The University of South Bohemia in České Budějovice
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Effect of predator exclusion on the fitness of *Cirsium arvense* via its interaction with pollinators and herbivores

Master’s thesis

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Annotation

The cascading effect of arthropod predators on the reproductive fitness of female plants of *Cirsium arvense* and their effect on pollinator and florivore behaviour was investigated using a manipulation experiment. The experiment was conducted on plants in their natural habitat in and around České Budějovice, Czech Republic. The experiment was designed in a causal framework using a Generative Simulation Model.

Declaration

I declare that I am the author of this qualification thesis and that in writing it, I have used the sources and literature displayed in the list of used sources only.

Yogita Karpate **Place:** České Budějovice **Date:** 10.04.2024

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CONTENTS

Introduction

1. INTRODUCTION

Plant fitness is affected by various abiotic factors and biotic interactions, both within and outside the trophic level. A plant's immobility makes the interactions with other trophic levels very important for its reproduction, specifically for pollination and seed dispersal. The reproductive fitness of sexually reproducing plants is driven by the strength of these mutualistic interactions (Vázquez et al., 2015). The effect of herbivory on plant fitness depends on the feeding guilds of herbivores and the strength of the interaction (Maron, 1998; Rusman et al., 2018). The net effect of all the interactions on plant reproductive fitness can be quantified in terms of plant fecundity, often represented by the seed set or the fruit set.

Pollinators and herbivores can impact plant fitness directly as well as indirectly. Direct effects could emerge from pollen limitation due to changes in pollinator behaviour or seed predation. The pollen limitation can also happen due to phenological mismatch between the pollinators, florivores, and plants, resulting from unpredictable weather changes (Mahoro, 2003). Herbivory can impact plant-pollinator interaction, thus indirectly impacting plant fitness due to limited pollination (McCall & Irwin, 2006; Mothershead & Marquis, 2000; Russell-Mercier & Sargent, 2015; Kessler et al., 2011). Predators, parasites, and parasitoids can also affect plant fitness indirectly via their interactions with pollinators or herbivores (Gómez & Zamora, 1994; Romero & Koricheva, 2011). Plant fitness is also affected by the changes in resource allocation resulting from competitive pressures from plants in the surroundings, coupled with the pressures exerted by herbivores (Vries et al., 2017). Besides biotic interactions, abiotic factors such as land-use patterns also impact plant fitness. Some studies consider the effect of historic land-use patterns on plant-insect interactions to be equally important as contemporary land-use practice (Hahn & Orrock 2015; Petanidou et al., 2013; Araújo et al., 2015; Barnes et al., 2017). Considering the complexity of multitrophic interactions, it is important to study integratively the direct effect of plant-pollinator, plant-herbivore, and indirect effect of predators on plant functions. The following sections explain these direct and indirect effects on plant fitness in more detail.

1.1 Effect of plant-pollinator interaction on plant fitness

Animal-mediated pollination is the most common reproduction strategy in angiosperms (Ollerton et al. 2011). According to the Cretaceous Terrestrial revolution theory (Lloyd et al., 2008), the diversity of angiosperms expanded rapidly during the Cretaceous, and insects coevolved with the angiosperms (Benton et al., 2022). This shows that animal-mediated pollination is not a recent adaptation but a very old plant strategy for maintaining genetic diversity. Angiosperms invest significant resources to attract pollinators, sometimes at the cost of lesser resource allocation to defence and growth (Briggs & Schultz, 1990). Studies have shown that the declining pollinator diversity and declining number of specialist pollinators could be correlated with a drop in the relative abundance of outcrossing plant species (Biesmeijer et al., 2006).

Plant traits that improve a plant's attractiveness and pollinator efficiency are selected during evolution (Sletvold et al, 2010; Glaettli & Barrett, 2008). The pollinator-meditated trait selection is even stronger in specialised plant-pollinator interactions (Hansen et al., 2012). Visual cues such as floral display, inflorescence size, clear nectar guides, and olfactory cues indicate resource quality and damage to these cues can directly affect plant fitness (Strauss et al., 1999). However, pollinator interactions are not always mutualistic. The interaction can fall anywhere along the spectrum of mutualism to commensalism depending on temporal, spatial, biotic, abiotic, and even genetic factors (Hoeksema & Bruna, 2015). Plants with larger floral displays are susceptible to reduced plant fitness via geitonogamy, a form of self-pollination in which the pollen is transferred to another flower in the same plant (de Jong et al., 1993; Johnson & Nilsson, 1999; Chittka & Thompson, 2001). Additionally, nectar robbers also visit flowers and can over-exploit plant resources and dissuade pollinators from visiting (Richman et al, 2017). Similarly, pollen exploiters could induce pollen limitation due to a reduction in pollen deposition in female flowers (Koski et al, 2018). Some studies claim that the presence of invasive plants and invasive pollinators can disrupt the pollination of native plants due replacement of native pollinators and an increase in visits to invasive plants (Traveset & Richardson, 2014; Albrecht et al., 2016; Aizen et al, 2014).

1.2 Effect of plant-herbivore interactions on plant fitness

Herbivores from different guilds affect plant fitness and biomass by consuming different plant tissues (Novotny et al. 2010). Plants deploy resources in defence against these herbivores, often at the cost of reproductive fitness (Strauss et al., 1999; Jacobsen & Raguso, 2018). Endophagous herbivores such as pre-dispersal seed predators (florivores) can reduce plant fitness directly whereas guilds such as folivores, both endophytic (miners, gallers etc) and exophytic (caterpillars, sawflies, etc.), nectar robbers, and florivores other than seed predators affect plant fitness indirectly. The extent of impact from different guilds could differ depending on different biotic and abiotic factors. However, the net impact on plant fitness would also depend on the extent to which herbivory impacts plant-pollinator interactions.

Endophagous insects feed on living plant tissue, but some researchers also include the insects that feed on non-living plant tissues in this category (Tooker & Giron, 2020). Endophagy is assumed to be an ancestral trait in some orders of insects such as Coleoptera and Lepidoptera, and a derived trait in Diptera (Tooker & Giron, 2020). Seed predation is a kind of endophagy mostly performed by larvae or nymphs and rarely found in adult insects (Tooker & Giron, 2020). Endophagous florivores such as seed predators can reduce plant fitness directly by predating on viable seeds and indirectly by changing pollinator behaviour (Hambäck, 2001) and inducing the release of volatile compounds that dissuade pollinators from visiting (Lucas-Barbosa et al, 2011). The survival of seed-predator larvae is affected by the shifting phenology of plants. The asynchronous phenology of plants and florivores can impact the florivory damage (Hamann et al., 2021) and its effect can be amplified by anthropogenic activities (Singer & Parmesan, 2010). Plants deploy defensive strategies to reduce damage from herbivory yet attract pollinators. Some plants do this by synchronising the emission of plant volatiles only with pollinator activity (Theis et al., 2007). Other plants emit volatile compounds to attract predators and parasitoids after detecting the damage from herbivory (Paré & Tumlinson, 1996; Kessler & Baldwin, 2001; Lucas-Barbosa et al, 2011).

1.3 Effect of pollinator-herbivore interaction on plant fitness

Plant fitness can be indirectly affected by the nature of the interaction between pollinators and herbivores. Herbivory damage can dissuade pollinator visits or could trigger volatile emissions that act as an olfactory cue for pollinators to differentiate between a damaged and a more rewarding plant (Kessler et al., 2011). Studies have proved that pollinators avoid visiting flowers damaged by florivores due to reduced attractiveness (Ferreira et al, 2013). Bud herbivory can also indirectly reduce pollinator visits by reducing the number of open flowers (Adler et al., 2001). To avoid herbivores, yet attract pollinators, some plants adjust their phenology to match that of pollinators (Theis et al., 2007). However, generalist herbivores might still adapt to the changes in the plant phenology.

Studies differ in their inferences on the indirect impact of pollinator-herbivore interactions on plant fitness. For instance, some studies have documented herbivory-induced indirect effects on plant fitness via a shift in pollinator behaviour (McCall & Irwin, 2006; Mothershead & Marquis, 2000; Russell-Mercier & Sargent, 2015; Kessler et al., 2011). One recent study claimed that florivores can detect plant volatile compounds better than pollinators (Sasidharan et al., 2023), implying a greater impact of florivores on plant fitness. However, there are some studies which show stronger pollinator-mediated selective pressure on floral traits (Bartkowska & Johnston, 2012).

1.4 Cascading effect of arthropod predators on plant fitness

The interaction of arthropod predators with pollinators and herbivores can affect plant fitness indirectly. Plants have evolved with different traits to attract predators and parasitoids of herbivores (Paré & Tumlinson, 1996; Kessler & Baldwin, 2001; Rudgers, 2004). Some of these traits are considered to have been coevolved with specific predators (Rudgers, 2004). Predators can affect the behaviour of phytophagous and endophagous herbivores, causing a positive impact on plant biomass (Moran et al, 1996), plant tissue damage (Romero et al, 2004; Trager et al, 2010) and plant fitness (Romero & Koricheva, 2011). However, the strength of the positive effect can vary with herbivore taxon and their relative abundance (Romero et al, 2004). Conversely, predatory insects can negatively impact plant reproductive fitness by dissuading pollinators from visiting flowers (Suttle, 2003). Therefore, the net effect of predators on plant fitness depends on the relative strength of the pressure of predators on pollinators and herbivores. However, some studies suggest that the positive impact of reduced plant damage by herbivores is stronger than the negative impact caused by reduced plant-pollinator interaction (Romero & Koricheva, 2011). In addition to the relative strength of interactions, the net effect on plant reproduction and biomass would also depend on whether the predators are generalists or specialists (Diehl et al., 2013), and if the predators are offered any rewards (Romero & Koricheva, 2011). Effect on plant functions can also vary with the functional richness of predators because the presence of one functional type can affect the other (Moran et al, 1996).

As explained in the previous sections, plants exert bottom-up pressure on the higher trophic levels using plant defenses. However, the relative strength of the bottom-up effect on arthropod predators and the top-down cascading effect of predators on plants varies with landscape and season (Dyer et al, 1999; Gratton et al, 2003). Parasitoids can also enhance plant fitness by feeding on herbivore larvae. The positive cascading effect via parasitoids could be stronger than predators (Romero & Koricheva, 2011).

1.5 Effect of abiotic environment and land use on multitrophic interactions

Biotic interactions at all levels are sensitive to changes in the abiotic factors. Some biotic interactions, especially those which are specialised in nature, can be more sensitive to changes in the environment than generalised interactions (Rocha & Fellowes, 2020). The environmental changes can be due to variations in climate or due to some anthropogenic interventions such as land use change. The effect of abiotic factors on biotic interactions can differ between land-use types (ref) and trophic levels (Xiao et al, 2016; Brys et al, 2004; Albrecht et al, 2012; Brys et al, 2004; Taki & Kaven, 2007).

Studies have mostly demonstrated the negative effects of land-use intensification on plant fitness via negative impact on mutualistic interactions (Klein et al., 2003). Variations in land-use intensification can also cause spatial variation in pollen limitation (Gomez et al, 2010). Similar to pollinators, disturbances in habitat can negatively affect herbivore composition. However, some studies show no effect on herbivory despite a reduction in herbivore diversity due to the replacement of specialist herbivores by fast-adapting generalist species (Rossetti et al, 2017) Although the florivores could be more sensitive to the changes in the environment than the plants (Hamann et al., 2021), they also tend to adapt easily (Cobb & Whitham, 1993). The effect of land use on predators could be taxon-specific. Mäntylä et al, (2011) showed a positive effect of bird predators on plant biomass irrespective of the land use and the climatic zone. However, the interactive effect of land-use type and scale can affect arthropod predators differently (Lemessa et al, 2015). Land-use intensity and climatic variations could also affect the phenology of arthropod predators (Hanson et al, 2017).

2. STUDY OBJECTIVE

The interactions described in the sections above are not operating in isolation, rather they are happening simultaneously in nature. However, these interactions have been generally studied separately (Lucas-Barbosa et al., 2011). An integrative approach towards studying multitrophic interactions could provide insights into the relative impact of direct and indirect effects on plant fitness. Additionally, studying these interactions at different spatial and temporal scales could help in comparing the drivers. In this study, we analysed the direct effects of plant-pollinator and plant-florivore interactions integratively with the indirect effects of predators on plant fitness. We also considered the indirect effect of pollinator-florivore interactions.

We present the results of this thesis in two integrated chapters, written as manuscripts for future submission to peer-reviewed journals. In the first part (Chapter 1), we used a causal framework to hypothesise the direct and indirect effects of interactions on plant fitness. To do so, we used Generative Simulation modelling to simulate hypothetical datasets fitting our hypotheses. We then used the Structural Causal Modelling (SCM) approach to understand if the statistical model can accurately estimate the causal effects that were hypothesised in the first step. Additionally, we used sensitivity analysis to understand the sensitivity of the response variable toward changes in the values of predictors used in the statistical model.

In the second part of our study (Chapter 2), we used the causal model that we verified in Chapter 1 to design an experiment for four sites of *Cirsium arvense*. We collected data on pollinator visitation, and florivore abundance, and calculated plant fitness. We compared the results from our field experiment with the inference from the causal model from Chapter 1.

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CHAPTER I

Applying generative simulations and sensitivity analysis to validate experimental design

Applying generative simulations and sensitivity analysis to validate experimental design

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ABSTRACT

Ecologists have been primarily relying on experiments to infer causality for natural phenomena. Experiments are designed to provide a controlled environment where only the hypothesised cause varies between observations. However, creating a perfectly controlled environment in nature or even in the laboratory is rarely possible. Several other sources of variability can lead to confounding effects that prevent causal inference. This problem can be dealt with by using the framework delineated under the Structural Causal Modelling (SCM) approach and specifically using Generative Simulation Modelling to produce simulated data that incorporates all known sources of variation in the system. In this study, we used generative modelling to investigate the drivers of the cascading effect of arthropod predators on plant fitness of a ruderal plant species, *Cirsium arvense*. We found that under our causal assumptions, statistical models accurately estimate the causal effects. We also used Local Sensitivity Analysis to predict the impact of changes in the hypothesised causes on plant fitness.

1. INTRODUCTION

Ecological statistical models are used either for data exploration, inferential analysis, or for prediction (Tredennick et al., 2021). Inferential analysis in ecological studies is aimed at determining the causality underlying a natural phenomenon. Ecologists have been using experiments in nature and laboratories to identify and quantify these causal relationships. However, the causal inference from experiments is highly sensitive to experiment design

(Kimmel et al., 2021). Common problems while designing experiments are replication and pseudoreplication, nonlinearity in the spatial variation of environmental drivers (Kreyling et al., 2018), and unobserved side-effects of treatment (Kimmel et al., 2021). Even after controlling for the intricacies of experiment design, inferential analysis of the observed data, without an in-depth understanding of the study system, could be misleading because the patterns generated from the experiment data might not imply causality (O'Sullivan & Perry, 2013).

