



# Native ants vary in their use of seeds from a recently introduced myrmecochorous exotic plant

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## Abstract

Myrmecochory is a common mutualism between ants and plants benefiting both partners: ants obtain a nutrient-rich food source, while plants enjoy a host of benefits ranging from enhanced dispersal to protected germination sites. However, this mutualism can be exploited by invasive myrmecochores, where native ants spread invasive plant seeds, possibly to the detriment of native plant assemblages. With the recent introduction of a potentially invasive myrmecochorous plant (*Thesium ramosum*) in Alberta, Canada, we tested ant interest in *T. ramosum*. To evaluate both general interest in *T. ramosum* as a food source, and preference for *T. ramosum* over other food sources, we collected colonies of four commonly occurring native *Formica* species and conducted seed removal trials and food preference trials. We then evaluated interest in and preference for *T. ramosum* seeds through assessing mean rate of seed removal and food item removal, total number of seeds and food items removed, and trends in seed and food item removal through time. We found that while all ant species tested showed interest in *T. ramosum*, interest level varied among species, and additional factors such as colony size and presence of host species in socially parasitic species influenced interest in *T. ramosum*. Considering native ant interest in *T. ramosum* as a food source, it seems plausible that *Formica* species may act as a dispersal vector for *T. ramosum*, potentially enhancing its invasiveness.

**Keywords** Formicidae · Myrmecochory · Invasiveness · Mutualism · Thesium

## Introduction

Myrmecochory, the ant-mediated dispersal of seeds, is a diverse and globally important mutualism (Beattie 1985; Hölldobler and Wilson 1990; Richardson et al. 2000; Bronstein et al. 2006; Bascompte and Jordano 2007; del Toro et al. 2012). Plants that have evolved myrmecochorous life habits produce a nutrient-rich structure on their seeds, the elaiosome, which acts as an attractant and reward for ants. Ant workers harvest the seeds and carry them back to their colonies, where the elaiosome is typically removed and fed

to workers and larvae. The seed is discarded into waste structures known as middens, either inside or close by the nest, where the nutrient-rich midden facilitates the germination of the seeds (Beattie 1985; Hölldobler and Wilson 1990). Myrmecochory is ubiquitous globally; it is estimated to have evolved over 100 times, and more than 11,000 species of plants are myrmecochores (Lengyel et al. 2009, 2010). Lineages that have evolved myrmecochory are more diverse than lineages without (Lengyel et al. 2009), suggesting that myrmecochory is both a powerful evolutionary adaptation and a driver of increased diversification.

Elaiosomes are typically lipid- and protein-rich; both lipids and proteins comprise an important part of ant diets and influence the production of reproductive castes (Bono and Heithaus 2002; Morales and Heithaus 1998; Clark and King 2012). However, the exact nature of the benefits of myrmecochory for plants are more complex and less clearly defined. There are multiple theories: (i) ant-mediated dispersal limits parental competition with young plants, and therefore increases the odds of newly germinated plants' survival; (ii) ants remove seeds that would otherwise be consumed wholesale by seed predators; (iii) seeds in ant

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mounds are more likely to survive fires than otherwise, which may be an adaptation in drier habitats; (iv) ant-mediated dispersal limits interspecific competition, as the ants remove the seeds to areas with fewer plants; and (v) ant mounds provide nutrient-rich microsites that facilitate germination (Beattie 1985; Hölldobler and Wilson 1990; Giladi 2006). These hypotheses are not mutually exclusive and evidence exists for multiple benefits. Xeric, dry habitats where wildfire is common in Australia and southern Africa support high biodiversity of myrmecochorous plants (Berg 1975; Bond and Slingsby 1983; Boyd 2001). Seeds germinate more rapidly and frequently in nutrient-rich ant mound microhabitats (Berg-Binder and Suarez 2012; Prior et al. 2014; Hanzawa et al. 1988; Gibson 1993); ants remove seeds away from parental plants at varying distances, but frequently far enough that they are not in competition with their parents (Andersen 1998; Gómez and Espadaler 2013; Higashi et al. 1989); and ant removal and burying of seeds has been shown to limit seed predation by small vertebrates (Smith et al. 1986, 1989; Higashi et al. 1989; Ohkawara and Higashi 1994). Considered together, these benefits imply that myrmecochory provides a significant advantage for plants that evolve this particular adaptation.

While myrmecochory is widely distributed, there are many areas where invasive myrmecochores have been introduced into an ecosystem. There are at least 47 species of introduced myrmecochores in the United States, with undoubtedly more uncounted (Pemberton and Delilah 1990). The effects of invasive plants on arthropod communities are highly variable and complex, but there is some evidence that invasive plants can negatively impact arthropod assemblages through competitive suppression of native plant species used as food sources (Herrera and Dudley 2003; Gratton and Denno 2006; Gerber et al. 2008). Conversely, invasive myrmecochorous plants could provide a benefit for ants, though they might damage other aspects of the ecosystem.

In the United States, native ants are known to forage and transport seeds from a wide variety of invasive plants, including *Cirsium arvense* and *Centaurea maculosa* (Alba-Lynn and Henk 2010; Berg-Binder and Suarez 2012; Jensen and Six 2006; Bossard 1991). In some circumstances, they forage preferentially on the invasive plant's seeds and ignore native plants, which contingent on the dispersing ant could enhance the invasive plant's spread (Jensen and Six 2006; Pearson et al. 2014). Such preferences may be due to a range of reasons, such as the sudden increase or high abundance of newly introduced plants, making them more easily accessible and apparent (Prior et al. 2015). This dynamic may also be enhanced by the character of the native ant assemblage; seed-collecting ant species display a range of myrmecochorous foraging behaviours, spanning collective harvesting and caching in the nest to individual foragers who remove the elaiosome and deposit seeds externally (Giladi 2006).

This behavioural variation influences dispersal quality, with caching behaviours associated with generally poor dispersal ability and elaiosome removal and external displacement associated with high dispersal quality (Giladi 2006). Dominance hierarchies in ant communities may also influence seed dispersal, with subordinate species typically displaying opportunistic individual foraging behaviour, perhaps to expand the diversity of food sources or avoid competitive interactions with other foragers (Ohkawara et al. 1996; Giladi 2004). Behavioural variation may also be linked to species trophic level: in a study on ant-seed interactions examining native ants and both invasive and native plants, the authors found that ant foraging type was a predictor of ant-seed interactions, with seed predators correlating with native plants and general omnivores correlating with invasive plants (Pirk and Lopez de Casenave 2017). Larger ants have also been recorded carrying a myrmecochorous plant's seeds back to their mounds while smaller ants stripped the elaiosome from the seed in place (Bossard 1991; Passos and Oliveira 2002). These findings suggest that an invasive myrmecochore's dispersal ability to some extent rests upon the ant species present.

Invasive myrmecochores benefit from these interactions as well, with increased rates of germination and seedling growth when associated with ants (Prior et al. 2014, 2015). In one study, invasive leafy spurge grew in greater concentrations on and around seed-dispersing native *Formica* mounds when compared to areas > 3 m away from the mound, suggesting that the seeds were benefitting from the nutrient-rich soil of the ant mounds (Berg-Binder and Suarez 2012). Ant-mediated dispersal of invasive plant seeds thus has the potential to enhance invasive plant spread, and more broadly, influence plant community dynamics in an area.

