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Seed fate in ant-mediated dispersal: Seed dispersal effectiveness in the *Ectatomma ruidum* (Formicidae)— *Zanthoxylum ekmanii* (Rutaceae) system

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Abstract

Plants are often dispersal limited relying on passive or active agents to find suitable microhabitats for germination. Seeds of pioneer tree species, for example, require light gaps for growth but have short median dispersal distances and often do not provide a food reward to encourage animal dispersal. Zanthoxylum ekmanii seeds are frequently moved by ants but evaluating the effectiveness of ant-mediated seed removal requires knowledge of the species moving the seeds, how far they are moved, and the deposition site. To assess the effectiveness of ants as seed dispersers of Z. ekmanii, we utilized the seed dispersal effectiveness framework. We tracked the movement of seeds from caches on the forest floor, revealing that foragers of Ectatomma ruidum moved 32.8% of seeds an average first distance of 99.8 cm with 68.3% of those seeds taken into a colony. The quality of deposition location was assessed using a seedling emergence study where freshly germinated seeds were buried at different depths. Seedlings were primarily able to emerge from the shallowest depths. Wax castings of E. ruidum colonies demonstrated that seeds brought into the colony were deposited in chambers that had larvae present and experienced more damage than seeds unhandled by ants. Foragers, however, did not have a strong enough bite force to rupture Z. ekmanii seeds likely because their muscle morphology is not structured to maximize force generation. Overall, E. ruidum may help fine tune deposition location, incorporating seeds into the topsoil, though few seeds will likely emerge if soil bioturbation is low.

Abstract in Spanish is available with online material

KEYWORDS

Barro Colorado Island, directed dispersal, myrmecochory, Neotropical pioneer tree species, Panama

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1 | INTRODUCTION

Plants are often dispersal limited relying on passive or active agents to find suitable microhabitats for germination. Pioneer tree species in particular face many barriers to recruitment of new individuals into a population. While they produce many small seeds, only a fraction of seeds reach suitable microsites for germination and establishment (Dalling et al., 2002). Seeds of pioneer species require high light microsites to break dormancy cues and germinate (Pearson et al., 2002). Treefall gaps are examples of suitable microsites (Hubbell et al., 1999) and can be relatively common in forests though unpredictable and spatially heterogeneous (Martínez-Ramos et al., 1988). Seeds of pioneer species reach these gaps by either being good colonizers or remaining viable for long periods of time waiting for conditions to become favorable. For example, Zanthoxylum (Rutaceae) is a widespread genus (Appelhans et al., 2018) whose seeds provide no obvious dispersal rewards. In Panama, some Zanthoxylum species have clear light gap requirements, and while they typically produce many seeds, they have a short (0.8 m) median dispersal distance (Dalling et al., 2002). This is possibly offset by their ability to remain viable in the seed bank for decades (Dalling & Brown, 2009). However, models suggest that long-term viability alone cannot explain pioneer tree population distributions suggesting that effective dispersal systems are still necessary (Dalling & John, 2008).

The Neotropical pioneer tree Zanthoxylum ekmanii Alain (formerly Z. belizense Lund.) is a dioecious tree that fruits January-March and is found in Central America (Croat, 1978). Its seeds can remain viable in the soil for at least 18 years (Dalling & Brown, 2009), and are not known to provide a food reward to attract dispersers. However, their seeds are attractive to ants, especially the common, ground dwelling ant Ectatomma ruidum (Ruzi et al., 2021). Ant-mediated seed dispersal is both phylogenetically and geographically widespread (Lengyel et al., 2010). Traditionally, this interaction is considered a mutualism, where plants benefit in multiple ways and the ants receive a food reward. However, the nature of this interaction has come under question, particularly from the ant's perspective (Gammans et al., 2005; Warren et al., 2019). This is particularly true for seeds of plants such as Z. ekmanii which are attractive to ants yet failed to provide a food resource (Ruzi et al., 2021). In contrast, there are many hypothesized benefits of ant dispersal to plants including directed dispersal into favorable microsites, increased dispersal distance, and protection from predators and pathogens (Giladi, 2006).

