

# EXPERIMENTAL POLLINATOR DECLINE AFFECTS PLANT REPRODUCTION AND IS MEDIATED BY PLANT MATING SYSTEM

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**Abstract**—There is growing concern that current pollinator decline will affect the reproduction of plant species, potentially driving a decline in plant population densities. We experimentally tested whether a reduction in flower visitation caused a reduction in fertilization rate in several species, and whether any reduction in fecundity of species depends on their degree of reproductive dependence on pollinators and their attractiveness for pollinators. We recorded visitation rate, fertilization rate, seed weight, flower size and density of nineteen insect-pollinated perennial herbs inside thirty 2 x 2 m dome-shaped cages covered with fishnet (experimental plots) and in thirty control plots in a Norwegian hay meadow. We used a bagging experiment to estimate the ability of the study species to produce seeds in the absence of pollinators. The visitation rate for fifteen of nineteen study species was lower inside cages than outside and only three of the fifteen species showed significantly reduced fertilization rates in the experimental plots. The magnitude of reduction in fertilization rate was positively related to the degree of pollinator dependence, but not to their attractiveness for pollinators or to the reduction in visitation rate. Seed weight was not affected by the experiment. The lack of an overall effect of reduced pollinator visitation on fertilization rate suggests that some species may be robust to a pollinator decline that could increase pollen limitation on plant reproduction. Our results suggest that species with greater pollinator dependence are more vulnerable to pollinator loss.

**Keywords:** blossom cover, degree of pollinator dependence, fertilization rate, pollinator decline, seed weight, visitation rate

## INTRODUCTION

The majority of plant species are dependent on animal pollination (Burd 1994; Dauber et al. 2010) and many of them experience pollen limitation on their reproductive success (Knight et al. 2005). Both low quantity and low quality of pollen may result in pollen limitation (Ashman et al. 2004; Wilcock & Neiland 2002). One component of quantitative pollen limitation is pollinator limitation (Aizen & Harder 2007) and this particular component has received considerable attention in relation to the recently documented pollinator decline (Biesmeijer et al. 2006; Goulson et al. 2008; Pauw 2007). Pollinator decline is caused by many factors, such as degradation of natural and semi-natural habitats, climate change, and changes in plant and pollinator distributions (Aguilar et al. 2006; Hegland et al. 2009; Kearns et al. 1998; Knight et al. 2005). These threats may all disrupt plant-pollinator interactions, leading to pollinator and ultimately pollen limitation, with potential large effects on plant population dynamics and plant community composition (Ashman et al. 2004). Even so, there is a lack of experimental studies of the effect of community-wide reductions in pollinator visitation rate and their effects on plant fecundity through pollen limitation (but see Lázaro et al. *submitted*).

In order to maintain seed production plant species that experience pollen limitation due to low visitation rates can either evolve higher attractiveness and/or alternative pollination modes (Ashman et al. 2004; Eckert et al. 2009). In particular, plant species which can use different pollination modes (e.g. selfing and wind pollination) may be reproductively assured against reductions in pollinator availability (Culley et al. 2002; Kennedy & Elle 2008). In addition to life history traits, such as breeding system, ecological factors may affect plant reproductive responses to pollinator decline. For instance, large individual flower displays and dense patches of plants often receive a higher number of visits (Ebeling et al. 2008; Grindeland et al. 2005; Hegland & Totland 2005) due to a higher attraction (Dreisig 1995) and/or foraging economy of pollinators (Goulson 2003).

A reduction in pollinator availability may not only affect the quantity of seeds produced but also their quality (Aizen & Harder 2007), since selfing in self-compatible plant species may result in reduced seed quality due to increased inbreeding (Dudash & Fenster 2000; Grindeland 2008). The seed quality in purely outcrossing plant species may also suffer from reduced pollinator availability if pollinators forage within restricted areas instead of moving long distances to find rewarding flowers (Dukas & Real 1993; Thomson et al. 1982). Such spatially restricted foraging may increase biparental inbreeding in self-incompatible plant species, since closely situated plants are often related (Waser & Price 1994).

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Here we use an experimental approach to study whether a reduction in flower visitation affects reproductive success in nineteen plant species in a mown hay meadow in south Norway. Furthermore, we examine whether the magnitude of reduction in fecundity of these nineteen species is related to the reduction in pollinator visitation rate, to their dependence on pollinators for fertilization and to their attractiveness for pollinators. We simulated a decline in pollinator visitation by using dome-shaped cages covered with fishnet (experimental plots) that effectively reduced flower visitation rates and estimated the reduction in plant fecundity (fertilization rate and seed weight) and visitation rates as the difference between the values obtained in experimental versus control plots. Our specific questions were: 1) does a reduction in visitation rate cause a reduction in plant fecundity?; and 2) is there a relationship between the reduction in visitation rate, the degree of pollinator dependence, the attractiveness of plant species for pollinators and the reduction in plant fecundity?

