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Abstract

Cultivating well-developed and marketable garden strawberries (*Fragria x ananassa*) and woodland strawberries (*Fragaria vesca*) depends on successful pollination by insects. Organic garden strawberry plantations harbour higher pollinator densities and have been shown to produce bigger and better developed fruits than conventional farms. However, organic cultivators sometimes face severe problems from herbivory by leaf beetles that forage on both leaves and flowers. This study aims to investigate how the damage on woodland strawberry by leaf beetle herbivores (*Galerucella tenella* and *G. sagittariae*) affect 1) the pollinators' response to damaged vs. undamaged flowers, 2) pollination success, fruit weight and fruit development and 3) the production of flowers and runners. Two different plant types of woodland strawberry were used; the commercial variety Rügen were herbivore damage was focused on the flowers and wild genotypes that received herbivory on both leaves and flowers. Pollinators (bees and hover flies) clearly preferred undamaged flowers and there was no significant difference between pollinator groups. Both plant types had a higher pollination success in undamaged flowers compared to damaged ones but it was only significant in the Rügen plants. Rügen plants produced lighter fruits after florivory but there was no obvious effect on fruit weight after herbivory and florivory in the wild genotypes. There were less deformations from undamaged control flowers in Rügen plants but none of the plant types showed any significant effects of herbivory on the number of deformations. Hand pollination was done on at least one flower of each plant in both plant types to control that damage by herbivores caused lower pollination rather than direct damages that caused a lower weight or more deformations. Hand pollination caused an increase in weight for Rügen plants and decrease of deformations for both plant types, suggesting that pollen was limited. The number of runners produced during the experiment decreased with herbivory-damage while the number of produced flowers was unaffected by damage. To establish a more reliable way to cultivate woodland strawberries without pesticides it could be advantageous to further investigate the plants' inherent defence to herbivores. There are also possibilities to decrease the amount of leaf beetles in cultivations through the hymenopteran parasitoid *Asecodes lucens* that should be further investigated.

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Introduction

Fruit development and pollination success

There is an increased market for organically grown crops, and one benefit received by cultivating organically is the increased abundance and diversity of pollinators (Horzschuh et al. 2008; Jonason et al. 2011). Garden strawberries depend on insect pollinators to produce well-developed fruits (Lopez-Medina et al. 2006, Albano et al. 2009), and the amount of fully developed fruits and their biomass has been shown to be higher in organic farms than conventional ones (Andersson et al. 2012). Better fruit yield is likely to be related to the higher abundance and diversity of pollinators.

On the other hand, herbivory damage by leaf beetles and other pest insects is common when garden- and woodland strawberries are cultivated without the use of pesticides. There are several ways herbivory could affect the flowers' attractiveness to pollinators, both directly and indirectly. Visual and olfactory cues from petals play important roles for the flowers' attractiveness to pollinators. For example, Kudoh and Whigham (1998) showed that two hymenopteran pollinators of *Hibiscus moscheutos* ignore flowers were the petals were completely experimentally removed. Herbivory can cause plants to produce smaller petals (Lehtilä and Strauss 1999, Strauss et al. 1999) or herbivores can reduce petals directly through florivory (McCall 2006), both of which may decrease pollination success. For example, Karban and Strauss (1993) showed that petals of *Erigeron glaucus* that were damaged by thrips received significantly less pollinator visits. Other kinds of florivory such as consumption of the anthers can also result in less pollinator visitations than undamaged flowers (Sõber et. al. 2010). In the Butterfly pea *Centrosema virginianuim* -reduced pollinator visitation caused by florivory results in fewer fruits and seeds (Cardel and Koptur 2010). It is important to investigate whether similar patterns exists in woodland strawberries *(Fragaria vesca)* to avoid reduced fruit yields in organic cultivations compared to conventional ones.

Defence compounds in garden strawberries can both be constitutively expressed or induced by herbivory (Amil-Ruis et al. 2011). Studies on other species show further examples of herbivory induced defence compounds that could also be applied to woodland strawberries. Damage caused by herbivores can increase the concentrations of certain chemical defences, where an increase of secondary compounds, e.g. nicotine, is induced by grazing insect herbivores (Euler and Baldwin 1996). An increase of defence chemicals can decrease the time pollinators spend on each flower, which also affects the pollination success negatively (Strauss et al. 1999). Because of their need for pollinator visits, outcrossing tobacco species has been shown to have lower amounts of nicotine than selfing species (Adler et al. 2012). Herbivory has also been shown to affect floral scents and hence decrease pollinator visitation (Kessler et al. 2011). Other results of herbivory that are likely to affect pollination success are florivory of nectar guides (Botto-Mahan et al. 2011), decreased amount of available nectar per flower, and a decrease of inflorescences per flower (Krupnick et al. 1999). Herbivory by *Galerucella* beetles in woodland strawberry plantations damages both leafs and petals (Jensen 2006) and it is thereby possible to affect attractiveness both through chemical and visual cues.

Pollinators and the difference between pollinator groups

Cultivated strawberries usually receive visits by various pollinator species, for example bumble bees, wild- and honeybees and hover flies. Although common pollinator groups are equally effective at fertilizing ovules during a single visit to strawberries (Albano et al.

2009) it is unknown whether there are interspecific differences in their selection of undamaged or damaged plants and flowers. Due to pollen limitation, supplemental pollinators, usually bumble bees or honey bees, are often bought in to woodland strawberry plantations to increase the pollination success and fruit production (Jensen 2006). Knowledge of which pollinators that are more tolerant to damaged flowers would be useful in organic cultivations of woodland strawberries. Less "picky" pollinator species could give a higher pollination success compared to other species during *Galerucella* outbreaks and hence reduce costs of herbivore damage. Despite the fact that all pollinator groups cannot be bought in and placed in a cultivation, this can still be important for the choice of location for a woodland strawberry cultivation and conservation management of pollinator diversity. Surveying pollinator behaviour for herbivore influence on flower development or for manually damaged flowers may also be very useful for breeding on an increased inherent defence in woodland strawberries. As mentioned above, defence compounds can decrease pollinator visitation and a difference between mechanical and herbivory-caused damages and undamaged flowers may reveal such effects.

Flower and runner production in wild populations

Wild woodland strawberry genotypes can normally reproduce both sexually with seeds and clonally by sending out runners. Both clonal reproduction and seeds can be very important for a species survival. A metapopulation model of *Hieracium pilosella,* that reproduces both sexually and clonally, showed that both modes are needed to maintain a healthy metapopulation and the allocation between these strategies depends on the quality of the habitat. Clones were important to expand locally and maintain an already established population, whereas seeds are necessary to spread into uncolonised areas and start new populations (Stöcklin and Winkler 2004). In garden strawberries, herbivory by the vine weevil *Otiorhynchus sulcatus* can result in a lower production of runners (Gange 2001). *Galerucella* beetles overwinter in the soil around its host plants and can stay in the same area for several years and generations (Olofsson and Pettersson 1992). If the plants can adjust their resource allocation to the reproduction system most efficient for each habitat, it is possible that they allocate energy to long distance dispersal when local conditions are unfavourable. In that case there should be a higher flower production and lower runner prodution for herbivory damaged plants compared to undamaged ones. Alternatively, damage through florivory on sexually reproductive parts could give a response to use the clonal reproduction system instead if it experiences less damage. It is also possible that costs related to herbivory for example through defence strategies are so high that there is a reduction in production of both runners and flowers.