The benefits of ant-mediated dispersal to plants vary depending on how the benefit is defined, the plant species, and the ecosystem studied. First, the stated benefits of directed dispersal are typically due to ant nests providing favorable microsites for germination or growth of the plants (Davidson & Morton, 1981; Farji-Brener & Werenkraut, 2017; Sankovitz et al., 2019). Second, dispersal away from the parent plant is beneficial if seeds experience speciesspecific negative density dependent mortality near the maternal plant (though negative effects also occur near conspecifics; Connell, 1971; Janzen, 1970). Typically, ants act as short distance dispersers fine-tuning seed deposition location after primary dispersal by a vertebrate. However, ant dispersal distances vary greatly depending on the species investigated ranging from a few centimeters up to 180 m (Gómez & Espadaler, 2013). Thirdly, seeds can benefit by avoiding predators when ants incorporate them into the soil where they are less likely to be consumed (Giladi, 2006). Burying seeds may reduce olfactory cues that rodents use to detect them (Paulsen et al., 2013). For example, Estrada and Coates-Estrada (1991) determined that the rate at which rodents detect seeds rapidly decreased with increasing burial depth. Giladi (2006) reviewed these proposed plant benefits finding the most support for the predator-avoidance hypothesis (81% of 27 studies), followed by the dispersal distance hypothesis (76% of 17 studies), and lastly directed dispersal hypothesis (46% of 46 studies). Despite evidence supporting each hypothesis coming from diverse taxa, research from tropical forests or from trees are generally underrepresented.

Neotropical pioneer tree species are an ideal system to examine the consequences of ant-mediated seed dispersal as their seeds require suitable microsites for germination and are dispersed by a variety of ants (Pizo & Oliveira, 1998; Ruzi et al., 2021). Here, we use the dispersal effectiveness framework (Schupp, 1993; Schupp et al., 2010) to examine the effectiveness of E. ruidum workers as a dispersal agent for seeds of the pioneer tree species Z. ekmanii. This framework breaks down seed dispersal effectiveness into quantitative and gualitative components. The guantitative component consists of the number of visits a dispersal agent makes and the number of seeds dispersed per visit, while the gualitative component consists of the quality of both the seed deposition location and seed handling (Schupp, 1993; Schupp et al., 2010). In endozoochorous systems where this framework was first developed, more than one seed is dispersed per visit, and the quality of handling relates to both how seeds are treated in the beak or mouth as well as gut passage time. However, ants may need to visit seeds multiple times before a single seed is successfully dispersed. The quality of ant handling refers to any processing or damage the seeds may accrue either during dispersal by foragers or by larvae attempting to eat the seeds.

We characterized the short-term seed fate of *Z. ekmanii* seeds by assessing the quality of dispersal by *E. ruidum* workers. We hypothesized that *E. ruidum* foragers act as effective dispersers of *Z. ekmanii* seeds, rescuing seeds from negative density dependent conditions by dispersing them away from the parent tree toward locations that enhance the survival of seed to seedling. To this aim, we addressed the following research questions regarding the qualitative component of the seed dispersal effectiveness framework: (a). Do *E. ruidum* workers conduct directed dispersal depositing seeds in their colonies at distances that are biologically relevant (i.e., outside the tree crown)? (b). Can seeds survive and emerge from the deposition location? (c). To what extent do *E. ruidum* damage the seeds that they remove?

2 | METHODS

2.1 | Study site and species

Seed caches were placed at five sites >350 m apart from each other on Barro Colorado Island (BCI) (9°10'N, 79°51'W), Republic of

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Panama. Sites were at least 20 m from conspecific trees (Ruzi et al., 2017; Zalamea et al., 2015). BCI is a seasonal lowland tropical rainforest, experiencing most of its annual 2600 mm rainfall during its wet season (~late April to ~early January, Windsor, 1990). Wax casting of ant colonies was conducted at Parque Soberania near Gamboa (9°11'N, 79°70'W), Republic of Panama.

The Zanthoxylum genus is widespread, with most species being found pantropical and some species found in temperate regions (Appelhans et al., 2018). Zanthoxylum ekmanii is found ranging from Southern Mexico through Panama and potentially into Colombia (Croat, 1978). On BCI, it is the most common of the four Zanthoxylum species (Dalling & Brown, 2009) with seeds commonly found within the soil seed bank (Dalling et al., 1997). It is a dioecious pioneer species that reaches 13-30 m tall, can grow one meter diameter at breast height, flowers in August-October, and fruits between January and March (Croat, 1978). As a canopy tree, its crown area has been measured at 211 m² (\pm 38 SD, Park et al., 2019). Their primary dispersal mode has been recorded as animal dispersal (Muller-Landau et al., 2008) with birds and monkeys as likely dispersers (Carlquist, 1966; Croat, 1978; Hladik & Hladik, 1969). Other Zanthoxylum species have been reported as potentially dispersed by birds (Díaz Vélez et al., 2017; Heleno et al., 2013; Nogales et al., 2017; Pillatt et al., 2010; Ponce et al., 2012), rodents (Clark, 1981), and some reptiles (Heleno et al., 2013; Nogales et al., 2017). Seeds generally fall still attached to their infructescences beneath the parent crown, are about 3.5 to 5 mm long (Croat, 1978) and are not known to have an elaiosome, aril, or fruit to act as a food reward. The only potential food reward comes from the outer layer of the seed coat, the sarcotesta, which is present in all members of the Zanthoxlum genus (Hartley, 2001).