## MATERIALS AND METHODS

### Study area and study species

The study site is located on a species-rich hay meadow at Ryghsetra (59°44'03''N, 10°02'48''E), in Buskerud county in Norway. The blooming season in this species rich community begins in early May and ends in mid-late August, and approximately 55 insect-visited plant species bloom during this period. We studied the nineteen species with the highest number of flowering individuals among the insect-pollinated perennial herbs occurring at Ryghsetra. However, the experiment reduced pollinator visitation in only fifteen species and only these species are used in analyses. From literature we know that most of the fifteen species, which we

studied in our study site, are probably able to produce seeds to a greater or lesser degree without pollinator visitation, with the exception of *Centaurea jacea*, *Primula veris*, *Trifolium pratense* and most likely also *Lathyrus linifolius* (Table I). From flower visitation observations conducted in control plots in 2006, we found that the flower visitor assemblage consisted of 72.7% bumblebees, 11.0% muscoid flies, 5.5% solitary bees, 4.7% hover flies, 2.4% ants, 1.6% butterflies, 0.9% honeybees, 0.5% beetles and 0.5% bee flies.

### Experimental manipulation of pollinator visitation

The hay meadow was large and relatively homogeneous, and we therefore placed thirty pairs of permanent plots (2 × 2 m) systematically along two parallel rows (separated by ca. 5 m) across the study site and marked the inner square of 1 × 1 m of each plot, at the beginning of the field season in 2006. In order to reduce any effect of the experimental setup (see below) we only conducted flower visitation observations inside this inner square meter. Plot pairs were separated by at least 3 m and plots within a pair were separated by 2 m. We randomly selected one plot of each pair for the experimental reduction of pollinator visitation (experimental plots hereafter), whereas the other plot was left unmanipulated and open for natural pollination (control plots hereafter). To obtain a reduction in pollinator visitation, we placed dome-shaped cages made of two 4 m long PVC-tubes bent diagonally over the experimental plots, and covered the domes with transparent nylon fishnet with a mesh width of 1.05 × 1.05 cm. The size of the cages was 2 × 2 × 1 m (l, w, h). To allow pollinators entering the cages an easy exit, we left the lowest approximately 10 cm without mesh, and had in addition another opening of 0.5 × 0.5 m on the top.

TABLE I. The fifteen study species and information from references about their plant family, compatibility system and the presence of apomixis in these.

Species	Family	Compatibility system	Apomixis	References
<i>Centaurea jacea</i>	Asteraceae	Self-incompatible	No	Hardy et al. (2001), Noyes (2007)
<i>Fragaria vesca</i>	Rosaceae	Self-compatible	No	Schulze et al. (2012), Nosrati et al. (2010)
<i>Galium mollugo</i>	Rubiaceae			
<i>Geranium sylvaticum</i>	Geraniaceae	Self-compatible		Ramula et al. (2007)
<i>Hieracium cymosum</i>	Asteraceae			
<i>Hieracium lactucella</i>	Asteraceae	Self-incompatible	Facultative apomictic	Krahulec et al. (2008), Koltunow et al. (1998)
<i>Hieracium pilosella</i>	Asteraceae	Self-incompatible	Facultative apomictic	Koltunow et al. (1998), Houlston & Chapman (2004)
<i>Knautia arvensis</i>	Dipsacaceae	Self-compatible		Vange (2002)
<i>Lathyrus linifolius</i>	Fabaceae			
<i>Leucanthemum vulgare</i>	Asteraceae	Self-incompatible	Not demonstrated	Andersson (2008), Noyes (2007)
<i>Linum catharticum</i>	Linaceae			
<i>Polygala vulgaris</i>	Polygalaceae	Self-compatible		Norderhaug (1995)
<i>Potentilla thuringiaca</i>	Rosaceae		Facultative pseudogamous apomictic	Dobeš et al. (2013)
<i>Primula veris</i>	Primulaceae	Self-incompatible		Wedderburn & Richards (1990)
<i>Trifolium pratense</i>	Fabaceae	Self-incompatible		Leduc et al. (1990)

In a pilot-study conducted in 2005, we checked potential biotic and abiotic side-effects of the cages, such as changes in herbivory, seed predation, photosynthetically active radiation and wind speed. No significant side-effects of the cages were found (all  $P \gg 0.05$ ; results not shown), except for a marginally significant reduction in wind speed inside the cages (19.4 %;  $P = 0.066$ ), that did not seem to affect the flying insects. The experimental set up and/or the reduced density of simultaneous foragers (Inouye 1978; Lázaro et al. 2011; Valido et al. 2002) might also affect the foraging behaviour of individual pollinators. If so, we would not know whether the results of our study were due to a reduction in pollinator availability exclusively or to related changes caused by the experimental set up itself. In another study (Lázaro et al. *Submitted*) we therefore checked whether the experimental setup altered the behaviour and composition of pollinators of *Centaurea jacea*, *Galium mollugo* and *Leucanthemum vulgare*, three of the most abundant and visited species in this community. We found no difference in the number of flowers contacted per individual visitor or visit duration inside the plots. There was no significant difference either in the composition of pollinators visiting these species in control and experimental plots, except for *Centaurea jacea*, which showed a minor decrease of bumblebees inside the experimental plots (72.2% vs. 82.8% of total pollinators for experimental and control plots, respectively; Lázaro et al. *submitted*). These results indicate that our experiment reduced the number of pollinator visits without affecting the biotic and abiotic environment of plants or the behaviour and composition of their pollinators. Our experimental set up therefore seems appropriate to test the effects of pollinator reduction on plant fecundity.

