**Title**

Climate warming increases insect-driven seed removal of two eliaosome-bearing invasive thistle species

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**Abstract**

Ants and other insects are often a source of localised secondary dispersal for wind-dispersed plants, and thus play an important ecological role in their spatial dynamics. However, there is limited information on how climate change will affect such dispersal processes. Here, we use field experiments to investigate how climate warming affects seed removal; this initiation of movement represents the first step in insect-driven secondary dispersal. We find that increased growing temperature influences seed attractiveness to insect dispersers in the invasive thistles *Carduus nutans* and *Carduus acanthoides*. Seeds from maternal plants grown at temperatures 0.6 °C higher than ambient are removed at higher rates by insect dispersers than their unwarmed counterparts. We also observe that seed elaiosomes in these two species play an important role in dispersal, as seeds without elaiosomes were significantly less likely to be removed by insects over the same time period. Significant interaction effects between elaiosome presence/absence and warming treatment were observed as well, though only for *C. acanthoides*, with the boost in seed removal from warming dampened when the elaiosome was present compared to when it was absent. These findings provide evidence that climate warming may alter aspects of the dispersal process such as seed removal, which could subsequently affect how plants disperse their seeds in a changing world.

**Introduction**

For sedentary organisms such as plants, seed dispersal is the only stage in the life cycle during which movement occurs. It is thus an essential component of plant spatial dynamics. While plant propagules are often dispersed by abiotic vectors such as wind, water, and gravity, they may also be dispersed biotically by organisms such as insects, birds, and mammals. Such dispersal of seeds by biotic vectors typically occurs not in isolation, but as part of a larger network of parallel and serial dispersal events, both primary and secondary in nature (Figure 1). Integration of these primary and secondary pathways and the vectors responsible into a total dispersal kernel (a probability distribution of how far seeds are dispersed when accounting for all possible dispersal vectors), can help us quantify how plant populations move or expand at a variety of different spatial scales (Nathan 2007). However, significant challenges in identifying these biotic dispersers and quantifying how they disperse seeds make it challenging to fully understand their role in seed dispersal, especially for smaller seeds that cannot be easily tracked through empirical means (Rogers *et al*. 2019).

For these smaller seeds, ants and other insects are often an overlooked source of localised secondary dispersal and are responsible for moving seeds after they have been dispersed by wind, water, gravity, or even another biotic dispersal vector (Vander Wall and Longland 2004). In particular, ant-mediated seed dispersal, or myrmecochory, has been shown to be a common occurrence in many plant species. In more than 80 plant families across the world, ant-dispersed plant species contain seeds that bear an elaiosome, a small structure on the seed achene that facilitates removal and dispersal by ants (Edwards *et al*. 2006). The elaiosome has a high lipid content and likely serves as a reward for the ant dispersing the seed (Brew *et al*. 1989; Hughes and Westoby 1990, 1992), as ants will typically take the seeds back to their nest and consume the elaiosome, then store the seed achene in a midden (Berg 1975; Culver and Beattie 1978). This dispersal can be advantageous for a variety of different reasons, including moving propagules to areas with better germination and growing conditions as well as less competition from other plants (Handel and Beattie 1990). Removal of a seed by ants is not guaranteed to result in successful dispersal of that particular seed, as seeds can be consumed or destroyed in the process; nonetheless, it is still a critical step in secondary dispersal because it represents the initiation of one or more secondary dispersal processes that move seeds further from the parent plant (Jongejans *et al*. 2015a).

Investigations of ant-mediated seed removal can be quite challenging, however. This challenge is further complicated by climate change, where changes such as increases in temperature may affect various characteristics that determine how seeds move or are moved. For example, changes in the number of seeds that plant can produce may result from elevated growing temperatures (Sato *et al*. 2006, Hedhly *et al*. 2009, Caignard *et al*. 2017) and increased CO2 levels (Edwards *et al*. 2001, Thürig *et al*. 2003, Way *et al.* 2010), thus affecting the number of seeds available for secondary dispersal. The dynamics of seed release can also be affected by increased growing temperatures, with some species more likely to release their seeds (Teller *et al*. 2016), also affecting the number of seeds available for secondary dispersal. Climate change may also cause shifts in the range or abundance of animal and insect species that remove seeds, as well as shifts in the traits that attract these species, with potential consequences for dispersal patterns (Ruxton and Schaefer 2012, Mokany *et al*. 2014, Sales *et al*. 2021). Even seed nutrient content has the potential to be affected by climate change and a variety of studies, especially on agricultural crops, have shown changes in nutrient allocations and chemical makeup due to factors such as increased temperatures or elevated CO2 (Thomas *et al*. 2003, Caldwell *et al*. 2005, Singh *et al*. 2013). However, there are still gaps in knowledge regarding how such climate-driven changes in seed shape, size, or nutrient allocations affect seed attractiveness to dispersers or how these dispersers move seeds. Addressing these gaps, though, must start with a better understanding of the underlying dispersal mechanisms themselves (Travis *et al*. 2013).

