**Journal name**

*Ecology*

**Manuscript type**

Article

**Title**

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

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**Data accessibility**

All novel data and code are on GitHub at <https://github.com/TrevorHD/InsectSeedRemoval> and will be archived on Zenodo upon acceptance for publication.

**Key Words**

Ants*, Carduus acanthoides*, *Carduus nutans*, climate change, dispersal, elaiosome, invasive species, myrmecochory, seed removal

**Abstract**

Ants and other insects are often a source of localized secondary dispersal for wind-dispersed plants and thus play an important ecological role in their spatial dynamics, but 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 above ambient were 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 over the same time period. Significant interactions between elaiosome presence/absence and warming treatment were also observed, 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 dispersal such as seed removal by secondary dispersers, with potential ramifications for dispersal in future climates since seed-bearing plants around the world may be subject to increased growing temperatures, and many of these plant species bear elaiosomes and experience seed dispersal by insects.

**Introduction**

For sedentary organisms such as plants, seed dispersal is the only stage in the life cycle during which movement of whole individuals occurs, and is thus an essential component of plant spatial dynamics. Plant propagules may be dispersed by abiotic vectors such as wind, water, and gravity, and/or by biotic vectors such as insects, birds, and mammals. Such dispersal of seeds by biotic vectors typically occurs as part of a larger network of parallel and serial dispersal events, both primary and secondary in nature (Figure 1). Integration of primary and secondary pathways and the vectors responsible into a total dispersal kernel, or probability distribution of how far seeds are dispersed when accounting for all possible dispersal vectors, can quantify how plant populations move or expand at a variety of 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 localized secondary dispersal following primary dispersal by wind, water, or other biotic vectors (Vander Wall and Longland 2004). In particular, ant-mediated seed dispersal, or myrmecochory, is common 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 distal end of 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 often 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 does not guarantee successful dispersal of that seed, as seeds can be consumed or destroyed in the process; nonetheless, it is a critical step in secondary dispersal because it initiates 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 seed production 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 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 various studies, especially on 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 climate-driven changes in seed shape, size, or nutrient allocations affect seed attractiveness to dispersers or how dispersers move seeds. Furthermore, for seeds with elaiosomes, it is largely unknown how the shape, size, or nutrient content of the elaiosomes themselves are affected.

Addressing these knowledge gaps 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, studying how they move seeds can facilitate a better understanding of how plant species spread. For secondary dispersal, such an understanding starts with quantifying seed removal, as this constitutes the first step of the secondary dispersal process (Jongejans *et al*. 2015b). Once we understand what factors affect seed removal, we can then address the mechanisms that generate the differences in seed traits to which ants respond, and also the effects of any differential removal on subsequent spatio-temporal plant population dynamics.

A common 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, LoPresti *et al*. 2022), where seeds are aggregated and the number of seeds removed is monitored. 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 challenges associated with documenting the ultimate fate of removed seeds (Vander Wall *et al*. 2005a), these experiments provide tractable starting points for identification of secondary dispersal vectors and quantification of their contributions to secondary dispersal.

Here, we seek to better understand secondary dispersal by investigating removal of seeds from two invasive, non-native thistle species, while noting that removal studies alone cannot distinguish ultimate seed fate (secondary dispersal or predation). We also seek to explore to what extent the elaiosome, as well as increased growing temperatures of maternal plants, affects seed removal rates and attractiveness to insect dispersers. Thus, we investigate four questions. First, at what rate are seeds removed by insects after a seed has experienced primary dispersal? Second, does the seed elaiosome significantly affect the rate of removal? Third, are seeds from maternal plants grown in increased 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 or not the maternal plant is warmed? These questions will allow us to better understand how climate warming may affect secondary dispersal of these important invasive species, with potential implications for the many other elaiosome-bearing plant species across the world that will be subjected to increased growing temperatures in future climates.