In four of the nineteen species (*Anthyllis vulneraria*, *Lotus corniculatus*, *Potentilla erecta* and *Vicia cracca*) the visitation rate was higher in experimental than control plots. We therefore excluded these four species from analyses since we focus on effects of reduced visitation rate.

### Visitation rate and plant fecundity

We observed flower visitation at Ryghsetra from 27 May to 19 July, 2006, covering the entire blooming season of the study species. Observations were done between 0800 and 1800 h on days without rain and when winds were calm. Both experimental and control plots of the same pair were observed simultaneously (or immediately after each other) using 20 minute observation periods ( $N = 247$ ). The order of observation of pairs was random, but we never observed the same pair more than once per day. We observed pollinator visitation to each flower or inflorescence (depending on the species; flowers hereafter) of all flowering species occurring within the inner square of the plots. Since pollinator observations were plot based, the observation time to each individual study plant species ranged from 1.7 hours to 15.3 hours (Table 2). A visit was defined to have occurred when there was contact between the visitor's body and the reproductive organs of the flower. We categorized

each visitor into bumblebees (*Bombus* spp.), solitary bees (e.g. Colletidae, Andrenidae, Halictidae; all of them of similar small size), honeybees (*Apis mellifera*), ants (Formicidae), hover flies (Syrphidae), muscoid flies (mainly Muscidae, Anthomyiidae), beetles (Coleoptera), butterflies (Lepidoptera) and beeflies (*Bombylius minor*). After each observation period we counted the number of open flowers of all plant species occurring within both the inner square and the entire plot.

We obtained the average visitation rate of each pollinator groups to all fifteen study plant species in each observation period by dividing the number of pollinator visits to each plant species by the number of open flowers of that plant species in the inner squares of each plot (Table 3). We also obtained the total visitation rate per flower and plot for all study plant species in each observation period, by dividing the total number of pollinator visits to each plant species by the number of open flowers of that plant species in the inner squares of the plots. Previous studies suggest that seed set may be related to the abundance of specific flower-visitor groups, rather than to the total abundance of all pollinators (Franzen & Larsson 2009; Steffan-Dewenter & Tschardtke 1999). However, total visitation by all pollinator groups and visitation by the most abundant pollinator group to each plant species were significantly correlated ( $r = 0.788$ ,  $n = 15$ ,  $P = 0.000$ ). Therefore, we only used the total visitation rate from all pollinators groups (visitation rate, hereafter) in the analyses where we study the relationships between the magnitude reduction in plant fecundity and the reduction in flower visitation.

To estimate fertilization rate, we haphazardly selected and marked one flower in three individuals per species and plot (unless fewer individuals were present) in both control and experimental plots. We marked flowers while they were still blooming to avoid bias due to subconscious selection of fruits. We collected the fruits or infructescences (depending on the species; fruits hereafter) of marked flowers, when fruits were dry and immediately before dispersal. Fruits were stored in paper bags until they were dissected in the laboratory and the number of undeveloped seeds and fully developed seeds was counted. The extent of seed predation was very low and only occurred in three species. In these species (*Centaurea jacea*, *Hieracium pilosella* and *Lathyrus linifolius*) we counted the number of seed as accurately as possible and only weighted undamaged seeds. We weighed the dry weight of all undamaged developed seeds per fruit together to the nearest mg. Fertilization rates were obtained by dividing the number of fully developed and aborted seeds by the total number of ovules. In *Primula veris*, we could not reliably determine the number of ovules, and therefore we calculated the fertilization rates of this species by dividing the number of fertilized seeds by the average number of ovules per flower (50 ovules; Wissman (2006)). When plant species had single-seeded fruits (e.g. *Potentilla*), an average was calculated for each infructescence to obtain a single measure of fertilization rate per individual plant.

TABLE 2 The fifteen study species and the reduction in visitation rate (VR), their degree of pollinator dependence (DPD) and log transformed blossom cover (logBC). For control (C) and experimental plots (E): the number of hours each species was observed (Obs. hour), and the number of plots (P) and number of individuals (I) that were used for the analyses of fertilization rate (FR) and seed weight (SW).