Ecological models that base their inferences on the patterns from experimental observations are called discriminative models (Perry et al., 2016). The common challenge with discriminative modelling is deciding on the variables to be included in the models and the selection of statistical models (Tredennick et al., 2021; Murtaugh, 2009). One commonly applied solution is looking for significant relationships and the most parsimonious model using a stepwise selection process (Whittingham et al., 2006). Although stepwise selection can result in some significant associations, the overall predictability of the model is only sometimes reliable because there is a chance of misinterpreting a significant correlation as a causal relation (Whittingham et al., 2006; Stephens et al., 2005; Pearl, 2009). This is because an observed pattern could be linked to many processes (O'Sullivan & Perry, 2013; McElreath, 2020). Other possible problems overlooked in this approach are confounding variable bias (Runge, 2023; Arif & MacNeil, 2023), overcontrol bias, collider bias, and selection bias (Arif & MacNeil, 2023; Elwert & Winship, 2014; McElreath, 2020). Predictions based on observational data are also a common practice in ecological sciences. However, predictive analysis on small datasets and without having solid causal reasoning, and a detailed dataset, could also lead to erroneous conclusions (O'Sullivan & Perry, 2013).

The problems with the standard approach of experimentation and modelling described above can be dealt with through simulation modelling. Simulation models can help in predicting the shortcomings of the experimentation method, controlling the biases, and selecting appropriate variables, ahead of executing the experiment. Generative simulation models are simplified versions of real-world systems (O'Sullivan & Perry, 2013), or experiments (Dowling, 1999). Generative simulation models, as defined by Dowling (1999), are like testing a theory using a computational experiment. This kind of simplified representation of a complex ecological system can be created by breaking the system into components, and the connection between components depicts their interactions (O'Sullivan & Perry, 2013). The relation between the system components can be defined mathematically, based on the domain knowledge (Dowling, 1999; Perry et al., 2016). In summary, the steps involved in generative simulation modelling are i) conceptualising the problem (conceptual model) ii) defining the conceptual model in mathematical form (mathematical model) iii) testing the conceptual and mathematical model using statistical methods (statistical model). The application of generative simulation modelling in ecology ranges from simulation of historical data in paleoecology (Perry et al., 2016), to prediction, design of experiments, and causal inference (O'Sullivan & Perry, 2013).

Generative simulation models used for causal inference are called causal models. Different causal models are used for different applications (Runge, 2023; Arif & MacNeil, 2023). A widely used causal modelling approach in epidemiology and social science, Structural Causal Modelling (SCM) (Pearl, 2009), has been lately becoming popular in ecology (Arif & MacNeil, 2023). SCM uses a graphical causal framework, known as a Directed Acyclic Graph (DAG), to describe the hypothesised causal relationships. DAGs consist of variables as nodes, and arrows connecting the variables define their relationship. DAGs are efficient in identifying confounding variables (Rohrer, 2018), backdoor (non-causal), and frontdoor (causal) paths. Once identified, the variables in the backdoor path can be controlled by using backdoor criteria in which all the non-causal paths that emerge from the treatment (cause) to the response variable (effect) are blocked (Elwert, 2013; Arif & MacNeil, 2023). While the front door paths are always kept open as they are the part of causal path emerging from treatment to response (Elwert, 2013; Arif & MacNeil, 2023). DAGs somewhat resemble Structural Equation Models (SEM), However, unlike SEM, DAGs are non-parametric, therefore, they can be applied to any ecological system, having linear or non-linear causal relationships. Another important feature that makes DAGs more flexible than SEM is that they can include all the causal paths regardless of whether each path is being tested or not (Kunicki et al., 2023).

Hypothetical datasets can be generated based on DAGs. Statistical analysis can be performed on these datasets using the same statistical tools used in discriminative models. The difference between the prediction from the statistical model and the known value from the generative simulation model informs about the ability of the model to infer causality. The lesser the deviation between the estimated value from the statistical model and the known value from the generative simulation model, the better the model in predicting causal linkages (Arif & MacNeil, 2023).

These models can be further tested for the stability of the response by using sensitivity analysis (Barabás et al., 2014). Sensitivity analysis is used to estimate the sensitivity of response variables (effect) against the variation in the values of predictors (Cause). The sensitivity of the models can be evaluated locally (Local sensitivity analysis, LSA), by changing one parameter at a time while other parameters remain constant, or globally (Global sensitivity analysis, GSA), by varying multiple parameters simultaneously (Xu et al., 2004). Although GSA can provide a more realistic understanding of the robustness, LSA is widely used for its simplicity (Xu et al., 2004).

We applied the SCM framework to support the design of our experiment to understand the cascading effect of arthropod predators on the fitness of a common Asteraceae species, *Cirsium arvense*. We created a DAG for our hypotheses and a generative model fitting the hypotheses. Then we compared the estimates from the statistical model with the hypothesised effects to understand the accuracy of prediction of causal linkages. We verified the reliability of causal inference by introducing variability in the predictors and repeating the comparison of estimates with the modelled effects for each case. Lastly, we used local sensitivity analysis to understand the sensitivity of the response variable (plant fitness) to changes in different predictors.

2. GENERATIVE SIMULATION MODEL AND CAUSAL EFFECTS

2.1 Directed Acyclic Graph and Experimental Design

We formed our hypotheses about the effect of multitrophic interactions on the reproductive fitness of female plants in *Cirsium arvense* based on domain knowledge. We identified the factors that could affect plant fitness (plant fecundity or total number of viable seeds), including both the experimental manipulation (predator exclusion) and other aspects of the plant populations, and herbivore and pollinator communities. These variables and their relationships are described in the DAG below (Fig 1). Our graphical model indicates that plant fitness is positively affected by an increase in pollinator visits, and negatively affected by an increase in florivore abundance. Pollinator visits are determined by the pollinator composition of the site. Similarly, florivore abundance is determined by the florivore composition of the site. Both pollinator visits and florivore abundance can be controlled by predators. Therefore, we hypothesised that predator exclusion will improve plant fitness via an increase in the number of pollinator visits, and suppress plant fitness via an increase in the abundance of florivore larvae. The probability of seed fertilisation will also be affected by proximity to the male plants and the number of male plants.

To test our causal assumptions, we used the same experiment design as planned for the actual field experiment: We generated a given number of plant individuals, each of them with an equal number of flowerheads. Each flowerhead had several flowers sampled from a uniform distribution with minimum and maximum number of flowers equal to 65 and 80. This range reflected the usual range of number of flowers in our study system. These plants were then clustered in a blocked design, where we applied different treatments to each plant within a block. Several replicates of such blocks were generated. These blocks were equally distributed between the planned number of sites. All the variables concerning the design of the experiment were used as input parameters for the generative model as detailed in the following section. The important point to note here is that some of the variables in our causal diagram had an effect only at the site or block level. For this reason, the generative model includes sites and blocks which are not shown in the DAG.

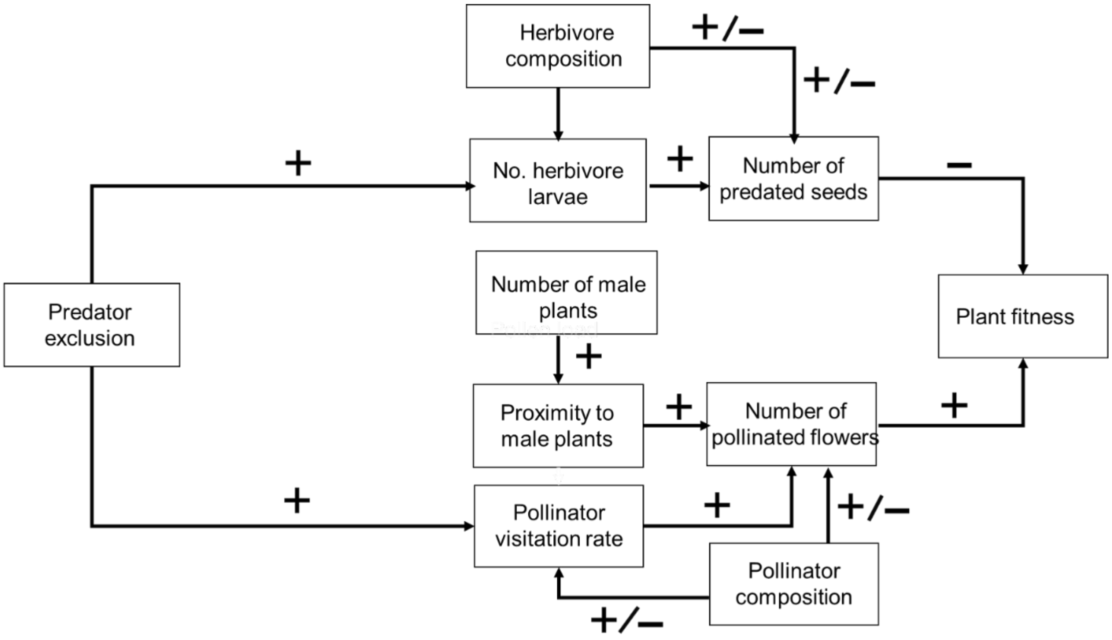


Figure 1: Directed acyclic graph for the processes we hypothesised would influence plant fitness in the experiment. Each rectangle represents one variable, with arrows indicating a causal link between them. The '+' and '−' signs represent the direction of the effect between two variables. On the extreme left, Predator exclusion is the experiment manipulation (treatment), while the other variables represent either measured or unmeasured predictors as described in the hypothesis. On the extreme right is the response variable, plant fitness which is fecundity (number of viable seeds) in this case.

2.2 Generative Simulation Model

We created a generative model fitting the hypotheses described in the DAG. The input parameters to the generative model were used to calculate the probability of fertilization for each flower. The variables related to experimental design were the number of sites, number of blocks per site, treatments, distance from male plants, and number of open flowerheads. We created two variables, site pollinator effect and site florivore effect, to simulate the variation in pollinator composition and florivore composition between sites. Similarly, we created a treatment effect variable to account for the relative effect of experimental manipulation compared to control (Eq 1).

To simplify the model, we assumed that the positive effect of predator exclusion on pollinators would be greater than the negative effect of predator exclusion on the florivores. This assumption was included in our model using multipliers ('A' for pollinators and 'B' for florivores) for the treatment effect of predator exclusion. The probability of fertilisation was calculated for each of these flowers as an additive effect of the site pollinator effect, site florivore effect, total treatment effect, and distance from male plants on the logit scale (Eq 1). This probability of fertilisation was employed on every flower in each flowerhead to determine the total number of viable seeds per flowerhead (Eq 2).

$$logit(p) = sp + sf + A * t - B * t + d) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. \; Eq\ (1)$$

$$N \sim Binomial\ (nf, p) \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. \; Eq\ (2)$$

In Eq (1), t is for treatment effect, sp is site pollinator effect, sf is site florivore effect, d is distance from male plants, and A and B are constants representing the magnitude of the effect of treatment on pollinators and florivores respectively. As explained in the previous section, we assumed that $A > B$. The total site effect was a combination of sp and sf. The total treatment effect was determined based on the values of A and B. Variables sp and sf were generated at the level of site, therefore, in the simulations, plants from the same site were assigned the same values for sp and sf. Distance from male plants, d, was generated at the level of block, hence plants from the same block shared this value. In Eq (2), N represents the total number of viable seeds per flowerhead which is a binomial function of the probability of fertilisation (p) and number of flowers per flowerhead (nf).

Our generative model is flexible enough to accept different values of input parameters. This is important because it allows us to test our model's behaviour for different parameter combinations. To simulate different values of treatment effect, we assumed the treatment effect of control to be fixed at 0 (on the logit scale) and varied the effect size of the treatment under evaluation (Table S1 & S4). Similarly, we assumed the effect of site pollinators to be fixed at 0 for one of the sites, simultaneously simulating a higher probability for the second and a lower one for the third site (Table S2). The same approach was employed to generate different impacts of site florivores under the assumption that the effect of florivores was 10 % of the impact of pollinators for all the sites (Table S3).

2.3 Statistical Model & Causal Effect

We used a Generalized Linear Mixed Effect model (GLMM) with a binomial family to estimate the odds of fertilisation (logit (p), where p is the probability of fertilisation) for each flower in a flowerhead, for all treatments in all the sites. We used treatment, site, and distance from male plants as fixed effects in our statistical model to understand the effect of treatments and spatial variation in insect composition on plant fitness. Our model used blocks, individual plants, and individual flowerheads as random factors (Eq 3). Post-hoc analysis was used to compare the odds of fertilization for treatment under evaluation with control. The estimated values from the statistical model were compared with the known value from the generative model to assess its accuracy.

$$logit\ (p) = S + T + d + (1|block) + (1|individual\ plant) + (1|flowerhead) \ldots Eq(3)$$

In Eq (3), total site effect is represented by S, total treatment effect is T and distance from male plants is D. As explained in the previous section, total site effect (S) corresponds to the combined effect of sp and sf, total treatment effect (T) corresponds to the value of $(A\text{-}B) * t$, and distance from male plants (d) is the same as in Eq (2).

2.4 Causal effect estimates

We compared the estimated effects of the variables in the statistical model (Eq 3) with the known effect from the generative model (Eq 1 & Eq 2) for three cases. For the first case, we assumed that the predator exclusion effect on florivores is 60% of that on the pollinators. This assumption is reflected in values of A and B in Eq (1) as 1 and 0.6 respectively and an expected net effect of $T = 0.8$ when $t = 2$. The site pollinator effect, site florivore effect, and distance from male plants were fixed at 0. We did recover a predator exclusion effect of 0.831 ± 0.03 and the effect of other parameters (control, site effects, and distance from male plants) close to 0. This result indicates that our statistical model can correctly estimate the effects of all the variables in this situation. This means that our statistical model fits our assumption of causal linkages between predators and net plant fitness. We repeated this exercise for multiple values for treatment effects to verify our findings. This finding was further used for the sensitivity analysis.

The second evaluation was done for spatial variation in site effect. As explained above, the total site effect encapsulates both site-pollinator and site-florivore effects. For this we varied the site pollinator effect, keeping the treatment effect of predator exclusion (*t*) fixed at 2, and no variation in the site-florivore effect. This time we assumed the predator exclusion effect on florivores to be 20% of the effect of pollinators, so the total treatment effect for predator exclusion was 1.6 in this case. The estimated treatment effect was 1.582 ± 0.03. For site pollinator effects, Sites A, B, and C were set to 0, 1, and -1 respectively and were estimated as -0.018 ± 0.03, 0.951 ± 0.03, and -0.986 ± 0.04. This result again indicated that our statistical model can estimate the known effects of spatial variation along with the treatment effect accurately, indicating that our statistical model can detect the causal effect of sites on plant fitness. Similar results can be generated by varying the site-florivore effect on plant fitness.

Finally, we used a model with three treatments to simulate the effect of a predator model in addition to control and predator exclusion. Same as the previous two cases, we used treatment effects for florivores as 20% of the effect on pollinators, and *t* was 0 for control, 1 for predator exclusion, and -1 for a predator model, hence the total treatment effect of predator exclusion was equal to 0.8, and -0.8 for predator model. The site effect for pollinators, florivores, and the distance from male plants was fixed at 0. The estimated effect for predator model and predator exclusion was 0.827 ± 0.03, and -0.806 ± 0.03. Therefore, we again verified for the third case that our statistical model can estimate the causal effect of multiple treatments on plant fitness correctly.

3. SENSITIVITY ANALYSIS

We used sensitivity analysis to determine the sensitivity of the response variable to the variation in the values of predictors. For our experiment, we used local sensitivity analysis to evaluate the sensitivity of the proportion of fertilised seeds of each flower, to changes in treatment effects for predator exclusion and predator model treatments, keeping the effect of control fixed at 0.