Considering the potential for ants to be significant dispersal vectors of invasive plants, we tested the preference of native ant species for invasive plant seeds in a newly emerged potential native ant-invasive plant mutualism in Alberta, Canada. We asked the following questions (1) do native ant species harvest *Thesium ramosum* seeds as a food resource? (2) Is there a difference in preference for *T. ramosum* seeds among ant species? (3) Do ant species prefer *T. ramosum* seeds to other food resources present in the park? To test these questions, we designed two sets of experiments: (1) seed removal trials, in which laboratory colonies of native ant species were offered *T. ramosum* seeds and their rate of harvest and removal was evaluated; and (2) food preference trials, in which these colonies were offered *T. ramosum* and other food items normally present in their habitat and their preferences were evaluated.

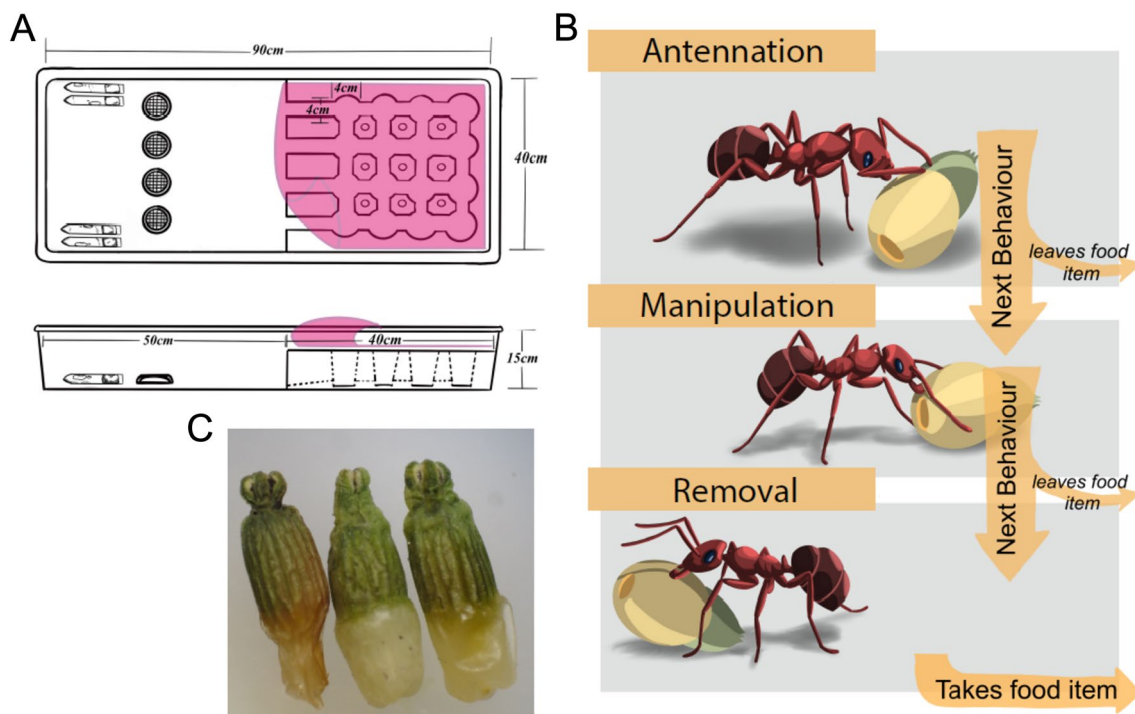
## Methods

### Study system

*Thesium ramosum* is an invasive plant native to south-western Asia that was first noticed in the study site (Fish Creek Provincial Park, Calgary, Alberta, Canada) in 2001, which also marked its first recorded appearance in Canada (Macdonald & Visser 2022). The genus *Thesium*, formerly included in Sanataleacea, was placed in its own family Thesiaceae by Nickrent et al. (2010) based on molecular and morphological evidence. *T. ramosum* is a hemiparasitic perennial herb which parasitizes other plant species using root nodules to leach nutrients from its hosts (Hendrych 1972). In a study conducted on forty plant species present in the park, *T. ramosum* was found to parasitize all of them (pers. comm. McLean, work in preparation). The effect of *T. ramosum* on the host plant is unknown, as is most of the plant's general biology, but the seeds of *T. ramosum* bear elaiosomes, suggesting that in its native range it is at least

partly reliant upon ants for dispersal (Fig. 1C). The plant flowers and seeds from late May until frost (September or October) in its introduced range in Canada (Macdonald & Visser 2022) but this may differ from its flowering and seeding period in its native range, which is unknown.

Alberta's native ant fauna is dominated by several genera: *Formica*, *Lasius*, *Camponotus*, and *Myrmica* (Glasier et al. 2013). These ant species are predominantly omnivorous or generalist predators; overall, there are few specialized granivorous ants in Canada and the northern United States (Wheeler and Wheeler 1988a, b; Glasier et al. 2013, 2016). However, *Formica* species opportunistically forage seeds from both native and introduced myrmecochorous plants (Berg-Binder and Suarez 2012; Jensen and Six 2006). In this experiment, we selected four native *Formica* species: *F. podzolica*, *F. argentea*, *F. obscuriventris*, and *F. aserva* to evaluate consumption and dispersal of *T. ramosum* seeds. Selection of these species was based on field observations of the species carrying *T. ramosum* seeds, and the extreme ecological dominance of these species in the park. *Formica* species in general form large colonies, typically polydomous



**Fig. 1** **A** Schematic of colony nest setup, indicating foraging arena (small circles denote location of Rodac plates during experiments, cylinders denote location of Falcon tubes filled with water); nest structure (red rectangle denotes transparent red film over nest to enable visual monitoring of within-structure behaviour); and dimensions of containers. **B** Schematic illustrating ant foraging behaviours measured in food item removal trials. Behaviours were assessed beginning with antennation, where the worker taps her antennae over the food item, to manipulation, where the worker manipulates the food item

with her mandibles, to finally removal, where the worker removes the food item to return to the colony. Arrows indicate the possible progression of each behavioural stage, where the worker may progress to the next stage of foraging, or is insufficiently interested in the food item and leaves it behind. **C** *Thesium ramosum* seeds showing white elaiosome; middle seed with fully ripe elaiosome and left and right seeds showing signs of desiccation. Each seed is ~5–6 mm long. Image by I.D. MacDonald, used with permission

and polygynous, and are often numerically and ecologically dominant where present (Debout et al. 2007; Romiguier et al. 2018). *F. aserva* and *F. obscuriventris* are typically larger-bodied though variable in body size, build thatched dome-shaped mounds, and may be more aggressive; while *F. argentea* and *F. podzolica* are smaller with minimal worker size variation, build low and broad dirt mounds, and are often less aggressive (Mackay and Mackay 2002).