Species	DPD	logBC	VR	Plot	Obs. hour	FR	SW
						P (I)	P (I)
<i>Centaurea jacea</i>	0.05	3.82	0.38	C	9.7	20 (72)	20 (71)
				E	11	18 (57)	18 (57)
<i>Fragaria vesca</i>	0.22	2.04	0.64	C	4.7	15 (45)	15 (45)
				E	5	13 (40)	13 (40)
<i>Galium mollugo</i>	0.34	1.85	0.15	C	16	30 (111)	30 (108)
				E	15.3	30 (114)	30 (107)
<i>Geranium sylvaticum</i>	0.68	2.49	0.7	C	3	6 (13)	4 (4)
				E	1.7	2 (4)	2 (2)
<i>Hieracium cymosum</i>	0	2.40	0.82	C	4	16 (36)	15 (28)
				E	3.7	16 (33)	14 (26)
<i>Hieracium lactucella</i>	0.22	2.30	0.52	C	5	20 (45)	19 (36)
				E	4.7	19 (35)	13 (19)
<i>Hieracium pilosella</i>	0	2.71	0.56	C	7	21 (47)	19 (36)
				E	7.3	21 (53)	21 (44)
<i>Knautia arvensis</i>	0.9	2.40	0.84	C	2.3	6 (13)	6 (13)
				E	3.7	12 (23)	12 (21)
<i>Lathyrus linifolius</i>	1	2.04	1	C	1.7	4 (7)	2 (2)
				E	1.7	6 (11)	2 (2)
<i>Leucanthemum vulgare</i>	0.61	3.33	0.61	C	9.7	28 (75)	28 (74)
				E	10	27 (63)	27 (63)
<i>Linum catharticum</i>	0.16	2.07	0.62	C	8	26 (69)	26 (69)
				E	9.3	27 (80)	27 (80)
<i>Polygala vulgaris</i>	0.57	2.54	1	C	10.3	24 (48)	23 (47)
				E	10.7	25 (53)	25 (53)
<i>Potentilla thuringiaca</i>	0.22	2.76	0.62	C	3.7	15 (35)	15 (35)
				E	4.3	16 (38)	16 (38)
<i>Primula veris</i>	1	3.70	0.64	C	5.7	8 (10)	5 (6)
				E	6.3	11 (11)	4 (4)
<i>Trifolium pratense</i>	1	3.42	0.12	C	3.7	9 (21)	8 (18)
				E	5.7	11 (26)	10 (18)

#### *Plant species characteristics: degree of pollinator dependence and blossom cover*

To test the ability of the study species to produce seeds in the absence of pollinators we conducted a bagging experiment where we used fifteen pairs of individuals outside the plots at Ryghsetra. Individuals in a pair occurred close together in space and were as similar in size as possible. One individual of each pair was haphazardly selected for the

bagging treatment, i.e. all or part of its flowers were bagged with mosquito net before anthesis, whereas the other was left unmanipulated and open for natural pollination. For plant species with many flowers and branches (e.g. *Potentilla* or *Trifolium*), we used pairs of branches instead of pairs of individuals to perform this experiment. We collected their seeds when ripe and calculated fertilization rates per treatment as explained above. We estimated the degree of pollinator dependence for each plant species as  $DPD = 1 -$

B/C, where B is the average fertilization rate of all fifteen bagged individuals (or branches) and C is the average fertilization rate of all fifteen unmanipulated individuals (or branches). A value of 0 indicate no differences in fertilization rate between bagged and unmanipulated flowers, and thus no dependency of pollinator visitation for fertilization, whereas a value of 1 indicates a complete dependency on pollinator visitation for fertilization. This experiment was performed for all the study species, except *Primula veris*. However, we set the degree of pollinator dependence of this species to 1, since this species is an obligate outbreeder (Wedderburn & Richards 1990). Table 2 shows DPD for each study species.

Flower density on its own might not be an appropriate measure of differences in pollinator attractiveness among plant species, since the attractiveness of a plant species also could be influenced by the size of its reproductive units (Bell 1985; Dudash et al. 2011; Eckhart 1991; Galen & Newport 1987). We therefore followed the procedure by Hegland and Boeke (2006) and combined flower size and flower density for each species into one standardized measure to obtain a variable that more accurately describes the attractiveness of plant species for pollinators (blossom cover, hereafter). Thus, we calculated blossom cover as the product of the average number of flowers per m<sup>2</sup> and the average area of one flower (see values for each species in Table 2). We used data from the entire 2 x 2 m plots to calculate the density of flowers. To calculate the area occupied by a flower, we measured the size of the flowers in the field on thirty hazarphardly selected individuals per species with a digital calliper. For flowers with circular outline (e.g. *Leucanthemum vulgare*) we used the formula of  $\pi r^2$ , where  $r$  = radius. For flowers with a depth dimension (e.g. *Lathyrus linifolius*) we used the formula  $2\pi r d + \pi r^2$ , where  $r$  = radius and  $d$  = depth (Hegland & Totland 2005).

### Data analyses

We used Generalized Linear Models (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp) to test whether the experiment significantly reduced the overall visitation rate as well as the visitation rate for each pollinator group to the study species. We performed separate analyses for overall visitation rate to each plant species as well as separate analyses for each pollinator group and study plant species. Due to the nature of the data, we used gamma distributions with log link functions for all the analysis except for visitation rate of bumblebees in *Centaurea jacea* and *Knautia arvensis* where we used normal distributions with link identity. We used individual observation periods as sample units and the treatment (experimental vs. control) as a fixed factor. Due to the very low visitation rate for some species, we only analysed the difference in visitation rate between control and experimental plots in species with more than 10 visits in total from a specific pollinator group to a study species.

To test whether the experiment significantly reduced fecundity (fertilization rate and seed weight) in the study species we used Mixed Effects Models (R 2.10.1; R Development Core Team 2009). We used Gaussian distributions since data fulfilled the assumptions of normality. We performed separate analyses for each response

variable and study species. In these analyses we used individual plants as sample units, plot as a random factor and the treatment (experimental vs. control) as a fixed factor.

