the first-generation imagoes began to emerge and grazed them down again (data not shown). At the peak of live first-generation imagoes after 40 days, there were 111±24.27 (S.E.) live imagoes in each 'miniature ley'. Several first-generation imagoes were already dead, giving a total of 164.0±13.61 live and dead imagoes in each 'miniature ley'. The lower population of imagoes and larvae in treatments with competition took a week longer to remove most/all lamina from most dock plants (Figure 3.4). As the introduced imagoes died and larvae pupated, the docks began to recover until first-generation imagoes emerged, mostly in the Italian ryegrass treatment (Figure 4) and grazed them somewhat down again before the end on the experiment (data not shown).

Differences in beetle population sizes between the grazing treatments throughout the rest of the experiment was reflected in leaf number and area. Except for after 14 days, there was no difference in the number of leaves of a notable size between the grazing treatments (Figure 3.3). On the other hand, from 41 days and till the end of the experiment, the leaf area was lower in the non-competing, grazing treatment than the grazing treatments with competition (Table 3.3). The non-competing, grazing treatment was the only one of the grazing treatments to have consistently fewer leaves, and lower leaf area from 27 – 28 days and until the end of the experiment when compared to the ungrazed treatments (Figure 3.3; Table 3.3). The grazing treatments with competition had fewer leaves of a notable size and lower leaf area than their ungrazed counterparts at both the assessment after 27 – 28 days and 42 days (Figure 3.3; Table 3.3). The interaction of the competition and grazing effects, where the combination showed less effects on leaf assessments than grazing alone indicated here were confirmed by the full model, where all leaf number (≥ 5 cm), area and weight assessments except the first leaf number assessment showed a strong interaction (Table S2; Table 3.3).

Table 3.4. Mean developmental stages (BBCH) of grass and clover (± S.E.) throughout the experiment, as well as fresh weight (mean ± S.E.) of grass and clover from the two harvests undertaken during the experiment and dry weight of grass and clover from the final harvest. Means are given per 'miniature ley' (n = 4).

Days after release of beetles	Competition with a ley mixture		Competition with Italian ryegrass	
	Ungrazed	Grazed	Ungrazed	Grazed
14 days:				
Grass BBCH mean value	22.70±0.50	23.55±0.24	25.30±0.44	25.28±0.71
Red clover BBCH mean	13.10±0.24	12.75±0.17	-	-
25-26 days, second harvest:				
Grass (+ red clover) fresh weight, g	841.9±36.7	843.9±51.5	768.5±32.1	845.9±22.8
42 days:				
Grass BBCH mean value	20.88±0.47	21.98±0.52	25.60±0.34	25.68±0.24
Red clover BBCH mean value	16.65±0.33	16.05±0.39	-	-
54-56 days, final harvest:				
Grass BBCH mean value	23.00±0.56	22.58±0.40	26.73±0.58	25.48±0.39
Red clover BBCH mean value	18.40±0.08	17.60±0.61	-	-
Grass (+ red clover) fresh weight, g	228.4±12.0	226.9±11.8	285.4±13.3	311.0±12.3
Grass (+ red clover) dry weight, g	56.92±2.05	56.16±2.94	63.26±2.10	63.22±3.68

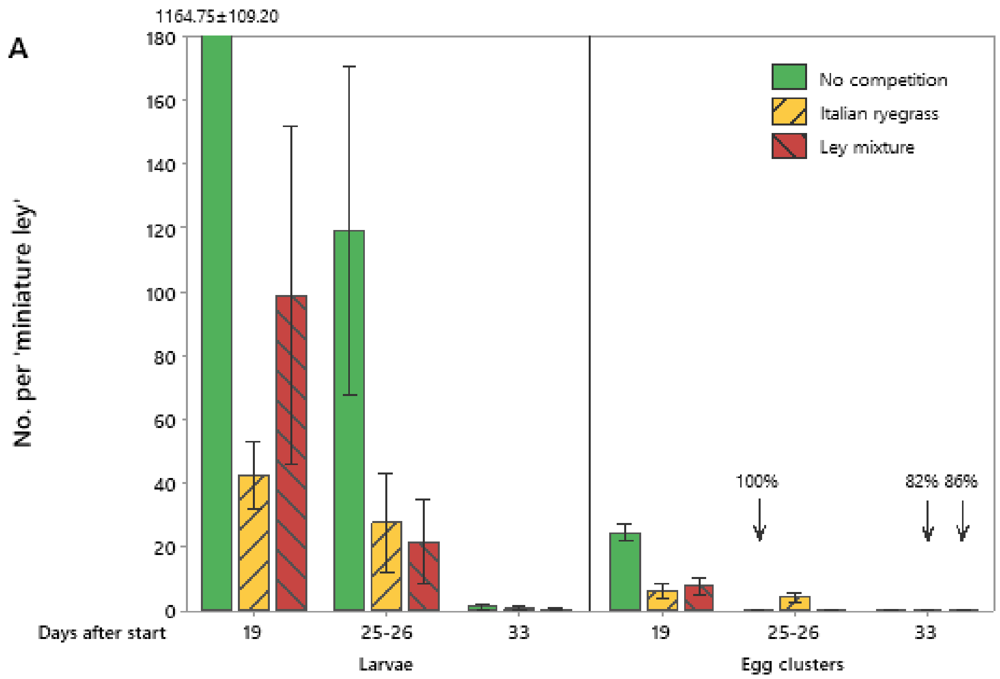
- : not relevant

However, both shoot and root dry weight of the grazed treatments with competition at the end of the experiment were lower than their ungrazed counterparts (Table 3.3). The difference was especially big in the root dry weight, with a 71.9 % and 72.9 % reduction in root weight in grazed treatments with respectively the ley mixture and Italian ryegrass. When grazed treatments are compared, the treatments with competition show a reduction in root weight of 66.5 % and 59.5 % for respectively the ley mixture and Italian ryegrass when compared to no competition.

There was found no difference in results between the two forage species mixtures examined when combined with grazing. All leaf number, area, and weight measurements, as well as root weight, show no difference (Figure 3.3; Table 3.3). However, an apparent higher number of larvae survived through to adulthood in the Italian ryegrass treatment (Figure 3.4).

3.2.4 Other aspects

Other factors that could have affected the dock plant development include fungi and non-coleopteran insects. Symptoms of fungal infection on the dock plants were visible already at the first true leaf stage, and most plants had symptoms at the time of transfer to the 'miniature leys'. The coverage of fungal lesions on the leaves stayed mostly stable until the end of the experiment, when it increased in the control treatment and showed a weak tendency to do the same in the other treatments, especially ungrazed treatments (personal observation). Fungal lesions created necrotic tissue and therefore affected the living leaf area measured. One 'miniature ley' of the ungrazed Italian ryegrass treatment had symptoms of herbivory on two of its dock plants in the first half of the experiment, and a pupa of *Noctua pronuba* L. (Noctuidae) was found in the soil at the end of the experiment. The herbivory from the larva(e) removed several leaves longer than five centimeters, that would otherwise have been counted. There were also many individuals of Sciaridae sp. (Diptera) in all miniature leys throughout the experiment, and their larvae were observed feeding on rotting leaves. There were clear signs of herbivory on the root crown of docks in the non-competing, grazing treatment, and this could have stemmed from either sciarid larvae or *G. viridula*.