Aims

The aim of this study was to investigate the effects of leaf beetle herbivory on pollinator behaviour, pollination success and reproductive strategy of woodland strawberries. This was investigated both in terms of cultivation values, for example decreased fruit production, as well as for plant reproductive success in wild genotypes such as pollination success, flower - and runner production and fruit development. Pollination success is an important part of these interactions and the behaviour of different pollinators is important to predict fruit production in cultivations and plant fitness in natural populations. To investigate the relationship between leaf beetle herbivores, woodland strawberries and pollinators I asked the following questions: 1) is there a preference for undamaged flowers compared to herbivore- or mechanically damaged flowers and is there a difference in flower preference between pollinator groups, 2) does herbivore damage on leaves and flowers of woodland strawberries affect pollination success, fruit weight and the number of deformations on fruits and 3) does herbivory affect the number of produced flowers and runners?

Methods

Study species

The commercial variety Rügen, *Fragaria vesca* and wild genotypes of woodland strawberries, *Fragaria vesca* were used in this study. Rügen plants are suitable for experiments focused on flowers since they all produce flowers and will produce flowers during a long season. Wild genotypes were used to see the natural response to herbivory in plants shaped by selection, where the ability to deal with herbivory can be important. Woodland strawberries are perennial and can be found in various habitats such as forest clearings, forest edges, along roads and paths and on stone walls. The wild genotypes produce plenty of runners during spring and summer that grow up to self-sufficient plants during the same season. Rügen is an old variety of woodland strawberries that does not produce runners. The harvest season is relatively long compared to other varieties and new flowers are produced during several months. The fruits are bigger than wild woodland strawberries with a sweet taste that makes it a popular variety in gardens and cultivations (Jensen 2006). Female plants can be found among wild genotypes of woodland strawberries; however most individuals are hermaphrodites (own obs.).There is a limited ability for self-pollination in both plant types (i.e. Rügen and wild genotypes) and insects are necessary to pollinate all pistils in a flower. The edible fruit consists of a fruit body with all seeds from the same flower. Around an unfertilised seed the fruit body will not swell up, and hence, successful pollination is required to avoid deformed fruits (Lopez-Medina et al. 2006).

Outbreaks of *Galerucella tenella* are common from the middle to south of Sweden while *G. sagittariae* are common in northern cultivations. Both species usually occur in low abundances and have a low effect on fruit production that cultivators can count on and be prepared for, but outbreaks with big problems and economic losses occur as well (Olofsson and Pettersson 1992). The adult beetles usually emerge in April to May, eggs are laid on the leaves in early June and larvae are found from the beginning of July (Olofsson and Pettersson 1992). Adult beetles forage on both leaves and flowers and can eat both seeds and fruit. Larvae mainly feed on the plants were they hatch but with less damage than adult beetles (Jensen 2006). Since they only have one generation per year the main damage is usually in the beginning of the season, leaving fruits ripened later undamaged. However, the first harvest is usually the one with the highest financial reward, so economic losses can be unsymetrically related to the decrease of produced fruits (Jensen 2006). Outside cultivations *Galerucella tenella* mainly feed and reproduce on *Filipendula ulmaria* in Sweden (Stenberg et. al. 2007) but other Rosaceae plants are also accepted (Stenberg and Axelsson 2007). *Galerucella sagittariae* are common on e.g. *Lysemachia, Comarum,* and *Fragaria* (Olofsson and Pettersson 1992).

Origin of materials

The Rügen plants were bought in June from a garden centre and replanted into 2-litre pots containing Hasselfors™ (Hasselfors, Örebro, Sweden) planting soil. 32 Rügen flowering plants were used. In addition, 150 wild genotypes of woodland strawberry were collected in April 2012 from an area consisting of managed coniferous forest outside Nora in

Västmanland, Sweden (59.481358, 14.886475). The plants were collected along paths and roads with a minimum of 15 metres between each individual to minimize the risk of selecting genetically identical individuals. At the time the plants were dug up they only had a couple of leaves, usually from the year before. The plants were then planted in 2-litre pots and covered with perforated (diameter 0.5 mm) polythene bags (Baumann Saatzuchtbedarf) to exclude insects to damage them before the start of the experiments. All plants were placed in Uppsala in a fenced garden that allows pollinating insects to enter but excludes bigger animals such as birds that could eat the ripened fruits.

All *Galerucella* beetles were collected from wild populations around Uppsala. *Galerucella tenella* can be sexed by their shape and size while *Galerucella sagittariae* individuals were sexed during mating.

Treatments

- Experiment 1.1. Herbivory effects on pollination and fruit development on Rügen plants

All Rügen plants produced flowers. The 32 plants received a herbivory treatment where *Galerucella sagittariae* larvae were placed directly on the flower's petals, to make it possible to focus on the result of damaged petals on the pollination success and fruit development. Every plant had at least one flower of each of the three herbivory treatments: 1) one *G. sagittariae* larva, 2) one *G. sagittariae* larva + hand pollination and 3) control-, open pollinated flowers. Hand pollination was done with a marten-hair brush with pollen taken from both its own anthers and anthers on flowers from other plants to mimic pollination by insects. The hand pollination was done to better explain possible effects on fruit development caused by larvae. A result where hand pollinated flowers have more deformations than control flowers would suggest that the damage is directly done by the larva itself rather than damaging flowers to that extent that the flowers become unattractive to pollinators.

Pollination success was measured by looking at the pistils after bloom. The pistils became darker after being pollinated (own obs.). That makes it possible to estimate the percent of successfully pollinated pistils on each flower, and gives a comparable value of pollination success directly after blooming. Hand pollinated flowers were estimated to have a pollination success of 80 – 100 % since they were repeatedly pollinated until at least 80 % of its pistils were pollinated. Every undeveloped part of a ripened fruit was counted as one deformation. However, a larger, undeveloped part of a fruit was counted as several deformations depending on how many "normal-sized" deformations it corresponded to. Both deformations and fruit weight were measured continuously as the fruits ripened with one day between each scoring. A mean for each herbivory treatment on each plant was calculated for the values of weight since there sometimes was one and sometimes several flowers with the same herbivory treatment on the same plant.

- Experiment 1.2. Pollinator response to herbivore-damaged flowers in Rügen plants

An observational study of natural pollinator behaviour was performed comparing visitation to damaged and undamaged (control) Rügen flowers. For each trial, half of the flowers were damaged by *G. sagittariae* larvae, while the other half was undamaged to avoid a bias in visits towards the more common kind. Wild pollinators were only present during sunny days with little wind, which limited the opportunities to study pollinator behaviour. Data on pollinator behaviour was collected between the 3rd of June and 16th of July 2012 during 5 different surveys. The type of pollinator was recorded and every individual was observed as long as it remained among the experiment plants. During that time all visits to damaged and undamaged flowers were counted. Flowers were mostly pollinated by hover flies *Syrphidae*, Bumble bees *Bombus,* family *Aphidae*, honey bees *Apis mellifera* and wild bees, superfamily *Apoidea*.