Second, we tested the sensitivity of the proportion of fertilised seeds to the variation in the site pollinator effect. We also analysed how the sampling effort (the number of collected flowerhead samples) influenced our power to detect differences between the treatments. Parameter values in these three cases are described in Table 1.

Table 1: Parameter values used for local sensitivity analysis for four evaluations. Each column represents one evaluation, and each row is for one parameter. Each column has all values fixed except the variable under evaluation. The values in square brackets show the range of values.

Parameters	Predator exclusion effect variation	Site pollinator effect variation	Predator model effect variation	Sampling effort variation
Treatment effect (control)	0	0	0	0
Treatment effect (predator exclusion)	[0,2]	0	1	0.2
Treatment effect (predator model)	NA	NA	[-1,1]	NA
Site pollinator effect (Site A, B, &C)	0	0, [0,1], [-1,0]	0	0
Site florivore effect	0	0	0	0
Sampling effort	3	3	3	[1,10]

3.1 Effect of variation in treatment effect

We analysed the sensitivity of the proportion of fertilised seeds per flowerhead to the variation in treatment effect for Experiment 1 (Table 1). Predator exclusion treatment effect (t) was varied between 0 and 2, where 0 meant no difference between control and predator exclusion effect, and 2 meant the effect of predator exclusion was two units higher than the effect of control on a logit scale. The results show that the proportion of fertilised seeds per flowerhead increased with the increase in the treatment effect of predator exclusion. In other words, the odds of seed fertilisation in predator exclusion will increase with the increase in the treatment effect of predator exclusion (Fig 2a). The differences between treatments became statistically significant with $p < 0.05$ from a treatment effect of 0.2.

3.2 Effect of variation in site pollinator effect

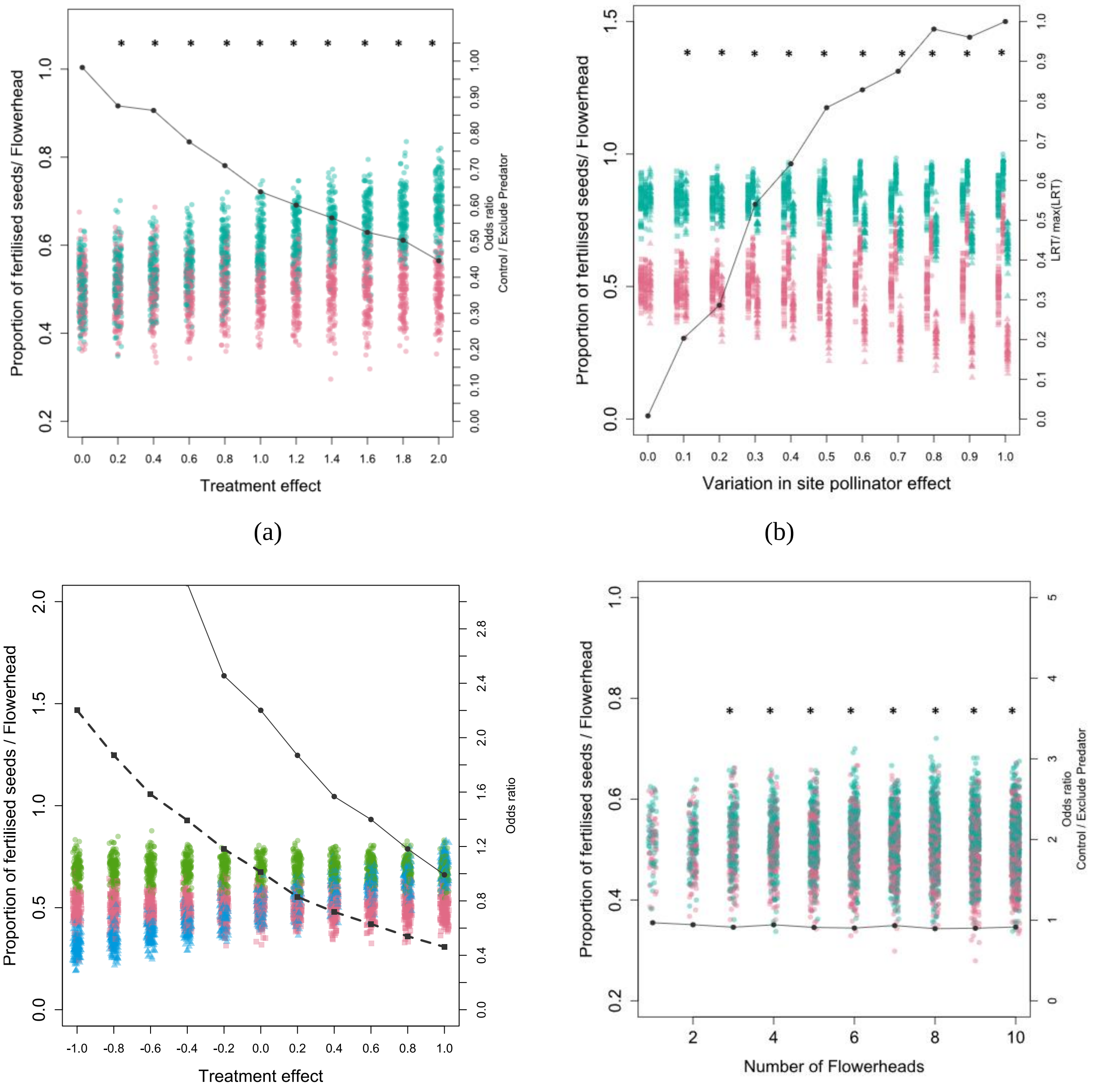
To test the impact of variation in the site pollinator effect, we kept the site-florivore effect, and distance from male plants as zero. The effect of site A was fixed at 0. The effect of site B and C were increasingly different from 0, positive for B and negative for C (Table S2). The sensitivity of plant fitness to the variability in site pollinator effect was tested, keeping the treatment effect of predator exclusion equal to 2 compared to control at 0. We calculated the likelihood ratio for the effect of site corresponding to each variation. The results in Fig 2b show that the likelihood of detecting an effect of site variation increases with increasing site variability, and is detectable with a $p < 0.05$ from site pollinator effect of site B and site C equal to 0.1 and -0.1, relative to effect of site A fixed at 0.

3.3 Effect of variation in predator model effect

We analysed the sensitivity of the proportion of fertilised seeds per flowerhead to the variation in treatment effect for the predator model, keeping control at a baseline of 0 and predator exclusion effect at 1. The proportion of fertilised seeds per flowerhead decreased with the increase in the treatment effect of the predator model. Fig 2c shows that the odds of seed fertilization for the predator model increase with an increase in the treatment effect. For this case, the odds of seed fertilisation increased with increasing treatment effect, as compared to control and predator exclusion. However, the odds were higher when compared with control as compared to predator exclusion. This is because the odds of seed fertilisation for predator exclusion treatment would be higher than control (Fig 2a) at predator exclusion effect (t) equal to 1. For this case, we could detect a significant difference in the control and predator model, and predator exclusion and predator model for all treatment effects ($p< 0.05$).

3.4 Effect of variation in the sample size of collected flowerheads

We tested the impact of sampling effort on detecting the effect of the treatment. To simulate this, we fixed all the values of all variables at 0, and the treatment effect of predator exclusion at 0.2 (threshold value for predator exclusion treatment effect). Like the case of variation in treatment effects, we assumed that the predator exclusion effect on florivores is 60% of that on the pollinators. We varied the sampling effort from 1 to 10. Our results showed that the odds of detecting an effect of treatment become significant after a sample size equal to 3 (Fig 2d).



(a)

(b)

(b)

(d)

Figure 2: Causal effect of different predictors on the proportion of fertilised seeds and odds of seed fertilisation (a) Effect of variation in predator exclusion treatment on the proportion of fertilised seeds. The secondary axis depicts the odds ratio between the control and predator exclusion treatments. The blue dots represent predator exclusion treatment and the pink dots represent control. This result is generated considering no variation in the site pollinator effect, site florivore effect, and distance from male plants.

(b) effect of site pollinator variation between three sites on the proportion of fertilised seeds and odds of seed fertilisation, keeping site florivore effect, and distance from male plants fixed at 0, and the treatment effect of predator exclusion (blue dots) and control (pink dots) at 0 and 1 respectively. The secondary axis represents the Likelihood ratio/max (Likelihood ratio) (c) effect of variation in treatment effect of predator model on the proportion of fertilised seeds and odds of seed fertilisation. The predator exclusion effect (green dots) is fixed at 1, control (pink dots) is fixed at 0, and the predator model effect (blue dots) is varied between [-1,1]. The odds ratio with respect to control is displayed with dashed lines, and the odds ratio with respect to predator exclusion is displayed with continuous lines. The site effects for pollinators, florivores and distance from male plants are fixed at 0 for this case. (d) effect of sampling effort on detecting the difference between treatments. For this, predator exclusion is fixed at 0.2 and control is fixed at 0. Site effects and distance from male plants are also fixed at 0. The continuous line represents the odds ratio between control and predator exclusion treatment. The asterisk (*) sign in all the plots represents significance.

4. DISCUSSION

Making causal inferences from observations or manipulative experiments is fraught with difficulties. This is because the main requirement for causal analysis is a closed system (O'Sullivan & Perry, 2013). Creating a controlled environment in most ecological studies is unrealistic. Moreover, it is also hard to validate if the artificially created controlled environment has a resemblance with the natural setup. This is where generative simulation models can make a difference. In the field of ecology, generative simulation models can be used as a tool which can help in designing better experiments. Tools such as sensitivity analysis can be used to test the sensitivity of model output against all the input parameters. This understanding can guide researchers about the precision with which different parameters should be measured to detect an effect in a field experiment. Additionally, this can provide an understanding of the sampling effort required to detect an effect of a cause (treatment).

We used a generative simulation model to design our experiment for testing the cascading effect of predators on the reproductive fitness of female plants in *Cirsium arvense*. We simulated datasets that fit our causal assumption as described in Fig 1. Our main intention in this analysis was to understand that if a causal linkage exists in our study system, then how precisely can our model predict these causal effects? Our approach is in line with some other studies on causal inference (Runge, 2023; Arif & MacNeil, 2023). We created separate experiments (Fig 2a-2d) for the specific questions we asked in this study (Chapter 2). Our results show that our models can estimate the causal effect precisely. In other words, if our hypothesis is true, and we design a real-world experiment and collect the data for the parameters used to create the model, we can detect the causal effect using this model. This approach is opposite to the widely used method in which multiple regression using all variables available is done based on how closely it fits the pattern. The fit of the model could increase with the complexity. However, model fit does not imply causality. To infer causality the models should be based on the causal assumptions, backed by the biological knowledge of the system.

Our model is a simplistic representation of our study system. We modelled the probability of fertilisation on a logit scale which ensured that the probability of seed fertilisation always stayed between 0 and 1. For simplicity, we modelled the values for the treatment effect and site effects also on a logit scale. At this stage, we directly used these effect values rather than going deeper into the mechanisms. As a next step, we can expand this model and make it more mechanistic by introducing parameters that generate treatment effects and site effects from ecological processes. For example, the treatment effect is the net indirect effect of predators on plant fitness. This net effect is being routed through the pollinators and the florivores. So, in the next level of this model, we can calculate the net treatment effect by introducing parameters such as the number of pollinators and florivores being predated by predators. This way the complexity of the model can be increased in a stepwise manner and thus we can build simulations that are closer to the interactions in the real world. Similarly, the complexity of other parameters such as the site pollinator effect or site florivore effect can be increased.

We backed our model with sensitivity analysis for the parameters that we hypothesised would have a causal effect on plant fitness. The sensitivity of plant fitness to variation in the treatment effect of predator exclusion provided us with an understanding of the threshold treatment effect, above which the chances of detecting the effect are high for our intended study design. In Fig 2a, the fertilisation increases above a treatment effect value of 0.2 (on a logit scale). Similarly, while checking the effect of the variability in pollinator effect between sites, we were able to quantify the effect of site variability and when it is detectable using our sampling design (Fig 2b). By introducing a third treatment, we demonstrated that our model is fit for the comparison of multiple treatment effects simultaneously (Fig 2c).

We also used sensitivity analysis to check the sensitivity of flowerhead samples to be collected for seed counting and florivore identification. According to the conventional approach, the more the number of samples, the better the ability to detect the effect. However, it is often difficult to figure out the right number of samples to be collected per plant. Field biologists often collect the number of samples that are logistically and financially feasible. In this study, we tried to understand if the number of samples collected would affect our detection ability. Our analysis (Fig 2d) shows that the detection of effect of treatment on plant fitness becomes significant from a sample size equal to 3. We used this result to plan the sample collection in our actual field experiment as detailed in Chapter 2 of this thesis. Through this, we tried to demonstrate how generative simulation models and tools such as sensitivity analysis could improve the overall quality of the experiment.

For this analysis, we varied one parameter at a time (local sensitivity analysis). This helped us detect the threshold values of the parameters under evaluation above which the detection of an effect is possible with our study design. Our results from the three cases (Fig 2a-2d) showed that our model is fit for detecting very small effects of parameters. We used this inference to describe our result from the actual field experiment (Chapter 2). We used a simplistic version of sensitivity analysis (LSA). However, this can be further developed into global sensitivity analysis in which multiple parameters can be varied together and thus the outputs could provide a more realistic understanding of the sensitivity of response corresponding to each combination of different predictors. However, global sensitivity analysis of biological systems is very complex, given the inherent complexity of the system.

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SUPPLEMENTARY MATERIAL

Table: S1 Treatment effect values for predator exclusion when the effect of control is fixed at a baseline of 0. The values of the treatment effect are on a logit scale. Row no. 1 shows no difference between predator exclusion and control, and then from the second row onwards, the effect of predator exclusion in increased by 0.1 on a logit scale. Eleven values were generated were used to simulate eleven separate datasets fitting the hypothesis in Fig 1.

S. No	Exclude Predator	Control
1.	0	0
2.	0.2	0
3.	0.4	0
4.	0.6	0
5.	0.8	0
6.	1.0	0
7.	1.2	0
8.	1.4	0
9.	1.6	0
10.	1.8	0
11.	2.0	0

Table: S2 Site pollinator effect values for three sites A, B, and C. The effect of Site A is fixed at 0, Site B effect is simulated to be greater than that of Site A, and Site C effect is simulated to be lower than Site A. The values of the site effect for all the sites are on a logit scale. Row no. 1 shows no difference between the sites. Eleven values were generated to simulate different combinations of site effects to test spatial variation in the effect of treatments on plant fitness. Site florivore effect is assumed to be lower (10%) than the site pollinator effect for this simulation.

S. No	Site A	Site B	Site C
1.	0	0	0
2.	0	0.1	-0.1
3.	0	0.2	-0.2
4.	0	0.3	-0.3
5.	0	0.4	-0.4
6.	0	0.5	-0.5
7.	0	0.6	-0.6
8.	0	0.7	-0.7
9.	0	0.8	-0.8
10.	0	0.9	-0.9
11.	0	1.0	-1.0

Table: S3 Site florivore effect values three sites A, B, and C. All values correspond to the values of site pollinator effect shown in Table 2. All values are assumed to be 10% of the site pollinator effect for this simulation model. The effect of Site A is fixed at 0, Site B effect is simulated to be greater than that of Site A, and Site C effect is simulated to be lower than Site A. The values of the site effect for all the sites are on a logit scale. Row no. 1 shows no difference between the sites. Eleven values were generated to simulate different combinations of site effects to test spatial variation in the effect of treatments on plant fitness.