Colonies were collected in early May through late June 2018. We collected four colonies per species for a total of 16 colonies. We only collected queens in four colonies, but collected brood (larvae and pupae) in 11 colonies, and in several colonies, winged reproductives were produced and later enclosed. Colonies ranged from 500 to 1000 workers. All lab colony details may be found in Table 1. In the laboratory, each of the 16 colonies were kept in separate large plastic 90×40×15 cm high containers: 40×40 cm of the plastic container was filled with plaster-of-Paris molded into a 4×4 grid of nest chambers connected by channels, while the remaining 50×40 cm of the container were left open as a foraging arena (Fig. 1A). The foraging arena is within the typical foraging range of *Formica* species, which does not typically exceed more than a few metres (Traniello et al. 1991). Within the foraging arena space, four 15 mL Falcon tubes were placed, two on each side, filled with water and stoppered with cotton balls to provide a water source for the colony. These tubes were kept full and replaced at regular intervals throughout the experiments every 2–3 days. Transparent red plastic film was used to cover the nesting chambers to allow for observation while keeping the nest dark for the colony. The container borders were coated with Fluon to keep the colony contained, and a mesh net was additionally placed over each container to prevent escapes. Each colony was provided with a 100 mm diameter Petri dish of Bhatkar diet, an artificial diet enriched with sugars, vitamins, and proteins, three times weekly (Bhatkar and Whitcomb 1970). Typically, *F. obscuriventris* recruited extremely quickly to the food source during feeding times, while the other three species foraged more sporadically. Colonies were maintained at room temperature (20–23 °C) in the lab for the duration of the experiments. At the completion of all experiments, colonies were repatriated to the park at their original locations.

### Seed removal experiments

All *T. ramosum* seeds were collected from *T. ramosum* plants growing in Fish Creek Provincial Park and were immediately frozen to preserve freshness. *T. ramosum* produces seeds beginning in late May and continues flowering and seeding through the summer until frost occurs (under drought conditions, however, flowering and seeding stops); we collected all seeds in early July with experiments following in

the weeks thereafter. Each seed was evaluated for the presence of an elaiosome; seeds without an elaiosome were not harvested. The seeds were thawed immediately prior to the experiments. Prior to *T. ramosum* seed removal trials, the colonies were starved for 48 h to encourage interest in foraging, though water was continuously available.

We placed 20 *T. ramosum* seeds each in the bottoms of four 65 mm diameter Rodac plates, spaced in an equidistant line approximately 25 cm from the nest chamber openings in the foraging arena. We observed the number of seeds removed at timed intervals: 15 min after seeds were placed in the foraging arena, 30 min, 1 h, 2 h, 3 h, 6 h, 24 h, and 48 h. After 48 h, the seeds were typically desiccated enough that the workers no longer showed interest in them. Food preference experiments were subsequently conducted; the timeline varied, but usually were conducted within one week after the seed removal experiments.

### Food preference experiments

We compared colony preference among four different food sources: seeds from *T. ramosum*, *Agropyron cristatum* (crested wheatgrass), *Thlaspi arvense* (stinkweed), and mealworms, the larval form of *Tenebrio molitor*. Both crested wheatgrass and stinkweed are prolific in Fish Creek Provincial Park and are probably known to the colonies collected. While they are both non-native plants, they have been present in the area for many decades and so have been assimilated into the local ecosystem. These plants were selected based on initial preference trials where we offered *F. obscuriventris* a spread of different plant seeds growing in the park; they typically preferred the crested wheatgrass and stinkweed to other plants. Neither plant's seeds bear elaiosomes, though elaiosome-bearing seeds from other species were offered as part of the trials.

As in the seed removal trials, all seeds of all plants were collected in Fish Creek Provincial Park and were immediately frozen to preserve freshness, then thawed immediately prior to the experiments. Mealworms were purchased from local pet stores and immediately prior to the trials were cut into 1–3 mm pieces for easier manipulation by the ants. Prior to the food preference trials, the colonies were starved for 48 h to encourage interest in foraging, though again water was continuously available.

We observed colonies for 30 min at a time, and subsequently checked the number of food pieces at 4 h and 24 h after the commencement of the trial. We placed four 65 mm diameter Rodac plates at a distance of approximately 25 cm from the nest chamber openings in the foraging arena, spaced equidistantly from one another. Each dish held either 20 *T. ramosum* seeds, 20 crested wheatgrass seeds, 20 stinkweed seeds, or 20 mealworm pieces from 5 mealworms; the order in which these were placed was randomized.

**Table 1** Lab colony data. In columns *Brood collected*, *Queens collected*, *Males collected*, *Hosts collected*, *Thesium present nearby*: Y indicates yes, and N indicates no

Colony number	Species	Date collected	Latitude	Longitude	Brood collected	Queens collected	Males collected	Hosts collected	Colony size	<i>Thesium</i> present nearby
C1	<i>Formica argentea</i>	May 23 2018	51.1111	114.0936	N	Y—6	N	N	M	Y
C2	<i>Formica obscuriventris</i>	May 24 2018	51.0764	114.2247	N	Y—4	N	N	S	N
C3	<i>Formica aserva</i>	May 23 2018	51.0894	114.2411	Y	N	N	N	S	N
C4	<i>Formica obscuriventris</i>	May 30 2018	51.0192	114.2169	Y	N	N	N	L	Y
C5	<i>Formica argentea</i>	May 22 2018	51.0478	114.2767	N	N	N	N	M	Y
C6	<i>Formica obscuriventris</i>	May 22 2018	51.0992	114.2142	N	N	N	N	L	Y
C7	<i>Formica aserva</i>	May 28 2018	51.0125	114.1919	N	Y—7	N	Y— <i>Formica alipetens</i>	M	Y
C8	<i>Formica aserva</i>	May 29 2018	51.0197	114.1942	Y	Y—4	N	Y— <i>Formica alipetens</i>	M	Y
C9	<i>Formica podzolica</i>	May 30 2018	51.0775	114.2267	Y	N	N	N	M	N
C10	<i>Formica podzolica</i>	June 6 2018	51.0928	114.1983	Y	N	N	N	M	N
C11	<i>Formica aserva</i>	June 5 2018	51.0942	114.115	Y	N	N	N	M	Y
C12	<i>Formica podzolica</i>	June 4 2018	51.1103	114.08	Y	N	N	N	M	Y
C13	<i>Formica argentea</i>	June 7 2018	50.9656	114.0369	Y	N	N	N	M	N
C14	<i>Formica podzolica</i>	June 5 2018	51.07	114.2253	Y	N	N	N	M	Y
C15	<i>Formica obscuriventris</i>	June 8 2018	51.1856	114.1281	Y	N	Y	N	L	
C16	<i>Formica argentea</i>	June 14 2018	51.1253	114.0914	Y	N	N	N	M	

In column *Colony size*: S indicates small, M indicates medium, and L indicates large. In column *Queens collected*, the number of queens collected is reported; in column *Hosts collected*, the species of host collected is recorded. Colonies C5 and C6 began producing male brood (workers laying non-fertilized eggs) roughly two weeks after they were collected; colonies C4 and C15, collected with brood, continued to produce male brood via worker reproduction after collection

During the observation period of 30 min, we recorded the number of times that antennation, manipulation, and removal occurred at each dish (Fig. 1B). Antennation involves the ant rapidly tapping its antennae over the piece of food, as an initial foray to determine if the food is edible and of interest. Manipulation is a further step after antennation, where the ant grasps the food piece in its mandibles and moves it around, assessing its suitability as a food source. The final stage in foraging is removal, where the ant physically removes the food piece from the Rodac plate to bring it back to the colony. While food pieces can be set down again after removal if the ant loses interest or finds the food piece too heavy or unwieldy to carry, we counted any complete removal from the Rodac plate as an instance of removal, because it indicated sufficient interest in the food item. At the end of the 30-min observation period, we tallied up the number of seeds and mealworm pieces left in each dish, and assessed this again at the 4-h and 24-h checkpoints.

