1   **Ant-mediated seed dispersal in a warmed world**

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3   Katharine L. Stuble[1†]

4   Courtney M. Patterson[1]

5   Mariano A. Rodriguez-Cabal[2]

6   Relena R. Ribbons[1]

7   Robert R. Dunn[3]

8   Nathan J. Sanders[1‡]

9

10   1 Dept. of Ecology & Evolutionary Biology, University of Tennessee, Knoxville, TN 37996,

11   USA

12   2 Dept. of Zoology, University of British Columbia, Vancouver, B.C. V6T1Z4, Canada

13   3 Dept. of Biology, North Carolina State University, Raleigh, NC 27695

14   † Current address / Corresponding author: Oklahoma Biological Survey, 111 E Chesapeake St,

15   Norman, OK, 73019 USA

16   ‡ Current address: Center for Macroecology, Evolution and Climate, Natural History Museum of

17   Denmark, University of Copenhagen, DK-2100 Copenhagen, Denmark

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Abstract

Climate change affects communities both directly and indirectly via changes in interspecific

interactions. One such interaction that may be altered under climate change is the ant-plant seed

dispersal mutualism common in deciduous forests of the eastern US. As climatic warming alters

the abundance and activity levels of ants, the potential exists for shifts in rates of ant-mediated

seed removal. We used an experimental temperature manipulation at two sites in the eastern US

(Harvard Forest in Massachusetts and Duke Forest in North Carolina) to examine the potential

impacts of climatic warming on overall rates of seed dispersal (using *Asarum canadense* seeds)

as well as species-specific rates of seed dispersal at the Duke Forest site. We also examined the

relationship between ant critical thermal maxima (CT_{max}) and the mean seed removal

temperature for each ant species. We found that seed removal rates did not change as a result of

experimental warming at either study site, nor were there any changes in species-specific rates of

seed dispersal. There was, however, a positive relationship between CT_{max} and mean seed

removal temperature, whereby species with higher CT_{max} removed more seeds at hotter

temperatures. The temperature at which seeds were removed was influenced by experimental

warming as well as diurnal and day-to-day fluctuations in temperature. Taken together, our

results suggest that while temperature may play a role in regulating seed removal by ants, ant

plant seed-dispersal mutualisms may be more robust to climate change than currently assumed.

Keywords: ants, climate change, myrmecochory, seed dispersal, warming

Introduction

Understanding how organisms will respond to ongoing changes in climate, leading to subsequent changes in key ecological processes, is essential in order to predict the structure and function of ecosystems in the future. For example, the alteration of interspecific interactions is one important mechanism by which climate change may ultimately alter the structure and function of ecosystems (Tylianakis et al. 2008, van der Putten et al. 2010, Walther 2010, Harley 2011). The majority of studies on the effects of climate change on interspecific interactions have focused on negative interactions, such as competition (Suttle et al. 2007), predator-prey interactions (Rothley and Dutton 2006, Barton and Schmitz 2009, Harley 2011), and herbivory (Bale et al. 2002). Though rare relative to studies that focus on physiological responses (Norby and Luo 2004, Dillon et al. 2010) or aggregate community-level responses (Klein et al. 2004, Villalpando et al. 2009, Fitzpatrick et al. 2011, Sheldon et al. 2011), empirical studies of the effects of climate on interactions among species have found that climate change can alter the nature and outcomes of interspecific interactions through a variety of mechanisms such as altered abundance and fitness levels of key species (Suttle et al. 2007), shifts in phenology (Both et al. 2009), and species range shifts (Harley 2011). Each of these mechanisms can decouple interspecific interactions by altering the frequency and intensity of interactions among species.

As with most studies in ecology, work on the effects of climate change on positive interactions is lacking, even though mutualisms play fundamental roles in structuring communities and ecosystems (Callaway 1995, Stachowicz 2001). Mutualisms including plant-pollinator interactions and mycorrhizal interactions have been altered by climate change (Parrent et al. 2006, Memmott et al. 2007, Hoover et al. 2012). However, the influence of climate change on other types of positive interactions is not as well studied.

63   One such mutualism that may be altered by climate change is the ant-plant seed dispersal

64   mutualism, which includes hundreds of ant species and thousands of plant species around the

65   world (Beattie and Hughes 2002; Gove et al 2007; Rico-Gray and Oliviera 2007, Lengyel et al.