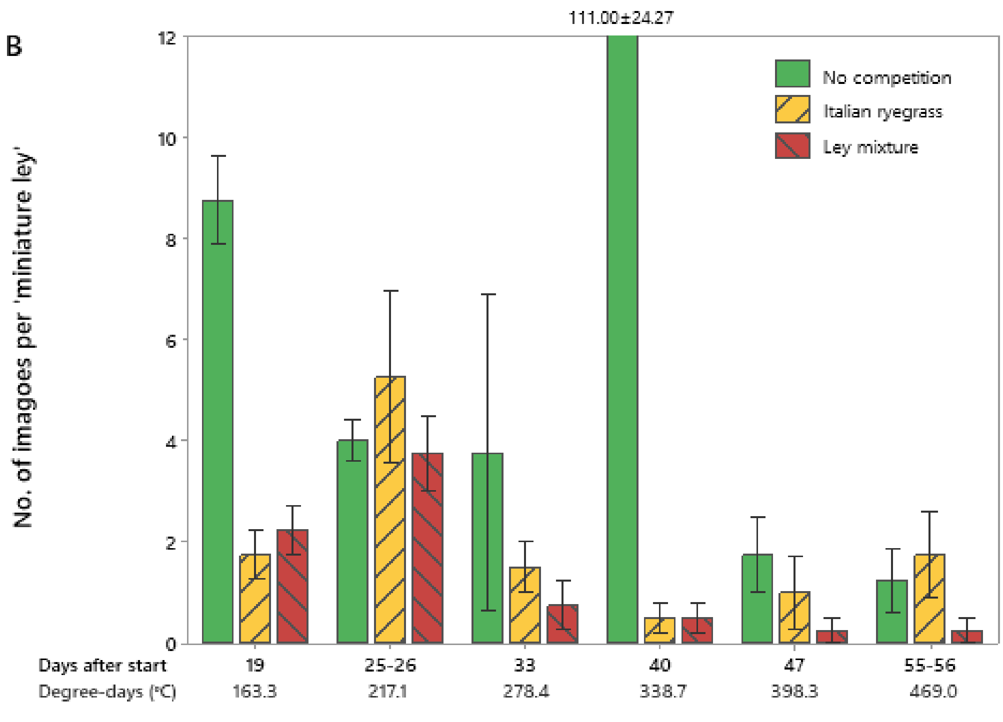


Figure 3.4. Mean number (± S.E.) of egg clusters, larvae (A) and imagoes (B) found per 'miniature ley' throughout the experiment (n = 4). Days are counted from the release of imagoes into the 'miniature leys'. Larvae and egg cluster numbers are only given up to 33 days as there were only registered one larva (in treatment with Italian ryegrass) and no egg clusters after this point. Average accumulated degree-days are given, with the lower developmental threshold of *G. viridula*, 7.9 °C (Kucherov & Kipyatkov, 2011) as base temperature. The arrows and corresponding percentages give the point in time where 100 % (no competition), 82 % (Italian ryegrass) or 86 % (ley mixture) of the dock plants have no/close to no living lamina left in the different treatments. Means ± S.E. are given for bars that are longer than the y-axis. Numbers for 25 - 26 days after start include *G. viridula* on forage plants because the sward was harvested then. Standard error of the mean is calculated from individual standard deviations.

3.3 Experiment 3

3.3.1 Dock population

The dock population was lower in site one compared to site two, and decreased over time in site one, thereby affecting the opportunities of dock beetles to disperse and find host plants. Figure 3.5 shows the distribution of dock plants in the fields in August. There was a consistently high number of docks observed throughout the field of site two, while the observed number of dock plants dropped from May to August in site one, ending with a small population of patchy distribution. The observed number of dock plants dropped after the first harvest, and due to a lack of dock plants, only around half of the possible (40) plants were examined in each annulus of the first site at the first two registrations (Table 3.5). The last registration of the first site was significantly reduced due to a lack of dock plants with leaves that could be examined for dock beetles, as well as a long sward that made