- **Experiment 1.3. Pollinator response to manually damaged flowers in Rügen plants**

An additional study of pollinator behaviour was done on the 30th of July when pollinators appeared more frequently. This was done both to improve the knowledge of pollinator behaviour of different groups visiting these plants (e.g. hover flies, bees and bumble bees) and to observe enough individuals for statistical analyses of flower preference. At this time almost no flowers damaged by larva were left, so damages had to be done manually by perforating the petals with a pencil. 32 Rügen plants were used where half of the flowers blooming were damaged and the other half were left undamaged as control. Every pollinator individual visiting the experiment plants were recorded to pollinator type and number of visits to damaged and undamaged flowers. In this experiment no bumble bees appeared during the surveys, and pollinators were divided in two groups; Hover flies *Syrphidae,* and Bees, superfamily *Apoidea* to compare behaviour between groups.

- **Experiment 2.1. Herbivory effects on pollination and fruit development in wild genotypes of woodland strawberry**

The wild genotypes of woodland strawberry were placed on a long table in blocks of three according to how big the plants were and how far the flowers were developed. This was done to diminish the effects of different plant sizes between the herbivory treatments and to have equal amounts of flowering plants in each herbivory treatment. These blocks stood in lines across the table and each block had one plant treated with *Galerucella tenella*, one plant treated with *Galerucella saggitariae* and one untreated, control plant. Every plant that received either *G. sagittariae* or *G. tenella* treatment had one female and one male beetle placed on them. The perforated polythene bags covering each plant made a small cage around each plant and prevented the beetles to escape or become eaten by predators. Most beetle pairs laid eggs but those who did not had an amount of eggs similar to the other plants added inside the cage to have an equal effect of larvae on all plants with the same herbivory treatment. Adult beetles were removed after two weeks and the bags were removed at the same time to allow pollination of the flowers.

Every plant that produced flowers had at least one marked flower that was open pollinated, and one hand pollinated. Hand pollination was performed in the same way as described for experiment 1, with pollen from its own and other woodland strawberry plants. This was done to be able to control that possible effects on flower or fruit development was an effect of pollination rather than a direct effect from herbivory.

Pollination success, deformations and fruit weight was measured in the same way as the Rügen plants. Pollination success was estimated as the percent of darkened pistils on each flower. Every undeveloped part of a ripened fruit was counted as one deformation. Deformations on fruits from the wild plants were similar to deformations on Rügen plants and could be measured in the same way. There were sometimes fruits that were completely undeveloped, probably due to a higher variation in self-pollination between the wild plants compared to Rügen plants. These deformations were also estimated to a corresponding number of deformations. Most flowering plants had replicates of flowers with the same herbivory treatment and a mean for each plant was used in the analyses.

- **Experiment 2.2. Herbivory effects on flower and runner production in wild genotypes of woodland strawberry**

All flowers (including buds) and runners on the wild genotypes of woodland strawberry were counted three times on each plant to know how flower production changed according to time and different herbivory treatments. Beetles were placed on the plants 27th - 28th of May. Flowers and runners were counted the first time at the 26th of May before any beetles were put out, at the 11th of June when bags were removed and finally at the 4th of July. Individual plant biomass was estimated one day after the adults were placed on the plants, at the 28th - 29th of May. Each leaf's width was measured and all leaves width for each plant was summed up as an estimate of biomass, comparable between plants. The damage by adult beetles and larvae was measured by eye as percent of the whole plant that was damaged. This was done at the 4th of July, at the same time as flowers and runners were counted the last time.

Statistics

- **Experiment 1.1. Herbivory effects on pollination and fruit development on Rügen plants**

All response variables used in anovas were tested for normal distributions and all samples for weight of Rügen plants were normally distributed. A Bartlett's test was done to control that there were no significant differences in the variances of the fruit weight between the three herbivory treatment groups. Bartlett's test and a variance test were used to compare variances of fruit weight and pollination success respectively. Data on pollination success was arcsin transformed to meet the test assumptions for normal distribution. To compare the percent of successfully pollinated pistils on flowers with larva versus open pollinated flowers a paired t-test was used. Hand pollinated flowers were not included in this test since they were repeatedly hand pollinated until they reached at least 80 % of successfully pollinated pistils on each flower. A two way-anova was performed to test the difference in fruit weight between the three herbivory treatments on Rügen flowers. Plant individual was added as a factor to control for differences between plant individuals. To evaluate which herbivory treatments that were significantly different from each other Tukey's test for honest significant differences (HSD) was used. The effect of different herbivore treatments on the number of deformations was tested with a GLM with poisson distribution.

The effect of mean pollination success on mean fruit weight for each plant and herbivory treatment was tested with linear regression analyses. The relationship between pollination success and deformations was investigated in a similar way but with a GLM with a poisson distribution. To make the data on deformations fit a poisson distribution the number of deformations for each fruit was included in the test, instead of using the mean of two fruits from the same plant as mentioned for fruit weight and pollination success. Pearsons correlation was used to test the relationship between deformations and fruit weight. For the tests regarding effects of pollination success on fruit weight and deformations and the relationship between fruit weight and deformations, all herbivory treatments were included and treated equally.

- **Experiment 1.2. Pollinator response to herbivore-damaged flowers in Rügen plants**

The preference for undamaged flowers by pollinators was tested with a GLM with quasipoisson distribution, due to its wide and unevenly spread data.

- **Experiment 1.3. Pollinator response to manually damaged flowers in Rügen plants**

The same model was used for experiments with manually damaged flowers. The proportion of visits to damaged flowers for all individuals in the bee group was compared to the same value for the hover fly group with a binomial proportion test (Crawley 2007). This is a way to compare proportions for groups with unequal number of total visits between groups.

- **Experiment 2.1. Herbivory effects on pollination and fruit development in wild genotypes of woodland strawberry**

The difference in damage on each plant between the herbivory treatments was analysed with a GLM. A quasipoisson distribution was used for the model since the data for percent of damage between different plants was wide and unevenly distributed. A mean value for pollination success was calculated for each plant. After an arcsin transformation of pollination values to make the values normally distributed, an anova was performed to evaluate the effect of different herbivory treatments. The factors that were included in the initial model were: number of flowers and runners produced during the experiment, the quota of flowers/runners (ln-transformed), leaf size (summed width), damage in percent and block. Their relevance to the response variable was tested and factors with a p-value higher than 0.1 were excluded from the model. Tukey’s test for honest significant differences (HSD) was then used to evaluate which herbivory treatments that were significantly different from each other.

The effect of the different plant herbivory treatments and hand-pollination on the number of fruit deformations was analysed with a GLM with poisson distribution. Summed leaf size was included in the model to control for plant size. The number of open flowers during the experiment may affect attractiveness to pollinators and was also included. The built in step function in R was used to find the model with the lowest AIC value, i.e. the model with the best fit using as few degrees of freedom as possible (Crawley 2007). Both leaf size and open flowers were then deleted from the model. Linear regression was used to examine the effects of herbivory treatment and hand pollination on fruit weight. To control for plant size the summed leaf size was included in the model. The number of open flowers was included to control for a possible decrease in fruit weight due to a higher number of flowers on the same plant or an increase in pollination success for being more attractive to pollinators.