Given the role that ants and other insects play in seed dispersal, a better understanding of how they move seeds can help us better understand how plant species dispersed by these insects spread. Such an understanding starts with quantifying seed removal, as this constitutes the first step of secondary dispersal (Jongejans *et al*. 2015b). A commonly used and straightforward experimental setup to quantify seed removal by secondary dispersers consists of so-called “cafeterias” or “depots” (e.g. Xiao *et al*. 2006, Fischer and Türke 2016, Gurney *et al*. 2015, Linabury *et al*. 2019), where seeds are aggregated in various locations and the number of seeds removed is closely monitored. When paired with video recording or exclusion treatments such as cages, seed removal experiments allow researchers to observe the types of animals or insects removing seeds and approximate their contribution of rates to seed removal (Jongejans *et al*. 2015b, Griffiths *et al*. 2018). In the face of numerous challenges associated with quantifying secondary dispersal processes and documenting the ultimate fate of removed seeds (Vander Wall *et al*. 2005a), seed removal experiments provide an easily-manageable starting point for further inquiry into identifying secondary dispersal vectors and quantifying their contributions to the secondary dispersal process.

Here, we seek to develop a better understanding of how seeds are moved by secondary dispersal vectors, namely ants, by investigating removal of seed from two invasive, non-native thistle species. We also seek to explore to what extent the elaiosome, as well as increased growing temperatures of maternal plants, affects seed removal rates and overall attractiveness to insect dispersers. We thus investigate four questions. First, what are the approximate rates of seed removal via insect after a seed has experienced primary dispersal? Second, does the seed elaiosome affect the rate of removal? Third, are seeds from maternal plants that experienced increased growing temperatures any more or less likely to be removed by insects than their unwarmed counterparts? And fourth, does the effect of the seed elaiosome on removal rates change based on whether the maternal plant is warmed or unwarmed? These questions will allow us to understand how climate warming may affect the secondary dispersal of these important invasive species.

**Materials and Methods**

***Study Species***

*Carduus nutans* L. and *Carduus acanthoides* L. are invasive thistles in the Asteraceae family that, while native to Eurasia, can now be found across the world and are listed as noxious weeds across much of the United States (Skinner *et al.* 2000). These thistles are extremely unpalatable to grazing animals (Trumble and Kok 1982), are highly successful due to high germination rates and the large numbers of seeds they produce (Desrochers *et al*. 1988). They thrive in highly disturbed areas such as pastures, roadsides, railways, and utility corridors. Both *C. nutans* and *C. acanthoides* display monocarpic perennial reproductive behaviour; while typically biennial, have both been shown to increase annualism under increased growing temperatures (Keller and Shea 2021). Reproduction in both species occurs exclusively by seed and dispersal occurs primarily by wind, with dispersal facilitated by a lightweight pappus attached to the distal end of the seed achene. Seeds from both species also display nutrient-rich elaiosomes on the distal end of the achene that likely play a role in ant-driven dispersal (Pemberton and Irving 1990). Ants and other insects have been observed moving seeds in previous seed removal experiments (Jongejans *et al*. 2015b).

***Seed collection and preparation***

*C. nutans* and *C. acanthoides* from which seeds were harvested were grown under control and ambient warming treatments as part of an experiment in Drees and Shea (*in prep.*) to estimate the effects of warming on the distribution of flower heights; that publication discusses the methods and experimental setup in greater detail. Individuals of each species were grown in a grid-like arrangement, and a subset of individuals within each species were randomly assigned a fibreglass open-top chamber. These chambers, built to specifications of the International Tundra Experiment Manual (Molau and Mølgaard 1996), have been demonstrated to increase the ambient temperature by approximately 0.6 °C on average over a year (Zhang *et al*. 2011) without significantly affecting other possible growth factors such as soil moisture and snow depth. When plants set seed, mesh pollen bags were wrapped around the flower heads to ensure that seeds from these invasive thistles did not escape and contaminate other parts of the study area, as seeds from these invasive thistles may stay dormant for years.