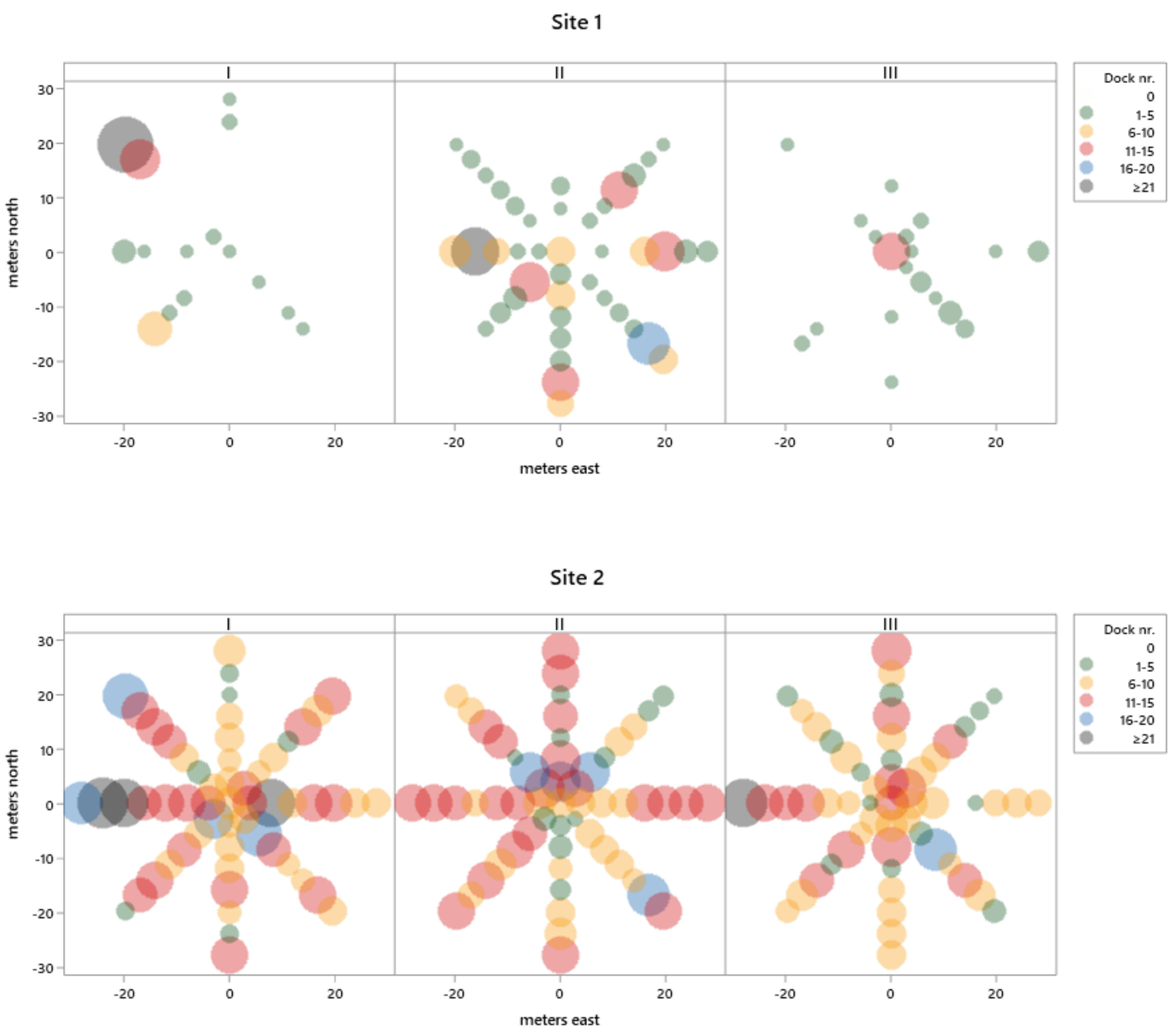


Figure 3.5. Spatial distribution and abundance of dock plants in the three plots at site one per August 9th - 12th and site two per August 13th - 27th. The size of the bubbles, as well as color, indicate the number of dock plants found per m^2.

the detection of imagoes more difficult (it was necessary to lift/move the grass, and this would cause any imagoes to drop to the ground). The decline in dock plants directly before the last registration was in a large degree due to larvae of *Trachea atriplicis* L. (Noctuidae). This noctuid moth had invaded the field in large numbers towards the end of July and consumed most all lamina of docks in taller grass (personal observation). The registration method chosen for practical purposes similarly affected the possibility of detecting present *G. viridula* by examining a smaller percentage of area for each concentric annulus (Table 3.5).

Table 3.5. Total number of beetles, larvae and egg clusters found at increasing distance from the release point (mean ± S.D.), and number of plants examined at increasing distance from the release point (mean ± S.D., averaged over all plots and registrations days). The maximum number of plants examined is 40. The examined percent of area in each annulus is also shown.

Days after release	Meters from center						
of beetles	0-2 m	4-6 m	9-11 m	14-16 m	19-21 m	24-26 m	27-29 m
Site 1:							
5-6 days	16.0±20.2	0.7±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
20-21 days	144.7±236.0	1.7±1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
37-39 days*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Plants examined**	-	22.5±5.1	23.0±5.6	21.7±10.1	22.0±10.2	16.0±5.5	13.0±6.6
Site 2:							
12-13 days	47.7±47.0	10.3±15.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
24-26 days	32.3±43.7	93.7±133.8	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
41-42 days	0.7±1.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
52-53 days	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Plants examined	-	39.8±0.4	39.5±1.4	39.4±1.3	38.8±2.1	39.0±2.4	38.9±2.2
Area of annulus examined (%)	100.0	40.0	20.0	13.3	10.0	8.0	7.1

*Missing half of the registration points. **Average of plants examined at the two first registrations.

(-) Not counted

3.3.2 Dispersal of *G. viridula*

The detected dispersal of *G. viridula* was generally short and differed between the two sites. In the first site, most all imagoes, larvae, and eggs were detected within two meters of the release point, and in the second site, there was more dispersal toward five and even ten meters from the release point at the furthest (Table 3.5). At both sites, the largest number of beetles were detected approximately three weeks after the release of the beetles, and this was also when most beetles were detected 5 – 10 meters from the release point in both sites (Table 3.5). Of the beetles detected after three weeks in both sites, 93 % were larvae, 6.5 % were egg clusters, and only 0.5 % were imagoes (data not shown). The beetles detected at the two last registrations of site two were first-generation imagoes, found within a meter from the release point (data not shown). There was no clear directional movement from the release point (Figure S1).

To be able to predict the probability of detecting *G. viridula* at increasing distance from the release point and thus their dispersal, a linear regression was used. Due to a large probability of finding many beetles when first discovering one at a registration point, the data were converted to binary (found/not found) data, so a linear regression would more realistically predict the probability of finding *G. viridula* at different times and distances. The following binary logistic regression equation was fitted:

P(detection) = exp(Y')/(1 + exp(Y')) where Y' = 4.387 - 0.643 meters from center – 0.1541 days after start + 0.0000 site one + 1.633 site two + 0.008563 days after start*meters from center

Figure 3.6 shows the predicted probability of detecting *G. viridula* at different times and distances from the center. The predicted probability of detecting *G. viridula*, and detecting them at increasing distances from the center, decreases as the time from release increases. The chance of finding *G. viridula* is predicted to be higher at all times and distances at site two than site one. According to the model based on the sampling method used in this experiment, there is a very low chance of detecting any *G. viridula* more than 12 or 15 meters from the center at respectively site one or two at any time (Figure 3.6).

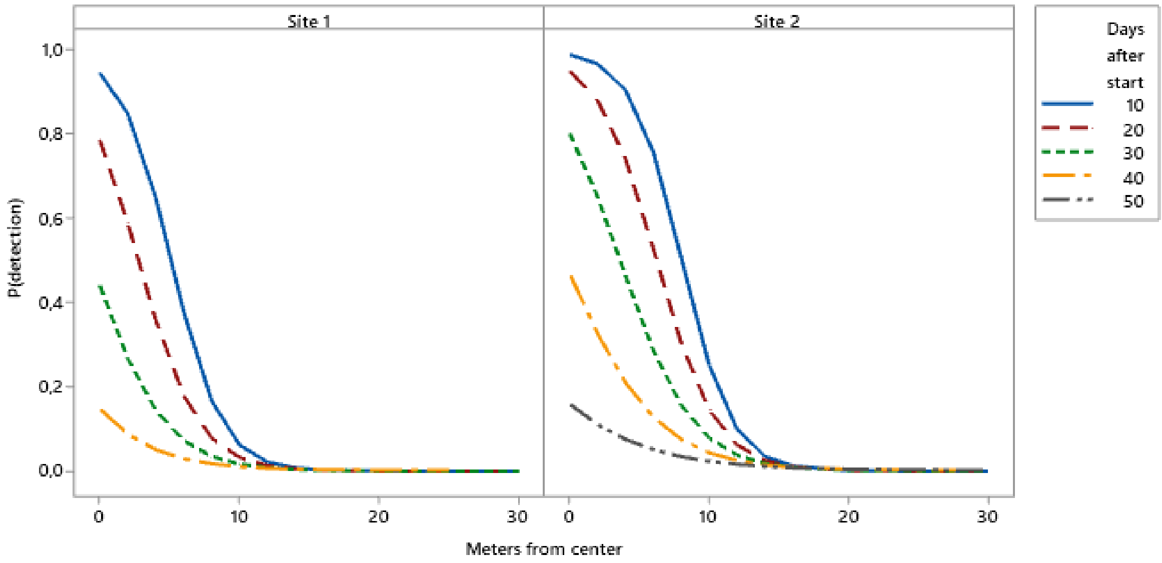


Figure 3.6. Predicted probability of detecting *G. viridula* at increasing distance from the release point over time. Values are predicted using a binary logistic regression equation.