To study the relationships between the magnitude reduction in plant fecundity, the reduction in flower visitation, blossom cover, and degree of pollinator dependence we used Multiple Linear Regression (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). We used Gaussian distributions since data fulfilled the assumptions of normality and log-transformed the 'blossom cover' predictor variable for a better fit of the models. In order to obtain a single measurement per treatment and species and avoid potential problems of pseudo replication, we averaged the data of visitation rate (VR), fertilization rate (FR) and seed weight (SW) first per plot and then by species. For all three variables we calculated the magnitude of reduction in the experimental plots as  $1 - E/C$ , where E is the average VR, FR or SW for the experimental plots and C is the average VR, FR or SW for the control plots. Negative values indicate an increase of VR, FR or SW in the experimental plots compared to the control plots, whereas positive values indicate a reduction in these variables in the experimental plots compared to the control plots. We conducted separate models for each fecundity variable (reduction in fertilization rate and seed weight). The fifteen plant species were the sample units in both models. We ran the full models containing the three continuous variables: 'visitation rate', 'log blossom cover', 'degree of pollinator dependence'. We did not include any interactions in the analysis due to the low number of samples (fifteen samples) and therefore few degrees of freedom.

## RESULTS

### Experimental reduction in visitation rates and plant fecundity

In the fifteen study species the overall visitation rate was on average 61% lower inside than outside the experimental plots (ranging from 12% to 100%), and 80 % of the species showed a more than 50% lower visitation rate inside cages (Table 2). The overall visitation rate was significantly lower in the experimental plot compared to the control plots (Table 3) for *Centaurea jacea* ( $\chi^2_1 = 13.7$ ,  $P = 0.000$ ), *Hieracium cymosum* ( $\chi^2_1 = 4.0$ ,  $P = 0.045$ ), *Knautia arvensis* ( $\chi^2_1 = 15.9$ ,  $P = 0.000$ ) and for *Leucanthemum vulgare* ( $\chi^2_1 = 14.8$ ,  $P = 0.000$ ). From the 20 cases where the specific pollinator group visited a specific study species more than 10 times, the difference between control and experimental plots were significant in 10 cases (Table 3). The visitation rate of bumblebees ( $\chi^2_1 = 13.2$ ,  $P = 0.000$ ), butterflies ( $\chi^2_1 = 10.1$ ,  $P = 0.002$ ) and hover flies ( $\chi^2_1 = 6.2$ ,  $P = 0.012$ ) was significantly lower in the experimental plot for *Centaurea jacea*. The visitation rate of flies ( $\chi^2_1 = 3.9$ ,  $P = 0.046$ ) was significantly lower in the experimental plot for *Hieracium cymosum*. The visitation rate of flies ( $\chi^2_1 = 5.5$ ,  $P = 0.019$ ) was significantly lower in the experimental plot for *Knautia arvensis*, and finally the visitation rate of flies ( $\chi^2_1 = 7.3$ ,  $P = 0.007$ ), butterflies ( $\chi^2_1 = 8.5$ ,  $P = 0.004$ ) and hover flies ( $\chi^2_1 = 14.2$ ,  $P = 0.000$ )