Once individuals completed their life cycle, they were cut down, and bagged seed heads were collected from all individuals that produced five or more viable flowers. During this process, seed heads from all individuals of the same species were mixed together to randomise possible differences in seed size, shape, nutrient content, and any other factors that vary between individuals and may affect seed attractiveness to insects. The mesh pollen bags were then removed from the decapitated seed heads, and the heads were placed in a large paper bag and allowed to desiccate in a dry, low-light environment at approximately 24 °C. After approximately a month of desiccation, the bags were sealed and shaken vigorously to separate the seeds from the seed heads and pappi from the seeds; separated seeds were then sifted to remove spines, pappi, and other plant debris, and were then stored in an airtight container.

After seeds were successfully extracted from the flower heads, they were irradiated to ensure they could be freely dispersed by ants and other insects without spreading these invasive thistles and contaminating the study site. Seeds from *C. nutans* and *C. acanthoides* were irradiated using a 1000 KR dosage of gamma radiation; this dosage was demonstrated by Jongejans *et al*. (2015b) to prevent any germination without affecting attractiveness of seeds to insects.

Once seeds were irradiated, a subset of seeds from each of the four combinations of species and warming/ambient maternal treatment were assigned to an elaoisome removal treatment. Elaiosomes were removed by holding the seed with a pair of forceps, aligning the flattest side of the seed parallel to the workbench, and then using a teasing needle to press the elaiosome against the workbench until it was severed from the seed achene. In instances where the elaiosome did not cleanly separate, the teasing needle was used to gently dig out the remainder of the elaiosome from the achene. Seeds not receiving the elaiosome removal treatment were handled with forceps in the same manner mentioned above, for approximately the same duration as the seeds whose elaiosomes were removed; this was done so that possible differences in experimental outcomes could not be attributed to differences in the way the seeds were handled. After seeds had their elaiosomes removed and the control seeds were handled appropriately, seeds were again placed in airtight containers based on treatment type.

Seed depots were then built in a controlled environment before being transported to the field site, where they would serve as points where insects could easily access and remove seeds. All depots were constructed using 95 mm diameter petri dishes with a 15 cm tall edge and placing black sandpaper at the bottom to mimic the appearance of soil (Jongejans *et al*. 2015b). Sandpaper was glued to the bottom of the petri dish to prevent warping or movement of the paper due to wind, moisture, sunlight exposure, or insect activity. Each depot was arrayed with 25 seeds of a given treatment combination located near the centre of the petri dish, though spread out enough so that seeds were not piled on top of each other.

***Experimental setup***

The seed removal experiment was conducted at the Russell E. Larson Agricultural Research Farm in Rock Springs, Pennsylvania, which is the same study site at which the experiment from which the seeds were derived was conducted. Seed depots were arranged over an 8 x 10 grid in a small field approximately 50 m from the location of the experiment from which the seeds were collected, with individual seed depots spaced 1 m apart. Seeds within a particular seed depot represented one of eight unique combinations of species (*C. nutans* vs *C. acanthoides*), warming treatment on the maternal plant (warmed vs unwarmed, with “maternal plant warming” henceforth referred to as “warming” for brevity), and elaiosome treatment (present vs absent); each row of eight depots contained a randomised arrangement of these eight unique treatment combinations, for a total of 10 depots for each treatment combination across the 8 blocked rows. Before placing each depot, the small patch of vegetation below was trimmed and lightly compacted so that the petri dish could be easily fit in the grass canopy, reducing the chance of depots being disturbed by wind and ensuring easy access by insects.

Once all seed depots were placed, each seed depot was photographed from directly above by an observer at 30-minute intervals for the first 12 hours, from 09:00 to 21:00 on 07 September 2020; after 12 hours had elapsed, depots were then photographed once at 24 hours, again at 36 hours, and then once more at 48 hours. In instances where the sandpaper bottom of the dish was disturbed, it was fixed to the bottom of the petri dish again, and the remaining seeds gently placed back around the centre of the seed depot; such instances were extremely uncommon, however. No rain was observed over the duration of the experiment, and temperatures ranged between approximately 7°C and 27°C.