S. No	Site A	Site B	Site C
1.	0	0	0
2.	0	0.01	-0.01
3.	0	0.02	-0.02
4.	0	0.03	-0.03
5.	0	0.04	-0.04
6.	0	0.05	-0.05
7.	0	0.06	-0.06
8.	0	0.07	-0.07
9.	0	0.08	-0.08
10.	0	0.09	-0.09
11.	0	0.10	-0.10

Table: 4 Treatment effect values for the predator model when the effect of control and predator exclusion are fixed at a baseline of 0 and 1. The values of the treatment effect are on a logit scale. The variation from [-1,1] for the predator model treatment indicates that its effect on plant fitness can be lower than control or it can reach maximum up to the effect of predator exclusion. Eleven values were generated were used to simulate eleven separate datasets fitting the hypothesis in Fig 1.

S. No	Predator Model	Exclude Predator	Control
1.	-1.0	1.0	0
2.	-0.8	1.0	0
3.	-0.6	1.0	0
4.	-0.4	1.0	0
5.	-0.2	1.0	0
6.	0.0	1.0	0
7.	0.2	1.0	0
8.	0.4	1.0	0
9.	0.6	1.0	0
10.	0.8	1.0	0
11.	1.0	1.0	0

CHAPTER II

Pollinators, not predators affect endophagous florivores in *Cirsium arvense*

Pollinators, not predators affect endophagous florivores in *Cirsium arvense*

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ABSTRACT

Plant fitness is a net result multiple biotic and abiotic factors operating interactively. Therefore, an accurate assessment of the impact of individual components requires studying multiple factors simultaneously. However, studies mostly focus on a single effect, and therefore inferring causal effects from their results could be erroneous. We applied an integrative approach to study the cascading effect of arthropod predators on female reproductive fitness of *Cirsium arvense*, via its impact on plant-pollinator and plant-florivore interactions. Additionally, we also studied the impact of pollinator-florivore interactions on female reproductive fitness. Our results show that the arthropod predators do not impact pollinator visitation rate or florivore abundance, implying that there would be no cascading impact on plant fitness. This was validated with an absence of any effect of predators on the proportion of fertilised seeds per flowerhead. However, we found that florivore survival in *Cirisum arvense* indirectly depends on pollinator visitation. Our study lays the ground for future studies that can focus on studying the drivers of the indirect effect of pollinators on florivores in different study systems.

1. INTRODUCTION

Plant fitness depends on abiotic conditions and biotic interactions. Plants benefit from mutualistic interactions with pollinators whereas interactions with herbivores reduce plant fitness. Herbivores from different guilds can have a direct or indirect impact on plant fitness. For instance, florivores such as seed predators reduce plant fitness directly whereas folivores have an indirect impact via changing resource allocation dynamics (Vries et al., 2017). The interactions between pollinators and herbivores could also impact plant fitness indirectly (Mahoro, 2003; McCall & Irwin, 2006; Mothershead & Marquis, 2000; Russell-Mercier & Sargent, 2015; Kessler et al., 2011). The net effect of plant-pollinator and plant-herbivore interactions on plant fitness can be positive, negative, or neutral depending on which interaction has a higher impact (Adler et al., 2001). Its evolutionary consequence could be the shift in plant traits or phenology because of opposing or aligned selective forces applied by pollinators and herbivores (Russell-Mercier & Sargent, 2015).

Cascading effects from predator-pollinator, predator-herbivore, and parasitoid-herbivore interactions can have a net positive or negative impact on plant fitness. These indirect effects will be positive when the predators/parasitoids control herbivores, or negative if predators dissuade the pollinators from visiting the flowers (Moran et al, 1996; Romero et al, 2004; Trager et al, 2010). The net indirect effect could change based on biotic factors such as insect community composition, plant community composition, abiotic factors such as land use and climate, and their interplay (Hahn & Orrock 2015; Araújo et al., 2015; Barnes et al., 2017).

Plant interaction with mutualists and herbivores has been generally studied separately (Lucas-Barbosa et al., 2011). However, studying interactions in isolation might not give a realistic understanding of the net impact of multitrophic interactions on plant fitness. Additionally, it is also important to understand the relative impact of direct and indirect effects and their drivers. Therefore, It is essential to integrate studies on plant-pollinator and plant-herbivore interactions (Adler et al., 2001; Lucas-Barbosa et al., 2011), and the cascading effect of predators, to estimate these consequences correctly. We used this integrative approach to understand the net effect of pollinators, florivores (predispersal seed-predators), and the indirect effect of predators and pollinator-florivore interactions on female plants of *Cirsium arvense*. We used fecundity as a measure of fitness for our study (Pincheira-Donoso & Hunt, 2017). We ask the following questions

1) What is the net effect of predator exclusion on plant fitness?
2) Does the net effect of predator exclusion vary spatially?
3) Does the predator presence affect the foraging behaviour of pollinators or herbivores?

4) Do pollinators and florivores affect each other indirectly?

1.1 Hypothesis

We hypothesise that plant fecundity will increase with an increasing pollinator visitation rate. The pollinator visitation rate will depend on the pollinator composition of the site and will increase with pollinator richness and abundance (Albrecht et al., 2012). Second, the seed predation rate will be determined by florivore abundance and composition. Our third hypothesis is that plant fitness will increase via increased pollinator visits in the absence of predators (Romero et al., 2011). Similarly, plant fitness will decrease via increased florivore abundance in the absence of predators (Horvitz & Schemske, 1984; Trager et al., 2010). Therefore, the net cascading impact of predators on plant fitness will be determined by the relative impact of pollinators and florivores. Our fourth hypothesis is that the florivores will prefer the plants that receive more pollinator visits (Parachnowitsch & Caruso, 2008), and therefore the seed predation will increase. And, pollinators will avoid the plants that florivores attack (Kessler et al., 2011; Lucas-Barbosa et al, 2011). We created a Directed Acyclic Graph (Fig 1) to represent all the hypotheses visually.

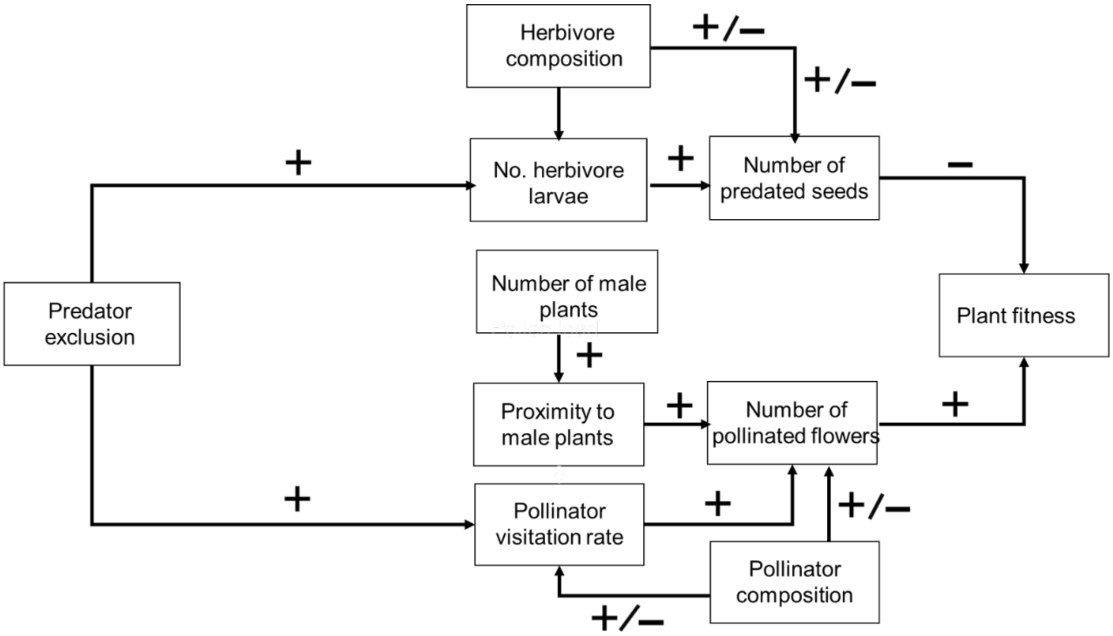


Figure 1: Directed acyclic graph for the processes we hypothesise to influence plant fitness in the experiment. Each rectangle represents one variable, with arrows indicating a causal link between them. The

‘＋’ and ‘－’ signs represent the direction of the effect between two variables. On the left side, Predator exclusion is the experiment manipulation, while the other variables represent either measured or unmeasured predictors as described in the hypothesis.

2. METHODS

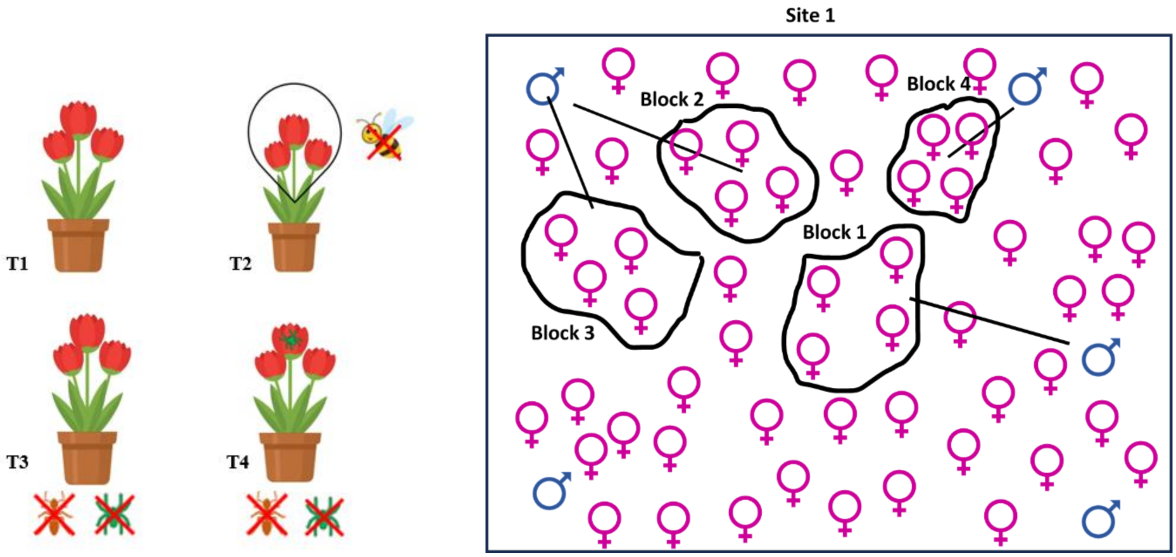
2.1 Model plant species

We used *Cirsium arvense*, a functionally dioecious ruderal plant species which is native to Europe (Tiley, 2010). It starts flowering in late spring and flowers throughout the summer. Each plant is either functionally male or female, however, each plant is morphologically hermaphrodite (Heimann & Cussans, 1996). This species also has a high resilience to abiotic stressors, so it can thrive in disturbed environments (Guggisberg et al., 2012; Tiley, 2010) and has fast vegetative propagation assisted by a widely spread deep-rooting system (Tiley, 2010). Vegetative propagation helps in rapid and easy colonization locally (Heimann & Cussans, 1996). It gets easily established in disturbed sites (Tiley, 2010). However, *Cirsium arvense* depends on sexual reproduction via pollinators to maintain genetic diversity within and outside the population (Heimann & Cussans, 1996). A large amount of nectar and emission of volatile compounds indicates that *Cirsium arvense* invests significantly to attract pollinators and other insects (Theis 2007, Sayed 2008; Tiley, 2010). The populations of *Cirsium arvense* occur in large patches that are mostly female-biased (Drunen & Dorken, 2012), surrounded by male plants at the periphery of the patches. Studies on populations of *Cirsium arvense* show that the distance between female and male plants (Drunen & Dorken, 2012; Tiley, 2010), and the sex ratio within the population (Heimann & Cussans, 1996) can affect plant fecundity. However, some studies do not consider the sex ratio to be an important factor (Drunen & Dorken, 2012).

2.2 Experimental design

We established a blocked experiment with four treatments: control, predator exclusion, pollinator exclusion, and predator model (Fig S7). This was repeated in four populations of *Cirsium arvense*, located at sites in the vicinity of České Budějovice in the Czech Republic (Fig S8). The experiment was established just before the flowering season, in the third week of June 2022. This ensured that the flowers were not pollinated before the experiment. In each site, 12 blocks of four plants close to each other were chosen to apply the treatments. The first plant was the control (T1, Fig 2a). Pollinators were excluded from the second plant to validate the dependency of *Cirsium arvense* on sexual reproduction. For this, we used a pollinator exclusion bag which covered three or more flowerheads in the plant (T2, Fig 2a). We removed predators from the third plant to understand the effect of predators on plant fitness. To exclude the predators, we applied adhesive traps to the plant stem (Brand: CHEMOSTOP ECOFIX) and manually removed any predators already established from the flowerheads (T3, Fig 2a). For the last treatment, we used an artificial predator model, a plastic spider painted green, to test the impact of predator presence on pollinator and florivore behaviour. The predator model was placed on the flowerheads or near the flowerheads of the fourth plant after removing the real predators using the method used in the predator exclusion treatment (T4, Fig 2a). We ensured the effective removal of predators from the second and fourth treatments by manually clearing the plants and grasses in the surroundings of our experiment. The manual clearance of surroundings was repeated periodically throughout the experiment period. Pollinator observation was conducted during the experiment. Additionally, once flowerheads were mature and before seed dispersal, they were collected for florivore sampling (Fig S9) and plant fitness measurement, as detailed below.

Each site was divided into 12 blocks of four plants placed throughout the population. Each block consisted of all four treatments, which were randomly assigned. The distance of the closest male individual to the centroid of each block was measured. In total, there were 48 blocks with 196 plant individuals in the four sites. Two sites were abandoned patches of agricultural land, surrounded by agricultural fields. The remaining two were located at the edge of fields that are regularly used for agricultural purposes. All the populations were female-biased, having male plants at the edge of the populations. In two sites that were spread along the edge of the agricultural fields, the male plants were located towards the extremes of the edges (Fig 2b). We did not survey the predator composition before establishing the experiment. However, from visual inspection, we found the main predators in each site were ants and spiders. We did not account for flying predators in this study.



(a) b)

Figure 2: Description of treatments (a) and experimental design (b). The first treatment (T1) is control, the second treatment (T2) is pollinator exclusion, the third treatment (T3) is predator exclusion, and the last treatment (T4) is predator model. The treatments were applied to four closely located female plants that form one block as described in (b). The patch of female plants was surrounded by male plants. The straight line depicts the distance of blocks from the closest male individuals.

2.3 Pollinators, florivores and plant fitness

Plant-pollinator interactions were recorded by observing pollinator activity in control, predator exclusion, and predator model treatments. We visually observed plant-pollinator interactions for 15 minutes in each plant. During the observation, we recorded the number of visited flowerheads per plant for each pollinator and the number of open flowerheads per plant at the observation time. The description of the pollinators was noted for identification. We also recorded videos and pictures in the field to help with identification. The observation lasted from morning 7 AM to 1 PM every day. The block order was selected randomly along the observation days. The observation continued for the entire flowering season which lasted till the end of July 2022. We recorded these interactions for 27.25 hrs across all sites (Table S1). The description of the pollinators, the pictures and videos were used off-field for identification. We used morphospecies for the pollinators that were not identified at the species level.