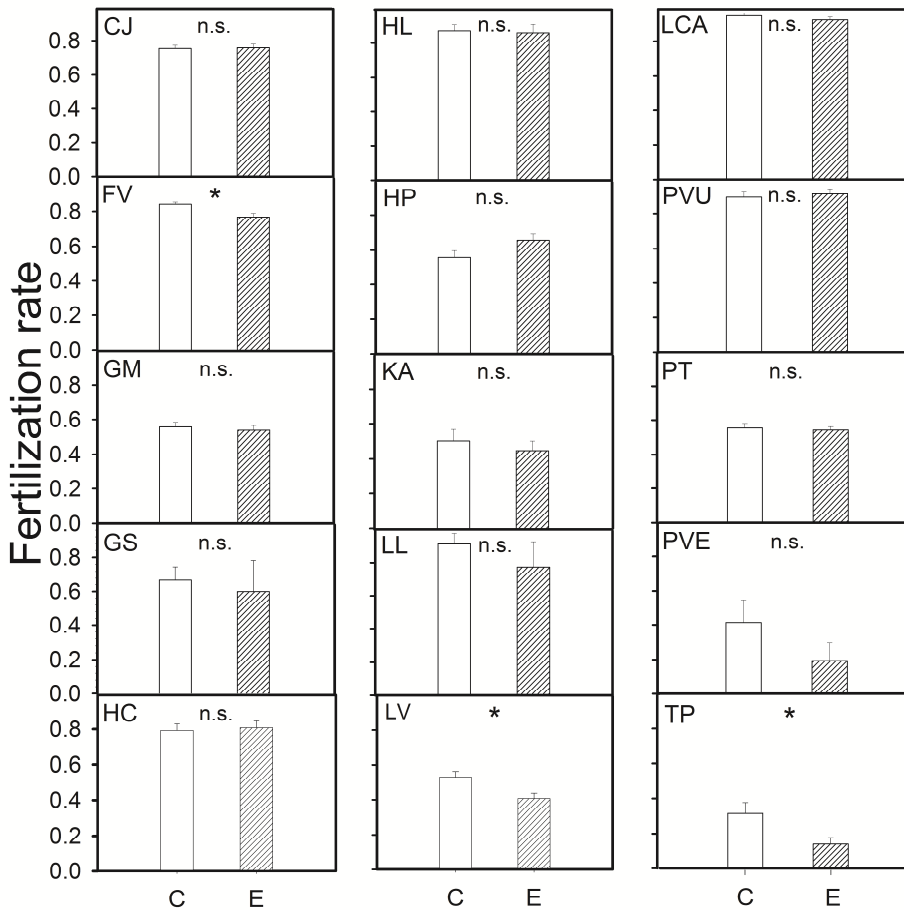


FIG. 1 Mean fertilization rate  $\pm$  SE in experimental (E) and control (C) plots for all study species. *Centaurea jacea* (CJ), *Fragaria vesca* (FV), *Galium mollugo* (GM), *Geranium sylvaticum* (GS), *Hieracium cymosum* (HC), *Hieracium lactucella* (HL), *Hieracium pilosella* (HP), *Knautia arvensis* (KA), *Lathyrus linifolius* (LL), *Leucanthemum vulgare* (LV), *Linum catharticum* (LCA), *Polygala vulgaris* (PVU), *Potentilla thuringiaca* (PT), *Primula veris* (PVE) and *Trifolium pratense* (TP). 'n.s.' indicates non-significant differences between treatments ( $P > 0.1$ ), whereas '\*' indicates significant statistical differences between treatments ( $P < 0.05$ ).

was significantly lower in the experimental plot for *Leucanthemum vulgare*.

Despite the relatively large reductions in visitation rates, the effects on plant fecundity were modest and most of the species showed no statistically significant differences in fecundity between experimental and control plots (Fig. 1). Nevertheless, the experiment significantly affected the fertilization rate of *Fragaria vesca* ( $F_{1,26} = 7.46$ ,  $P = 0.01$ ), *Leucanthemum vulgare* ( $F_{1,53} = 6.49$ ,  $P = 0.01$ ) and *Trifolium pratense* ( $F_{1,18} = 4.91$ ,  $P = 0.04$ ), which all had significantly lower fertilization rate in the experimental plots (Fig. 1). Other species, also showed lower fertilization rate in the experimental plots than in control plots, but the differences between treatments were not statistically significant (Fig. 1). For some of them, such as *Geranium sylvaticum*, *Knautia arvensis*, *Lathyrus linifolius* and *Primula veris* (from  $F_{1,8} = 1.57$ ,  $P > 0.25$  to  $F_{1,36} = 0.07$ ,  $P > 0.79$ ), the lack of significance is most likely due to low sample sizes since the differences between treatments seems large and the 95% confidence interval are wide (Fig. 1; Table 2; Figure S1 in Electronic Supplemental Material). The experimental reduction in visitation rate did not cause a significant reduction in the seed weight of any of the study species (from  $F_{1,37} = 3.38$ ,  $P = 0.07$  to  $F_{1,39} = 0.01$ ,  $P = 0.93$ ).

*Relationships between reduction in plant fecundity, reduction in visitation rates, degree of pollinator dependence and blossom cover*

The degree of pollinator dependence was significantly and positively related to the magnitude of reduction in fertilization rate ( $F_{1,11} = 21.1$ ,  $P = 0.001$ ; Fig 2A). The magnitude of reduction in fertilization rate was neither related to blossom cover ( $F_{1,11} = 1.99$ ,  $P = 0.186$ ; Fig 2B) nor to the reduction in visitation rate ( $F_{1,11} = 2.52$ ,  $P = 0.141$ ; Fig 2C). The reduction in seed weight was not significantly related to any of the predictor variables ( $P > 0.61$  in all cases).

## DISCUSSION

Pollinator decline and its potential effects on ecosystem services have recently received considerable attention (for review see Potts et al. 2010). Although several studies on single plant species have used correlative approaches to study how differences in pollinator visitation lead to variation in pollen limitation (Gomez et al. 2010; Moeller et al. 2012) and seed set (Jennersten 1988; Pauw 2007), very few studies have experimentally manipulated pollinator availability directly (Fontaine et al. 2008; Fontaine et al. 2006). We partially reduced pollinator availability to an entire plant community to more realistically simulate potential effects of pollinator decline on plant fecundity. Our results show that even large experimental reductions in pollinator visitation rates do not necessarily reduce plant fecundity. We found that the effects of a reduction in pollinator visitation on plant fecundity varied among species and these differences appear to be driven by differences in their degree of pollinator dependence.

As expected, the effects of reduced pollinator visitation on fecundity increased with the plants' dependence on

*Potentilla thuringiaca* (PT), *Primula veris* (PVE) and *Trifolium pratense* (TP).