### Statistical analysis

While we attempted to standardize colony replicates, since colonies were removed from Fish Creek Provincial Park and were not lab-reared, there were some differences among colonies. The differences initially assessed included colony size; presence of reproductive castes; presence of brood; and presence of host species in socially parasitic species (see Table 1). During initial data exploration, it was apparent that the presence of brood and reproductives did not affect the removal of seeds or food items as their means and variances were virtually identical. Given the limited number of data points for including all factors and the asymmetric distribution of brood and reproductives among the colonies, we removed brood and reproductives from later analysis. *F. aserva* is an opportunistic social parasite; unlike obligate social parasites, *F. aserva* colonies can function without host species, but commonly augment their colonies with host species. While we made an effort to only collect *F. aserva* colonies that had no hosts, we subsequently discovered individual hosts in two of four colonies; both host species were identified as *F. altipetens*. Additionally, we evaluated presence or absence of *T. ramosum* in a 15 m radius around the colony's original location, as prior exposure to *T. ramosum* in their natural habitat might have some influence on whether colonies were more likely to incorporate it as a food source.

We evaluated differences in the rate of food item removal among species and other variables listed above for both the food preference trials and the seed removal trials. To better assess potential differences among species preferences, we evaluated changes in the rate of removal three ways: (1) mean rate of removal of food items, calculated as the mean rate per hour at each time period and subsequently averaged;

(2) final total number of food items removed; and (3) trends in food removal over time. All data were tested for normality and homogeneity of variances using the Shapiro–Wilk test and Levene's test, respectively. Where data were normal and variances were homogeneous, we used analysis of variance (ANOVA) and subsequently planned comparisons using post-hoc Tukey's tests to assess directionality of differences among levels; where data were non-normal, we used the non-parametric Kruskal–Wallis (K–W) test which is a distribution-free test based on rankings (Sokal & Rohlf 1995). Following K–W tests, we assessed differences among levels within treatments using post-hoc focused multiple comparisons based on the Wilcoxon rank-sum test (Field et al 2012). These results were expressed as differences with respect to the critical difference at  $\alpha=0.05$  in all cases. To assess whether there was a trend in food removal over time, the non-parametric Jonckheere–Terpstra (J–T) test was used. Similar to the Kruskal–Wallis test, it tests for differences among medians of each group but also includes information on whether there is an ordered trend in the medians, in this case each time interval (Field et al 2012; Jonckheere 1954).

We additionally assessed potential differences in behavioural observations among species (the number of times a food item was antennated or manipulated). All behavioural data were tested for normality and homogeneity of variances using Shapiro–Wilk and Levene's tests; no behavioural data were found to be normal, so all behavioural data were analysed using Kruskal–Wallis tests followed by post-hoc focused comparisons. All data were analysed using R version 4.0.2, using the *car* (Fox and Wiesberg 2019), *clinfun* (Sehan 2018), *pgirmess* (Giraudoux 2012), and *statsr* (Rundel et al 2021) packages.

## Results

### Seed removal trials

We found that all ant species assessed would remove at least some *Thesium ramosum* seeds, though there was a significant difference among species in all three metrics assessed: mean rate of removal, final number of seeds removed, and change in number of seeds removed over time. *Formica obscuriventris* showed the greatest preference for *T. ramosum*, though *F. aserva* and *F. argentea* also showed some degree of preference for the seeds. *F. podzolica* showed very little interest in the seeds in general, though some colonies did remove a number of seeds. Early interest in the seeds to some degree predicted overall interest; if the colony initially ignored the seeds, they were less likely to remove many overall. Seeds tended to desiccate 24–48 h after initiating the trial, at which point the workers mostly ignored the seeds. The seeds were typically removed to random chambers

within the nest; very few colonies formed any sort of centralized midden structure, but generally deposited the seeds in small piles of 2–5 seeds.

The final number of *T. ramosum* seeds removed was significantly affected only by ant species ( $H(3) = 10.441$ ,  $p = 0.0152$ ). Focused comparisons revealed that *F. obscuriventris* removed significantly more seeds than did *F. podzolica* (difference = 9.625, critical difference = 8.8817) (Figs. 2A, S1A, Table S1). When assessing the mean rate of *T. ramosum* seed removal, we found that both ant species ( $F(3, 10) = 13.27$ ,  $p = 0.00081$ ) and colony size ( $F(2, 10) = 11.50$ ,  $p = 0.00256$ ) significantly influenced the mean rate of seed removal. Planned comparisons showed that *F. podzolica* removed seeds at a significantly slower mean rate than the other three species ( $t = -3.555$ ,  $p = 0.0052$ ) (Figs. 2B, S1B, Table S2). Similarly, large colonies removed seeds significantly faster than did medium ( $t = -2.458$ ,  $p = 0.0338$ ) or small colonies ( $t = -4.650$ ,  $p = 0.00091$ ) (Fig. S1C, Table S2).

The number of seeds removed over time significantly increased for all ant species; *F. argentea* ( $JT = 349.5$ ,  $p = 4 \times 10^{-4}$ ), *F. aserva* ( $JT = 311$ ,  $p = 0.0044$ ), *F. obscuriventris* ( $JT = 297.5$ ,  $p = 0.004$ ), *F. podzolica* ( $JT = 328$ ,  $p = 0.0016$ ) (Fig. 3, Table S3).

### Food preference trials: food item removal

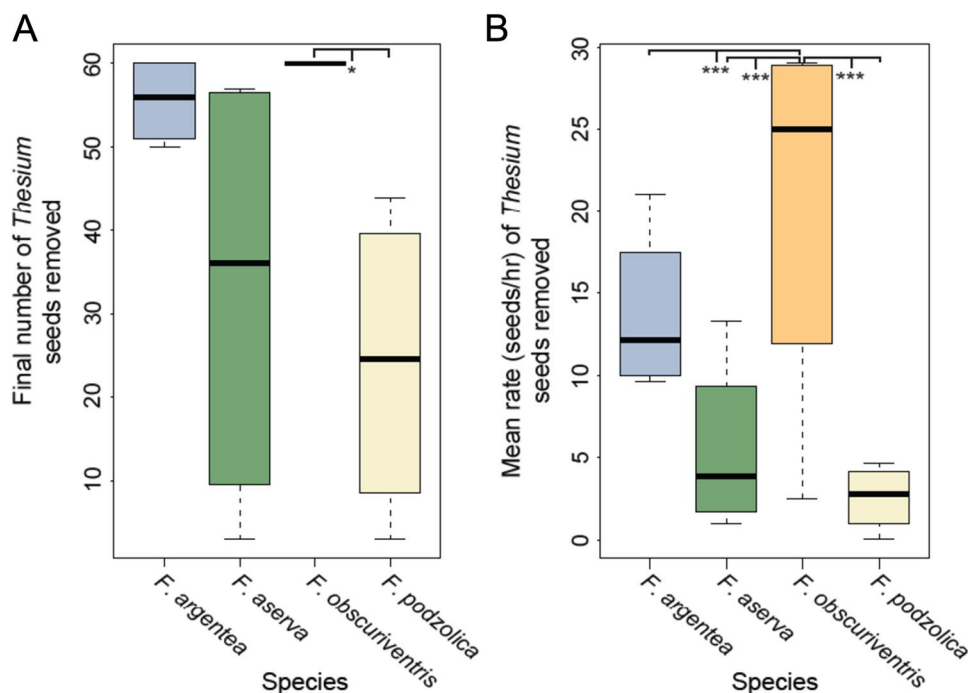
Number of food items removed within the first thirty minutes of observations was influenced by species ( $H(3) = 24.83$ ),  $p = 1.67 \times 10^{-5}$  and colony size ( $H(2) = 24.55$ ),  $p = 4.658 \times 10^{-6}$ ) (Table S4). *F. obscuriventris* removed more food items