**Methods and materials**

***Study Species***

*Carduus nutans* L. and *Carduus acanthoides* L. are invasive thistles in the Asteraceae family that, while native to Eurasia, have spread across the world and are widely listed as noxious weeds in the United States (Skinner *et al.* 2000). The thistles are unpalatable to grazing animals (Trumble and Kok 1982), successful due to high seed production and germination (Desrochers *et al*. 1988), and thrive in disturbed areas such as pastures, roadsides, railways, and utility corridors. Both species are monocarpic perennials, but show increased 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 (Weiss 1908, Pemberton and Irving 1990, Mayer *et al*. 2005). 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 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, but essentials are noted here. Individual plants were grown in a grid-like arrangement, and a subset of individuals within each species were randomly assigned a fiberglass 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 growth-related factors such as soil moisture and snow depth. Mesh pollen bags were wrapped around senescing flower heads to ensure that seeds did not escape.

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 individuals of the same species and warming treatment were mixed together to randomize across possible differences in seed size, shape, nutrient content, and other factors that vary between individuals and may affect seed attractiveness to insects. The pollen bags were then removed from the seed heads, and the heads were placed in paper bags and allowed to desiccate in a dry, low-light environment at approximately 24 °C. After approximately a month of desiccation, seeds were separated from their pappi and flower heads, and stored in an airtight container. Seeds were then irradiated to ensure they could be freely dispersed by ants and other insects without further spreading these invasive thistles. Irradiation was performed with a 1000 KR dosage of gamma radiation; this dosage was demonstrated by Jongejans *et al*. (2015b) to prevent any germination without affecting attractiveness to insects (O’Hanlon *et al.* 2019).

Once seeds were irradiated, a subset of seeds from each of the four combinations of species and warming/ambient maternal treatment was 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 handling, seeds were again placed in airtight containers based on treatment type.

Seed depots were built in a controlled environment before deployment at the field site, where insects could easily access and remove seeds. All depots were constructed using petri dishes with a 95 mm diameter and 15 mm edge; the edge on the petri dishes reduces the likelihood that seeds are scattered by mechanisms other than direct seed removal, such as wind or vibrations from insect visitors. Black sandpaper was used to mimic the appearance of soil (Jongejans *et al*. 2015b), and 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 center of the petri dish, though spread out enough so that seeds were not piled on top of each other.

A portion of the irradiated seed was also weighed to examine whether warming of the maternal plant had any significant effects on seed mass. Because the mass of individual *C. nutans* and *C. acanthoides* seeds is so low (typically a few milligrams each), seeds were weighed in groups of 20 to ensure that measurement error due to contamination, scale oscillation, etc. would be minimal compared to the measured mass. For each of the four combinations of species and warming treatment, 30 groups of 20 seeds with their elaiosomes intact were weighed.

***Experimental setup***

The seed removal experiment was conducted at the Russell E. Larson Agricultural Research Farm at Rock Springs, in the in the Valley and Ridge physiographic province of Pennsylvania. The study site is a former pasture located at the base of a deciduous-forested mountain ridge, and has not been grazed for more than 20 years. Common ground cover species at this site include grasses such as *Arrhenatherum elatius*, *Dactylis glomerata*, *Elymus repens*, and dicots such as *Plantago lanceolata*, *Trifolium pratense*, and *Trifolium repens*, among others (Rauschert and Shea 2012).

Seed depots were arranged over an 8 x 10 grid in a small field approximately 50 m from where the seeds were collected, with the depots spaced 1 m apart. Each seed depot represented one of eight unique combinations from three fully crossed two-level factors of species (*C. nutans* vs *C. acanthoides*), warming treatment on the maternal plant (warmed vs unwarmed, with “warmed maternal plant” henceforth referred to as “warmed” for brevity), and elaiosome treatment (present vs absent); each row of eight depots contained a randomized 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 vegetation below was trimmed and lightly compacted so that the petri dish easily fit in the grass canopy, reducing the chance of depot disturbance by wind and ensuring easy access for insects.

Once all seed depots were placed, each 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, depots were photographed at 24 hours, 36 hours, and 48 hours. In the rare instances where the sandpaper bottom of the dish was disturbed, it was re-attached and the remaining seeds gently placed back around the center of the seed depot. No precipitation 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 color between the seeds and the black sandpaper on the seed depots. 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 2022). 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*. 2022) to fit a generalized 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 primary interest, models were fit separately for each of the two species to facilitate ease of model interpretation, with elaiosome and warming treatments, and their interaction, encoded as fixed effects; treatment block was encoded as a random effect. To examine significance of the treatments at different points in time, models were fit separately at the 6-, 12-, and 24-hour marks similar to analyses performed by Jongejans *et al*. (2015b), and conditioned on the number of seeds at the previous time 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. Survival curves from observational data across all timesteps were constructed, and two-sided Kolmogorov-Smirnov tests were used to test whether survival curves for a given combination of species and one treatment differed between levels of the other treatment. The GLMs we fit are conceptually similar to a survival model that allows for a time-varying hazard; results from fitting parametric survival models are qualitatively similar (Appendix S1).