- **Experiment 2.2. Herbivory effects on flower and runner production in wild genotypes of woodland strawberry**

The response to different herbivory treatments for the number of flowers produced during the experiment period, 11th of June - 4th of July, was analysed in an anova after the values for produced flowers had been ln-transformed. To control for biomass, leaf size was included in the model. Other costs for the plant include runner production, so the total number of runners produced during the season was also included in the model. The effect of leaf damage was related with the effect of different herbivory treatments and therefore leaf damage was removed from the model. After that the step function in R was used to find the best fitting model.

The number of runners produced during the experiment was ln-transformed and tested with linear regression for the effect of herbivory treatment, leaf size, damage and flower production. In this case damage had a much stronger effect on the response variable than herbivory treatment, so treatment was excluded. And again, the built-in step function in R was used to find the simplest and most suitable model for the remaining variables.

Results

- **Experiment 1.1. Herbivory effects on pollination and fruit development on Rügen plants**

Undamaged flowers received significantly more pollen than damaged ones (t = 2.298, df = 31, p = 0.028) (fig. 1) and hand pollination resulted in at least 80 % of the pistils being successfully pollinated. There was a clear effect on fruit weight between the different herbivory treatments (F = 12.87, p < 0.001) (table 1). The fruit weight of woodland strawberries from damaged flowers was significantly lower compared to control flowers and hand pollinated, damaged flowers (fig. 2). No such difference was found between damaged flowers that had been hand pollinated and control flowers (fig. 2). Damaged flowers had less, but not significantly less, deformations than control flowers and significantly less deformations than damaged flowers that had been hand pollinated (table 2).

Table 1. Two-way anova for the effect of herbivory treatment on woodland strawberry fruit weight. The different herbivory treatments were (1) herbivore-damaged flowers, (2) herbivore-damaged, hand pollinated flowers and (3) undamaged, control flowers. Significant p-values are indicated by bold font.

	df	MS	F	p
Treatment	2	0.9508	12.87	**< 0.001**
Plant	31	0.1363	1.845	**0.0244**
Residuals	53	0.0739		

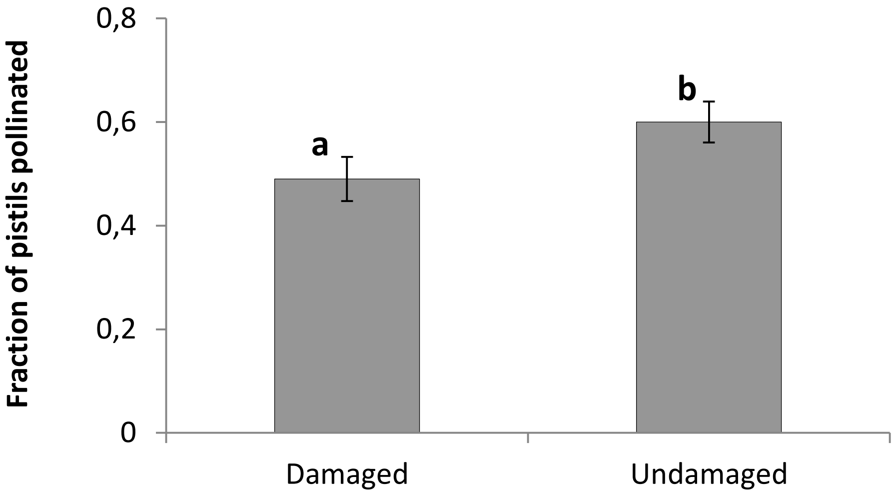

Figure 1. Pollination success in Rügen plants, estimated as the fraction of pistils per flower that were successfully pollinated. The herbivory treatments are damage by *Galerucella sagittariae* and undamaged control flowers. The bars show original (not arcsin transformed) values (± SE). n = 32. A significant difference is indicated by different letters.

Table 2. GLM with poisson distribution showing the effects of different herbivory treatments on the number of fruit deformations in Rügen plants. Control flowers and herbivore-damaged, hand pollinated flowers are compared to herbivore-damaged flowers. Significant p-values in bold.

	Estimate	**SE**	**z**	**p**
(Intercept)	0.3483	0.121	2.872	0.004
Damaged + hand pollinated flowers	-0.389	0.189	-2.064	**0.039**
Control flowers	-0.268	0.184	-1.456	0.145

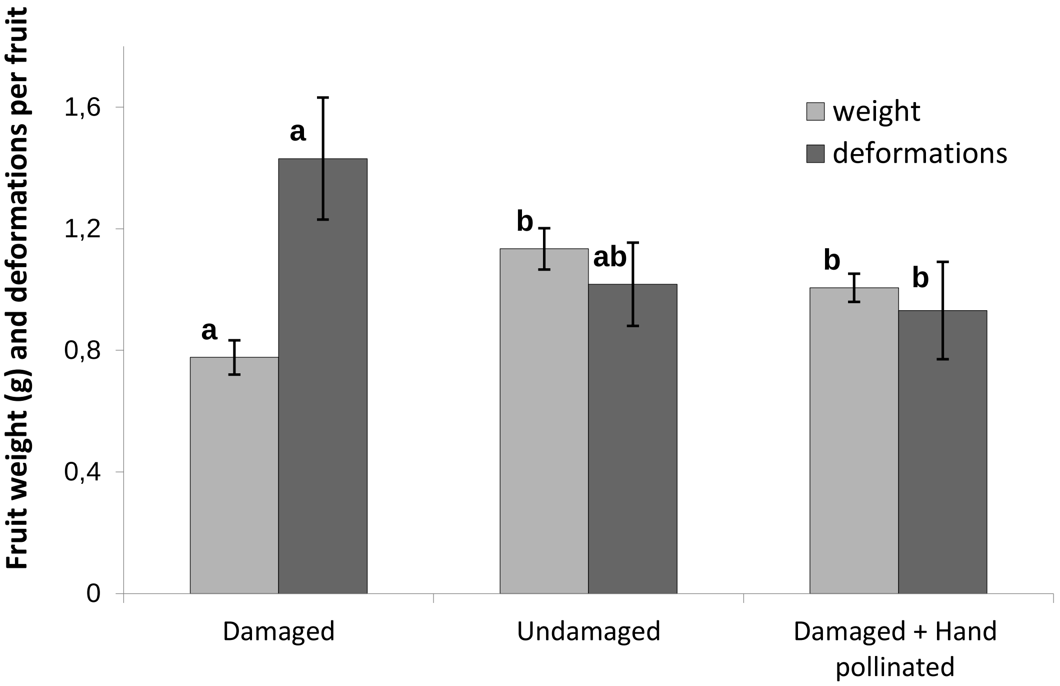

Figure 2. Fruit weight (g) and number of deformations for Rügen woodland strawberries from three different flower treatments (herbivore-damaged, undamaged control and herbivore-damaged hand pollinated). Error bars denote SE. Significant differences within series are indicated by different letters. “ab” indicates an unsignificant difference to both a and b. Note that fruit weight and deformations are shown on the same y-axis.