Zanthoxylum ekmanii seeds experience physiological dormancy being unable to immediately respond to germination cues due to chemicals inhibiting embryo growth (Dalling et al., 2011; Long et al., 2015), and are members of the physiologically dormant defense syndrome having permeable seeds that are defended chemically (Zalamea et al., 2018). Based on the experiments by Zalamea et al. (2018), *Z. ekmanii* seeds do not lose viability after 30 months of burial in the absence of predators, and whole seed extracts have been demonstrated to inhibit the growth of two fungal pathogens. In addition, *Z. ekmanii* seeds have thick seed coats (mean = 268.8 micrometers, SD = 58.0, N = 35, (Zalamea et al., 2018).

Seeds of *Z. ekmanii* were collected during the dry season in the Barro Colorado Nature Monument (BCNM) (Zimmerman et al., 2007). Seeds were rinsed with distilled water and allowed to dry in an air-conditioned (~ 22°C), dehumidified dark room to prevent molding. Only seeds that sunk in water were used in the study as these are likely to be viable seeds (e.g., Daneshvar et al., 2017) while most of the seeds that floated were empty (P-C. Zalamea personal communication; see Methods S1, Table S1).

Ectatomma ruidum is the most common ant species at the study sites and workers were frequently observed removing *Z. ekmanii* seeds in 2013 and 2014 (Ruzi et al., 2021). *Ectatomma ruidum* is a diurnal foraging omnivorous ant species known to consume insects and plant-based resources (Lachaud, 1990; Pratt, 1989). *Ectatomma ruidum* workers are relatively large (Weber's length of 2.780 mm (Ruzi et al., 2021)), tend to forage individually though they can recruit other foragers if the resources are large or of high-quality (Pratt, 1989), and are easily identified on site.

2.2 | Seed cache setup

Seed caches were placed on the forest floor at five sites during the 2015 and 2016 dry season, and observed from 1000 to 1200 h. For each year, only seeds collected that year were used. Seed caches consisted of five Z. ekmanii seeds (2015 = 15 caches, 2016 = 10 caches). Caches were placed haphazardly along a randomly chosen edge of a rectangular plot (9 m by 15 m; Ruzi et al., 2017, 2021; Sarmiento et al., 2017; Zalamea et al., 2015), with no plot side used more than once within a given year. Approximately 0.75 m² of leaf litter was cleared around the caches to increase visibility around the cache to track ants / seeds. Sites were baited from 0930 to 1000 h with Quaker oatmeal cookiesTM (granola flavor) to encourage ant activity back into the area after the litter was cleared. This may have increased ant activity in the area which could have resulted in greater seed removal than under conditions when baits are not used. However, there was no statistical difference in twohour seed removal based on comparison with data from Ruzi et al. (2017, 2021) who placed seed caches without baits (see Methods S1). Observations were split into "first" and "second" movements if a seed was deposited at one location and then moved to another (Figure 1a).

2.3 | Distance to and location of seed deposition location (a)

Seed caches were used to estimate removal distances to deposition location. Seeds were each marked with an individual-colored dot of enamel modeling paint (Testors[®]) to facilitate tracking individual seeds. This paint had no observable impact on seed removal by ants of other species (Magalhães et al., 2018; Passos & Oliveira, 2002). A screw with the same color paint as the seed was placed wherever the seed was deposited. At 1200 h, the straight-line distances of the seed movements were measured with respect to the original location for the first movement and from screws for subsequent movements. The locations of screws were categorized as either being dropped, brought into a colony, or location unknown. Occasionally multiple seeds were removed at once, so not all possible movements, distances, and deposition locations were observed. Distances and deposition location were recorded for all movement numbers (Figure 1b,c). We used the crown area measured by Park et al. (2019) and assumed a circular tree crown to determine the distance seeds would need to be moved to no longer be under the crown. This distance was 8.2 m (\pm 3.5 SD) if seeds were initially deposited at the center of the crown.



FIGURE 1 Representative drawing of straight-line dispersal distances and deposition locations of Z. ekmanii seeds relative to north (N). The pink seed demonstrates a first movement of 221 cm and a second movement of 60 cm. Two different E. ruidum colonies took seeds. Color refers to the paint marking on the seed. Drawing is not to scale (a). Histogram of straight-line dispersal distances by E. ruidum by movement number (bin width =15 cm; first movement number: N = 39; second movement number: N = 9; third movement number: N = 1) (b) and percentages of seeds that went into either colonies, were dropped, or were lost based on the total number of seed destinations recorded (N = 51; based on frequency of all deposition locations recorded) (c)

2.4 Quality of seed deposition location (b)

To assess the quality of the deposition location by determining chamber depths, location of seed deposition within nests, and distribution of ants within colonies, 13 colonies were wax casted in 2015 and 2016 following methods by Tschinkel (2010). These casts were melted down after chamber depths were measured. Six of the 13 casts were provisioned with Z. ekmanii seeds on the same day as casting. Chambers were numbered by following the tunnel from the nest entrance deeper into the soil; therefore, shallower attached chambers were given lower numbers than deeper chambers. Chamber depths were measured as the distance from the soil surface (i.e., colony entrance) to the bottom of the chamber.