To understand the effect of the treatments on plant-seed predator interactions, we collected three mature flowerheads per treatment from all the blocks towards the end of the anthesis. We collected 609 flowerheads for all four sites (Table S2). We counted the number of seed predators per flowerhead and then stored them in a 70% ethanol solution. Later These samples were identified up to the species level. We assigned morphospecies to the ones which we could not identify beyond family.

We measured the fecundity per flowerhead to estimate the net effect of pollinators, seed predators and the cascading effect of predators on plant fitness. For this, we counted the number of fertilised seeds, the number of unfertilised seeds, and the number of seeds damaged by seed predators for each of the three flowerheads collected from every plant. From this data, we calculated the proportion of fertilised, and the proportion of damaged seeds per flowerhead. The proportion of fertilised seeds represented the net fitness per flowerhead.

From pollinator observations and sampled florivores, we estimated the richness of pollinators and florivores in each site. To account for possible sampling incompleteness, we used the abundance-based Chao1 index (Chao & Shen, 2003) instead of observed richness. We estimated the effect of treatments on pollinator behaviour by their impact on pollinator visitation rate per flowerhead per hour. We compared the proportion of damaged seeds per flowerhead to understand the effect of treatments on the seed predators. The impact of treatments on net plant fitness was estimated by comparing the proportion of fertilised seeds per flowerhead between the treatments.

2.4 Statistical analysis

We used a Generalized linear mixed-effect model with a binomial family to estimate the effect of treatments, sites, site pollinator richness, site seed predator richness, and distance from male plants on the odds of pollination and seed predation for each flower. Blocks, individual plants, and individual flowerheads were used as random factors in our model. We have validated this model using a generative simulation modelling approach (Chapter 1). As explained in Chapter 1, this model was built using a causal framework in which we analysed the effect of cause (treatment) on the response (plant fitness). As described in the results our statistical model is fit for detecting the effect of predator exclusion on plant fitness above treatment effect of 0.2.

3. RESULTS

3.1 Pollinator visitation and florivore abundance

The observed pollinator richness of sites A, B, C, and D was 12, 15, 14, and 15. The Chao1 index for these sites was 13, 22, 15, and 17.5. The estimated richness of all sites, except Site B, is the same as the observed richness. This indicates our sampling was sufficient to estimate the richness in the different sites, allowing us to use local pollinator richness as a predictor in our models. Overall, the highest number of visits were from families Apidae, followed by Syrphidae, and Sarcophagidae. Additionally, other families from orders Coleoptera, Diptera, Lepidoptera and Hymenoptera were recorded (Table S3). In terms of the abundance distribution of visits for the different pollinator families, there is some variation between sites, but the overall pattern remained consistent. Pollinators from Apidae visited the most in all the sites, except Site D, where plants received more Syrphidae visitors than Apidae (Fig 3d-3g). Pollinator visitation rate was not dependent on distance from male plants in our experiment (Fig S1).

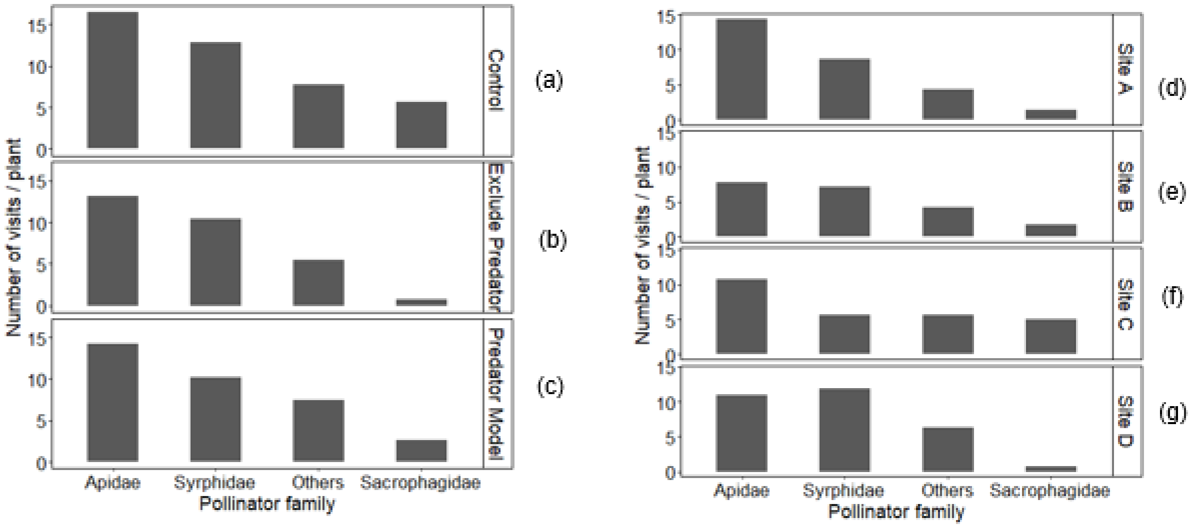


Figure 3: Total Number of pollinator visits to *Cirsium arvense* flowers submitted to three treatments in four sites in Southern Bohemia, Czech Republic. (a) - (c) are different treatments (Control, Exclude predators

and Predator model, respectively). (d) - (g) are different sites. Number of visits/plants in all the plots is plotted on the log scale.

We applied the same methodology to estimate the florivore richness per site. We found that the florivore richness in sites A, B, C, and D was 9, 7, 6, and 4 and observed The Chao1 estimate of richness for these sites was 12, 7.5, 6, and 5 respectively. This again indicates that the sampling was sufficient to use florivores richness as a predictor in the models. The florivores belonged mainly to the families of Cecidomyiidae, Syrphidae, Platygastaridae, and Tephritidae. Insects from the Cecidomyiidae group were of high abundance in all sites (Fig 4e-4h). The species richness of florivores in the control plants was greater than in the plants from where pollinators were excluded (Fig 4a-4d). This result validates our fourth hypothesis that the florivores will be more attracted to the plants that receive more visits from pollinators.

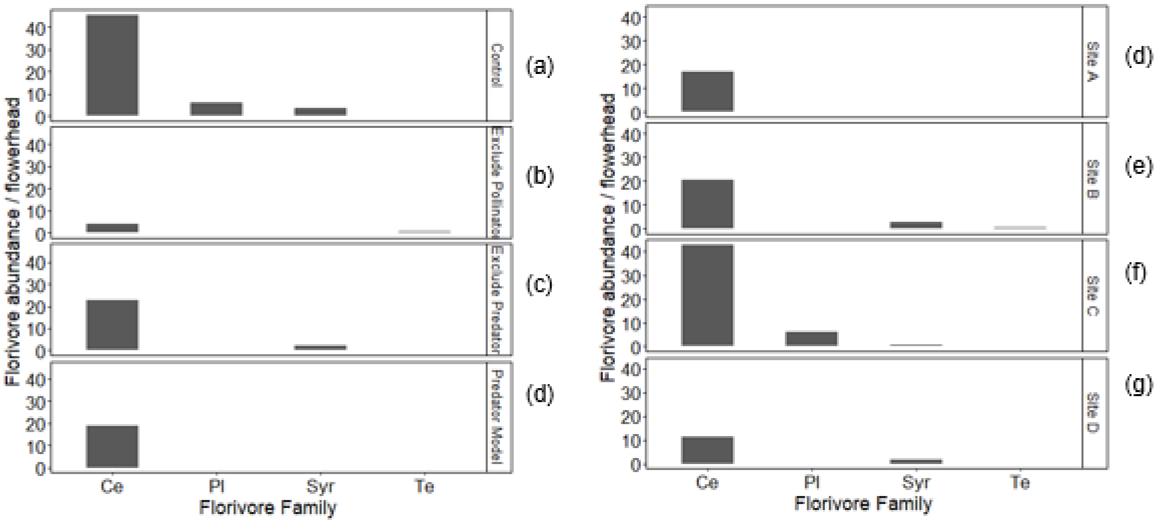
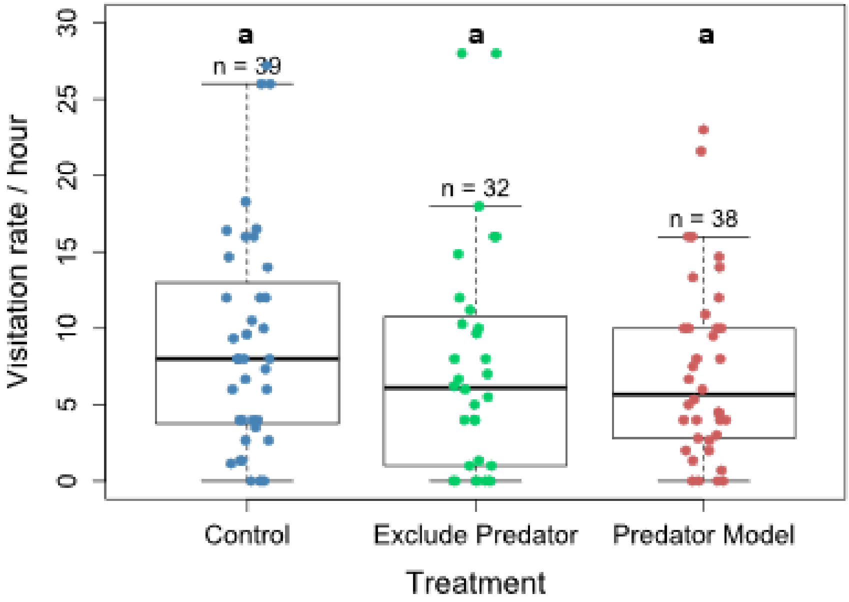


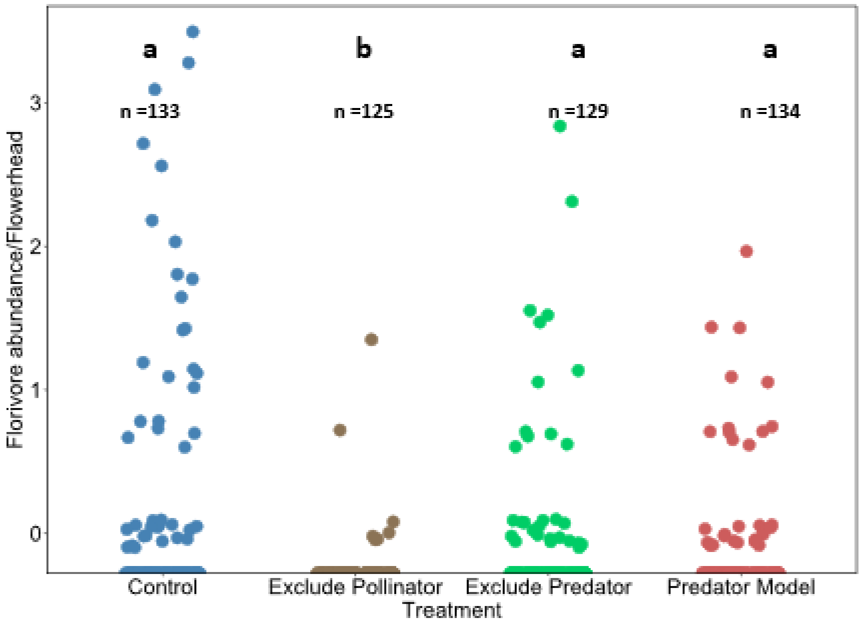
Figure 4: Florivore abundance per flowerhead of *Cirsium arvense* in three treatments in four sites in Southern Bohemia, Czech Republic. (a) - (d) are different treatments (Control, Exclude predators, Exclude pollinators, and Predator model, respectively). (e) - (h) are different sites. Florivore abundance/flowerhead in all the plots is plotted on the log scale. Florivore families plotted along the x-axis: Ce (Cecidomyiidae), Pl (Platygastaridae), Syr (Syrphidae), and Te (Tephritidae)

3.2 Effect of predator and pollinator exclusion on pollinator visitation rate and florivore abundance

The pollinator visitation rate per hour per flowerhead was not affected by predator exclusion, with the null model without treatment or site as the best-performing one (Table S5). In Fig 5a, we showed that predator exclusion and predator model treatments do not differ from the control, and there was no spatial variation in this pattern (Fig S2). Similarly, predator exclusion and predator model did not affect the florivore abundance in each flowerhead. However, we noticed a significant reduction in florivore abundance when the pollinators were excluded from the plants (Fig 5b). This pattern is repeated in all the sites (Fig S3). These results are supported by our model selection, with the model including only treatment as the best performing (Table S6), and multiple pairwise comparisons showing that only pollinator exclusion was different from the other treatments (Table S7).



(a)

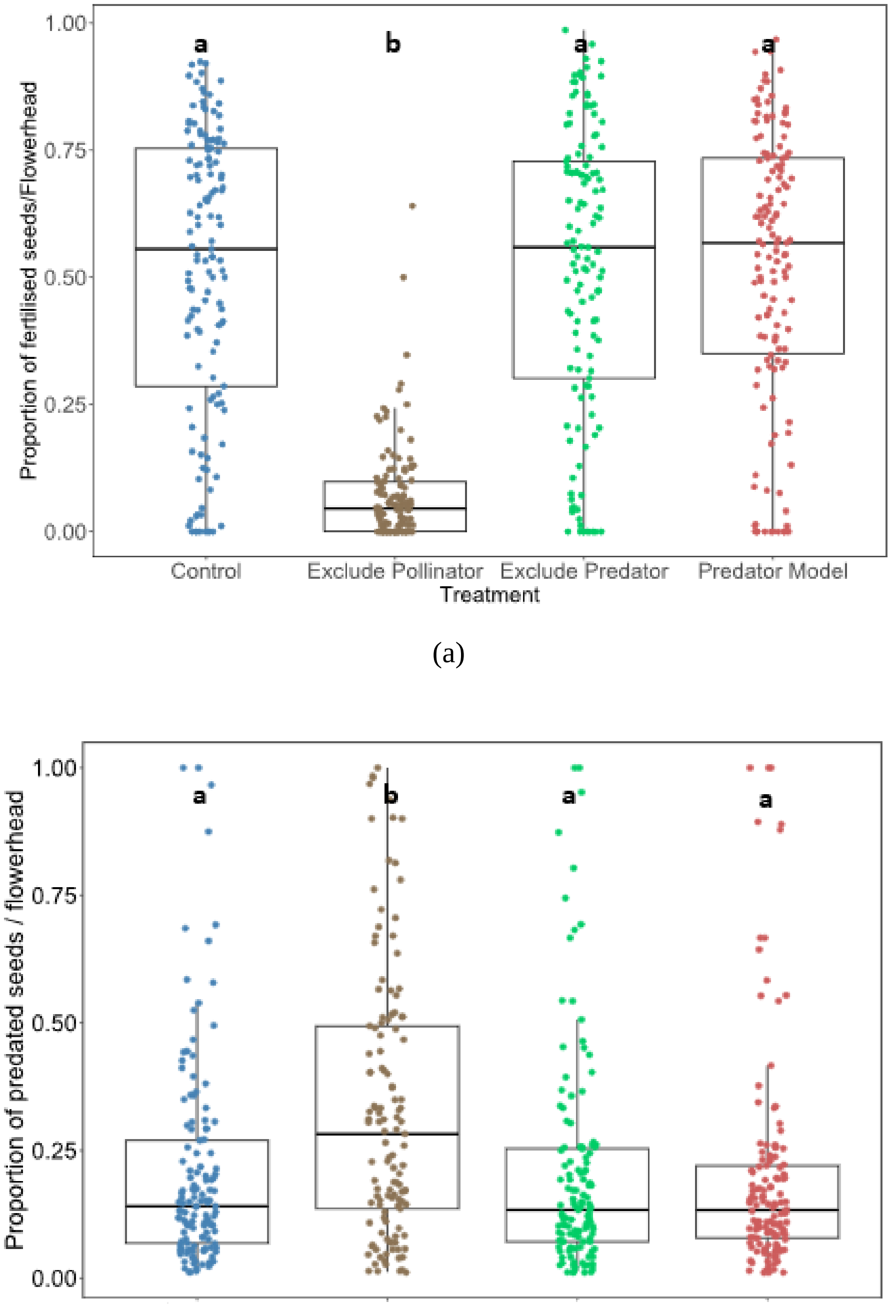


(b)

Figure 5: Pollinator visitation rate/hour/plant, separated by treatments. Each point represents the number of visits per plant (a) Florivore abundance per flowerhead, separated by treatments. Each point represents one flowerhead (b).