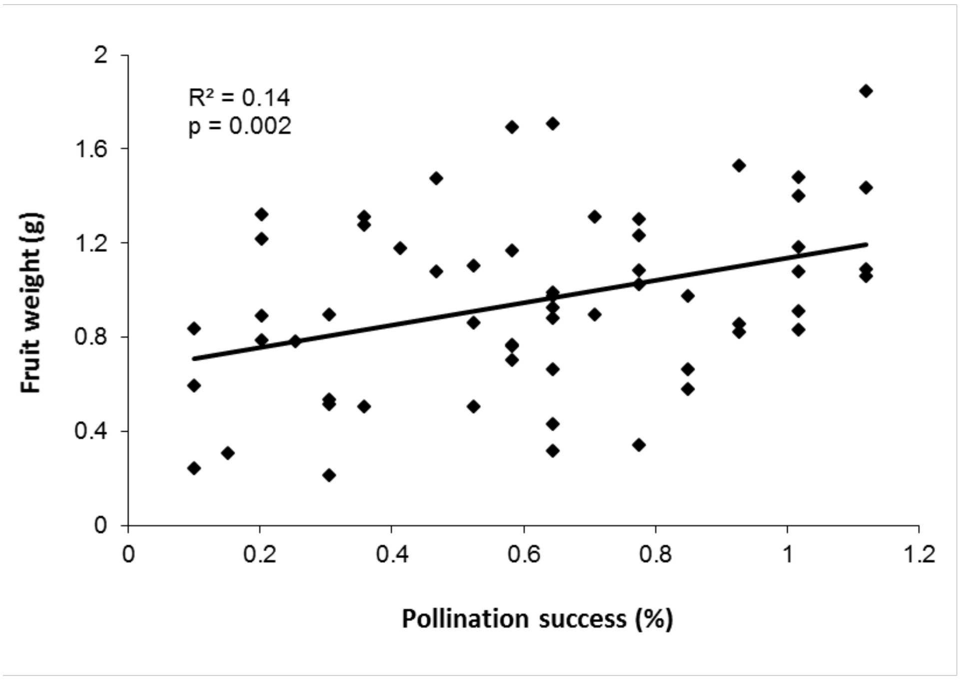

Figure 3. Relationship between pollination success (arcsin-transformed) and fruit weight of woodland strawberries in Rügen plants. Data for all herbivory treatments are included. Pollination success was measured as the fraction of pistils that were successfully pollinated.

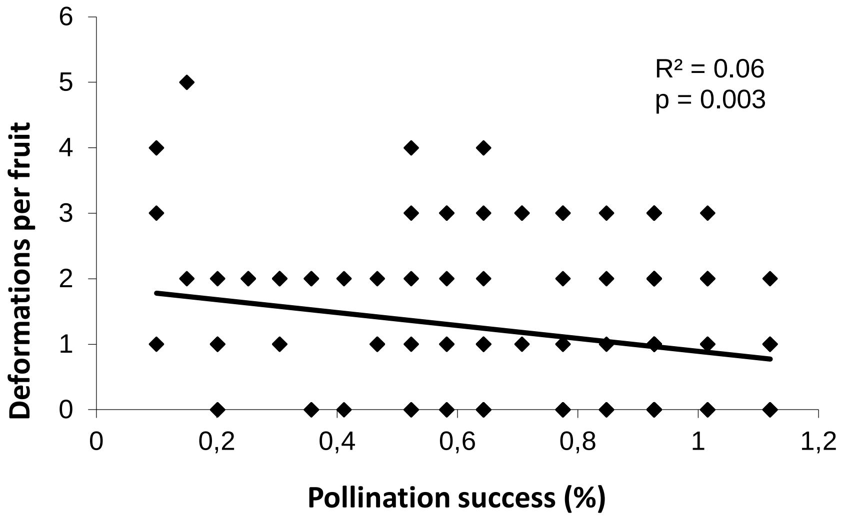

Figure 4. Relationship between deformations on woodland strawberry fruits and pollination of flowers in Rügen plants. Data from all herbivory treatments are included. Pollination success was measured as the fraction of pistils that were pollinated on each flower, showed as arcsin-transformed values.

The fruit weight of ripe fruits was significantly affected by how successful the pollination was for the same flower ($r^2 = 0.14$, $t = 3.191$, $p = 0.002$) (figure 3). The number of deformations was negatively and significantly related to the pollination of the same flowers (table 3, figure 4). There was also a significant, negative correlation between fruit weight and deformations ($r = 0.564$, $t = 6.304$, $p < 0.001$, df = 85).

Tabel 3. GLM with poisson distribution for the effect of pollination on the number of deformations on woodland strawberry fruits in Rügen plants. Pollination was measured as the percentage of pistils on each flowers that appeared to be successfully pollinated. Df = 144. The Pollination data were arcsin-transformed prior to analysis. Significant p-values are indicated by bold font.

	Estimate	SE	z	p
(Intercept)	0.6991	0.1936	3.611	< 0.001
Pollination	-0.7916	0.2637	-3.002	**0.003**

- Experiment 1.2. Pollinator response to herbivore-damaged flowers in Rügen plants

Flowers damaged by larva were significantly less visited than undamaged ones, where the damaged ones had an average of 0.8 visits (SE = ± 0.3) per pollinator while the undamaged ones had an average of 11.6 visits (SE = ± 2.21) per individual pollinator (table 4).

Table 4. GLM with quasipoisson distribution showing the effect of damaged versus undamaged flowers for pollinator visitation. Significant p-values are indicated by bold font.

	Estimate	SE	t	p
(Intercept)	-0.5596	0.8074	-0.693	0.501
Undamaged flower	2.6741	0.8348	3.203	**0.008**

- **Experiment 1.3. Pollinator response to manually damaged flowers in Rügen plants**

The flower-preference experiment with manually damaged plants showed a more even distribution than experiment 1.2 between the visits to damaged and undamaged flowers. Damaged flowers were visited 1.5 times on average and undamaged ones were visited 3.9 times on average per individual pollinator (fig. 5).

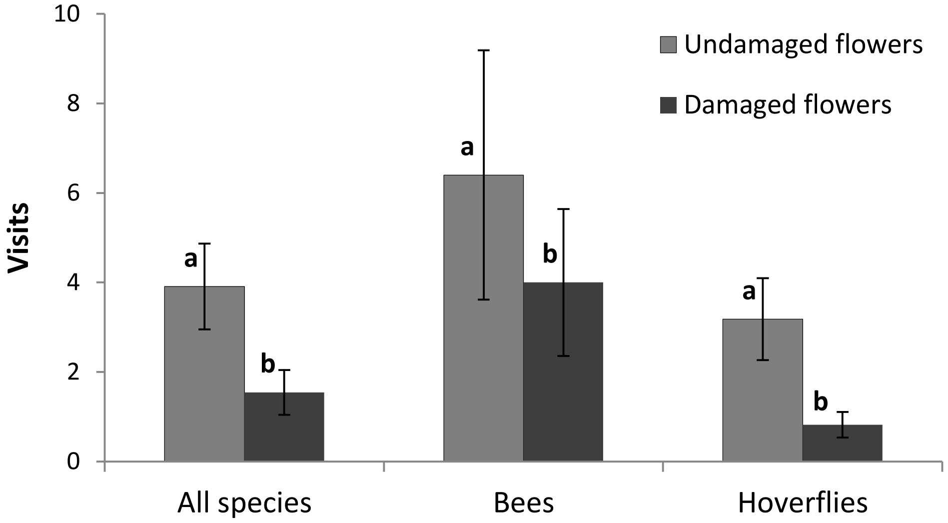

Figure 5. Mean number of visits to each flower type when half of the flowers in bloom were damaged manually and the other half was left untreated. The left bars show grouped data including all pollinators: the middle and right bars show the pollinators divided in two groups; Hoverflies *Syrphidae* and bees, *Apoidea* (wild- and honey bees included). n = 22, n = 17 for hoverflies and n = 5 for bees, respectively. Error bars denote SE.