Chamber depths were compared to the proposed maximum emergence depth for Z. ekmanii. This value was estimated using the following formula from Bond et al. (1999):

maximum emergence depth = 27.3 * seed weight^{0.334}.

Ten replicates of 50 fresh seeds each were massed on a microbalance to the nearest 0.1 mg (Table S2). To assess whether seeds of Z. ekmanii could emerge from burial depths in ant nests, a seedling emergence study was conducted in the 2018 dry season. Soil was collected from beneath two mature Z. ekmanii trees on BCI and sieved through a 0.2 mm sieve to collect seeds. Seeds were placed in paraffin sealed petri dishes lined with two layers of moistened paper towel in an ambient air greenhouse (temperature: approximate 22-34°C; humidity: approximately 45-94%). These seeds were checked daily for germination. Seeds were buried in clear plastic tubing (2.5 cm diameter) either the day or one day after germination occurred. Plastic tubing with seeds were placed in a plastic shelf that had a white Styrofoam sheet above and below it and covered with two layers of shade cloth to reduce sunlight. Each tube was filled with sieved soil to the five cm mark ensuring room for root growth before seeds were added and more sieved soil poured over the seeds until reaching the desired burial depth. Burial depths consisted of 0 (N = 4), 2 (N = 14), 5 (N = 12) and 7 cm (N = 8) below the soil surface. Soil density was also calculated and compared to the reported

density in Cavelier (1992) and potential impact of soil biota on germination evaluated (see Methods S1). Tubes were watered with distilled water as needed.

2.5 | Assessment of seed damage (c)

To assess the handling damage caused by ants to seeds, the percentage of seed coat damaged was compared between seeds that presumably have never encountered ants and seeds that had been retrieved from wax casts. Seeds that had presumably never encountered ants were collected from infructescences that had fallen under the parent crown. Images were taken using Leica Application Suite Core (version 4.9.0, Leica Microsystems, Switzerland), a Leica M205 C stereo microscope (467 nm resolution) that had an attached Leica DRC 425 digital camera (5 megapixel). Multiple images per seed taken at different focal depths were stacked using Zerene Stacker (version 1.04, Zerene Systems LLC) using the align and stack all (PMax) setting. Seeds were oriented in the same plane, so that as much surface area as possible would be visible in the stacked images. Stacked images were assessed in ImageJ (version 1.52a, Schneider et al., 2012) by calculating the total seed surface area and any damaged areas. The percentage of damaged area per seed was used in analyses, so that the amount of damage was standardized to seed size.

To determine if *E. ruidum* workers have the mandible strength to damage seeds, bite force measurements on four workers were taken using a modified piezoelectric sensor and software (FlexiForce, ELF system, Tekscan) to record real time mandibular pressure (10 g force required to start recording). The sensor was modified by removing excess plastic around the edge to better allow ants access to the sensor while biting. The exposed edge of the sensor was protected with parafilm. The maximum force reached was recorded for three to five bites, each 20 sec long. All bites were recorded in a single session and ants were not returned to the colony. The average fracture resistance of seeds was taken from Zalamea et al. (2018) and is 68.3 N (SD = 24.3, N = 100).

2.6 Statistical analyses

Analyses were conducted in R (version 3.6.1, R Core Team, 2019) and RStudio (version 1.2.2019, RStudio Team, 2019) with data manipulated using *tidyverse* (version 1.3.0, Wickham et al., 2019) and *plyr* (version 1.8.4, Wickham, 2011). Figures were visualized using *ggplot2* (version 3.2.1, Wickham, 2016).

To assess dispersal distances and deposition location of seeds, trials from 2015 to 2016 were pooled together. To determine whether the distances moved by ants was enough to leave the parent crown if deposited in the center near a tree trunk, we used a one sample Wilcoxon signed rank test using the *wilcox.test* function in the base R *stats* package as seed removal distances were not normally distributed (Table S3).

A one sample t-test was used to compare chamber depth to the estimated maximum emergence depth. Each chamber number in which seeds were found was tested independently from one another and the assumption of normality was checked using Shapiro-Wilk tests.