3.3 Effect of predator exclusion and pollinator exclusion on plant fitness

The proportion of fertilised flowers per flowerhead did not vary between control, predator exclusion and predator model treatments. This indicates that predators do not affect female plant fitness in *Cirsium arvense*. However, the proportion of fertilised flowers per flowerhead significantly drops when pollinators are excluded from plants (Fig 6a). This shows that *Cirisum arvense* depends on pollinators for sexual reproduction. The proportion of predated seeds per flowerhead also is not affected by predator exclusion or predator model (Fig 6b). This indicates that florivore performance is not affected by predators in this system.



(a)

(b)

Figure 6: Proportion of fertilised seeds per flowerhead, separated by treatments (a) The proportion of predated seeds per flowerhead, separated by treatments Each point in both the plots represents one flowerhead (b).

3.4 Indirect effect of pollinators on florivores

The proportion of fertilised seeds in control plants was greater than the pollinator exclusion treatment in all the sites (Fig 6a). This indicates that *Cirsium arvense* depends on pollinators for seed fertilisation and this effect does not vary spatially. This result is in line with our first hypothesis that plant fecundity will increase with increasing pollinator visitation rate. We also found that the proportion of predated seeds in pollinator exclusion treatment was higher than in the control plants (Fig 6b). However, the number of florivores that emerged from flowerheads in the pollinator exclusion plants was lower than in the control group (Fig 5b). This result contradicts the second hypothesis, that seed predation will increase with the number of florivores. This pattern also repeats in all four sites, indicating no spatial variation in the pattern of seed predation. This result also indicates that the fitness of *Cirsium arvense* is more dependent on pollinators than on the florivores. It also shows that endophagous florivores in *Cirsium arvense* depend on pollinators to complete their life cycle.

4. DISCUSSION

Our experiment shows that the proportion of fertilised seeds significantly reduces when the pollinators are experimentally removed, as expected for a dioecious species (Fig 6a). This indicates that pollinator-meditated sexual reproduction could be a strategy to maintain genetic variability within and outside the population in *Cirsium arvense* (Tiley, 2010). This could be especially important in *Cirsium arvense* because it engages extensively in vegetative propagation and the importance of sexual reproduction has been downplayed in this species (Heimann & Cussans, 1996). However, we have shown that pollination is essential for seed production and thus long-distance dispersal. This finding is important, especially in the context of partially self-compatible plant species because it shows that despite the ability of self-fertilisation, plants rely on pollinators for reproduction. Other studies on self-compatible plant species also show a positive effect of pollinator richness on plant fecundity (Klein et al., 2003).

Further, we noticed a higher number of visits by pollinators from the Apidae family, followed by Syrphidae in all four sites (Fig 3d-3g). At the same time, the florivore abundance does not impact the pollinator visitation rate (Fig S6a) or the proportion of fertilised seeds (Fig S6b) in any of the sites. These findings suggest that the fecundity of *Cirsium arvense* is driven by pollinators and not florivores. Some other studies have also found that higher visitation by certain groups can have a stronger positive impact on plant fecundity (Albrecht et al., 2007; Albrecht et al., 2012; Genung et al., 2017).

Various studies have proved a positive correlation between pollinator richness and plant reproductive success (Klein et al., 2003; Albrecht et al., 2007; Albrecht et al., 2012;). The four sites in our experiment were very similar in terms of pollinator richness. Consequently, we could not verify if the pollinator visitation rates were driven by the pollinator richness from our experiment. Moreover, the pollinator visitation rates for treatments were not significantly different in any of the sites. Similarly, the florivore richness of all four sites was nearly identical. Therefore, the effect of florivore composition on florivore abundance or the proportion of damaged seeds could not be verified in this study.

This similarity in the pollinator and florivore community could be driven by the contemporary and historical land use in the sites and the neighbourhoods (Senapathi et al., 2017; Cusser et al., 2018), with a stronger effect of the historical legacy of the sites (Hahn & Orrock, 2015), and neighbourhoods (Cusser et al., 2018). The pollinator and florivore composition in the sites can also be governed by the neighbourhood insect diversity (Albrecht et al., 2007), or by the association effect with the neighbourhood plant communities (Underwood et al., 2020). Seasons, neighbourhood plant biomass, and the phylogenetic distance between *Cirsium arvense* to neighbourhood species could drive the magnitude and intensity of the neighbourhood effect (Mutz et al., 2022).

We did not find any cascading effect of predators on pollinator visitation rate in our experiment. This could be because pollinators from some families, especially the large-sized pollinators, show weaker responses to the predator presence (Romero et al., 2011). Another possible reason could be the predation risk of ants and sit-and-wait predators might not be very significant in the pollinator groups present in the experiment sites (Romero et al., 2011). In line with our hypothesis, as predators show no effect on pollinator visitation (Fig 5a), we see no cascading effect on plant fitness (Fig 6a).

The reasoning applied to pollinator groups could also be valid for the florivores. We assume that the predators present in the experiment sites do not have any effect on the seed-predator families (Trager et al., 2010), therefore predators do not pose any effect on florivore abundance in our experiment. Contrary to our results, some studies claim that perennial plants benefit more from indirect defences from predators than annual plants (Trager et al., 2010). This could be due to the difference in experiment methods from other studies (Trager et al., 2010). In addition to this, our results show no evidence of different effects of predators on pollinators vs. florivores.

Whether plant fitness is indirectly affected by the interaction between pollinators and florivores, most of the literature suggests a stronger impact of herbivores on pollination. These impacts can be mediated by changes in chemical composition, phenology, or morphological traits of the plants that affect attractiveness to pollinators, such as floral traits and quantity and quality of the resources offered (Mothershead & Marquis, 2000; Kessler et al., 2011; Hambäck, 2001; Russell-Mercier & Sargent, 2015; Adler et al., 2001). Herbivore damage to flowers can reduce pollinator visitation rate (Hambäck, 2001, Mothershead & Marquis, 2000) or pollination efficiency, and therefore cause a cascading effect on plant fecundity or other aspects of plant fitness (Russell-Mercier & Sargent, 2015). Studies have also suggested that herbivores can impact plant fitness indirectly by altering the growth-reproduction-defence trade-off dynamics in plants (Schwachtje & Baldwin, 2008). The magnitude and intensity of these indirect effects could be governed by the effect of abiotic factors such as land use and climate, resource availability, and biotic factors such as insect guild (Araújo et al., 2015), and interactions of these factors on pollinators and herbivores respectively (Barnes et al., 2017; Senapathi et al., 2017; Cusser et al., 2018; Araújo et al., 2015).

However, the results from our experiment show the opposite: a stronger impact of pollinators on florivores. We found that the proportion of predated seeds was higher in the pollinator exclusion treatment (Fig 6b), whereas the florivore abundance was higher in control plants (Fig 5b). To better understand this seemingly contradictory result, we can consider the life cycle of a florivore along the anthesis of the plant. First, florivores lay their eggs in flower buds. The larvae then grow inside the flowerheads, feeding on flower tissues and seeds, and they emerge in the adult stage. In the absence of pollination, these florivores feed on unfertilised seeds and thus have a very low probability of completing their lifecycle due to undernourishment. Conversely, the higher the proportion of fertilised seeds, the more likely are florivores to be able to complete their life cycle. This result implies that florivores could select the flowers that are more likely to be visited by pollinators. Florivore dependence on pollinators is also reported in some other study systems (Strauss & Irwin, 2004; Parachnowitsch & Caruso, 2008). The underlying mechanism for florivore dependence on pollinators could be linked with the plant volatiles, florivore composition, pollinator composition, attributes of plant population, or other factors. In a nutshell, it is important to consider multiple factors and interactions between them to know the underlying mechanism of the indirect effects of pollinator-florivore interaction on plant fitness (Adler et al., 2001).

5. CONCLUSION

Our results show that predators have no cascading effect on plant fitness in *Cirisium arvense*. The results from the generative simulation model show that our design is efficient in detecting very small effect sizes for treatment or site differences. Thus, if we assume that our generative model reflects well the processes in this system, we have good evidence that there is no predator cascading effect in this study system. Rather, we found an indirect effect of pollinators on florivore fitness. Future studies could focus on the drivers of this indirect effect, for instance, biotic factors such as floral traits, or other plant traits. The abiotic environment or the land-use characteristics could also drive these interactions. Our methodology can be extended to study other plant species, and plant communities, to understand how the pattern of indirect effects of pollinators on herbivores, and vice-versa, change with study systems and regions.

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SUPPLEMENTARY MATERIAL

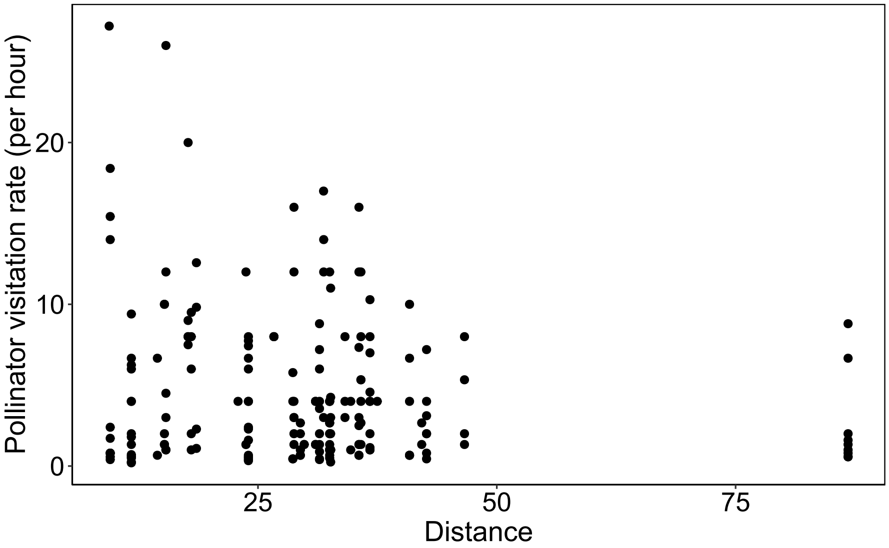


Figure S1: Pollinator visitation rate per hour to distance from male plants measured in meters. Each point represents one plant.

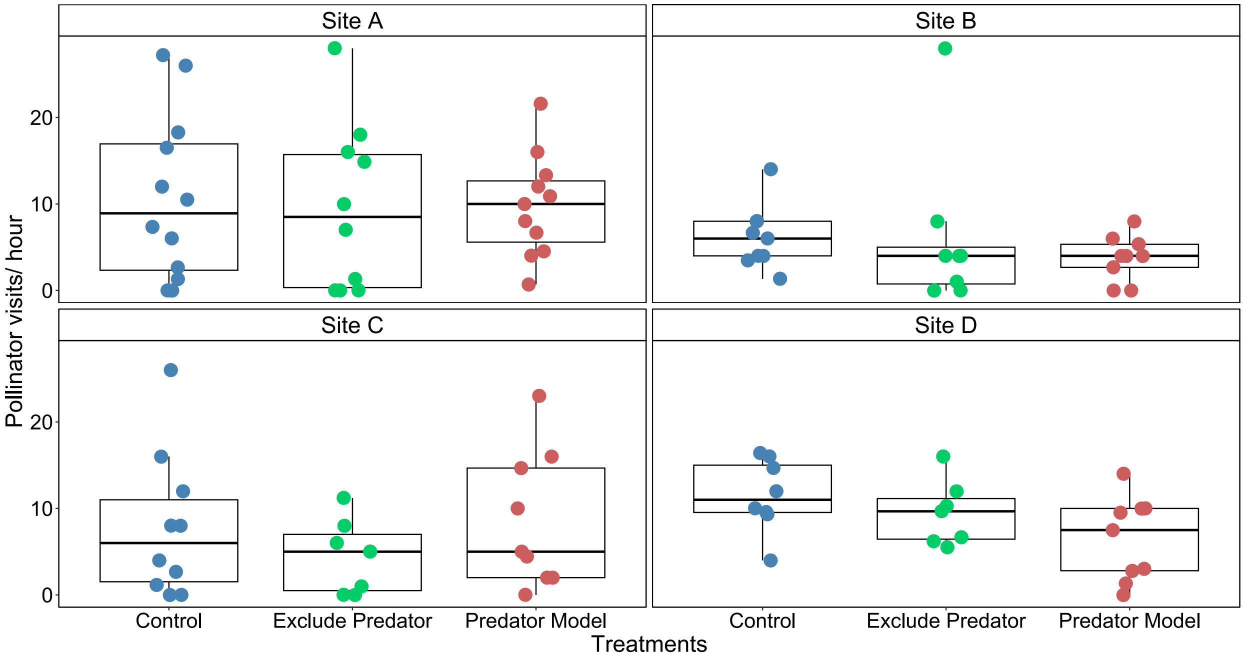


Figure S2: Pollinator visitation rate per hour for treatment control, exclude predator, and predator model separated by sites