during this time period than *F. argentea* (diff = 22.00), *F. aserva* (diff = 23.59), and *F. podzolica* (diff = 28.28, crit diff = 17.37 in all cases). Large colonies also removed more food than medium (diff = 27.80, crit diff = 14.52) and small colonies (diff = 27.77, crit diff = 20.34).

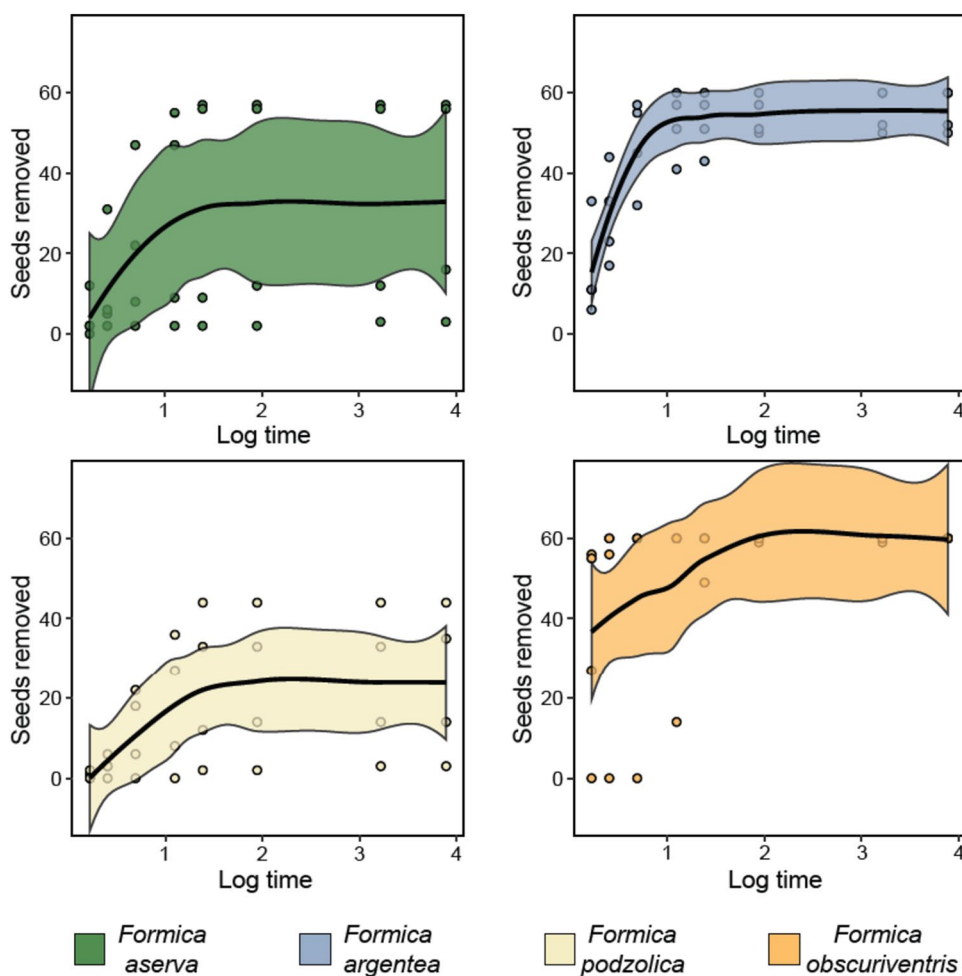
Several factors influenced the final number of food items removed (Figs. 4A, S2A–C, Table S5). Food type had a significant effect on the final number of food items removed ( $H(3) = 36.66$ ,  $p = 5.43 \times 10^{-8}$ ). *T. ramosum* was removed at higher numbers than either crested wheatgrass (diff = 35.06), and stinkweed seeds (diff = 37.75, crit diff = 29.93 in both cases). The total number of mealworms removed was significantly higher than both crested wheatgrass (diff = 53.29) and stinkweed seeds (diff = 55.98, crit diff = 29.93 in both cases) (Figs. 4A, S2A, Table S5). Colony size also had an effect on the final number of food items removed ( $H(2) = 49.90$ ,  $p = 1.46 \times 10^{-11}$ ), with larger colonies removing greater numbers of food items than medium (diff = 70.06, crit diff = 25.01) or small colonies (diff = 74.10, crit diff = 35.06) (Fig. S2B, Table S5). Species again influenced the final number of food items removed ( $H(3) = 51.75$ ,  $p = 3.39 \times 10^{-11}$ ), with *Formica obscuriventris* removing more items in total than *F. argentea* (diff = 57.28), *F. aserva* (diff = 65.09) and *F. podzolica* (diff = 69.96, crit diff = 29.93 in all cases) (Fig. S2C, Table S5). Time also significantly affected the number of food items removed ( $H(2) = 12.78$ ,  $p = 0.00168$ ), with significantly more food items removed at 24 h than at 30 min (diff = 33.49, crit diff = 23.52) (Fig. 4C, Table S5).

Several factors (species, food type, colony size and presence of *Thesium* around the original nest) influenced

**Fig. 2** Both final number of seeds removed (A) and mean rate of seed removal (B) differed significantly among species. Parentheses and asterisks among boxplots indicate significant differences in subsequent pairwise comparisons (\* indicates a p-value ranging between 0.01 and 0.05, \*\* indicates a value between 0.001 and 0.01, \*\*\* indicates a value 0.00099 or smaller). Black lines within the boxes indicate medians, boxes indicate first to third interquartile range, and bars (whiskers) indicate non-extreme minima and maxima of data (values within 1.5 times the interquartile range from the box edge), with dots indicating outlier data points



**Fig. 3** The number of seeds removed over time significantly increased for all species tested. Time intervals were log-scaled to highlight the early period of high seed removal rates. Solid line indicates average number of seeds removed per colony; coloured regions indicate 95% confidence interval