To assess whether the fixed effect of burial depth (categorical variable) impacted the ratio of seedling emergence to seeds placed (response variable), we conducted a linear mixed model (LMM) with replicate as a random effect. The LMM was conducted using the *lme* function in the *nlme* package (version 3.1–140, Pinheiro et al., 2019) with *p* values obtained using the *anova* function. Tukey posthoc means separation tests were conducted on significant fixed effects using the *emmeans* function in the *emmeans* package (version 1.4.3.01, Length, 2019).

A Welch two sample *t*-test assuming unequal variances was used to determine if there was a difference in mean surface area percentage that exhibited processing or damage between unhandled seeds and ones recovered from wax casts.

3 | RESULTS

Overall, workers from all ant species moved an average of 58% of seeds from caches in two hours in both study years (2015 = 58.67% mean $\pm 11.46\%$ *SE*, 44 seeds out of 75 placed; $2016 = 58.00\% \pm 13.81\%$, 29 seeds out of 50 placed). Workers of other ant species were also observed moving seeds and their interactions are summarized in Table S4. Here, we focus on the results of seed removal by workers of *E. ruidum*. Combining data from both years, *E. ruidum* workers were responsible for moving 32.8% of placed seeds (41/125) a first movement, 7.2% (9 seeds) a second movement, and 0.8% (1 seed) a third movement.

3.1 | Seed removal distance and deposition location (a)

The Z. ekmanii seeds dispersed by E. ruidum workers experienced a first movement distance of 99.8 \pm 91.1 cm (mean \pm SD, N = 39 seeds, 2 seeds lost during tracking) and a second movement distance of 61.5 \pm 70.9 cm (N = 9 seeds, Figure 1b). The only seed moved a third time by an E. ruidum worker was moved four centimeters. Based on first distance movements, E. ruidum workers were unable to move seeds from under the tree crown (V = 766, p < .001).

Pooling all movements together, a total of 51 destinations were recorded: 32 seeds were taken into an *E. ruidum* colony, 15 seed were dropped while being followed, and 4 seeds were lost while following other *E. ruidum* workers moving seeds (Figure 1c, Table S4). *Ectatomma ruidum* workers moved seeds in colonies during 12 of the total 25 trials. Of these, nine of the 12 times seeds were moved into a colony they went into one *E. ruidum* colony. However, they were sometimes taken into two (twice) or four (once) different colonies (e.g., Figure 1a).

When seeds were taken into four different E. ruidum colonies, they were originally taken into three colonies and then one seed reappeared and was taken into the fourth unique E. ruidum colony.

3.2 Seedling emergence (b)

Wax casts revealed colonies typically had at least three (max six) chambers at average depths of 7.72 (min = 3.8), 9.92 (min = 5.7), and 13.58 (min = 6.7) cm for chambers 1-3, respectively. Notably, chambers 1-4 sometimes were shallower than the estimated maximum emergence depth of 70.26 mm from Bond et al. (1999) depending on the colony (Figure 2b, Table S5). Workers tended to be present in all chambers while larvae were mostly present in the three shallowest chambers and occasionally in chamber four. Zanthoxylum ekmanii seeds were present in chambers 1-3 (Table S5, Figure 2a). Of the chambers where seeds were found, the first chamber was the only one not significantly different from the estimated maximum emergence depth (Table 1).

Burial depth ($F_{3,20} = 7.68$, p = .001) significantly influenced the likelihood of seedling emergence. Both the 0 cm and 2 cm depths had the highest proportion of seedlings emerge (Figure 2c), with only one emerging from the 5 cm depth. The soil bulk density used in the study was significantly greater than the reported density in Cavelier (1992) (0.82 vs. 0.74 g per cm³ this study vs. Cavelier, 1992; Methods S1, Table S6).

Ant handling (c) 3.3

Thirty-seven seeds were recovered from the six colonies that were provisioned with seeds prior to wax casting (Table S5). Seeds were found mainly in chambers 1-3 of casts, with only one seed not found in a chamber (Figure 2a). Only one seed was recovered with an exposed embryo which was likely damaged during the sectioning of the wax cast. This seed was not included in analyses. There was a significant difference in the percentage of seed surface area damaged between seeds recovered from inside a colony (N = 36) and those that have not interacted with ants (N = 41) (t-test: t = -5.04, df = 48.9, p < .001; Figure 3a). Seeds handled by ants had a pitted look (Figure 3b,c) while unhandled seeds were smooth (Figure 3d). Eighty-three percent of seeds recovered from chambers in the wax casts were found in chambers that also had larvae present. The maximum bite force from the four ants ranged from 0.060-0.092 N (Table 2). Ectatomma ruidum workers produced a much smaller bite force than the 68.3 N recorded in Zalamea et al. (2018) needed to rupture Z. ekmanii seeds.