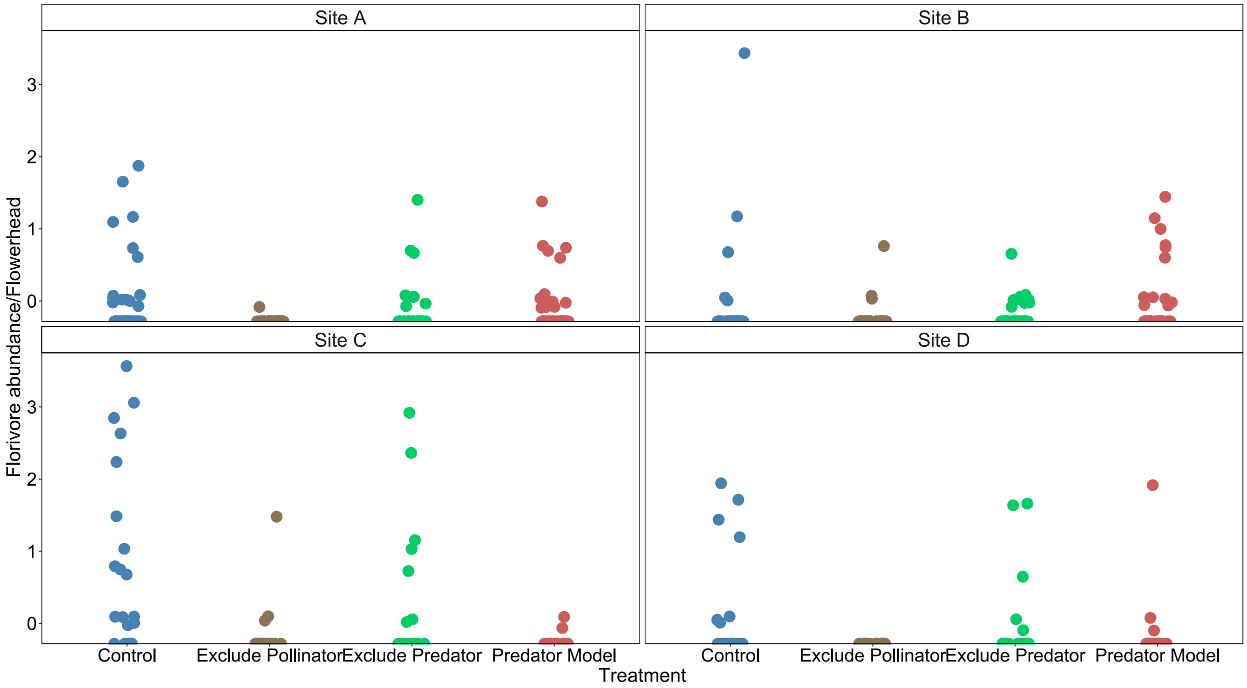


Figure S3: Florivore abundance per flowerhead for treatment control, pollinator exclusion, predator exclusion, and predator model separated by sites. The florivore abundance on the y-axis is plotted on a log scale

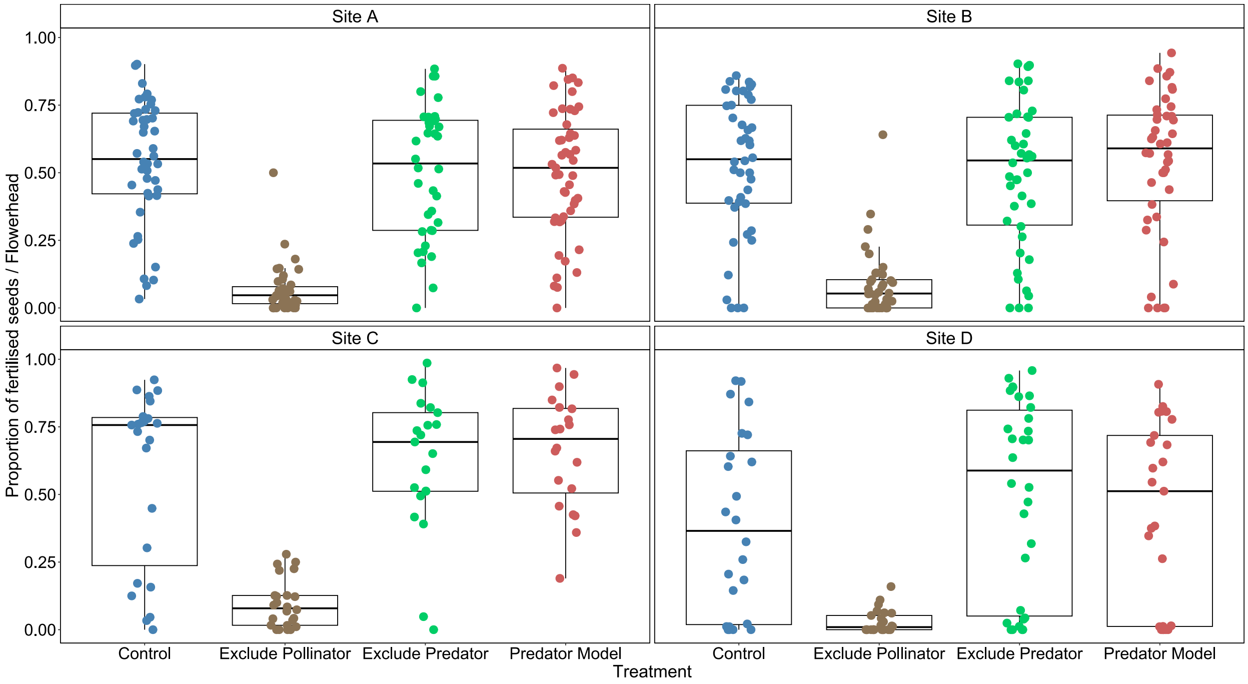


Figure S4: Proportion of fertilised seeds per flowerhead for treatment control, pollinator exclusion, predator exclusion, and predator model, separated by sites.

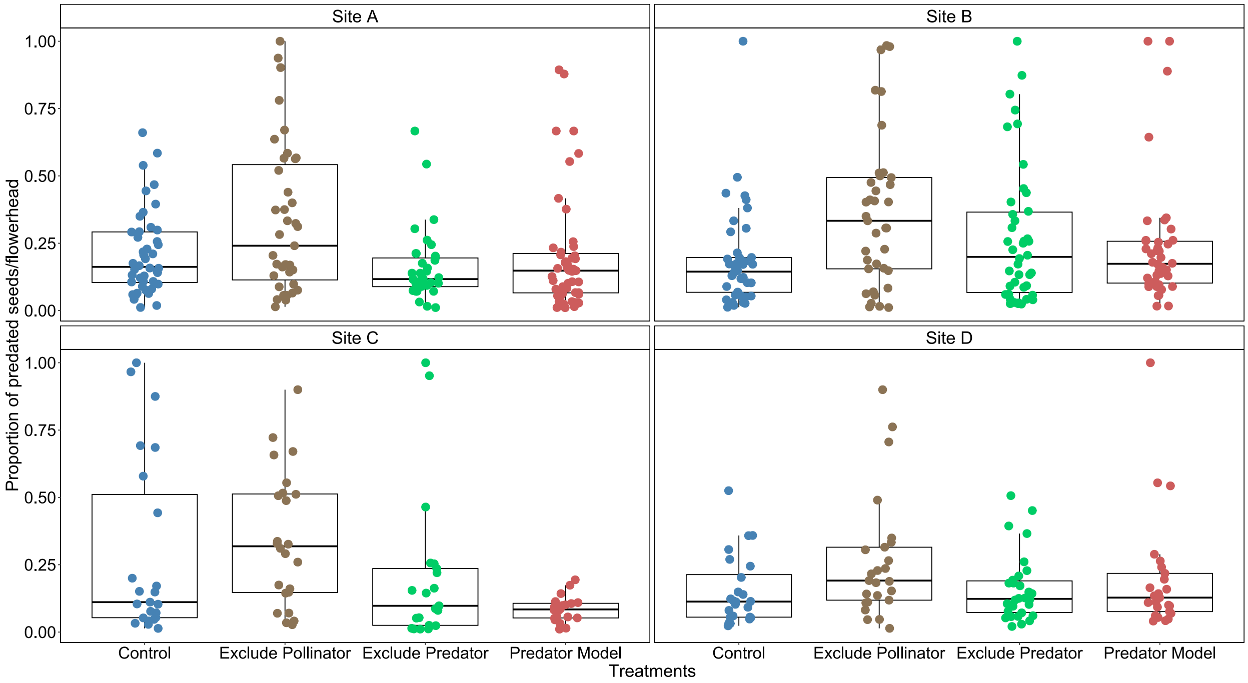


Figure S5: Proportion of predated seeds per flowerhead for treatment control, pollinator exclusion, predator exclusion, and predator model, separated by sites.

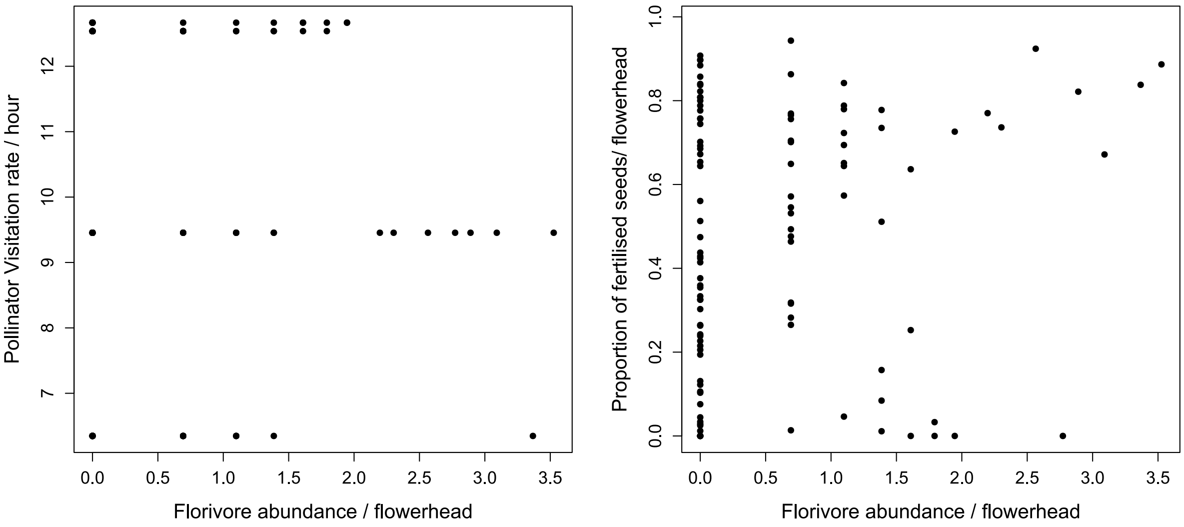


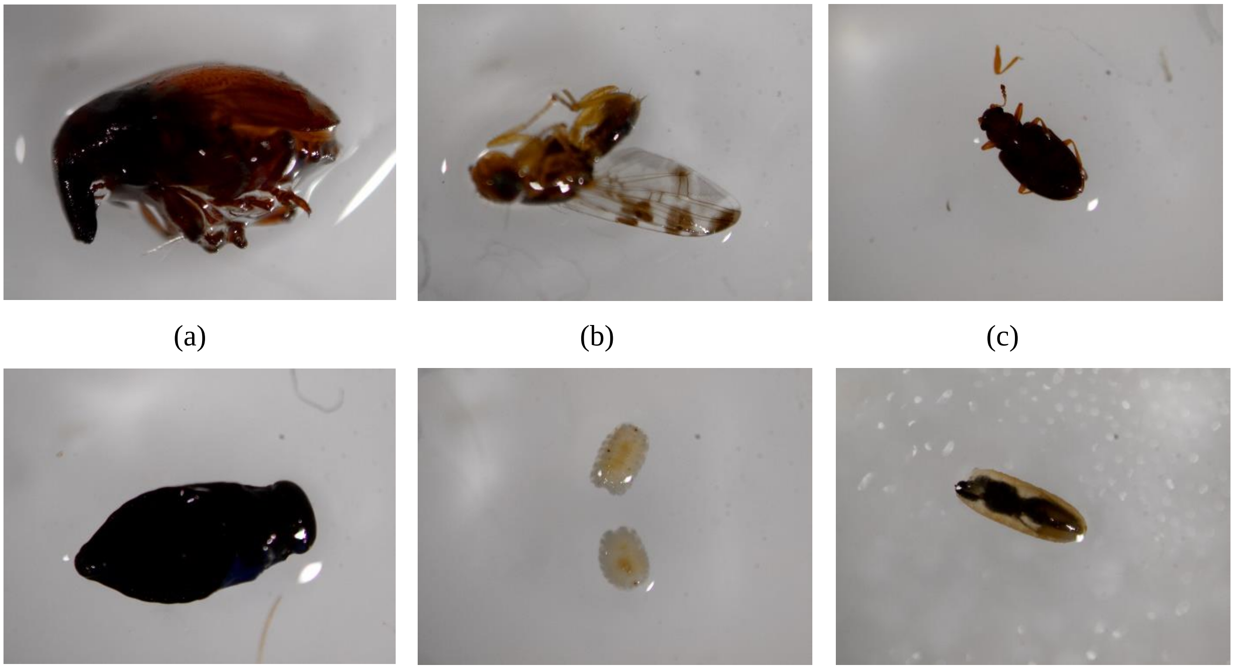
Figure S6: Pollinator visitation rate per hour vs. florivore abundance/flowerhead (a) Proportion of fertilised seeds per flowerhead vs. florivore abundance per flowerhead (b).

(a) (b)

Figure S7: Treatments established on *Cirsium arvense* populations in four sites. Predator model treatment (T4): The actual predators were removed and the surroundings were cleared of long grasses and plants. An artificial predator model of a sedentary arthropod predator (painted in green) was placed on plants (a). Pollinator exclusion treatment (T2): several flowerheads in a plant individual were covered with a pollinator bag. A wire mesh was attached to each pollinator bag to ensure that the bunch of flowerheads did not touch the bag (b).

(a) (b)

Figure S8: Experiment with all four treatments established on *Cirsium arvense* populations in sites. The experiment was established in Site D. The plants were arranged longitudinally along the edge of a rapeseed field. The male plants (not visible in the picture) were located across the dirt path at this site (a). Experiment established in Site A. The female plants were scattered around in a patch of an old abandoned field. The male plants (not visible in the picture) were located along the edges of the patch (b).



(a) (b) (c)

(d) (e) (f)

Figure S9: Florivore and parasitoid samples collected from all four sites. Curculionidae (a), Tephritidae (b), Coleoptera (c), Platygastridae (d), Cecidomyiidae larvae (e), and Hymenoptera (parasitoid) (f)

Table S1: Number of hours of pollinator observations in the four sites for the three treatments.

Site	Control	Exclude Predator	Predator Model
Site A	3	2.5	2.75
Site B	2.25	2	2.25
Site C	2.5	1.75	2.25
Site D	2	1.75	2.25

Table S2: Number of flowerheads collected in the four sites for the four treatments.