4 Discussion

4.1 Applied developmental stages of *G. viridula*

In Experiment 1, it was found that applied third instar larvae consumed a larger percentage of the four oldest *R. longifolius* leaves present and did so quicker than when gravid females were applied. The higher efficacy of third instar larvae in 2009 probably contributed to a lower survival of docks in the following year and lower shoot dry weight per plot in treatments with third instar larvae than in treatments with gravid females. The gravid females were not able to reduce either leaf area remaining in 2009, dock survival or shoot dry weight in 2010 when compared to the control. Still, they did reduce the leaf area remaining when compared to no herbivory (100 % leaf area remaining). *Rumex longifolius* survival to the following year was 44 – 60 % when gravid females were applied. This survival may have been reduced in 2010 compared to if the dock plants had not been grazed by *G. viridula*, as *R. longifolius* have been found to have an equal or higher regenerative ability than its close relatives *R. obtusifolius* and *R. crispus* (Fykse, 1986) and in a similar experiment with one-month-old docks transplanted into an established ley, *R. obtusifolius,* and *R. crispus* were found to have more than 70 % survival one year after transplantation (Hongo, 1989).

There were, however, some weaknesses with the design of Experiment 1, which means that the results must be cautiously interpreted. Firstly, the plots within blocks were not randomized. Non-randomized plots most probably had an insignificant effect on nutrient availability for the dock plants, as they were relatively closely planted in the middle of a newly sown field with the same soil texture throughout the field. Any effect on temperature and irradiance would also be expected to be low as there was little shadowing from trees, except in the evening when the shadow would be cast over an entire block at a time, affecting all treatments the same.

Secondly, the cages of control plots were without lids. The lack of lid could have affected the temperature inside the control cages in comparison to the other treatments, and thereby affected the relative growth of the control treatment in relation to the other treatments (Criddle et al., 1997). The lack of lid allowed escapes of *G. viridula* from at least one plot to affect all control plots. There were not believed to be any *G. viridula* present in the experimental site before the experiment, and *G. viridula* eggs in the control plots most likely stemmed from escaped imagoes from one or more of the plots with gravid females, most probably plots with 25 gravid females as they were closest to the control plots. It is unknown whether more than two treatments were affected by loss or introduction of escaped *G. viridula*. It is unlikely that plots with third instar larvae were affected significantly by escaped imagoes, however, as the first four dock leaves were consumed quite rapidly, and imagoes of *G. viridula* have been found to avoid feeding or to oviposit on dock leaves where there are, or have been more than 33.3 second or third instar larvae dm^{-2} (Schindek & Hilker, 1996). The *G. viridula* in the control plot consumed almost 80 % of each of the oldest four leaves of each plant, approximately as much as *G. viridula* in the treatment with 15 gravid females consumed. The damage produced in the control plots negatively affected the ability to compare to a control, especially when it came to survival and shoot dry weight the following year, as this was not a direct measurement of herbivory.

Thirdly, as only gravid females and no males were placed in each plot, there is a possibility that some only had unfertilized eggs. It is unknown how early the gravid females used in this experiment were separated from the males, but it was described by Uno Andersen (personal communication) that after winter hibernation during rearing for this experiment, females were separated from the males as they developed swollen abdomens, and this may have been the procedure in the last generation as well. Osborne (1879) observed that *G. viridula* could lay several hundred unfertilized eggs, though none of them hatched, and this could be the case for at least some imagoes in this instance as no larvae were ever described to be found in plots with gravid females. Also, it has been noted for several insects that oviposition is delayed in unfertilized females (Richardson, 1925, p. 2-3), and no eggs were found until 25 days after the release of the beetles, when *G. viridula* has been found to start ovipositing five to eight days (at 18 – 28 °C) after eclosion (Honek et al., 2003). A large proportion of unfertilized eggs could have lowered the effect of applying gravid females on dock seedlings in this experiment.

Finally, the chosen mode of leaf assessment in 2009 gave an indication of herbivory on each plant but did not account for differences in size between the leaves, or for the development of more leaves that were not grazed, as the same leaves were measured throughout the season, provided they could be discerned. If the experiment were to be repeated, it would be desirable to count the number of live leaves and non-destructively measure leaf area as well, either through a length x width measurement multiplied with a calculated conversion factor as used by Keary & Hatcher (2004) or through a program such as WinFOLIA™ used in Experiment 2.

Although there were methodological weaknesses in this experiment, it is still possible to see a tendency of third instar larvae removing leaf area faster than gravid females and reducing the survival of docks after a year more than gravid females. The true difference between the effect of gravid females and third instar larvae may, however, be lower than found in Experiment 1, as other experiments have found an effect on shoot and root dry weight of applying 16 and 8 – 16 gravid females per 16 dock plants (when under competition from grasses) on *R. obtusifolius* and *R. crispus*

(Bentley & Whittaker, 1979; Cottam et al., 1986). From Experiment 2, it was found that applying gravid females at the same density as in the treatment with 25 gravid females on *R. longifolius* lead to a strong decrease in dock leaf and root growth. When comparing accumulated degree-days between the two experiments, the leaf response variables in Experiment 2 were reduced over time in a curve more like percentage leaf area remaining of third instar larvae, than gravid females in Experiment 1, with leaf response variables after 217.1 degree-days comparable to the percentage leaf area remaining of third instar larvae after 133.3 degrees-days, and a more or less stable level after that. Whereas in treatments with applied gravid females in Experiment 1, there was a stable decline in percentage leaf area remaining throughout the experimental period in 2009. The results from Experiment 2 indicate that applying gravid females can lead to the same curve of defoliation as third instar larvae, with a somewhat delayed effect.

The difference in herbivory/relative effect of herbivory between the two experiments may have been partly because the docks were competing with different plant species. But seeing as the final root and shoot dry weight was the same on grazed, competing plants in Experiment 2, regardless of which species the docks were competing with, it seems likely that much of the difference stems from the delay in egg-laying found in Experiment 1. In Experiment 1, no eggs were found until after 240.9 degree-days, while the peak number of larvae registered had already been reached in Experiment 2 by 163.3 degree-days. The lack of egg clusters found before 25 days (240.9 degree-days) may be because of several factors. Firstly, the possible low fertility of the females, as previously mentioned, could have caused this, which would be a flaw in the experiment. Secondly, because of differences in lower developmental threshold and sum of effective temperatures between populations (SaSKa et al., 2014), as the beetles in Experiment 1 were collected in Øystre Sildre, and the beetles in Experiment 2 were collected in Ringsaker. However, Kucherov & Kipyatkov (2011) found that it was very difficult to demonstrate geographic variation in the thermal reaction norms of *G. viridula* but noted that they could not draw any definite conclusions on whether there were differences in thermal reaction norms between populations. Or thirdly, because of high predation rates of *G. viridula*, which could be expected in natural conditions (Martinková & Honek, 2004; Smith & Whittaker, 1980a). The cages should have limited predation, but as imagoes were able to escape, one can expect that predators were able to get in, and some may have become trapped inside the cages as they were built. However, predation is expected to be lower in less complex habitats (Langellotto & Denno, 2004) such as this newly sown ley (Smith & Whittaker, 1980a), and Smith and Whittaker (1980b) found that *G. viridula* was able to complete the highest number of generations on regularly cut grassland. It would be of interest to repeat the field experiment with males introduced along with gravid females of the Øystre Sildre population as well as the Ringsaker population to examine whether the difference in effect stemmed from differences between populations, differences between experimental conditions in the glasshouse and field, including predation and competing species, or from unfertilized gravid females in Experiment 1 as well as lack of males to additionally graze down plants.