4 DISCUSSION

We used the seed dispersal effectiveness framework (Schupp, 1993; Schupp et al., 2010) to assess short-term seed fate for Z. ekmanii.



FIGURE 2 Boxplots of the relative abundance of seeds per chamber averaged across six wax castings (a). Chamber depths both by colony (circles) and average (black lines) (b). Red circles are an example of the chamber depths from one colony (SAR843). The calculated maximum emergence depth line is set at 7.026 cm. Along the y-axis is a simplified depiction of what an wax caste may look like with the tunnel as the long rectangle and the ovals for chambers. Boxplots of the percentage of seedlings emerging by burial depth (c). Letters denote significant differences based on a Tukey post-hoc means separation test. The line in the middle of the boxplot represents the median value, the bottom and top of the box represents the 25th and 75th percentiles of the data, respectively, the whiskers extent to the most extreme data point that is not an outlier, and the points represent outliers (Wickham, 2016). Outliers are those that are $1.5 \times$ the inter-quartile range which is the distance between the 25th and 75th percentiles

Ectatomma ruidum was responsible for moving over half of the seeds relocated by ants, highlighting the importance of this species as a seed dispersal agent in this system. Ants are not typically longdistance dispersers and are more likely responsible for fine-tuning the deposition location of seeds, especially in the case of diplochory

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		Mean denth + SD	Shapiro-Wilk		t-tests		
Chamber	N	(cm)	W	р	t	df	р
1	13	7.7 ± 2.3	0.90	.14	1.10	12	.29
2	13	9.9 ± 2.4	0.93	.30	4.38	12	<.001
3	10	13.6 ± 3.9	0.91	.29	5.25	9	<.001

TABLE 1Summary statistics fromShapiro-Wilk test for normality and onesample t-tests for chamber depths versusestimated maximum emergence depth of7.026 cm



FIGURE 3 Boxplots of percentage of seed surface area damaged by *E*. *ruidum* that were either recovered from a colony wax caste (N = 36 seeds) or had presumably never interacted with ants (control: N = 41 seeds) (a). Image of *E*. *ruidum* damaged seeds, one of which still has some wax on the seed (b, c). Image of a control seed that still shows some damaged (d). Boxplots are plotted the same way as described in Figure 2

TABLE 2 Bite force data for four *E. ruidum* workers in grams (g). Values in bold and italic are the max bite for that individual in grams. Both max force and standard deviation on bite force generated converted into newtons (N)

		Bites (g)						
Individual	No. of Bites	1	2	3	4	5	Max bite (N)	SD bites (N)
SAR851_01	5	4.69	6.09	2.81	5.16	6.09	0.060	0.013
SAR948_01	3	9.37	8.44	8.44	NA	NA	0.092	0.005
SAR961_01	5	5.62	5.16	6.56	9.37	2.34	0.092	0.025
SAR1314_01	3	7.97	5.62	5.16	NA	NA	0.078	0.015

when dispersal is performed in at least two steps (Camargo et al., 2016; Christianini & Oliveira, 2010; Gallegos et al., 2014; Giladi, 2006). This appears to be the case here as seeds were moved short distances. However, seeds experienced directed dispersal with most deposited into a colony. Within the colony, seeds were placed at depths too deep for seedling emergence to occur reliably. However, given the long-term viability of *Z. ekmanii* seeds (Dalling & Brown, 2009), opportunity remains for either further dispersal or germination after bioturbation to occur. Together, the data suggest that *E. ruidum* is a secondary dispersal agent over relatively short distances; however, seeds cached within the colony will not likely increase seedling emergence rates over short time scales if the colony is the final deposition location.

There is an interesting scaling effect with ant-mediated seed dispersal. In herbaceous communities and shrubs, where elaiosomes are common (Beattie, 1983; Leal et al., 2015; Lengyel et al., 2010; Rico-Gray & Oliveira, 2007), a one meter dispersal event could represent several canopy widths from the maternal plant. Even in plant species without elaiosomes, plant growth form is linked to whether ants are effective seed dispersers (Anjos et al., 2020). For a tree, moving one meter often still leaves you beneath the crown; however, not all seeds will fall near the tree trunk meaning that less distance may be needed to no longer be under the parent crown. *Ectatomma ruidum* fits this model of short distance dispersal moving seeds an average distance of 99.8 cm with a maximum distance of 405.7 cm. These values are similar to those reported in the literature for *E*.

ruidum (mean 70 cm, max 385 cm, Zelikova & Breed, 2008) and other Ectatomma spp. (Ectatomma edentatum: mean 71 cm, max 400 cm, Magalhães et al., 2018). Though this dispersal distance is larger than the 42 cm reported for the Ectatomma genus in Gómez and Espadaler's (2013) review of myrmecochorous dispersal distances, it is shorter than the average distance seeds of both myrmecochorous (2.24 m, Gómez & Espadaler, 2013) and non-myrmecochorous (2.39 m, Anjos et al., 2020) species are dispersed. Ectatomma ruidum workers can conduct sequential movements, seeds may be moved farther than the average first distance movement reported here. In addition, as *E. ruidum* prefer to nest in open areas avoiding areas beneath tree canopies (Santamaría et al., 2009), it is possible that seeds are moved to microsites that have more light which could be beneficial for pioneer species.