Site	Control	Exclude pollinator	Exclude Predator	Predator Model
Site A	51	43	45	55
Site B	50	40	46	43
Site C	31	27	30	27
Site D	30	31	33	27

Table S3: Number of visited flowerheads by different pollinator families separated by three treatments, control, predator exclusion, and predator model in the four sites

Site	Treatments	Family	Visited flowerheads
Site A	Control	Apidae	169
Site A	Exclude Predator	Apidae	102
Site A	Predator Model	Apidae	111
Site A	Control	Calliphoridae	1
Site A	Exclude Predator	Calliphoridae	1
Site A	Control	Empididae	3
Site A	Exclude Predator	Empididae	1
Site A	Predator Model	Megachilidae	1
Site A	Exclude Predator	Pieridae	2
Site A	Exclude Predator	Sarcophagidae	2
Site A	Predator Model	Sarcophagidae	2
Site A	Control	Scarabaeidae	2
Site A	Predator Model	Scarabaeidae	1
Site A	Control	Sciaridae	1
Site A	Control	Syrphidae	33
Site A	Exclude Predator	Syrphidae	16
Site A	Predator Model	Syrphidae	12
Site B	Control	Andrenidae	1
Site B	Predator Model	Andrenidae	1
Site B	Control	Apidae	33
Site B	Exclude Predator	Apidae	9
Site B	Predator Model	Apidae	9
Site B	Control	Calliphoridae	1
Site B	Control	Cantharidae	4
Site B	Exclude Predator	Cantharidae	1
Site B	Predator Model	Cantharidae	5
Site B	Control	Megachilidae	1
Site B	Predator Model	Nitidulidae	1

Site B	Control	Sarcophagidae	6
Site B	Control	Syrphidae	27
Site B	Exclude Predator	Syrphidae	8
Site B	Predator Model	Syrphidae	6
Site C	Control	Andrenidae	1
Site C	Control	Apidae	66
Site C	Exclude Predator	Apidae	16
Site C	Predator Model	Apidae	42
Site C	Exclude Predator	Asillidae	2
Site C	Predator Model	Asillidae	5
Site C	Control	Cantharidae	3
Site C	Exclude Predator	Empididae	1
Site C	Predator Model	Empididae	1
Site C	Control	Nitidulidae	1
Site C	Exclude Predator	Nitidulidae	1
Site C	Control	Sarcophagidae	23
Site C	Predator Model	Sarcophagidae	7
Site C	Control	Scarabaeidae	1
Site C	Predator Model	Scarabaeidae	1
Site C	Control	Syrphidae	9
Site C	Exclude Predator	Syrphidae	5
Site C	Predator Model	Syrphidae	6
Site D	Control	Apidae	42
Site D	Exclude Predator	Apidae	30
Site D	Predator Model	Apidae	38
Site D	Predator Model	Curculionidae	1
Site D	Exclude Predator	Nitidulidae	2
Site D	Exclude Predator	Nymphalidae	1
Site D	Control	Pieridae	3
Site D	Exclude Predator	Pieridae	3
Site D	Predator Model	Pieridae	2

Site D	Control	Sarcophagidae	2
Site D	Exclude Predator	Sarcophagidae	1
Site D	Exclude Predator	Scarabaeidae	1
Site D	Predator Model	Scarabaeidae	1
Site D	Control	Sciaridae	1
Site D	Control	Syrphidae	46
Site D	Exclude Predator	Syrphidae	53
Site D	Predator Model	Syrphidae	58

Table S4: Number of florivores for different florivore families separated by four treatments, control, predator exclusion, pollinator exclusion, and predator model in the four sites

Site	**Treatments**	**Family**	**Number**
Site A	Control	Latridiidae	1
Site A	Exclude Pollinator	Latridiidae	1
Site A	Exclude Predator	Latridiidae	1
Site A	Control	Others (Coleoptera)	2
Site A	Exclude Pollinator	Others (Coleoptera)	1
Site A	Exclude Predator	Others (Coleoptera)	1
Site A	Control	Cecidomyiidae	30
Site A	Exclude Pollinator	Cecidomyiidae	4
Site A	Exclude Predator	Cecidomyiidae	19
Site A	Predator Model	Cecidomyiidae	25
Site A	Exclude Predator	Syrphidae	1
Site A	Control	Tephritidae	1
Site A	Exclude Predator	Tephritidae	1
Site A	Predator Model	Tephritidae	2
Site A	Control	Auchenorhyncha	1
Site A	Exclude Pollinator	Others (Hemiptera)	1
Site A	Exclude Predator	Others (Hymenoptera)	1
Site A	Predator Model	Platygastridae	2

Site B	Predator Model	Curculionidae	1
Site B	Control	Cecidomyiidae	77
Site B	Exclude Pollinator	Cecidomyiidae	7
Site B	Exclude Predator	Cecidomyiidae	19
Site B	Predator Model	Cecidomyiidae	14
Site B	Control	Syrphidae	6
Site B	Exclude Predator	Syrphidae	5
Site B	Control	Tephritidae	2
Site B	Exclude Pollinator	Tephritidae	2
Site B	Predator Model	Tephritidae	3
Site B	Control	Nematocera	1
Site B	Exclude Pollinator	Nematocera	1
Site B	Control	Hymenoptera	1
Site B	Control	Platygastridae	1
Site B	Exclude Predator	Platygastridae	2
Site B	Predator Model	Platygastridae	1
Site C	Control	Curculionidae	2
Site C	Control	Cecidomyiidae	92
Site C	Exclude Pollinator	Cecidomyiidae	6
Site C	Exclude Predator	Cecidomyiidae	64
Site C	Predator Model	Cecidomyiidae	39
Site C	Control	Syrphidae	2
Site C	Exclude Predator	Syrphidae	2
Site C	Control	Tephritidae	1
Site C	Exclude Predator	Tephritidae	1
Site C	Control	Platygastridae	22
Site C	Exclude Predator	Platygastridae	1
Site C	Predator Model	Thysanoptera	1
Site D	Control	Cecidomyiidae	12
Site D	Exclude Predator	Cecidomyiidae	14
Site D	Predator Model	Cecidomyiidae	15

Site D	Control	Syrphidae	6
Site D	Predator Model	Tephritidae	1
Site D	Control	Platygastridae	1
Site D	Exclude Predator	Platygastridae	1
Site D	Predator Model	Platygastridae	1

Table S5: Models to evaluate the drivers of pollinator visitation rate per plant per hour, dAIC, and df values corresponding to each model. These models are tested using Generalized Linear Mixed Effect (GLMM) models with site, treatments, distance from male plants, site pollinator richness index (Chao 1 index) as fixed effects and block as a random effect. We tested 13 different models and selected the best-fitting model using AIC-based model selection. Null model is the most parsimonious model in this case.

S.No.	Model	dAIC	df
1.	**Visitation rate ~ 1 + 1|Block**	**4.0**	**3**
2.	Visitation rate ~ Distance + 1|Block	0.0	4
3.	Visitation rate ~ Richness index$_{\text{pollinator}}$ + 1|Block	1.1	4
4.	Visitation rate ~ Richness index$_{\text{pollinator}}$ + Distance + 1|Block	0.1	5
5.	Visitation rate ~ Treatment + 1|Block	6.8	5
6.	Visitation rate ~ Site + 1|Block	3.5	6
7.	Visitation rate ~ Site + Richness index$_{\text{pollinator}}$ + 1|Block	3.5	6
8.	Visitation rate ~ Treatment + Richness index$_{\text{pollinator}}$ + 1|Block	4.1	6
9.	Visitation rate ~ Treatment+ Distance + 1|Block	4.1	6
10.	Visitation rate ~ Site + Distance + 1|Block	2.8	7
11.	Visitation rate ~ Site + Treatment + 1|Block	6.6	8
12.	Visitation rate ~ Site + Treatment+ Distance + 1|Block	5.7	9

13.	Visitation rate ~ Site + Treatment+ Distance + Richness index$_{pollinator}$ + 1|Block	5.7	9

Table S6: Models to evaluate the drivers of florivore abundance per flowerhead, dAIC, and df values corresponding to each model. These models are tested using Generalized Linear Mixed Effect (GLMM) models with negative binomial family. Site, treatments, site florivore richness index (Chao 1 index) are used as fixed effects. Block, and individual plants are used as random effects. Insects in all the models represent florivore abundance per flowerhead. We tested seven different models and selected the best-fitting model using AIC-based model selection. Model with treatments is the best-fitting model in this case.

S.No.	**Model**	**dAIC**	**df**
1.	**Insects ~ 1 + 1|Block + 1|Individual**	**32.2**	**4**
2.	Insects ~ Richness Index$_{florivore}$ + 1|Block + 1|Individual	33.7	5
3.	Insects ~ Treatment + 1|Block + 1|Individual	5.9	7
4.	Insects ~ Site + 1|Block + 1|Individual	30.9	7
5.	Insects ~ Site + Richness Index$_{florivore}$ + 1|Block + 1|Individual	30.9	7
6.	Insects ~ Treatment + Richness Index$_{florivore}$ + 1|Block + 1|Individual	6.8	8
7.	Insects ~ Site + Treatment + 1|Block + 1|Individual	0.0	10

Table S7: Post-hoc analysis of models for florivore abundance. Contrasts between treatments, corresponding odds ratio, and p values. A higher odds ratio represents higher florivore abundance per flowerhead for the treatment in the numerator. This result indicates that the florivore abundance is not different between control, predator exclusion, and predator model treatments. However, the florivore abundance in pollinator exclusion treatment is significantly lower than in other treatments.

Contrasts	**Ratio**	**SE**	**p.value**
Control / Exclude Pollinator	13.345	6.047	**< 0.0001**
Control / Exclude Predator	2.094	0.760	0.1750
Control / Predator Model	2.521	0.927	0.0581

Exclude Pollinator / Exclude predator	0.152	0.074	**0.0005**
Exclude Pollinator / Predator Model	0.189	0.089	**0.0024**
Exclude Predator / Predator Model	1.204	0.448	0.9596

Table S8: Models to evaluate the odds of seed predation, dAIC, and df values corresponding to each model. These models are tested using Generalized Linear Mixed Effect (GLMM) models with a binomial family. Site, treatments, site florivore richness index (Chao 1 index), and florivore abundance per florwerhead (Insects) are used as fixed effects. Block, individual plants, and individual flowerheads are used as random effects. The models evaluate probability of seed predation on a logit scale. We tested thirteen different models and selected the best-fitting model using AIC-based model selection. The model with treatments is the most parsimonious in this case.

S.No.	Model	dAIC	df
1.	**logit (seed predation) ~ 1 + 1|Block + 1|Individual + 1|Flowerhead**	**13.0**	**4**
2.	logit (seed predation) ~ Insects + 1|Block + 1|Individual + 1|Flowerhead	12.2	5
3.	logit (seed predation) ~ Richness Index$_{\text{florivore}}$ + 1|Block + 1|Individual + 1|Flowerhead	14.9	5
4.	logit (seed predation) ~ Site + 1|Block + 1|Individual + 1|Flowerhead	14.1	7
5.	logit (seed predation) ~ Treatment + 1|Block + 1|Individual + 1|Flowerhead	0.0	7
6.	logit (seed predation) ~ Site + Richness Index$_{\text{florivore}}$ + 1|Block + 1|Individual + 1|Flowerhead	14.1	7
7.	logit (seed predation) ~ Site + 1|Block + 1|Individual + 1|Flowerhead	14.1	7
8.	logit (seed predation) ~ Treatment + Insects + 1|Block + 1|Individual + 1|Flowerhead	0.3	8

9.	logit (seed predation) ~ Site + Insects + 1|Block + 1|Individual + 1|Flowerhead	13.6	8
10.	logit (seed predation) ~ Treatment + Richness Index$_{\text{florivore}}$ + 1|Block + 1|Individual + 1|Flowerhead	1.8	8
11.	logit (seed predation) ~ Treatment + Site + 1|Block + 1|Individual + 1|Flowerhead	0.1	10
12.	logit (seed predation) ~ Treatment + Site + Insects + 1|Block + 1|Individual + 1|Flowerhead	0.7	11
13.	logit (seed predation) ~ Treatment + Site + Insects + Richness Index$_{\text{florivore}}$ 1|Block + 1|Individual + 1|Flowerhead	0.7	11

Table S9: Post-hoc analysis of models for seed predation probability. Contrasts between treatments, corresponding odds ratio, and p values. A higher odds ratio represents higher florivore abundance per flowerhead for the treatment in the numerator. This result indicates that the seed predation probability is not different between control, predator exclusion, and predator model treatments. However, the seed predation probability in pollinator exclusion treatment is significantly higher than in other treatments.

Contrasts	Ratio	SE	p.value
Control / Exclude Pollinator	0.513	0.111	**0.01**
Control / Exclude Predator	1.150	0.250	0.9173
Control / Predator Model	1.152	0.251	0.9152
Exclude Pollinator / Exclude predator	2.244	0.483	**0.0010**
Exclude Pollinator / Predator Model	2.248	0.485	**0.0010**
Exclude Predator / Predator Model	1.002	0.217	1.0000

Table S10: Models to evaluate the odds of seed fertilisation, dAIC, and df values corresponding to each model. These models are tested using Generalized Linear Mixed Effect (GLMM) models with a binomial family. Site, treatments, site pollinator richness index, site florivore richness index (Chao 1 index), distance, and pollinator visitation rate per hour per plant are used as fixed effects. Block, individual plants, and individual flowerheads are used as random effects. The models evaluate probability of seed fertilisation on a logit scale. We tested twenty different models and selected the best-fitting model using AIC-based model selection. Null model is most parsimonious in this case.

S.No.	Model	dAIC	df
1.	**logit (seed fertilisation) ~ 1 + 1|Block +1|Individual + 1|Flowerhead**	**0**	**4**
2.	logit (seed fertilisation) ~ Distance + 1|Block +1|Individual + 1|Flowerhead	1.1	5
3.	logit (seed fertilisation) ~ Richness Index$_{pollinator}$ + + 1|Block +1|Individual + 1|Flowerhead	1.3	5
4.	logit (seed fertilisation) ~ Richness Index$_{florivore}$ + 1|Block +1|Individual + 1|Flowerhead	2.0	5
5.	logit (seed fertilisation) ~ Visitation rate + 1|Block +1|Individual + 1|Flowerhead	2.0	5
6.	logit (seed fertilisation) ~ Visitation rate + Richness Index$_{pollinator}$ + + 1|Block +1|Individual + 1|Flowerhead	1.5	6
7.	logit (seed fertilisation) ~ Distance + Richness Index$_{pollinator}$ + 1|Block +1|Individual + 1|Flowerhead	1.6	6
8.	logit (seed fertilisation) ~ Distance + Richness Index$_{florivore}$ + 1|Block +1|Individual + 1|Flowerhead	1.8	6
9.	logit (seed fertilisation) ~ Treatment + 1|Block +1|Individual + 1|Flowerhead	3.5	6
10.	logit (seed fertilisation) ~ Visitation rate + Richness Index$_{florivore}$ + 1|Block +1|Individual + 1|Flowerhead	4.1	6
11.	logit (seed fertilisation) ~ Site + Richness Index$_{florivore}$ + 1|Block +1|Individual + 1|Flowerhead	3.2	7

12.	logit (seed fertilisation) ~ Site + 1|Block +1|Individual + 1|Flowerhead	3.2	7
13.	logit (seed fertilisation) ~ Site + Richness Index$_{pollinator}$ + 1|Block +1|Individual + 1|Flowerhead	3.2	7
14.	logit (seed fertilisation) ~ Site + Visitation rate + 1|Block +1|Individual + 1|Flowerhead	3.2	7
15.	logit (seed fertilisation) ~ Treatment + Distance + 1|Block +1|Individual + 1|Flowerhead	4.7	7
16.	logit (seed fertilisation) ~ Treatment + Richness Index$_{pollinator}$ + 1|Block +1|Individual + 1|Flowerhead	4.8	7
17.	logit (seed fertilisation) ~ Treatment + Richness Index$_{florivore}$ + 1|Block +1|Individual + 1|Flowerhead	5.6	7
18.	logit (seed fertilisation) ~ Treatment + Visitation rate + 1|Block +1|Individual + 1|Flowerhead	5.6	7
19.	logit (seed fertilisation) ~ Site + Distance + 1|Block +1|Individual + 1|Flowerhead	4.0	8
20.	logit (seed fertilisation) ~ Treatment + Site + 1|Block +1|Individual + 1|Flowerhead	6.8	9