The difference between individual pollinator groups (figure 5) was not significant (χ^2= 3.7974, df = 1, p = 0.051), but both groups tested separately and all individuals grouped together all showed a significant preference for undamaged flowers (table 5, figure 5).

Table 5. Pollinator preference for manually damaged versus undamaged flowers. The table shows the result for three individual GLM models with quasipoisson distribution. Individual differences for all pollinators are controlled for. Significant p-values are indicated by bold font.

	Estimate	**SE**	**t**	**p**
(Intercept), all	0.125	0.556	0.225	0.824
Damage, all	0.928	0.217	4.286	**<0.001**
(Intercept), Bees	1.242	0.144	8.598	0.001
Damage, Bees	0.470	0.109	4.301	**0.013**
(Intercept), Hover flies	-0.194	0.666	-0.291	0.775
Damage, Hover flies	1.350	0.361	3.739	**0.002**

- **Experiment 2.1. Herbivory effects on pollination and fruit development in wild genotypes of woodland strawberry**

All three herbivory treatments differed significantly from each other in leaf damage (table 6). Plants treated with *G. tenella* had a mean of 7.47 % leaf damage, plants with *G.*

sagiattariae had 3.86 % and the control plants without beetles was damaged to 0.79 % of their total leaf area.

Table 6. GLM with quasipoisson distribution showing the leaf damage between herbivory treatments. The different herbivory treatments are (1) damage by *Galerucella tenella*, (2) damage by *G. sagittariae* and (3) undamaged control plants. Significant p-values are indicated by bold font.

	Estimate	**SE**	**F**	**P**
(Intercept)	1.303	0.201	6.497	< 0.001
G. tenella	0.661	0.270	2.451	**0.017**
Control	-1.539	0.536	-2.874	**0.006**

The pollination success was nearly significantly affected by herbivory treatment type when leaf size was controlled for (table 7, figure 6). Plants treated with *G. tenella* had a lower proportion of pistils pollinated than control plants and plants treated with *G.sagittariae*. There was no considerable difference between plants treated with *G. sagittariae* and control plants. The herbivory treatments did not have an effect on number of deformations, but hand pollinated flowers had a significantly lower amount of deformations (table 8, figure 7). There was no significant difference in fruit weight between the herbivory treatments. Neither did hand pollination or leaf size have any effect on the fruit weight, but the number of open flowers, i.e. the number of flowers produced during the experiment period, had a significant positive effect on the fruit weight (table 9, figure 7).

Table 7. Ancova table showing the effect of treatment on pollination success. Pollination success was defined as the proportion of pistils on each flower that was successfully pollinated, and a mean for each plant was used in an Ancova. The different treatments are (1) damage by *Galerucella.tenella*, (2) damage by *G. sagittariae* and (3) undamaged control plants. Values of aggregated leaf size from each plant are included in the model. Significant p-values are indicated by bold font.

	df	**M S**	**F**	**p**
Leaf size	1	0.653	4.499	**0.038**
Treatment	2	0.424	2.983	0.059
Residuals	56	0.148		

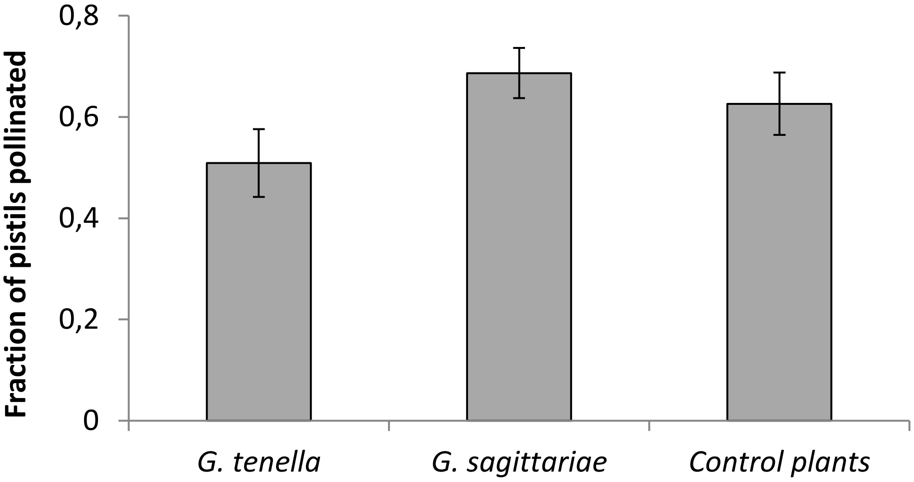

Figure 6. Pollination success for three different herbivory treatments in wild woodland strawberries. Pollination success is estimated as the fraction of pistils per flower that were successfully pollinated. The different herbivory treatments are (1) damage by *Galerucella.tenella*, (2) damage by *G. sagittariae* and (3) undamaged control plants. The bars show original (not arcsin transformed) values (± SE). $n_{\text{G. tenella}} = 15$, $n_{\text{G. Sagittariae}} = 25$, and $n_{\text{control}} = 19$.

Table 8. GLM with poisson distribution for the effect of herbivory treatment and hand pollination on the number of deformations on fruits in wild genotypes of woodland strawberry plants. The different herbivory treatments are (1) damage by *Galerucella tenella*, (2) damage by *G. sagittariae* and (3) undamaged control plants. Treatment 1 and 3 are compared to treatment 2. Significant p-values are indicated by bold font.

	Estimate	**SE**	**Z**	**p**
(Intercept)	-0.503	0.389	-1.295	0.195
Treatment	0.060	0.166	0.360	0.719
Hand pollination	-0.546	0.265	-2.061	**0.039**

Table 9. Linear model for the effect of herbivory treatment and hand pollination on fruit weight in woodland strawberries. The different herbivory treatments are (1) damage by *Galerucella tenella*, (2) damage by *G. sagittariae* and (3) undamaged control plants. Summed leaf size and number of open flowers (the number of flowers produced during the experiment period) are included in the model. Significant p-values are indicated by bold font.