Our dispersal distances may reflect the high *E. ruidum* colony density on BCI (1.06 entrances per m², Pratt, 1989). In addition, *E. ruidum* can move seeds that were initially moved by other ants and move seeds from one *E. ruidum* colony in another direction (4 observations) including moving seeds from one colony into another (1 observation). *Ectatomma ruidum* workers engage in thievery, where foragers from one colony are able to infiltrate another colony and intercept food items brought into the nest (Guénard & McGlynn, 2013), potentially explaining our observations that seeds removed into one colony can move to another. If seeds remain attractive over long time scales, these secondary dispersal events can shape dispersal kernels as shown by Jansen et al. (2012) with rodents stealing cached seeds from other rodents.

Consistent with directed dispersal, E. ruidum workers brought over half of the seeds they moved into a colony. However, colonies themselves vary in their suitability regarding seedling emergence. While chambers 1-3 of some colonies were shallower than the estimated maximum emergence depth, our seedling emergence study suggests that freshly germinated Z. ekmanii seeds may have a much shallower maximum emergence depth than expected. A similar finding was described by Renard et al. (2010) for seeds of Manihot esculenta subsp flabellifolia, a myrmecochorous plant found in French Guianna savannas that is dispersed by Ectatomma brunneum. The predicted maximum emergence depth (11.7 \pm 1.4 cm) was deeper than the depths seedlings could emerge from though seeds were often found even deeper within E. brunneum colonies. Bond et al. (1999) generated the formula for calculating emergence depth by planting seeds of 17 species (seed mass: 0.1-100 mg). However, they tested burial depth in sand, which we did not add to the substrate we grew Z. ekmanii seeds in. In addition, seeds of Z. ekmanii have thick seed coats (Zalamea et al., 2018); therefore, the mass of the intact seeds does not accurately represent the amount of stored energy available for seedling growth before photosynthesis occurs. Using the embryo would have estimated a shallower, but potentially, more accurate estimated maximum emergence depth. In addition, fresh seeds of Z. ekmanii have a low germination probability though that increases with time buried in the soil (P-C. Zalamea unpubl. data) and initially viable seeds can remain viable for long periods of time (Dalling & Brown, 2009; Zalamea et al., 2018). If E. ruidum workers

are engaging in thievery and are moving seeds, potentially discarding them in waste piles (likely outside of the nest, S.A. Ruzi personal observation), seeds may not remain in colonies long enough to germinate. Therefore, colonies may not be the final deposition place and may not dictate the microhabitat seedlings must be able to emerge from.

Ectatomma ruidum workers did not produce enough mandibular force to rupture Z. ekmanii seeds but were able to remove the outer layer of the seed coat. In the genus Pheidole, there are both minor and major worker castes, and the differences in head size between these castes is greater in seed harvesting species than in species with other diet preferences (Holley et al., 2016). These major workers have been proposed as specialized seed millers (Wilson, 1984) as their larger heads fit larger mandible closer muscles (Paul & Gronenberg, 1999). The angle at which these closer muscles attach to the apodeme region of the mandible determines whether speed (0°) or force (41–44°) is maximized. For *E. ruidum* workers, this angle is 22.7 \pm 3.4°, while *Pogonomyrmex badius* (a dimorphic ant known for milling seeds) workers is 38.4 \pm 8.2° (data from Paul & Gronenberg, 1999). Therefore, *E. ruidum* worker closer muscles are not configured to maximize force output for crushing seeds.

Seeds that had been taken into *E. ruidum* colonies have more surface area damage or processing marks than those that had never encountered ants. However, all the damage observed was confined to the sarcotesta, while the inner layer of the seed coat (sclerotesta) remained intact. *Ectatomma ruidum* foragers were observed biting the seed multiple times to get a better hold prior to dispersing it from the cache location (S.A. Ruzi personal observation) and may have scraped off the sarcotesta of the seed coat during that handling time. Alternatively, this damage could come from handling by larvae, as larvae conduct most of the food digestion in ant colonies (Cassill et al., 2005; Dussutour & Simpson, 2009) and seeds were almost always found in chambers with larvae. Some seeds that had never encountered ants did have some damage but not to the extent of ant handled seeds.