**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 S2, 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 S2, Figure S2), though mostly at night, where they would consume the seeds on the depot rather than carry them away. 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, none of them were observed removing any seeds and they were not identified further.

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, 84.4% (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 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 S2, Figure S3); 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 for most of the timesteps at which models were fit. Here, seeds from warmed maternal plants were typically 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 also show that seeds from warmed maternal plants were removed faster 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 S2, Figure S4), explicit comparisons to demonstrate whether differences in seed removal rates between species exist were not made since such a comparison was not a focus 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 S2, Figure S5 for visualization of marginal effects). This dampening effect on warming can be seen in Figure 3 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.

For each species, there were significant differences in seed mass between the warmed and unwarmed treatment groups for both *C. nutans* (d.f. = 58, *t* = 4.506, *n* = 60, *p* < 0.001) and *C. acanthoides* (d.f. = 58, *t* = 2.832, *n* = 60, *p* = 0.006). A group of 20 warmed *C. nutans* seeds weighed on average 3.4 0.7 mg more than a group of 20 unwarmed seeds, where plus/minus terms indicate one standard error, representing an approximately 5.6% increase in mass. For *C. acanthoides*, the same number of warmed seeds weighed on average 1.4 0.5 mg more compared to the unwarmed seeds, representing an approximately 4.5% increase in mass.

**Discussion**

All else equal, there are a wide variety of factors in the dispersal process that can be affected by climate change. Here, we focus on quantifying rates of seed removal post-primary dispersal, including a novel investigation into the effects of increased maternal plant growing temperatures on seed removal rates. We provide evidence that seed removal, a critical first step in secondary and higher-order dispersal pathways for *C. nutans* and *C. acanthoides*, is affected by climate warming while also demonstrating the importance of the elaiosome in this process. Our results suggest that a majority of *C. nutans* and *C. acanthoides* seeds dispersed via wind may also experience secondary dispersal and/or consumption, and that ants are responsible for the majority of seed removal events. This process is rapid, with 84.4% of all seeds removed over the course of only 24 hours (see also Jongejans *et al*. 2015b). We further demonstrate that the elaiosome plays an important role in enhancing removal by ants, as seeds of both species had significantly higher removal rates when the elaiosome was left intact. This agrees with previous studies that suggested that *C. nutans* and *C. acanthoides* elaiosomes 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). Furthermore, we demonstrate that seeds from warmed maternal plants experienced higher rates of removal by insects. This result is especially interesting because it provides the starting point for a research agenda that can further investigate the specific changes in seed traits driving the increase in seed attractiveness to insect dispersers, any mechanisms driving such changes prior to seed removal, and the implications of increased seed removal for subsequent rates of population spread.

Thus, by demonstrating an effect of maternal plant warming on rates of seed removal by insects, our study is a necessary precursor to several additional lines of investigation that can better delineate the exact causes of increased seed attractiveness in *C. nutans* and *C. acanthoides*. One avenue of research would involve investigating which specific seed traits are affected by maternal plant warming. Though we demonstrate that warming increases average mass of seeds as a whole, it is unknown whether or not the mass of the elaiosome itself increases or if this increase is entirely confined to the seed achene. There may also be changes in nutrient content, size, or shape for the elaiosome and/or seed that could also affect attractiveness to insects, and further work could not only examine which of these seed traits that are changing, but may also help delineate the specific mechanisms during the seed development process that drive how warming affects these seed traits.

Follow-up studies can even shed more light on the seed removal events themselves, using improved recording methods to address in greater detail how insects interact with the seeds. For example, rather than fixed-interval snapshots of seed depots every 30 minutes, continuous video recording could be employed. Not only would such recordings provide a finer time scale to determine the exact times at which seeds were removed, but they would also permit counting the number of interactions, and potentially identification of the insect species involved in seed removal in different habitats.