	Estimate	**SE**	**T**	**p**
(Intercept)	0.340	0.046	7.455	< 0.001
Treatment	-0.011	0.016	-0.679	0.499
Hand pollination	0.017	0.025	0.676	0.500
Leaf size	0.001	0.001	0.740	0.461
Open flowers	0.003	0.001	2.017	**0.046**

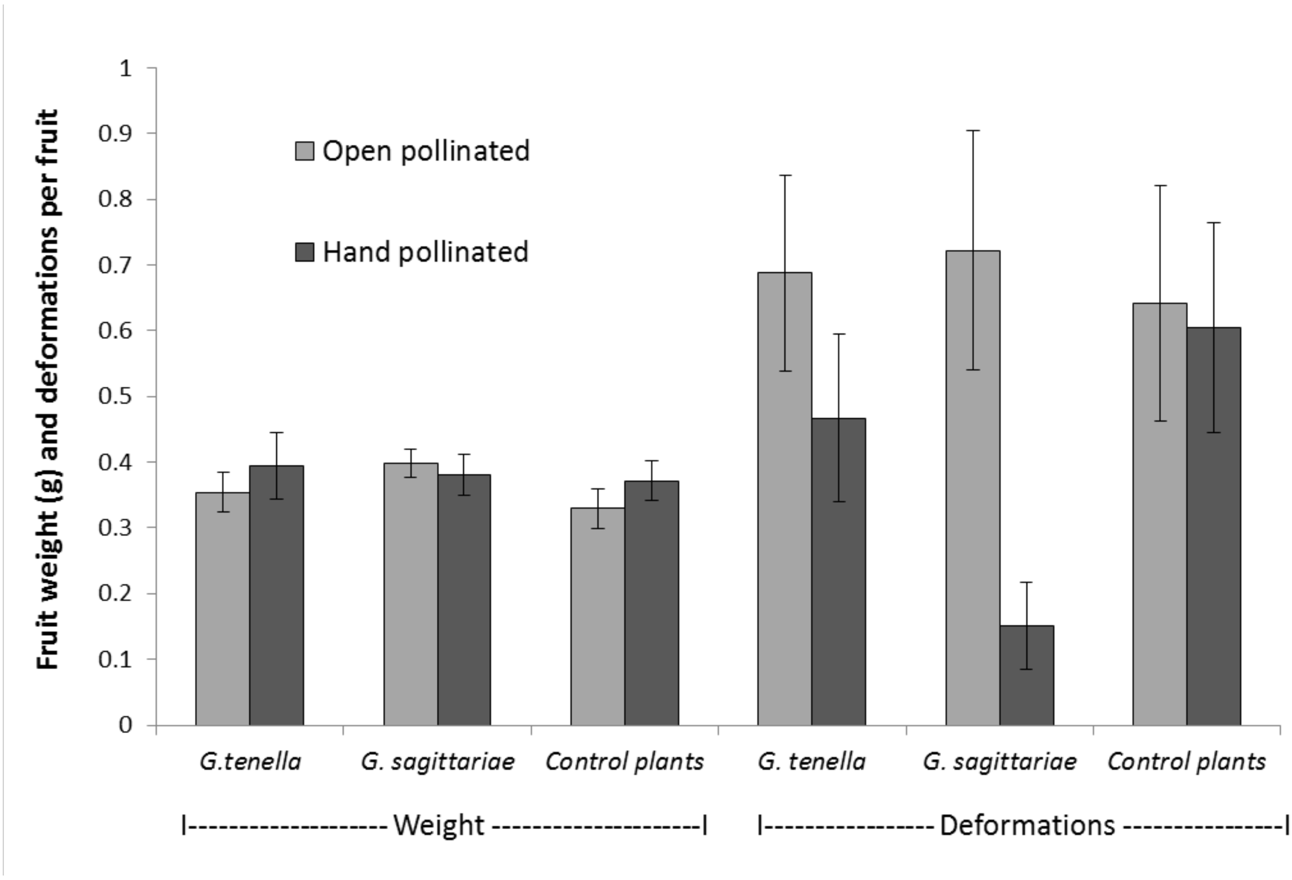

Figure 7. Fruit weight and deformations in three different herbivory treatments in wild genotypes of woodland strawberry. Every plant had both open pollinated and hand pollinated flowers. The different herbivory treatments are (1) damage by *Galerucella tenella*, (2) damage by *G. sagittariae* and (3) undamaged control plants. The bars show the mean fruit weight in grams and mean number of deformations per fruit for each herbivory treatment (± SE). Note that fruit weight and deformations are shown on the same y-axis. $n_{G. tenella} = 15$, $n_{G. Sagittariae} = 25$, and $n_{control} = 19$.

- **Experiment 2.2. Herbivory effects on flower and runner production in wild genotypes of woodland strawberry**

The number of flowers produced during the experiment was not affected by herbivory treatment but there was a significant interaction between herbivory treatment and runners on the number of produced flowers. However, the most important variable for flower production was the summed leaf size (table 10). The number of runners was significantly affected by damage, (which in turn is related to herbivory treatment). Even here, the summed leaf size also turned out to be a significant factor (table 11, figure 8).

Table 10. Ancova table for the effects of herbivory treatment, leaf size and runners on the number of flowers produced during the experiment. The different herbivory treatments are (1) damage by *Galerucella tenella*, (2) damage by *G. sagittariae* and (3) undamaged control plants. Leaf size is the sum of all leaf sizes on one plant added together. Significant p-values are indicated by bold font.

	df	MS	F	p
Treatment	2	2.131	1.926	0.156
Leaf size	1	10.85	9.808	**0.003**
Runners	1	5.630	5.089	**0.028**
Treatment:Leaf size	2	2.783	2.515	0.091
Treatment:Runners	2	4.853	4.387	**0.017**
Residuals	51	1.106		

Table 11. Linear model for the effects of damage and leaf size on the number of runners produced during the experiment. Leaf size is the sum of all leaf sizes on one plant added together. Damage is the total amount of herbivore-damage on each plant measured in percent. Significant p-values are indicated by bold font.

	Estimate	**Std. error**	**T**	**p**
Intercept	1.312	0.240	5.467	< 0.001
Damage	-0.054	0.030	-2.714	**0.009**
Leaf size	0.013	0.005	2.509	**0.015**

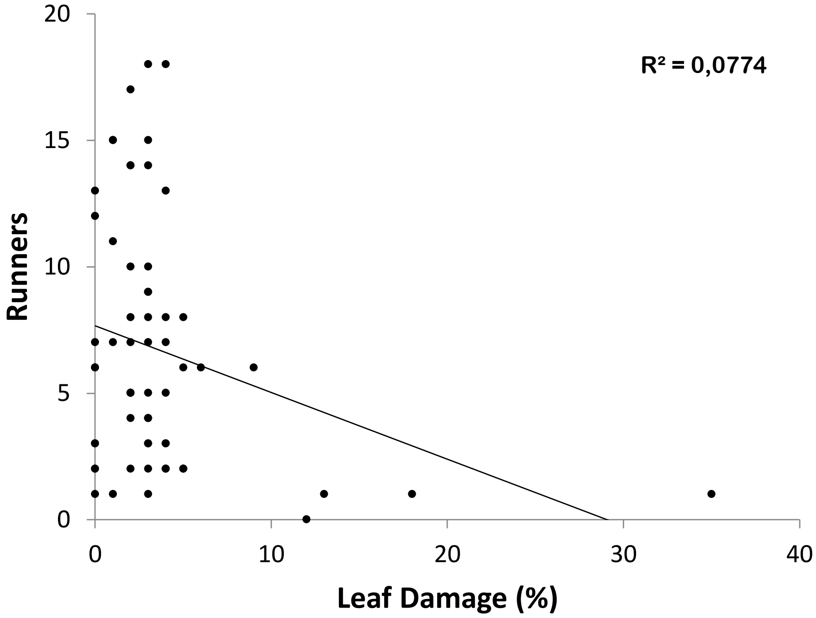

Figure 8. Relationship between the number of runners produced during the experiment period and herbivore-damage on the same plant. The plants have been damaged by either *Galerucella tenella* or *G. sagittariae* or have been left untreated as control plants. The leaf damage is measured as the total percent of leaf damage on each plant. n = 60.