***Image processing***

All images were processed manually by counting the number of seeds for each seed depot at each recorded time; this was enabled by the contrast in colour between the seeds and the black sandpaper on the seed depots (Figures 2, 3). Seeds were counted as removed only if they were completely removed from the seed depot; thus, even in instances where seeds were disturbed or scattered across the depot, they were still marked as present. For each image, the number of seeds was scored twice by the same observer across independent sessions to ensure accuracy of the data.

***Statistical Analyses***

All statistical analyses were conducted in R version 4.2.2 (R Development Core Team 2009). To assess significance of the warming and elaiosome removal treatments, we used the **glmer** function from the package **lme4** version 1.1-31 (Bates *et al*. 2012) to fit a generalised linear mixed-effects model to the data, using a logit link and treating the number of seeds removed at a given time as a binomial response. Since between-species comparisons were not of interest, models were fit separately for each of the two species to facilitate ease of model interpretation, with elaiosome and warming treatments encoded as fixed effects; interactions between treatments were encoded as fixed effects as well, and treatment block was encoded as a random effect. To examine significance of the treatments at different points in time, the models were fit separately at the 6-, 12-, and 24-hour marks akin to similar analyses performed by Jongejans *et al*. (2015b), and conditioned on the number of seeds at the previous mark. Models were not fit at the 48-hour mark due to convergence issues, as many seed depots had zero seeds remaining at this point in the experiment. Survival curves from observational data across all timesteps were constructed, and two-sided Kolmogorov-Smirnov tests were used to determine whether survival curves for a given combination of species and one treatment differed between levels of the other treatment.

**Results**

Over the course of the 48 hours that seed removal was documented, both photographs and direct observation suggest that insects were responsible for seed removal; no birds or mammals were documented in the marked study area or were observed removing any seeds. Throughout the entire duration of the experiment, seed removal by ants was observed and made up the vast majority of direct observations of seed removal, with the ants dragging individual seeds across the flat surface of the seed depot (Appendix S1, Figure S1) before carrying the seed over the outer walls; these observations were especially common at the 30-minute observation periods during the daytime, but also occurred at several of the observation periods after sunset. Crickets were also observed on the seed depots (Appendix S1, Figure S2), though mostly at night, where they would consume the seeds on the depot rather than carry them away and consume elsewhere. Grasshoppers frequently visited the depots during the daytime hours but were not observed consuming or removing seeds, though they consumed or attempted to remove the adhesive tape used to mark the depots. Though a variety of other small insects were observed at the depots as well, they were not identified; none of them were observed removing any seeds.

Observed rates of seed removal from depots were high, though varied between treatment combinations. On average, 24.7% (3.6%) of seeds were removed after 6 hours, 48.4% (4.5%) after 12 hours, 85.5% (3.2%) after 24 hours, and 94.6% (1.5%) after 48 hours, where plus/minus terms indicate one standard error. Rates of removal for warmed *C. nutans* with elaiosomes were particularly high compared to the overall averages, with 55.6% (13.1%) of seeds were removed after 6 hours, 79.6% (9.2%) after 12 hours, 98.0% (2.0%) after 24 hours, and 98.4% (1.6%) after 48 hours. For both *C. nutans* and *C. acanthoides*, removal of seeds with no warming treatment and no elaiosomes lagged behind that of the other treatment combinations (Appendix S1, Figure S2); however, removal at the end of the experiment was still high, with 87.2% 5.4%) removed for *C. nutans* and 88.9% (7.1%) removed for *C. acanthoides*.

Mixed-effect model coefficient estimates from Table 1 suggest that both warming treatment and elaiosome removal impacted the proportion of seeds removed, with significant effects in most of the timesteps at which models were fit. Here, seeds from warmed maternal plants were usually more likely to have been removed than those from unwarmed maternal plants, and seeds with their elaiosomes intact were more likely to have been removed than those without their elaiosomes. This reinforces trends seen in survival curves plotted from the observed seed counts over time, where seeds with their elaiosomes intact were generally removed at a faster rate than seeds without their elaiosomes for all combinations of warming and species, except for warmed *C. acanthoides* (Figure 2). Survival curves from the seed count data also show that seeds from warmed maternal plants were removed at a faster rate than seeds from unwarmed maternal plants for all combinations of elaiosome presence/absence and species, except for *C. acanthoides* with intact elaiosomes (Figure 3). While survival curves for warming/elaiosome treatment combinations were generally similar between species (Appendix S1, Figure S5), explicit comparisons to demonstrate whether or not significant differences in seed removal rates between species exist were not made since such a comparison was outside the scope of this investigation.