The findings in this study are supported by Renner (1969) (not seen, cited by Voigt et al., 2011) that found third instar larvae to consume up to 90 mg [most likely fresh weight, as the corresponding dry weight was found to be 2.9 mg by Bentley & Whittaker (1979)] of *R. obtusifolius* leaves day^{-1} and females to consume up to 170 mg day^{-1}. Their consumption rates would give a stronger immediate effect on the remaining leaf area of applying third instar larvae at a very high density than gravid females at a low density, which first gives a strong effect once a sufficient number of first-generation larvae are hatched and developed. The ultimate effect on survival until the next summer of dock plants, which was found here to be lower in treatments with third instar larvae than treatments with gravid females, depends on plant dry weight directly before winter (Weaver & Cavers, 1979). From Experiment 2 it was strongly indicated that when there was a less immediate restriction on shoot growth in the grazing treatment without competition versus with competition, the plant weight would stay significantly higher till the end of the experiment, even though the shoot growth was approximately equally suppressed between the grazing treatments throughout the rest of the experiment. This effect would also apply in Experiment 1 with a delayed effect by applying gravid females versus a more immediate effect of the third instar larvae.

4.2 Applied densities of a given developmental stage of *G. viridula*

In Experiment 1 with its mentioned weaknesses, there was found no significant difference in effect on the percentage of leaf area removed in the first season, survival, or shoot weight in the following season between 5, 15, and 25 gravid females per 16 plants. There was, however, a trend towards lower survival and higher shoot weight of *R. longifolius* at higher densities of gravid females. In a small study by Bentley & Whittaker (1979) one-month-old seedlings of *R. crispus* and *R. obtusifolius* was in one experiment grown with intraspecific competition for three months with grazing by *G. viridula* applied at a density equivalent to eight gravid females per 16 plants, and in another experiment grown alone for one month with grazing by *G. viridula* applied at a density equivalent to 16 gravid females and 32 males per 16 plants. In both experiments, *R. obtusifolius* and *R. crispus* were also grown with interspecific competition and grazing by *G. viridula* applied at a density of eight females and 16 males (males were only applied in the second experiment) per 16 dock plants. For dock plants exposed to an equivalent of eight gravid females per 16 plants, there was no difference in plant growth from the ungrazed control when grown without competition, but when grown with competition grazed plants showed reduced dock growth in three of four cases. In the second experiment, both species showed reduced plant growth when grown alone with grazing by an equivalent of 16 gravid females and 32 males per 16 plants. A very small experiment using 13 approximately two-month-old plants of *R. obtusifolius* found a tendency towards more reduction of dock growth after 20 days of grazing at higher densities of applied gravid females and males (Kwon & Nam, 2000). Densities applied were zero beetles, 16 gravid females and 32 males per 16 plants, and 32 gravid females and 64 males per 16 plants. When comparing these two additional studies, there appears to be a tendency of more reduced dock growth at higher densities of gravid females, at least of *R. crispus* and *R. obtusifolius*. The question is, therefore, whether the differences found between these studies stem from true differences between dock species on the ability of *G. viridula* to reduce growth, or if it is a result of the experimental designs with accompanying methodological weaknesses.

When comparing increasing densities of applied third instar larvae, there was a significantly higher effect of 250 larvae per 16 plants compared to 100 larvae on percentage of leaf area of the first four leaves remaining throughout the first season, and survival and shoot dry weight of docks the following season, but the effect was not increased further by applying 500 larvae. Of examined densities, 250 larvae per 16 first-year plants is therefore found in this experiment to be the most optimal density for application of third instar larvae on *R. longifolius* as almost all dock plants died before the following summer, and one would not gain anything by doubling the density. It may even have a negative effect to use 500 third instar larvae per 16 plants rather than 250, as there were tendencies toward a higher survival rate and shoot dry weight of *R. longifolius* the following season. This tendency may be because the dock leaves were grazed down too fast for the larvae to complete their development to pupae.

4.3 Combined effect of competition and grazing versus either factor alone

Dock plants grown under competition showed a very strong reduction in all response variables compared to the non-competing, ungrazed control. Docks exposed to grazing as well as competition showed a further strong reduction in most leaf area variables throughout the experiment, and a reduction of 71.9 – 72.9 % in root dry weight compared to their respective ungrazed, competing controls.

After the second grass (+ clover) harvest toward the end of the experiment, there was much less grass growth, and to some degree dock growth, which may have affected the competition pressure on dock plants and the relative ability of *G. viridula* to reduce dock growth. The reduced growth could have stemmed from sciarid fly larvae, depletion of nutrients in the soil, and too low light intensity. There were sciarid fly larvae in all cages, which could have damaged grass and dock roots, as in addition to feeding on decomposing plant matter, some species can feed on healthy roots (Mohrig et al., 2012; Sundbye, 2011). However, neither grass, clover, or dock roots showed any clear signs of significant damage by insects at the experiments end in any treatment, except for root crowns of docks in the non-competing grazing treatment, which showed clear signs of herbivory. Seeing as there were no equivalent damages in any other treatments, it seems likely that the damage stems from *G. viridula* which were desperate for food as there were around 1 000 larvae in each cage before the plants collapsed and 160 imagoes in each cage that emerged after pupation without, or with a very limited food source. This consumption of the root crown is, therefore, likely a result of the experimental design. Decreasing content of nutrients, especially nitrogen in the soil due to the removal of plant shoots and runoff of nutrients from the soil, may have reduced grass and dock growth (Willey, 2015, p. 107), and seems somewhat likely, as the clover growth was less reduced than grass growth. However, nitrogen depletion is unlikely to have affected the results of this experiment significantly, as Hatcher et al. (1997) found that the reduction of *R. obtusifolius* by *G. viridula*, *U. rumicis* or their combination was independent of soil nitrogen levels within the measured range of 0 – 400 kg ha^{-1} $year^{-1}$ of added N, and in this study, N was added at approximately 465.5 kg ha^{-1} $year^{-1}$. There could have been an effect of light intensity on grass, clover, and dock growth, as even though the artificially added PPFD was measured to be approximately at the recommended level of 200 µmol m^{-2} s^{-1} (Durner, 2013, p. 143) without netting cover which likely lowered the PPFD some, the daily light integral was likely under the recommended 26 mol m^{-2} day^{-1} for productive growth (Durner, 2013, p. 143) towards the end of the experiment, as the days became significantly shorter towards December (Islam et al., 2005). However, any reduction in growth would be equal between all treatments, and in the period of lower grass growth after the second grass (+ clover) harvest, there were no larvae and few imagoes present to graze competing docks, as to disproportionally affect grazed dock plants.