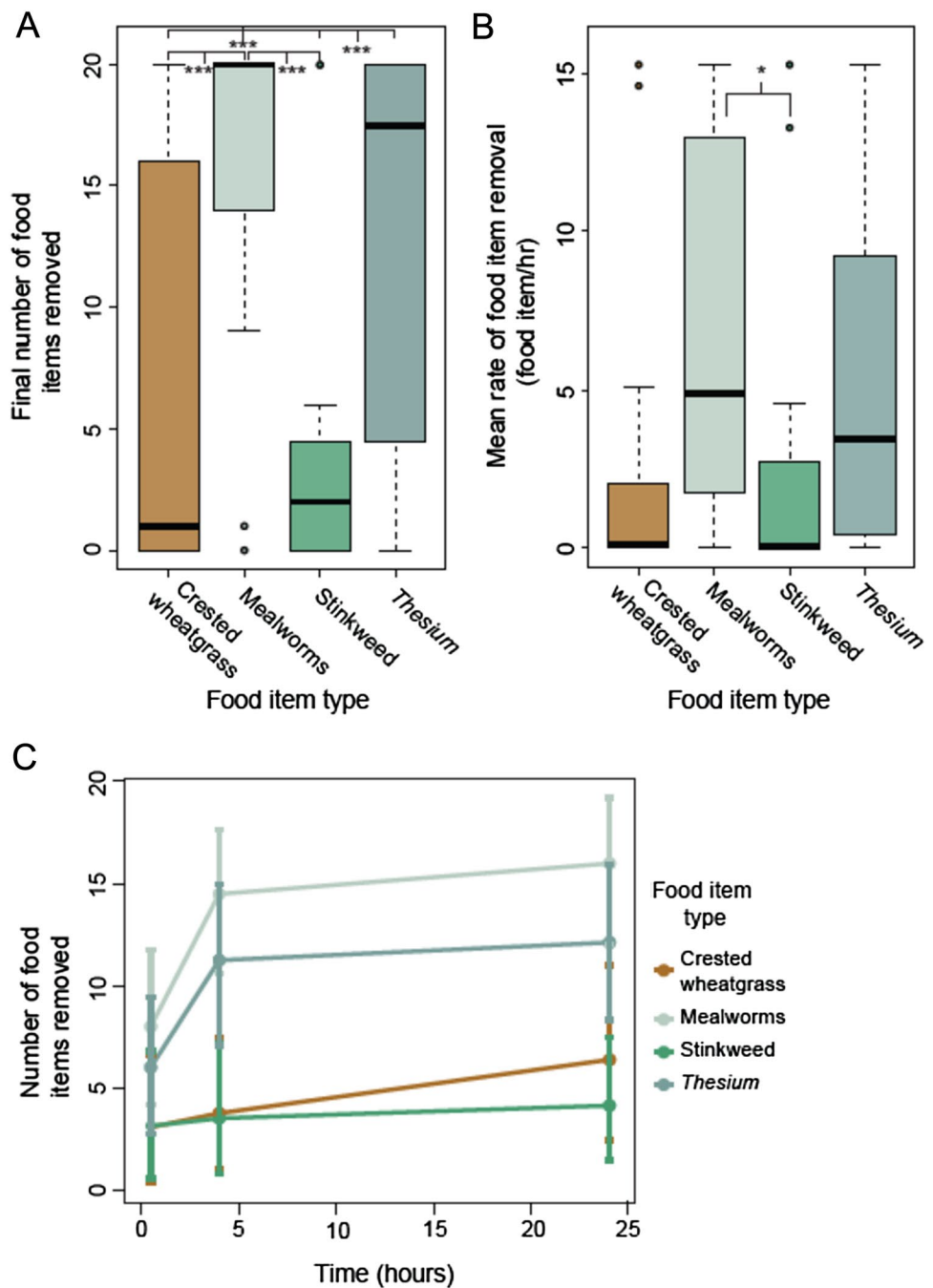
the mean rate of food item removal (Figs. 4B, S3A-D, Table S6). Mean rate of removal of food items showed only that mealworms were removed more quickly than stinkweed seeds (diff = 17.44, crit diff = 17.37). Colony size additionally affected the mean rate of removal for all food items ( $H(2) = 21.87$ ,  $p = 0.000018$ ): larger colonies again removed food at faster rates than either medium (diff = 27.44, crit diff = 14.52) or small colonies (diff = 29.06, crit diff = 20.34) (Fig. S3B, Table S6). The presence of *T. ramosum* around the original colony collection site had a significant effect ( $H(1) = 4.28$ ,  $p = 0.040$ ); colonies that had *T. ramosum* on or nearby the nest structures removed all food items at a faster mean rate than did colonies without *T. ramosum* nearby (diff = 9.65, crit diff = 9.20) (Fig. S3C, Table S6). Finally, species had a significant effect on removal rate ( $H(3) = 22.00$ ,  $p = 0.000065$ ); *Formica obscuriventris* removed all food items more rapidly than *F. argentea* (diff = 21.94), *F. aserva* (diff = 24.28) and *F. podzolica* (diff = 27.66, crit diff = 17.37 in all cases (Fig. S3D, Table S6).

### Food preference trials: behavioural observations

All behavioural trends were assessed within the first thirty minutes of constant observation. We found no significant difference in antennation rates among food item types, but some differences among other variables assessed (Fig. S5, Table S7). Species ( $H(3) = 18.86$ ,  $p = 0.0003$ ), presence of *T. ramosum* around the original nest ( $H(1) = 12.62$ ,  $p = 0.0004$ ), and presence of hosts ( $H(1) = 15.17$ ,  $p = 1.0 \times 10^{-5}$ ) all significantly affected antennation rates of food items (Fig. S5A-C, Table S7). *F. aserva* antennated significantly more frequently than did *F. argentea* (diff = 18.66) or *F. podzolica* (diff = 28.03, crit diff = 17.37) (Fig. S5A, Table S7). The presence of hosts in *F. aserva* nests also significantly affected antennation rates, as colonies with hosts antennated significantly more frequently than those without (diff = 23.18, crit diff = 11.69) (Fig. S5B, Table S7). Additionally, species with *T. ramosum* near their original nesting sites antennated significantly more frequently (diff = 16.64, crit diff = 9.20) than those without *T. ramosum* near their nests (Fig. S5C, Table S7).



**Fig. 4** Final number of food items removed (A), mean rate of food item removal (B), and the number of food items removed through time C all varied significantly by food item type. Parentheses and asterisks among boxplots indicate significant differences in subsequent pairwise comparisons (\*indicates a p-value ranging between 0.01 and 0.05, \*\*indicates a value between 0.001 and 0.01, \*\*\*indicates a value 0.00099 or smaller). Black lines within the boxes indicate medians, boxes indicate first to third interquartile range, and bars (whiskers) indicate non-extreme minima and maxima of data (values within 1.5 times the interquartile range from the box edge), with dots indicating outlier data points



Food type ( $H(3) = 8.97$ ,  $p = 0.0297$ ) and presence of *T. ramosum* around the original nest ( $H(1) = 10.69$ ,  $p = 0.0011$ ) significantly affected the number of times ants manipulated food items (Figs. 5A, S6A, S6B, Table S8). Mealworms were manipulated significantly more often than crested wheatgrass seeds (diff = 18.88, crit diff = 17.37) (Figs. 5A, S6A, Table S8). Colonies that had *T. ramosum* around the original nest site (diff = 15.11, crit diff = 9.20) manipulated food items significantly more often than did colonies without *T. ramosum* nearby (Fig. S6B, Table S8).