Interactions between elaiosome and warming treatments were also observed in the mixed effects models. For *C. acanthoides*, significant negative interactions at all three time points suggest that presence of the elaiosome dampens the increase in seed removal facilitated by warming – that is, seeds with elaiosomes intact experience less of an increase in removal rates under warming than seeds where the elaiosome is removed (Table 1; refer to Appendix S1, Figure S4 for visualisation of marginal effects). This dampening effect on warming can be seen in Figure 2 as well, where juxtaposed survival curves clearly indicate lower seed removal for unwarmed *C. acanthoides* when the elaiosome is absent, but not necessarily when it is present. For *C. nutans*, evidence for interactions between elaiosome and warming treatments was not as clear; while there was a significant negative interaction similar to the one above at the 6-hour marks, positive interactions were observed at the 12- and 24-hour marks but were not significant.

Paragraph about seed mass.

**Discussion**

The prevalence in the ecological literature of studies regarding seed removal and secondary dispersal indicates a growing interest in identifying and quantifying the movement of seeds by organisms such as insects, animals, and humans. For example, the seeds of the mahaleb cherry *Prunus mahaleb* are ingested, moved, and defecated by a variety of mammals such as foxes and badgers as well as birds such as crows, thrushes, warblers, and robins (Herrera and Jordano 1981; Guitián *et al*. 1992; Jordano *et al*. 2007). Multiple dispersal pathways exist in that system, with seeds consumed directly from the tree by birds, and also consumed from the ground to undergo secondary dispersal by birds and mammals after primary dispersal via gravity. The acorns of various oak trees (*Quercus* sp.) are often dispersed and cached by a variety of rodents such as mice and squirrels (Jensen and Nielsen 1986; Vander Wall 2001; Gómez *et al*. 2008). Some species can even be dispersed by carnivores when they prey on frugivores or granivores with seeds in their digestive tract (Hämäläinen *et al*. 2017). Humans are also often involved in the process of dispersing plant propagules, as numerous plants can be dispersed when propagules attach to clothing (Wichmann *et al*. 2009; Ansong and Pickering 2014), are caught in air currents generated by passing vehicles (Von Der Lippe *et al*. 2013) or on the vehicles themselves (Veldman and Putz 2010; Taylor *et al*. 2012), as a contaminant of horticultural stock (Hodkinson and Thompson 1997), and as impurities in agricultural produce such as grains (Shimono and Konuma 2008; Michael *et al*. 2010; Wilson *et al*. 2016). Here, we provide evidence of ant-mediated seed removal and dispersal, or myrmecochory, and demonstrate the importance of the elaiosome in these processes.

Our results indicate that the rate of seed removal from the ground is very high, with approximately 85.5% of all seeds removed over the course of only 24 hours. These results are similar to observed removal rates of *C. nutans* and *C. acanthoides* in a similar study by Jongejans *et al*. (2015b), who demonstrate that up to 88% of seeds are removed in 24 hours when there is no impediment to the movement of insect dispersers. These extremely high rates of seed removal suggest that the majority of *C. nutans* and *C. acanthoides* seeds dispersed via wind also experience some sort of dispersal or consumption via insects or animals. Jongejans *et al*. (2015b) also demonstrate that rates of seed removal were significantly lower when ant access to seed depots was restricted, indicating that ants were responsible for the majority of seed removal events; our observations of a disproportionately large number of ant visits to seed depots further support these results.

We also demonstrate that the elaiosome plays an important role in attracting seed removers, as seeds of both species had significantly higher rates of removal when the elaiosome was left intact compared to when it was removed from the seed achene. Previous studies have suggested that these elaiosomes in *C. nutans* and *C. acanthoides* are involved in myrmecochory, attracting ants and playing an important role in how they disperse seeds (Pemberton and Irving 1990, Alba-Lynn and Henk 2010); the differences in seed removal rates between elaiosome-present and elaiosome-absent seeds in our study lend further support to evidence of myrmecochory in these two thistle species. In addition to removal of the elaiosome significantly impacting seed removal rates, we find that exposing the maternal plant to increased growing temperatures also increases the rate at which its seeds are removed by insect dispersers, with seeds from warmed plants experiencing higher rates of removal. Why this is the case is unclear, as the effects of increased growing temperature on factors such as seed size or nutrient content, which likely play a role in how attractive insect dispersers find seeds, are not clear for *C. nutans* and *C. acanthoides*. Additional analyses will be necessary to explore the link between increased growing temperatures and increased seed removal in order to highlight the underlying mechanisms responsible for these changes.