Another avenue of research would involve examining post-removal seed-related factors besides physical traits and chemical composition. For example, does warming also affect germination rates or seed bank longevity, both of which have direct implications for rates of population growth and spread? Though we can easily observe seeds being removed from a controlled experimental area, it is difficult to find where and how far dispersers move seeds after removing them from seed depots – seeds successfully dispersed by ants can be cached underground and out of sight, and the small size of these seeds makes them difficult to track in general. To address this challenge, there exists a variety of techniques to assess where animal-dispersed seeds are moved, with each having its own set of strengths and weaknesses. Larger seeds can hold electronic devices and be tracked via 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 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 may also alter seed scent or taste. For ant-dispersed seeds such as *C. nutans* and *C. acanthoides*, pigment may not be useful if seeds are taken underground to ant nests and are 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 seeds that germinate, and presents 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 difficulty tracking small seeds such as those from *C. nutans* and *C. acanthoides*, while our study sheds light on the initiation of 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 seed fate after removal can be challenging, as removed seeds are typically exposed to some mixture of dispersal and predation. While some seeds are fully consumed or rendered unviable post-removal, others can be left intact by ants and scatter-hoarding animals (Hulme 1998, Vander Wall *et al*. 2005a, Penn and Crist 2018). Caching may protect seeds from further predation, however, the proportion of seeds dispersed or consumed is hard to assess. 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 may not only overestimate actual rates of seed predation, but also underestimate secondary dispersal of seeds, as seed removal may instead initiate 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, and we make no claims about post-removal seed fate. Modeling of possible outcomes to assess their potential impacts would be a fruitful way to explore the importance of different fates (Vellend *et al*. 2006).

A better understanding of seed removal rates thus has implications for spatial plant population dynamics. Given that most *C. nutans* and *C. acanthoides* seeds were removed after only 48 hours, almost all seeds already dispersed by wind may travel even further provided they are not subject to predation, and current estimates of how quickly these species spread (e.g., Skarpaas and Shea 2007, Jongejans *et al*. 2011, Zhang *et al*. 2011, Teller *et al*. 2016) might be conservative as they only account for primary dispersal by wind. Furthermore, the warming-induced increases in seed attractiveness to insect dispersers that we demonstrate here may increase rates of dispersal, thus increasing rates of population spread and increasing the burden on land managers trying to control these species in future climates. 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 thus plays a critical role in the movement of propagules. Though further research is needed, our study is a first step to fully understanding the potential for climate warming to increase secondary seed dispersal, and the resultant rate of population infilling and spread. Given that many plant families have elaiosome-bearing species and will be subject to increased growing temperatures, potential warming-induced shifts in secondary dispersal may be seen on a global scale.

**Acknowledgments**

The authors thank C.S. Bjorå, O. Pedersen, and I. Nordal for helpful discussions on relevant myrmecochory literature, C. Davison for assisting in gamma irradiation of thistle seeds at the Penn State Breazeale Reactor, and E. Hanks for advice on statistical analyses. J. Darlington, M. Dwyer, and other personnel at the Russell E. Larson Agricultural Research Center provided field logistical support. E. Hanks, L. Leites, and C. Lowry helped provide feedback on the manuscript. T.H.D. was supported by the Alumni Professor in the Biological Sciences endowment to K.S.

**Author Contributions**

T.H.D and K.S. designed the field experiment. T.H.D. conducted the field experiment, collected data, performed analyses, and led writing of the initial manuscript. T.H.D. and K.S. made contributions to subsequent versions of the manuscript.

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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 from the previous timestep that were removed.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | ***C. nutans* (*n* = 40)** | | |  | ***C. acanthoides* (*n* = 39)** | | |
|  |  |  |  |  |  |  |  |
| **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 Captions**

**Figure 1.** An illustration of primary, secondary, and higher-order dispersal pathways for a hypothetical system of biotic and abiotic dispersal vectors. Note that while this system is purely hypothetical and is not representative of all systems found in nature, dispersal networks can be as complex as those shown here.

**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.

A black background with arrows and lines

Description automatically generated

**Figure 1**



**Figure 2**



**Figure 3**