Preliminary observations noted that many of the foragers who interacted with food items did not immediately remove items of interest, but remained on the items, presumably feeding from them. Food type ( $H(3) = 9.39$ ,  $p = 0.025$ ), ant species ( $H(3) = 24.83$ ,  $p = 1.67 \times 10^{-5}$ ), colony size ( $H(2) = 24.55$ ,  $p = 4.66 \times 10^{-6}$ ), and presence of *Thesium* by nest sites ( $H(1) = 7.42$ ,  $p = 0.006$ ) significantly affected the number of ants on food items in the first 30 min of the trial (Figs. 5B, S7A-D, Table S9). Workers remained on mealworms significantly more frequently than on crested wheatgrass (diff = 17.75, crit diff = 17.37) (Figs. 5B, S7A,

**Fig. 5** Mean rate of manipulation of food items by workers varied significantly by food item type (A); as did number of workers present on food items at the 30-min-mark during the trial (B). Parentheses and asterisks among boxplots indicate significant differences in subsequent pairwise comparisons (\*indicates a  $p$ -value ranging between 0.01 and 0.05, \*\*indicates a value between 0.001 and 0.01, \*\*\*indicates a value 0.00099 or smaller). Black lines within the boxes indicate medians, boxes indicate first to third interquartile range, and bars (whiskers) indicate non-extreme minima and maxima of data (values within 1.5 times the interquartile range from the box edge), with dots indicating outlier data points

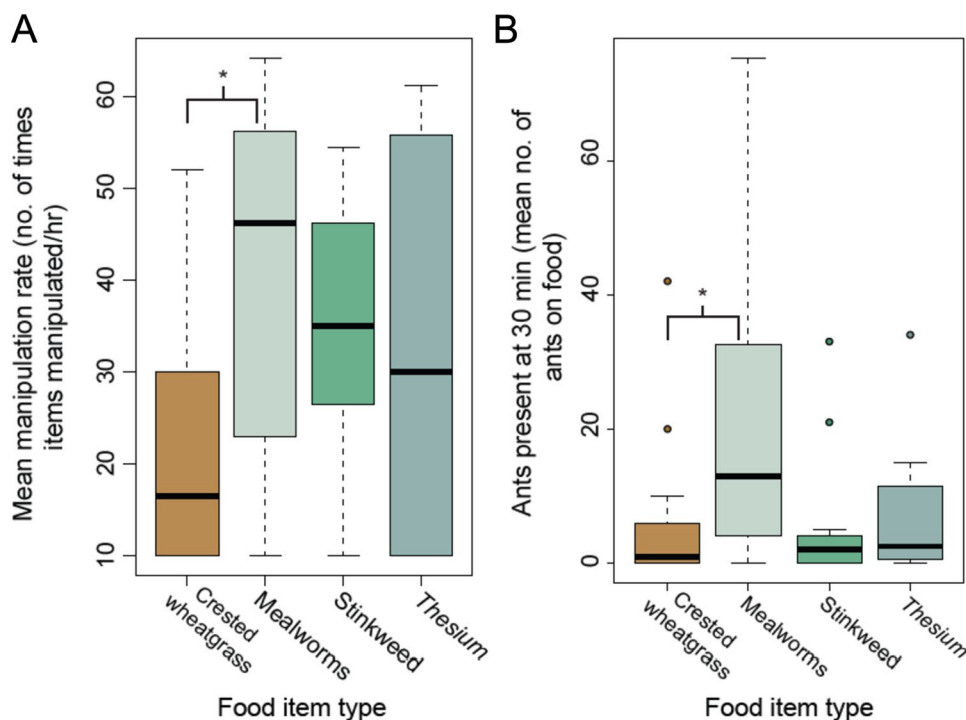


Table S9). Similar to other behavioural observations, *F. obscuriventris* was observed on food items significantly more often than either *F. argentea* (diff = 22.00), *F. aserva* (diff = 23.59), or *F. podzolica* (diff = 28.28, crit diff = 17.37) (Fig S7B, Table S9). Ants from large colonies were more often observed on food items than those from medium (diff = 27.80, crit diff = 14.52) or small colonies (diff = 27.77, crit diff = 20.34) (Fig. S7C, Table S9). Lastly, colonies with *Thesium* growing on or about their original nest sites were observed on food items more frequently than those without (diff = 12.57, crit diff = 9.20) (Fig. S7D, Table S9).

## Discussion

Our experiments were designed with three questions in mind: (1) do native ant species harvest *Thesium ramosum* seeds as a food resource? (2) Is there a difference in preference for *T. ramosum* seeds among ant species? (3) Do ant species prefer *T. ramosum* seeds above other food resources present in the park? We found several general trends with respect to these initial questions. All ant species tested removed *T. ramosum* seeds to at least some extent. However, preference for *T. ramosum* differed significantly among the four ant species tested: *Formica obscuriventris* removed more *T. ramosum* seeds than did other species, and in general *F. podzolica* removed seeds significantly slower than all other species. When comparing preference for *T. ramosum* seeds above other food sources, we found that colonies removed mealworms more rapidly than seeds of two other

plants present in the park, and final numbers of mealworms and *T. ramosum* seeds removed were significantly greater than the other two species of seeds. Behavioural patterns followed a similar trend: workers were observed antennating and manipulating mealworms more frequently than seeds.

We additionally found several factors that influenced seed removal and food preference aside from ant species. Generally, larger colonies removed more seeds and food items more rapidly; species who had known prior exposure to *T. ramosum* (measured by presence of *T. ramosum* on or about their original nesting site) removed more seeds and food items more rapidly; and the presence of hosts in functionally parasitic colonies also influenced interaction with seeds and food items, increasing antennation rates.

## Variables influencing ant interest in *Thesium ramosum*

We found consistent statistical support that *F. obscuriventris* preferred *T. ramosum* seeds to a greater degree than the three other ant species tested; in particular, *F. podzolica* demonstrated minimal interest in the seeds. *F. obscuriventris*' definitive interest in the seeds could be a result of multiple different factors: typically we found that their colonies in the park were larger than the other species'; they were much more active foragers in general than the other species while in the lab; and several of the larger lab colonies were also *F. obscuriventris* colonies. While there is little known in general about the ecology of *F. obscuriventris*, observations of their colonies in Nevada noted that the species

frequently formed large foraging columns from their nest sites (Wheeler and Wheeler 1986), which was also observed in Michigan (Talbot 1964). We additionally observed this ourselves in Fish Creek Park while scouting for potential *F. obscuriventris* nests. It is possible that this high level of extremely active foraging predisposed the colonies to remove *T. ramosum* at a higher rate than other species. Similarly, *F. obscuriventris* were more active foragers in the food preference trials and frequently removed most or all of the food items presented; this removal could be a reflection of active foraging in general.

*F. podzolica* was comparatively uninterested in *T. ramosum*; though the colonies removed a few seeds on average, for the most part they left the *T. ramosum*, other seeds, and mealworm pieces untouched. *F. podzolica* differs both in its morphology and nesting habits from *F. obscuriventris* and *aserva*, being smaller-bodied and forming low-slung dirt mounds compared to thatched domed mounds, but is morphologically highly similar to *F. argentea*, and more closely related (Borowiec et al. 2021). However, foraging behaviour between *F. podzolica* and *F. argentea* was quite different. *F. podzolica* is known to be a smaller-bodied, less aggressive species similar to other *F. fusca* group species (Mooney 2006), though there is minimal research on *F. argentea* behaviours. Thus, it is unclear why the two species should be so divergent in their foraging behaviour.