Our study is an important first step in quantifying secondary seed dispersal of thistle seeds by ants, but important challenges remain. While we can easily observe seeds being removed from a controlled experimental area, it is significantly more difficult to find where and how far dispersers move seeds after removing them from the seed depots. While not used in our study here, there exist a variety of techniques to assess where animal-dispersed seeds are moved, with each having its own set of strengths and weaknesses. Some seeds are large enough to store electronic devices and can be tracked using telemetry (e.g., Pons and Pausas 2007, Hirsch *et al*. 2012), and can thus be found without visual cues, which can be particularly useful in areas with dense vegetation or when seeds are cached underground. Seeds of various sizes can be painted or dyed with a fluorescent material or pigment that makes visual location easier (e.g. Levey and Sargent 2000, Reiter *et al*. 2006, Thomson 2007), but addition of pigment to seeds can significantly change their mass depending on the size of the seed and amount of pigment applied (Lemke *et al*. 2009), which could possibly alter the dynamics of seed removal or dispersal; such pigmentation could also possibly alter seed scent or taste. For ant-dispersed seeds such as *C. nutans* and *C. acanthoides*, fluorescent pigment may not be helpful in tracking seeds if they are taken underground to ant nests, where they are then no longer visible from the surface. Mapping emerging seedlings within the vicinity of a seed depot can also provide some information on secondary dispersal distances, though for invasive species such as *C. nutans* and *C. acanthoides* there are ethical concerns about allowing dispersal of viable seeds from an experiment into the environment (Jongejans *et al*. 2015b); hence our use of gamma irradiation to render the seeds unviable. Also, this method would only provide dispersal distance information for a fraction of dispersed seeds because it only examines the seeds that germinate, and would present the added challenge of ensuring that a particular seedling can be traced back to its source so that dispersal distance can be accurately quantified.

Given the difficulties with tracking small seeds such as those from *C. nutans* and *C. acanthoides*, while our study helps shed light on the first part of the secondary dispersal by quantifying seed removal after primary dispersal, it does not document the fate of the seeds after they have been removed. Accurately assessing the fate of seeds after they are removed can be quite challenging, as removed seeds are typically exposed to some mixture of dispersal and predation; for example, while some seeds are consumed in a destructive manner after removal or are otherwise rendered inviable, others can be left intact by ants and scatter-hoarding animals (Hulme 1998, Vander Wall *et al*. 2005a, Penn and Crist 2018). Often, such caching and burial can improve chances of germination by protecting seeds from predation after secondary dispersal, though seeds germinating after being cached at high densities may compete as seedlings (Hulme and Kollmann 2005). However, it is rarely known exactly what proportion of seeds are dispersed or cached without harm and what proportion experience predation. Despite this uncertainty in seed fate, many studies have conflated seed removal with predation, and often treat removed seeds as if they are consumed without substantial evidence to support this assumption (Vander Wall *et al*. 2005b). Such an assumption of seed fate may not only overestimate actual rates of seed predation, but also vastly underestimate secondary dispersal of seeds, as seed removal may not necessarily entail destruction but could instead be the first part of a series of secondary dispersal events (Vander Wall *et al*. 2005b). For *C. nutans* and *C. acanthoides*, the likely case is that some proportion of removed seeds are destroyed while others are dispersed, with a fraction of the dispersed seeds successfully germinating. For this reason, we have framed our results in terms of seed removal, since our uncertainty about the proportions of removed seeds that are consumed or dispersed limits us from making any claims about post-removal seed fate. Modelling of possible outcomes to assess their potential impacts would be a fruitful way to explore the importance of different fates (Drees and Shea, *in prep.*).