When docks act as weeds in grassland, they will be growing in interspecific competition with the sward, but seeing as many experiments that have been performed on biocontrol of docks by *G. viridula* have been performed without competition from a sward (Grossrieder & Keary, 2004; Hatcher et al., 1994; Hatcher, 1996; Whittaker, 1982), it is of interest to compare the responses of docks grown with and without competition to grazing by *G. viridula*.

In Experiment 2, except for the first assessment, where there was a larger effect of grazing with competition than grazing alone on both dock leaf response variables, there was either no difference in dock leaf response variables between grazing treatments, or there was a larger effect of grazing alone. This effect seemed to stem from an initial growth spurt of the plants that were not exposed to competition before eggs started to hatch. On these larger plants, many more larvae grew to the third instar, which consume more than three times the daily amount of *Rumex* spp. leaf mass as second instar larvae (Bentley & Whittaker, 1979; Renner, 1969, not seen. Cited by Voigt et al., 2011) and were therefore able to quickly defoliate the plant and lead to a collapse of the plants. They could have collapsed either as a direct result of herbivory, or as a defense mechanism of the plants to starve out the larvae, seeing as there were lamina remaining when they collapsed (Kant et al., 2015). More larvae were also able to complete their development in the grazing only treatment and thereby keep the docks defoliated after the initial collapse of the plants as new imagoes emerged when some plants developed new leaves, even though all imagoes died a short period after emergence due to lack of leaves to feed off. However, despite the fact that root crowns of non-competing, grazed plants were partially consumed by insects, most likely by *G. viridula,* the root dry weights of competing plants with grazing were 59.5 – 66.5 % lower than plants that had only been grazed, most probably an effect of the first weeks when docks that were only grazed were able to grow larger and store more assimilates in the roots before being grazed down to the same level as the other grazing treatments.

Dock plants were also affected by fungi. Already at the two-leaf stage, red lesions had developed, most likely of *Ramularia rubella* (Bonord.) Nannf. as it is very common on *R. longifolius* in Norway (Hatcher et al., 2008) or of *Venturia rumicis* (Desm.) G. Winter, which is also common on at least *R. obtusifolius* and *R. crispus* in Europe (Hatcher et al., 2008). As the experiment progressed, ungrazed docks had more observed lesions than docks that were grazed, an effect of beetle grazing also found by Hatcher & Paul (2000). Fungal infection possibly reducing root and shoot dry weight (Hatcher et al., 2008) in addition to negatively affecting leaf area measured. Together with the damages caused by the *N. pronuba* found in one of the ungrazed 'miniature leys', this could have affected the final difference in root and shoot dry weight between ungrazed and grazed plants. However, seeing as these damages serve to reflect natural conditions where dock plants are commonly infected by fungal pathogens (Hatcher et al., 2008) and are being grazed by other herbivores (Martinková & Honek, 2004), they serve to produce results of this experiment performed in an artificial environment which are more realistic to field conditions.

The results of Experiment 2 show that grazing by *G. viridula* at a density of 25 gravid females per 16 plants has a larger effect on *R. longifolius* growth when docks are competing with forage species compared to grazing or competition alone. The synergistic effect of competition and grazing on dock root and shoot dry weight found on *R. longifolius* are reflected in other experiments on *R. obtusifolius* and *R. crispus* growth*.* Bentley and Whittaker (1979) found that even modest levels of grazing (density equivalent to eight applied gravid females per 16 plant) could lead to a significant reduction in *R. obtusifolius* and *R. crispus* growth when plants were under interspecific competition (this was found in two experiments, one of which was described as heavy grazing, but had the same grazing pressure on plants grown under interspecific competition as the first experiment with "light grazing"), compared to ungrazed plants under interspecific competition. However, the same level of grazing on plants grown with intraspecific competition did not result in a reduction of plant growth compared to an ungrazed control. Cottam et al. (1986) similarly found that grazing of first-year *R. obtusifolius* by *G. viridula* at an applied density of 8 to 16 gravid females and 8 males per 16 plants in a field experiment was only able to reduce the growth of dock plants competing with grass. In a study using grazing by *G. viridula* applied at third instar larvae*,* infection by *U. rumicis* and competition from perennial ryegrass on *R. obtusifolius*, Keary & Hatcher (2004) found that herbivory, when docks were under competition from perennial ryegrass, was able to reduce shoot and root dry weight of docks.

The voltinism of *G. viridula* is also found to be positively affected when *G. viridula* is grazing on docks in regularly cut grassland compared to dock monoculture and even uncut grassland (Smith & Whittaker, 1980b). Fewer generations were connected with the synchronization of flowering and leaf loss for up to two months following flowering in uncut habitats (Smith & Whittaker, 1980a). This effect on voltinism may suggest that *G. viridula* will be a more effective biocontrol agent in cut grassland than uncut pasture.

4.4 Difference in reduction of dock growth when grazed, under competition from different plant species

Although the final root dry weight of ungrazed *R. longifolius* was significantly lower in Experiment 2 when grown in competition with the ley mixture than when grown in competition with Italian ryegrass, suggesting a stronger competitive effect of the ley mixture than Italian ryegrass, there was no difference between forage species mixtures on the root or shoot weight of grazed dock plants between the two competition levels.

Although there were no consistent differences in leaf response variables of docks between the two forage species mixtures throughout the experiment, which would suggest a higher competitive ability of the ley seed mixture than Italian ryegrass, only the last assessment was able to document the entire dock plant and thereby give a full picture. The number of developed leaves which would have given a clearer picture had also been recorded, but as the experiment progressed, it varied between 'miniature leys' and individual plants how fast a leaf decomposed. As the leaves were not marked as they developed with which number they were, the number found soon became meaningless. If the experiment were to be repeated, it would be desirable to count the number of live leaves, as that would best describe the current photosynthetic ability of the plant and would most likely have given a better understanding of the difference in root dry weight between treatments.

4.5 Dispersal of *G. viridula* over time from a central release point

In the 39 – 53 days following the release or gravid females in the perennial leys at the two farms, many *G. viridula* were found at five meters from the beetle release point, but no *G. viridula* were ever found further from the release point than ten meters. Using the model fitted with the results found here, there is a very low chance of ever detecting beetles further from the release point than 15 meters. The results found here coincide with a previous experiment that was part of a larger study where imagoes released were on average recaptured three meters from their release point and never more than seven meters from their release point (Whittaker et al., 1979), but is significantly shorter than the dispersal expected from the results of another study, where imagoes were recaptured eight meters from their release point already after two days (Smith & Whittaker, 1980a). In the first study they also shortly noted in conjunction with another experiment, with some conflicting dates in the table and text, that 23 days after the closest *G. viridula* was found 35 meters from the study grid, *G. viridula* was discovered two meters from the study grid, and was found inside the study grid after a further 20/50 days (Whittaker et al., 1979). This described dispersal did not appear to be a systematic survey but indicates that *G. viridula* is able, though not necessarily expected, to disperse over longer distances than ten meters.