Research on ant-mediated seed dispersal often focuses on dispersal of myrmecochorous plants that mediate seed dispersal by having an elaiosome attached to the seed (Beattie, 1985; Handel & Beattie, 1990). While myrmecochorous plants occur world-wide in several plant lineages, together they make up ~4.5% of angiosperm species (Lengyel et al., 2010), leaving a knowledge gap in ant-mediated dispersal for the vast majority of angiosperms. Antmediated seed dispersal can play an important role for these other plant species (Anjos et al., 2020) despite many of these interactions being previously overlooked because ants have yet to be observed interacting with them (e.g., Barroso et al., 2013; Christianini & Oliveira, 2010; Magalhães et al., 2018; Passos & Oliveira, 2002; Pizo & Oliveira, 1998). Ants can be a temporally stable disperser of aril bearing seeds (e.g., Campagnoli & Christianini, 2021), and thus may be an important selective force on the evolution of seed traits. Elaiosomes themselves are defined by their function (i.e., attract ants to initiate seed dispersal) and not by the many different fruit and seed tissues they can be derived from which are often called

by other names (e.g., an aril arises from the funicle, Gorb & Gorb, 2003). Notably, the sarcotesta is among possible seed tissues that give rise to an elaiosome (Gorb & Gorb, 2003). Alternatively, chemical cues from the seed coats of some species may elicit seed removal (Ruzi in prep). These chemicals may mimic food rewards by having chemical cues on their seed coat that act as a behavioral releaser (e.g., 1,2-diolein in elaiosome bearing systems (Marshall et al., 1979)). Without providing a food reward, plants may be exploiting the behavior of ants.

5 | CONCLUSION

Ectatomma ruidum workers disperse the seeds of Z. ekmanii that do not provide a known food reward though further investigation on whether the sarcotesta is acting as a reward is needed. While E. ruidum consistently removed seeds, these seeds do not move far through single dispersal events. However, these dispersal events were tracked over a two-hour time period, which is extremely short compared with the amount of time these seeds remain viable (Dalling & Brown, 2009). If these seeds remain attractive to ants for years, it is possible that these single dispersal events poorly estimate the absolute distance a seed moves. Seeds brought into the colony are kept at depths where it is unlikely germinating seedlings would survive. However, diel temperature fluctuations are needed for seeds of larger pioneer tree species (> 2 mg) to germinate (Pearson et al., 2002). Therefore, seeds of Z. ekmanii are to not likely to germinate at the deeper burial depths indicated by the wax casts as the magnitude of diel temperature fluctuations decreases with increasing depth (Pearson et al., 2002). In addition, as Z. ekmanii seeds experience physiological dormancy, they are unable to immediately respond to germination cues (Dalling et al., 2011). As a result, seeds will likely only germinate after some time and if the soil has been perturbed. For example, tip-up mounds caused from fallen trees have a higher abundance of pioneer tree seedlings than other areas within gaps with seedling species composition reflecting the seed bank composition in the same location (Putz, 1983). In a survey of dead trees conducted in the late 1970s, 25% of 310 trees resulted in tip up mounds with trees that were taller, had lower height to width ratio, and had denser, stronger wood more likely to produce tip up mounds than either snap or break off at ground level (Putz et al., 1983). In addition, larger gaps are more likely to be disturbed again than forest areas with smaller gaps, suggesting that treefall gaps on Barro Colorado Island may be somewhat stable (Young & Hubbell, 1991). Overall, canopy disturbances on Barro Colorado Island happen at rates of 1.8 to 2.1% per year (Cushman et al., 2022). Both the potential negative impacts (e.g., damage and deep caching at depths) or positive impacts (e.g., incorporation into the seed bank and further dispersal) of ant dispersal of seeds increases our awareness that ants may influence the seed bank of Neotropical pioneer tree species. However, these interactions need to be studied in the context of other seed traits, such as dormancy cues and long-term seed viability, and ecological processes, such as bioturbation of soil.

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CONFLICT OF INTEREST

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

AUTHOR CONTRIBUTION

Following contributor roles taxonomy: SAR and AVS involved in conceptualization, funding acquisition, methodology, and writing—review and editing. SAR involved in data curation, formal analysis, investigation, software, validation, visualization, and writing—original draft. AVS involved in resources and supervision.

DATA AVAILABILITY STATEMENT

All data and scripts needed to complete these analyses are available at the GitHub repository (https://github.com/sruzi24/Ruzi_and_ Suarez_seed_short_term_seed_fate) and Dryad Digital Repository (https://doi.org/10.5061/dryad.t1g1jwt4c, Ruzi and Suarez, 2022.

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SUPPORTING INFORMATION

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