A better understanding of seed removal rates also has implications for spatial plant population dynamics. For example, we demonstrate that the overwhelming majority of *C. nutans* and *C. acanthoides* seeds were removed after only 48 hours, indicating that almost all seeds that undergo primary dispersal have the possibility to travel even further from the parent plant than they already have, provided they are not subject to predation. This may mean that our current estimates of how quickly invasive species like *C. nutans* and *C. acanthoides* spread might be conservative, depending on the extent of seed predation, as current estimates of spatial spread (e.g. Skarpaas and Shea 2007, Jongejans *et al*. 2011, Zhang *et al*. 2016, Teller *et al*. 2016) only account for primary dispersal. Secondary dispersal may also affect the germination and recruitment of new individuals by moving seeds to locations that may be more favourable to germination than their original location. As with primary dispersal, the additional dispersal distance from the parent plant may reduce density-dependent mortality associated with predation or parent-offspring competition (Janzen 1970, Connell 1971). Ultimately, while seed removal may result in the destruction of otherwise viable seeds, it also serves as the initiation of the secondary dispersal processes and plays a critical role in the movement of propagules, though further study is needed to fully understand its impacts on population spread.

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**Table 1.** Estimates (1 standard error), -scores, and -values for the coefficients of the mixed-effects GLM with logit link fit to the proportion of seeds removed at 6, 12, and 24 hours. The intercept (baseline) represents seeds from unwarmed maternal plants and without elaiosomes. Estimates must be transformed using the inverse logit function to yield the proportion of seeds removed (see the main text for results).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | ***C. nutans*** | | | |  | ***C. acanthoides*** | | |
|  | |  | |  |  |  |  |  |  |
| **6 Hours** | | **Estimate** | | ***z*** | ***p*** |  | **Estimate** | ***z*** | ***p*** |
| Intercept | | -3.394 0.515 | | -6.596 | <0.001 |  | -3.827 0.451 | -7.281 | <0.001 |
| Warming | | 2.273 0.321 | | 7.085 | <0.001 |  | 1.965 0.334 | 5.887 | <0.001 |
| Elaiosome | | 2.374 0.321 | | 7.398 | <0.001 |  | 1.775 0.336 | 5.290 | <0.001 |
| Warming:Elaiosome | | -0.924 0.385 | | -2.399 | 0.016 |  | -2.397 0.418 | -5.733 | <0.001 |
|  | |  | |  |  |  |  |  |  |
| **12 Hours** | |  | |  |  |  |  |  |  |
| Intercept | | -1.662 0.426 | | -3.904 | <0.001 |  | -3.295 0.552 | -5.974 | <0.001 |
| Warming | | -0.158 0.275 | | -0.574 | 0.566 |  | 3.263 0.403 | 8.090 | <0.001 |
| Elaiosome | | 1.670 0.263 | | 6.338 | <0.001 |  | 2.980 0.385 | 7.748 | <0.001 |
| Warming:Elaiosome | | 0.584 0.401 | | 1.455 | 0.146 |  | -3.374 0.456 | -7.402 | <0.001 |
|  | |  | |  |  |  |  |  |  |
| **24 Hours** | |  | |  |  |  |  |  |  |
| Intercept | | 0.191 0.438 | | 0.436 | 0.663 |  | 1.604 0.764 | 2.100 | 0.036 |
| Warming | | 0.835 0.324 | | 2.576 | 0.010 |  | 1.222 0.387 | 3.158 | 0.002 |
| Elaiosome | | 0.962 0.329 | | 2.922 | 0.003 |  | 1.428 0.398 | 3.584 | <0.001 |
| Warming:Elaiosome | | 0.363 0.659 | | 0.551 | 0.582 |  | -2.401 0.608 | -3.951 | <0.001 |

**Figure 1.** An illustration of possible primary, secondary, and higher-order dispersal pathways for a hypothetical system of biotic and abiotic dispersal vectors.

Diagram

Description automatically generated

**Figure 2.** Observed number of seeds remaining when elaiosome is present (E+) or absent (E-), conditioned on species (CN *Carduus nutans*, CA *Carduus acanthoides*) and warming treatment applied to the maternal plant. Error bars represent standard error on the mean; -values are obtained from Kolmogorov-Smirnov tests, with low -values indicating significant differences between the two survival curves.



**Figure 3.** Observed number of seeds remaining when maternal plant is warmed or unwarmed, conditioned on species (CN *Carduus nutans*, CA *Carduus acanthoides*) and elaiosome presence (E+ present, E- absent). Error bars represent standard error on the mean; -values are obtained from Kolmogorov-Smirnov tests, with low -values indicating significant differences between the two survival curves.