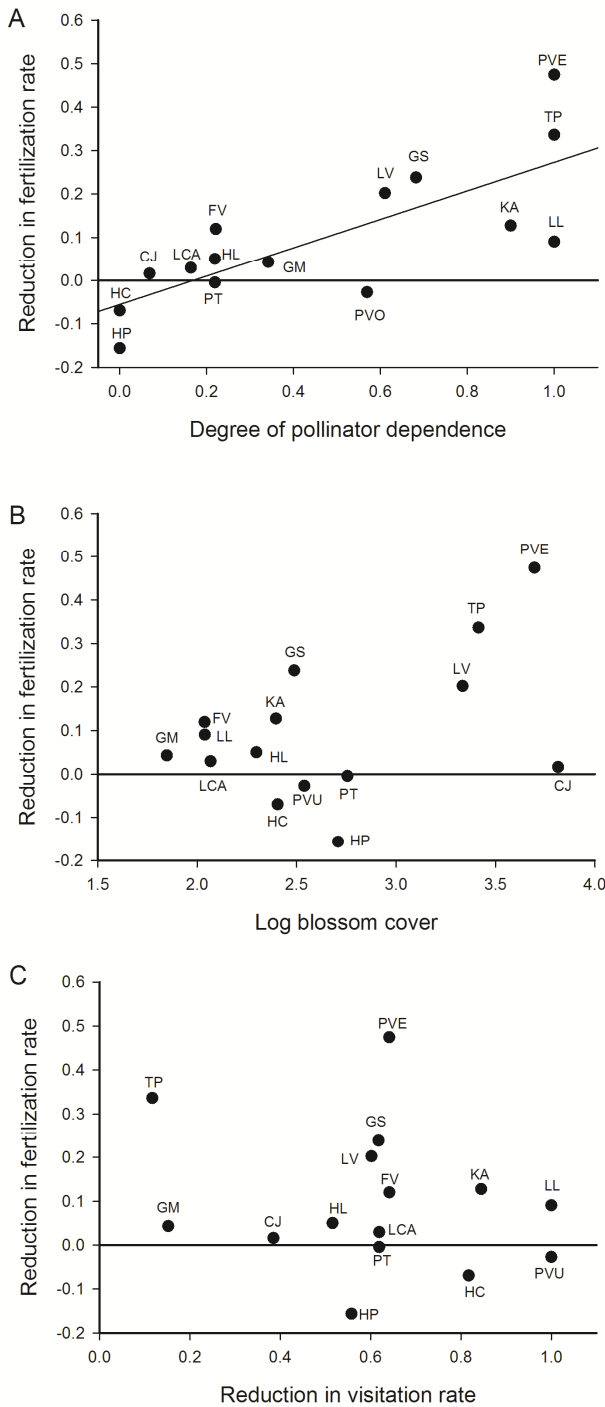


FIG. 2 Relationship between the reduction in fertilization rate and: A) the degree of pollinator dependence, B) blossom cover and C) reduction in visitation rate. *Centaurea jacea* (CJ), *Fragaria vesca* (FV), *Galium mollugo* (GM), *Geranium sylvaticum* (GS), *Hieracium cymosum* (HC), *Hieracium lactucella* (HL), *Hieracium pilosella* (HP), *Knautia arvensis* (KA), *Lathyrus linifolius* (LL), *Leucanthemum vulgare* (LV), *Linum catharticum* (LCA), *Polygala vulgaris* (PVU),

pollinators. This result supports the hypothesis that the ability to use different pollination modes, such as the capability of both selfing (Kalisz & Vogler 2003) and/or wind pollination (Totland & Sottocornola 2001), can serve as reproductive assurance when pollinator availability is low (Culley et al. 2002). In our study species, selfing is the most likely alternative to insect pollination due to their showy flowers and morphological features (e.g. closed flowers) which makes wind pollination improbable. Previous studies have also found that obligate outcrossing animal-pollinated species are more prone to pollen limitation (Knight et al. 2005), more affected by disturbances (e.g. habitat fragmentation) which reduce pollinator availability (Aguilar et al. 2006) and have recently declined more than plant species capable of selfing (Biesmeijer et al. 2006). Nevertheless, the recent review by Potts et al. (2010) highlights the need for more mechanistic evidence for the consequences of pollinator loss. We believe that our experiment and results provide such mechanistic evidence and support the hypothesis that plant species with a high degree of pollinator dependence are more vulnerable to declines in pollination services than predominately selfing species (Potts et al. 2010). However, if a reduction in visitation rate leads to an increase of the self-fertilization rate, then plant species with low pollinator dependence may also suffer from reduced pollinator availability since inbreeding depression can occur in later stages of recruitment (Husband & Schemske 1996). Inbred seeds are often smaller than outcrossed seeds (Lienert & Fischer 2004; Olesen et al. 1998) and larger seeds often have better germination, greater survival, and give rise to larger seedlings than smaller seeds (Baker et al. 1994). We, therefore, used seed weight as an estimate of seed quality, but did not find any effect of the reduction in visitation rate on seed weight. However, an effect on germination and survival (Husband & Schemske 1996) or decreased genetic diversity over time (Rusterholz & Baur 2010) cannot be discarded for species with low dependence of pollinators. For instance, a reduction in visitation rate may not affect the number and weight of seeds in aposporous apomicts species, such as *Hieracium* spp.. However, an increased number of asexually produced seeds in facultative apomictic species may reduce the amount of genetic variation within populations (Houliston & Chapman 2004). Future studies on population dynamics, outcrossing rate and genetic variability will hopefully shed light on the effect of pollinator decline on self-compatible and facultative apomictic species.