Discussion

Pollinator response to damaged flowers and difference between pollinator groups

There was a clear preference for undamaged flowers compared to damaged ones in both *G. sagittariae* larva damaged flowers and manually damaged flowers and for both pollinator groups (i.e. bees and hover flies) (table 4 and 5, figure 5). These results agrees with studies with other plants where flowers were damaged manually (Kudoh and Whigham 1998) and by florivory (Karban and Strauss 1993). Unfortunately it was a very rainy summer with low abundances of pollinators in the experiments. That obstructed the comparison of flower preference between different groups of pollinators at some points. For example, bumble bees that are common in woodland strawberry cultivations only occurred earlier in the season. At that time it was not warm and sunny enough to have enough of other pollinators to classify them into several groups for comparison.

The fact that pollinators clearly avoid both herbivore- and manually damaged flowers does not lead to a conclusion that pollinator preference is only based on visual impressions. It is still possible that manual damage induces defence mechanisms. One part of the plant can have a higher defence without increasing defence compounds on the whole plant (Lauer and Rossi 2011). In the wild genotypes of woodland strawberry where herbivores were less centred on the flowers, there was still a decrease in pollination success for plants treated with *G. tenella* but not for those treated with *G. sagittariae* (figure 6, table 7). *G. sagittariae* was the species used in both pollination success experiments and pollinator preference experiments in Rügen plants where both tests show negative effects of flower damage. There could of course be differences caused by plant types or the higher variation in wild plants. Another more likely explanation to the different results is the difference in treatment where pollinator visitation in relation to herbivory works on a much smaller scale than the whole plant, as found in the Rügen experiments. Briefly, for this experiment of pollinator preference there may be effects of both visual impressions and induced defences and the herbivore-damage effects ranges only to the individual flower that received the treatment.

Pollination success and fruit development

Pollination success was clearly affected by florivory/herbivory in the Rügen plants and to a nearly significant degree in wild genotypes of woodland strawberry, but the latter was only affected by *G. tenella* and not *G. sagittariae*. Like the study by Cardel and Koptur (2010) where the effects of florivory are examined on fruit and seed production in the Butterfly pea, fruit production does also seem to be affected by florivory through pollination success in woodland strawberries. Rügen plants produced significantly heavier fruits from undamaged flowers but no such differences were found for the different herbivory treatments in wild genotypes. Flowers in Rügen plants that were damaged by herbivores produced fruits with more deformations compared to undamaged control flowers, but there was no significant difference in deformations between damaged and control flowers. Neither were there any significant difference in deformations per fruit between the three herbivory treatments in the wild genotypes of woodland strawberries. However, there was a decrease of deformations in hand pollinated flowers for both plant types which suggests that pollen was limited and other results might be found in an area with a higher abundance of pollinating insects (table 2 and 8, figure 2 and 7).

There is a positive relationship between pollination success and fruit weight and a negative relationship between pollination success and deformations within the Rügen plants. This supports the method used for measuring pollination success in this study. It also supports the hypothesis that fruit weight of woodland strawberries is negatively affected by herbivores through its pollination success. The negative correlation between deformations and weight emphasizes the economic loss caused by deformations in woodland strawberry production.

The negative effects of fruit development may be less significant for the population dynamics and individual reproduction success of wild woodland strawberry than the negative effects experienced by woodland strawberry farmers. Producing runners is also an effective way to reproduce and maintain a local population. However, fruits with severe decreases in size or big deformations may become less attractive to seed dispersers (e.g. birds). Examples of this consequence are not very common, but Christensen and Whitham

(1991) found that Pinyon Pine trees attacked by an insect herbivore received less visits of birds foraging on cone seeds than tree stands without herbivore attacks. In this case it is more likely that the birds ignored groups of trees with lower amounts of cones as a result of the herbivory but similar effects may be found in woodland strawberry populations with *Galerucella* outbreaks. As mentioned in Stöcklin and Winkler (2004), long distance dispersal by seeds are important to maintain a viable metapopulation. Considering the additive effects of losses in reproductive success already achieved by a lower pollination success together with a possible decrease in seed dispersal, there could be important indirect effects of leaf beetle herbivory on metapopulation dynamics.

Herbiory-damage effects on flower and runner production

There was no significant effect of damage on the number of flowers produced during the season but the amount of produced runners was lower with increasing damage. This may be a result of less resources left for reproduction, but the fact that the amount of flowers is still unaffected could be a strategy to reproduce in the way most likely to be successful the next year. A plant that is subject to herbivory could also show stronger adaptations for the next season, and a longer study of the same plants could show adaptations of that kind.

Differences in reproduction strategies between plants that experience different amounts of herbivory may also be more obvious when following generations are examined. Steets and Ashman (2010) showed clear maternal effects on the number of flowers produced in *Impatiens capensis*, where maternal plants with a higher level of herbivory produced offspring with more flowers. *Impatiens capensis* has two types of reproduction strategies: flowers that are built for cross-pollination (chasmogamous) and selfing flowers (cleistogamous). Despite no significance, they found a decrease in the proportion of selfing flowers when the maternal plant had experienced a higher amount of herbivory. Considering these results, there could be more effects of herbivory in the reproductive systems of woodland strawberries in offspring and following generations, e.g. towards more selfing or more flowers.

Male plant fitness costs such as pollen amount was not measured in this study but could be another cost that may affect pollination success in a population or plantation of woodland strawberries. For example, Lehtilä and Strauss (1999) found that several aspects of male fitness in *Raphanus raphanistrum* was more affected by herbivory than female fitness in the same plants.

Conclusions

The results from this study show substantial effects of *Galerucella* herbivory on pollinator behaviour, with important implications for pollination success and fruit quality in the woodland strawberry. To establish a more reliable way to cultivate woodland strawberries without pesticides it could be advantageous to further investigate the plants inherent defence. Studies of other plant species have shown an indirect defence where predators of pest species are attracted to the plant with volatile chemicals (Dicke and Sabelis 1987, Turlings et al. 1990, Halitschke et al. 2008). In a study with garden strawberry (*Fragaria x ananassa*), the attractiveness to plants with and without herbivore-damage were tested on three predatory mites but without similar results (Himanen et al. 2005). The parasitoid *Asecodes lucens* has been shown to reduce *G. tenella* larvae and increase seed sets in

Filipendula ulmaria (Rosaceae) and *A. lucens* was attracted to the plant by its scent (Stenberg et al. 2007). *Asecodes lucens* also parasitizes *G. tenella* in garden strawberry plantations but a significant amount of parasitized larvae are only found in older plantations (Stenberg 2012). A reliable method of attracting this parasitoid could decrease the negative effects of *G. tenella* in woodland strawberry cultivations substantially. If woodland strawberries can attract *A. lucens* during herbivory and if breeding can increase these attractive compounds remains to be investigated. Contemporaneously with experiments of increased defence compounds it would be useful with more detailed studies of how pollinators react to different kinds of defence. For example, there could be stronger effects of induced defences than those present before a herbivore attack and differences in the reaction to different compounds. There could also be different reactions to herbivory defence between species or groups of pollinators that are not found in this study.

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