One of the more intriguing behaviours we observed was the increased interest in food items in *F. aserva* colonies that had host members. Colonies with hosts had elevated rates of antennation of food items compared to colonies without, and we observed hosts interacting with the *T. ramosum* seeds at higher rates than other members of the colony, though this was an incidental observation during the food removal trials and was not statistically evaluated. *F. aserva* colonies are facultatively dulotic, meaning that colonies can function without hosts to carry out foraging and nest maintenance tasks, though the fitness of the colony may be decreased (Mori et al. 2001; Savolainen and Deslippe 2001; Borowiec et al. 2021). The host species in both *F. aserva* colonies with hosts was *F. altipetens*, which we did not encounter in the park outside of their presence in these colonies. Despite dulotic behaviour being broadly studied, we could not find any information on colony diets changing as a result of host species being introduced. It is possible that the *F. altipetens* hosts were sourced from colonies that had been using *T. ramosum* as a food source for longer than the *F. aserva* colonies; however, since adult workers are not taken in raids but only late-stage brood and pupae, it is unclear how these foragers could have previously learned *T. ramosum* seeds as a food source. It may also be the case that host foragers forage further afield and longer than parasite foragers, exposing them to more types of food sources, but without further experimentation this is pure speculation.

The role of elaiosomes in western North American ecosystems is comparatively understudied to that of eastern North American deciduous or mixed forest ecosystems. Many spring ephemerals in eastern North American ecosystems have elaiosome-bearing seeds and their spread is at least partially mediated by ants (Beattie and Culver 1981; Clark 2022). However, a few western North American plant species are known to bear elaiosomes; the introduced weed genus *Euphorbia* (leafy spurge) is known to have elaiosomes and is present in Fish Creek Provincial Park. *Formica obscuripes* is known to harvest leafy spurge elaiosomes and may facilitate the spread of the plant, with the plant being found on or about *F. obscuripes* nests (Pemberton 1988). Interestingly, in preliminary food trials where we offered many types of seeds to *F. obscuriventris* in order to determine seed interest for our food trials, leafy spurge seeds were included and foragers showed minimal interest in them. It is possible that prior exposure to the plant is required for the foragers to learn it as a food source; while the plant is present in the park, there is no guarantee that the foragers from one particular colony would have encountered leafy spurge elaiosomes previously. Alternatively, just as interest in *T. ramosum* is to some extent species-specific in our findings even between closely related taxa, interest in leafy spurge elaiosomes may similarly vary among species.

Prior exposure to *T. ramosum* may also partially explain colony interest in the seeds. We found that colonies previously exposed to *T. ramosum* harvested food items at a higher rate than naive colonies. Foraging workers have been shown to recruit to and harvest familiar food resources at a faster rate than novel resources (Johnson 1990; Howard et al. 1996). Additionally, foragers exhibit learning behaviour; foragers preferentially select foods to which they have been previously exposed even when offered other edible foods (Nelson et al. 2020). *T. ramosum* has been present in Canada and the park since 2001; though the exact extent of the spread is not known, it is likely that many of the colonies in the park have had ample time to identify *T. ramosum* seeds as a food source, even if few other plants in the park have elaiosomes to assist with prior learning. Considering that our colonies harvested more food items after exposure to *T. ramosum* seeds, it seems likely that prior exposure to *T. ramosum* increases their interest in the seeds.

### Preference for *Thesium ramosum* over other food items

The marked preference for mealworms over other food items offered indicates a possible preference for protein sources. Protein typically comprises a lower proportion of *Formica* diets than other nutrient sources such as carbohydrates (Bernstein 1976), but composition of colony diet fluctuates throughout the year and is contingent on factors such

as colony life stage and resource availability, with protein often being foraged at higher rates when brood or reproductives are present in colonies (Cook et al. 2011; Dussutour and Simpson 2009). Most of our colonies were either collected with brood or began producing some shortly thereafter; we did not find any evidence that brood influenced food item removal, but workers from all colonies may have increased protein foraging given the timing of collection and experiments. Seasonality can affect foraging preferences and nutrient regulation, especially considering that reproductive cycles in colonies are temporally constrained (Cook et al. 2011); while we conducted these experiments during the summer, we do not have other seasonal groups with which to compare and so cannot speculate if seasonality was a factor here.

The colonies did show some preference for *T. ramosum* seeds over other types of seeds, though not over mealworms. Elaiosomes are typically lipid-rich structures; the chemical composition of *T. ramosum* elaiosomes is currently unknown, but likely similar to other elaiosomes. When compared to seeds, elaiosomes generally have higher proportions of amino acids and fatty acids (Fischer et al. 2008). This chemical composition makes them more attractive to foraging ants than the more carbohydrate-heavy seeds, particularly for larval nutrition; the presence of elaiosomes in colony diets has been associated with brood provisioning and the production of reproductive alates (Fischer et al. 2008; Warren and Giladi 2014; Warren et al. 2019). We did not find evidence of brood and reproductives influencing seed removal or food item removal, but most of our colonies already had or began producing brood, which may have influenced their interest in *T. ramosum* elaiosomes. It has also been proposed that elaiosome fatty acid composition evolved to mimic that of insect prey, thus attracting carnivorous and omnivorous ants that might otherwise avoid harvesting seeds (Hughes et al. 1994).

The Alberta ant assemblage is composed mostly of omnivorous species with some predaceous species; there are few specialized granivorous species in the province, none of which we recorded in the park (Glasier et al. 2013). Elaiosome foraging may thus be an opportunistic part of their diet, and perhaps linked to the timing of brood and alate production in native species. While little is known about the exact timing of brood production in Alberta *Formica* species, some *Formica* are known to produce alates in June through August (Mackay and Mackay 2002); during our fieldwork we found many reproductive alates from several *Formica* species, and all four species studied here were collected at least once with brood. The flowering and seeding period of *T. ramosum* does overlap with the likely brood and alate production period of local *Formica* species, suggesting that *T. ramosum* elaiosomes may be used as lipid sources for larval consumption. However, while we found that our

*Formica* colonies preferred mealworms over other seeds and to some degree preferred *T. ramosum* seeds over others, we did not find any evidence of increased *T. ramosum* seed collection by colonies with brood versus those without. It may be that colonies collected without brood were still primed to collect protein- and lipid-rich food sources given their phenology, but we cannot be certain this is the case.

## Conclusions

Overall, we find that common ant species in the park use *T. ramosum* seeds as a food source and may prefer it to other seeds present in the park, though to what extent is dependent on a multitude of factors. This interest and preference indicate it is likely that native ant species in Fish Creek Provincial Park are facilitating the spread of *T. ramosum* to some extent. Given its status as a hemiparasitic plant, such dispersal may impact the composition of plant communities within the park. However, our laboratory experiments only established native species' interest in seeds, and not in situ dispersal dynamics. Subsequent experiments conducted in the field with *T. ramosum* populations are needed to evaluate these dispersal dynamics more conclusively.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11829-024-10046-9>.

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**Data availability** All data and code required to reproduce the analyses and evaluate the conclusions in this manuscript are available in the appended supplementary materials.

## Declarations

**Competing interests** The authors declare that they have no competing interests.

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