Plants in small populations and flower patches of low density often have lower reproductive success than plants in dense and large populations (Dauber et al. 2010; Fischer & Matthies 1998; Hensen & Wesche 2006; Jennersten 1988; Jennersten & Nilsson 1993; Kunin 1997; Oostermeijer et al. 1998). A recent inter-specific study has shown that seed set of species also increase with their conspecific flower density (Lázaro et al. 2013). This is because plants at low density may be less attractive to pollinators (Dauber et al. 2010; Hegland & Totland 2005; Kunin 1997) and/or because



there is a decreased likelihood of receiving outcrossed and compatible pollen when the abundance of conspecifics is low (Jennersten & Nilsson 1993; Karron et al. 1995; Kunin 1997). Therefore, we expected species with lower blossom cover to be more affected by a reduction in pollinator availability, because when pollinator availability is low competition theory predicts low competition among pollinators for flowers (Alley 1982), and pollinators may choose the most abundant species in order to forage economically (Stephen & Krebs 1986). However, we did not find a relationship between the reduction in fertilization rate and blossom cover. Therefore it seems that attractiveness of plant species to pollinators does not change even if pollinator availability is reduced and thus pollinators compete less for food resources. We also expected the reduction in plant fecundity to increase with the reduction in flower visitation rate, but we found no such relationship between these two variables. At the within-species level, some studies have shown a strong positive relationship between seed production and visitation rate (Jennersten 1988; Pauw 2007), whereas others both at the within-species level (Dauber et al. 2010) and at the inter-specific level (Lázaro et al. 2013) have failed to find this intuitive relationship. However, the relationships present at the within species level might not occur at the among species level, because plant species may require a different visitation intensity to obtain a similar proportion of fertilized seeds due to, for example, large differences in ovule number among species.

The lack of an effect of reduced pollinator visitation on fertilization rate at the community level suggests that some species may be robust to a pollinator decline that could increase pollen limitation on plant reproduction. However, our approach has important limitations. First, the presence of resource limitation in this hay meadow could cancel out any difference in fertilization rate created by differences in visitation rate. However, although this possibility cannot be completely discarded, we used fertilization rate instead of seed set as a response variable in order to minimize an eventual effect of resource limitation. Experimental supplemental pollination, coupled with resource addition, would be required to reveal if pollen limitation occur in our study species. Second, sample size for some study plant species may be not high enough to detect significant effects in fertilization rate. At least four of the study species had very reduced fertilization rate in the experimental plots (Fig.1), but the 95% confidence intervals showed that the sample size was too small to detect a meaningful statistically significant difference in fecundity (Figure SI in Electronic Supplemental Material). Sampling adequately in studies at the community level in which several variables have to be recorded at time is always challenging, because plant species are not distributed homogeneously and show different abundances. Increasing sample size of less abundant species would help to improve future studies in the line of ours. Third, the relative low number of plant species in the study prevented us from including interactions in the model. However, one could expect a significant interaction between the reduction in visitation rate and the degree of pollinator dependence on fertilization rates, since low visitation rates might reduce more fertilization rates in plant species that are

pollinator dependent more than in autogamous species. Studies with a larger number of study species would be needed to confirm whether this interaction exists. Lastly, for four of the nineteen study species the experiment increased the visitation rate in the experimental plots compared to the control plot. The sample size of three of them (*Anthyllis vulneraria*, *Lotus corniculatus* and *Vicia cracca*) was rather low and this might explain the increase in visitation rate. However, *Potentilla erecta* was the study species which was observed for the longest time (16.7 hours in control and 17.3 hours in experimental plots) and showed a higher number of visits in the experimental plots compared to the control plots. It seems that the main reason for the increase in visitation rate in the experimental plots is a strong relationship between the number of visits and the number of *Potentilla erecta* flowers in the plots ( $\chi^2 = 246.28$ ,  $P < 0.0001$ ). Unfortunately, the number of *Potentilla erecta* flowers was significantly higher in the experimental plots compared to control plots ( $\chi^2 = 30.42$ ,  $P < 0.0001$ ) and this is a likely cause of the increased amount of visits in the experimental plots compared to the control plots.

### Conclusion

A large decline in pollinator availability, such as predicted under the global pollination crisis (Allen-Wardell et al. 1998; Biesmeijer et al. 2006; Kearns et al. 1998), may not necessarily cause a uniform large reduction in plant fecundity. Our experimental reduction of pollinator visitation did not imply a significant reduction in fecundity which indicates that some plant species may be quite robust to a pollinator decline. Also, plant species varied in their response to a pollinator decline, where the species most dependent on pollinators for reproduction were most strongly affected. However, the accumulated effects of annually small reductions in fecundity on population dynamics and persistence, and whether such differences in response to pollinator decline may translate into community-wide changes in species composition, are largely unknown. Experimental studies like ours are crucial to understand how pollinator declines may influence plant fecundity, population dynamics and ultimately the structure of plant communities.

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