Experiment 3's ability to detect dispersal was, however, limited, as a maximum of 40 plants were examined for every annulus, and already in the third annulus 9 – 11 m from the center, only 20 % of the annulus area was examined. It is, therefore, reasonable to assume that there were more *G. viridula* in each plot that were not found, and some may have dispersed further than ten meters. Few docks and a long sward forced the experiment to end early in site one, as no *G. viridula* possibly present were able to be detected, negatively affecting the results. As *G. viridula* has never been observed to fly (Smith & Whittaker, 1980a; Whittaker et al., 1979), few dock plants possibly affected the dispersal and survival of *G. viridula* in the ley as well, since they could not easily fly to find a suitable dock plant. Also, the releases of the beetles were late in the season, so that no second generation was begun, where at least in site two this resulted in a prolonged period with no egg clusters or larvae after the first-generation imagoes emerged, which are the easiest stages to discover, as imagoes drop to the ground when disturbed. The lack of egg clusters and larvae further reduced the ability to detect dispersal and affected the resulting model to expect fewer *G. viridula* at increasing distances over time.

Though other experiments have found higher dispersal rates (Smith & Whittaker, 1980a; Whittaker et al., 1979), this experiment indicates that *G. viridula* is expected to disperse between five and ten meters and no more than 15 meters from a release point within one generation in a perennial ley of diverse vegetation, highly infested with *Rumex* spp., while showing a shorter dispersal in a perennial ley with more patchy distribution. Using another method of registration, and a higher number of released imagoes, one may expect to find a higher number of beetles further out. However, it is at least possible to say that 12 released gravid females will spread evenly out a minimum of five meters from a release point in a ley with approximately uniform dock distribution in one generation.

4.6 Implications for the use of *G. viridula* as a biocontrol agent of *R. longifolius*

If *G. viridula* is to be used as a biocontrol agent of *R.* longifolius in Norwegian grassland, it is evident that applying third instar larvae, at a higher density than gravid females will produce the largest and most effective reduction in growth and survival of *R. longifolius*. The question is, however, if that will be practical if green dock beetles are to be used as biocontrol agents via the inundative method, as one would need to breed a higher number for release in a given area, and would need to release them at closer intervals (m), as the larvae most probably disperse more poorly than imagoes.

Even though the effect of applying gravid females was low in Experiment 1, the effect of applying imagoes at a density of 25 gravid females and 25 apparent males per 16 *R. longifolius* seedlings was very high in Experiment 2. However, the effect was somewhat delayed in comparison to applying third instar larvae. The high effect of gravid females and males found in Experiment 2 indicates that gravid females may be a suitable developmental stage for application in the field when factoring in that fewer *G. viridula* would have to be bred for release in a given area. In Experiment 2, it was also found that a higher number of individuals of *G. viridula* were able to complete a generation in the non-competing grazing treatment than the competing grazing treatments where non-competing *R. longifolius* plants were much larger than competing plants after two weeks. This difference in first-generation imagoes indicates that there is a different optimum of applied gravid females when dock plants are of different sizes, as the high initial number of beetles and larvae in the competing treatments seemed to graze down the dock plants before most larvae were large enough to pupate. The full potential of the 25 gravid females was therefore not utilized, and more larvae could perhaps have completed their development to first-generation imagoes if fewer applied imagoes had consumed the available dock plants. The emerged imagoes in the non-competing treatment died of starvation, however, but in nature, they would have the opportunity to disperse to look for new dock plants. Further research is needed, preferably a larger field experiment with males introduced along with gravid females on which number of applied imagoes per dm^2 of dock leaf will reduce *R. longifolius* growth and survival to the following year the most when grown under competition from grass/forage species.

From Experiment 1, it was found that 250 third instar larvae per 16 *R. longifolius* seedlings was the optimal applied density of the examined densities. If third instar larvae are found to be a practical developmental stage for release into fields, there should also be further research refining the number of larvae applied per dm^2 of *R. longifolius* leaves.

In another experiment performed in 2009 by (Andersen et al., 2011, unpublished) on control of second-year *R. longifolius* by application of 250 third instar larvae from two populations of *G. viridula*, it was found a tendency towards a higher effect of beetles from Tromsø over Øystre Sildre on the percentage of leaf area consumed. The difference between populations indicates that if green dock beetles are to be used as biocontrol agents, in addition to different developmental stages and numbers, different populations should also be examined for their efficacy in reducing dock growth, to produce the optimal effect.

In Experiment 2, it was found that combined competition and grazing gave the largest reduction in *R. longifolius* growth when compared to grazing and competition alone, and the ultimate reduction of dock growth, when grazed by G. viridula, was unaffected by with which forage species *R. longifolius* was grown in competition. This difference between grazed and ungrazed docks suggests that grazing by *G. viridula* may be able to compensate for the difference in competitive ability of forage species mixtures on *R. longifolius* and produce the same dock root dry weight between at least some forage species used in grassland. Although Experiment 2 was a glasshouse experiment, and therefore do not reflect exact field conditions, it strongly indicates that *G. viridula* in optimal density will be able to reduce the impact of *R. longifolius* on the grass/ley yield. Schulz (2013) calculated the monetary loss of milk production at different contents of *R. obtusifolius* in ryegrass silage and determined the economic threshold for spraying with a selective herbicide at a dry matter content of 3 – 5 % *R. obtusifolius* in ryegrass silage, or 7.4 % coverage of the grassland. As docks older than one year have not been found to be killed by *G. viridula* (Andersen et al., 2011, unpublished) or even by weekly mechanical defoliation (van Evert et al., 2020) which only reduced growth and only in some cases showed reduced reproduction when grazed by *G. viridula* (Bentley et al., 1980), it seems that application of *G. viridula* on dock plants will be most effective in reducing growth and survival when applied in their seedling stage/first year. As most established *R. longifolius* plants can be eliminated through deep plowing before ley renewal (Ringselle et al., 2019), it would be of interest to examine if applied *G. viridula* at a certain time interval and density can keep *R. longifolius* emerged after ley renewal under the economic threshold for chemical control for a longer period, through an experiment on seedlings which extends a few years from the establishment of the ley.

The dispersal field trial indicates that if green dock beetles are to be used as biocontrol agents that rapidly spread over a given area and suppress dock growth, they would have to be released at relatively close intervals (m) in a ley with relatively uniform dock distribution and most probably placed within dock patches in leys with more patchy distribution.

Green dock beetles are oligophagous (Martinková & Honek, 2004), and despite their low dispersal rates in the short term, released *G. viridula* have the potential to disperse to and affect surrounding habitats. Therefore, before any mass-release of *G. viridula* in the field to control docks, their potential impact on local flora and fauna should be examined.

5 Conclusion

Gastrophysa viridula has been found in this study to have a promising potential as a biocontrol agent of *R. longifolius* in Norwegian grasslands via the inundative method. Applying third instar larvae at a density of 250 larvae per 16 *R. longifolius* seedlings appears to be the most effective examined stage and density to reduce dock growth and survival, but one experiment also found a strong effect of applying 25 gravid females and males per 16 *R. longifolius* seedlings on shoot and root growth, implying that gravid females can also show an adequate effect on *R. longifolius* suppression. The final reduction of dock growth when grazed appears to be unaffected by which of the two forage species mixtures are used in the ley. Dispersal of *G. viridula* over the development of one generation was found to be short and indicates that imagoes would have to be released at a relatively close interval in leys with uniform distribution of docks, and within dock patches where the dock distribution is patchy to cover a given area quickly.

Further research is needed to refine the optimal density of applied imagoes or larvae and to determine the effect of applying *G. viridula* over several years in a ley, the dispersal of *G. viridula* over time, the most optimal population to be used and the impact of *G. viridula* on local flora and fauna.

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7 Supplemental data

Table S1. Tukey pairwise comparisons between all levels of competition and *G. viridula* on the number of true dock leaves at the beginning of the experiment (11th of October).

Tukey Pairwise Comparisons: grass

Grouping Information Using the Tukey Method and 95% Confidence

grass	N	Mean	Grouping
0	8	6,7500	A
1	8	6,5000	A
2	8	6,0625	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: beetles

Grouping Information Using the Tukey Method and 95% Confidence

beetles	N	Mean	Grouping
1	12	6,58333	A
0	12	6,29167	A

Means that do not share a letter are significantly different.

Table S2. ANOVA table with P-values for the responses of the six treatments throughout the experiment. All responses are given per plant. Values are transformed using the Box-Cox algorithm, where the calculated λ value determines the mode of transformation. Transformations used are Log_e (λ = 0.00), $\sqrt[2]{}$ (λ = 0.50), $\sqrt[3.394262]{}$ (λ = 0.29), $\sqrt[5.49489]{}$ (λ = 0.18) and $\sqrt[3.234068]{}$ (λ = 0.31).

Fixed factors	Df Num	Number of leaves longer than 5 cm			
		14 days after start	27-28 days after start	42 days after start	54 days after start
Competition	2	2e-10***	<1e-16***	2e-14***	2e-09***
G. viridula	1	2e-05***	<1e-16***	4e-16***	1e-12***
C**G. v*	2	0.335 n.s	<1e-16***	8e-15***	9e-12***
λ		0.50	0.00	0.00	0.00
		Leaf area measurements (cm^2)			
		Two longest leaves (13 days)	Leaves harvested (27-28 days)	Two longest leaves (41 days)	All leaves (55-56 days)
Competition	2	3e-10***	2e-11***	1e-11***	5e-08***
G. viridula	1	2e-08***	<1e-16***	9e-16***	3e-11***
C**G. v*	2	6e-04**	7e-12***	2e-13***	1e-11***
λ		0.50	0.00	0.29	0.18
		Dry weight measurements (mg)			
			Leaves harvested (27-28 days)	Shoot (55-56 days)	Root (57-58 days)
Competition	2		2e-11***	1e-11***	8e-12***
G. viridula	1		<1e-16***	8e-14***	1e-12***
C**G. v*	2		9e-12***	9e-14***	2e-07***
λ			0.00	0.31	0.00

***Significant at P < 0.0001, **significant at P < 0.001, n.s not significant.

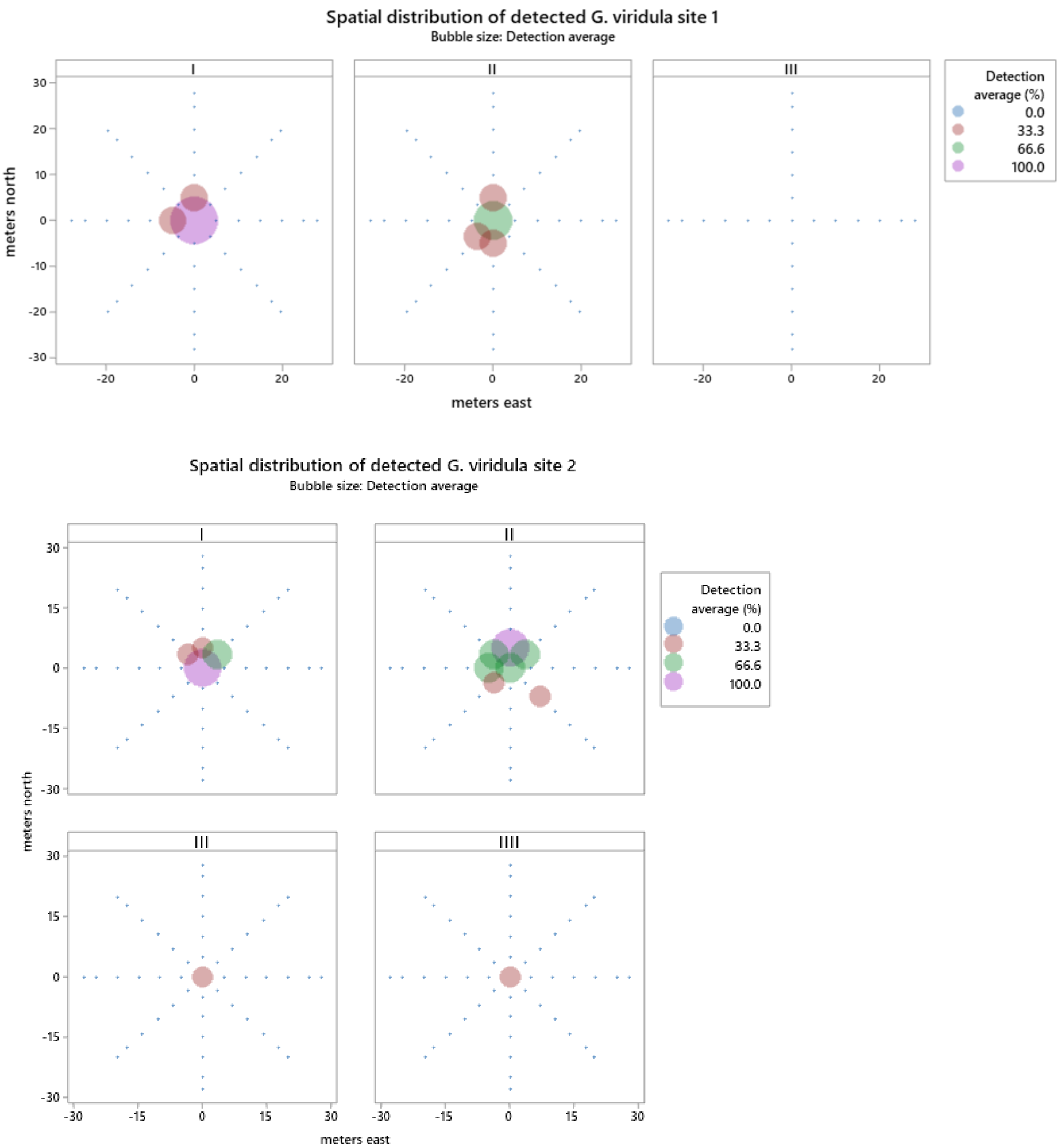

Figure S1. Spatial distribution of observed G. viridula at site one 5-6 days (I), 20-21 days (II) and 37-39 days (III) after release of beetles and at site two 12-13 days (I), 24-26 days (II), 41-42 days (III) and 52-53 days (IIII) after release of beetles. Bubble size is the detection (found/not found) average over the three replicates.

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