66   2010). In deciduous forests of the United States approximately thirty percent of understory herb

67   species might be ant-dispersed (Beattie and Culver 1981), and a proposed keystone seed-

68   dispersing ant species, *Aphaenogaster rudis,* is responsible for upwards of 90% of ant-mediated

69   seed dispersal (Ness et al. 2009; Zelikova et al. 2008). Such specialization in interactions can

70   make interaction networks more vulnerable to disruption as a result of low levels of functional

71   redundancy within a system (Aizen et al. 2012). Myrmechorous plant species that rely on a

72   single ant species for seed dispersal may be at increased risk for disruption by ongoing climatic

73   change (Pelini et al. 2011a, Warren et al. 2011) if that ant species is negatively affected by

74   warming. Inversely, systems in which multiple species are responsible for removing seeds may

75   prove to be more resistant to disruptions because of functional redundancy in the system

76   (Peterson et al. 1998). However, despite the importance and ubiquity of ant-plant seed-dispersal

77   mutualisms in ecosystems around the world and the importance of temperature in regulating ant

78   foraging, experiments examining the consequences of climatic change on plant-seed dispersal

79   mutualisms are rare (but see Pelini et al. 2011a).

80   To examine the potential for climate change to alter seed dispersal mutualisms, we

81   experimentally manipulated temperature at two sites in the eastern United States (North Carolina

82   and Massachusetts) and examined overall rates of seed removal as a function of temperature. At

83   the North Carolina site, we also examined species-specific rates of seed dispersal. We test two *a*

84   *priori* predictions:

85   *Prediction 1:* Rates of seed removal by ants would decrease as a result of experimental warming

86   at the southern site where species, including *A. rudis*, are closer to their critical thermal maxima

87   (CT_{max}) (Deutsch et al. 2008, Diamond et al. 2012a, Diamond et al. 2012b, Huey et al. 2012), a

88   pattern previously documented in this study system (Diamond et al. 2012a). Rates of seed

89   removal can be expected to increase at the northern site, where most species, including *A. rudis*,

90   are well below their CT_{max} (Diamond et al. 2012a) and perhaps even below their thermal optima.

91   *Prediction 2:* Species with higher CT_{max} would remove seeds more readily under warmer

92   conditions as compared to species with lower CT_{max} as these species are more tolerant of higher

93   temperatures and have been found to be more active as temperatures increase (Stuble et al.

94   2013).

95

Methods

Site description

98   This experiment was conducted at Duke Forest (southern site) in North Carolina and

99   Harvard Forest (northern site) in Massachusetts, USA, in order to examine the potential impacts

100   of climate change on seed dispersal mutualisms near the southern extent and northern extent of

101   the ranges of several ant species. The Duke Forest site consists of a closed-canopy oak-hickory

102   (*Quercus spp.*, *Carya spp.*) forest with a mean annual temperature of 15.5°C and approximately

103   1140 mm of precipitation annually. The Harvard Forest site is in a closed-canopy oak-maple

104   (*Quercus spp.-Acer spp.*) forest with a mean annual temperature of 7.1°C and 1066 mm of

105   precipitation a year. Though not present in the chambers, several myrmecochorous plant species

106   are common in these forests. These species include *Asarum canadense*, *Hexastylis arifolia*,

107   *Trillium* spp., *Violia rotundifolia*, and *Sanguinaria canadensis*, among others. Seeds of these

108   plant species are typically dispersed by ants in the spring (Thompson 1981).

109   The two sites share ~30 ant species, with the North Carolina site near the southern range

110   edge and the Massachusetts site near the northern range edge for many of these species (Pelini et

111   al. 2011b). The most abundant ant species at both sites is the proposed keystone seed disperser *A.*

112   *rudis* (Ness et al. 2009) (or at least a species in the *A. rudis* complex). *Crematogaster lineolata,* a

113   behaviorally aggressive species, can be abundant at the southern site in warmer months while

114   *Formica subsericea* and *Camponotus pennsylvanicus* (also behaviorally aggressive) are the two

115   next most abundant ant species at the Harvard Forest site (Stuble et al. 2013b). For the purposes

116   of this study we are combining *A. rudis, A. picea,* and *A. carolinensis* into the *A. rudis* complex

117   due to the difficultly of identifying these closely related species in the field.

118   At each site, there are twelve experimental open-top warming chambers (Fig. 1). Each

119   chamber is 5 m in diameter and 1.2 m tall with a 2-3 cm gap at the bottom to allow ants and

120   other organisms to move in and out. The chambers are approximately 1000 ant body lengths in

121   diameter. The chambers contain about 1 colony per square m, and the average foraging distance

122   of an *A. rudis* colony is ~70cm. Moreover, during the summer of 2012, we watched 72 *A. rudis*

123   workers visiting baits and returning to their colonies. Out of those 72 observations, only 1

124   worker visited a bait in the chamber and returned to a nest outside the chamber (L. Nichols,

125   unpublished data). So, most of the activity we see is from ants in the chambers. Nine chambers at

126   each site are warmed from 1.5°C to 5.5°C in 0.5°C steps using air warmed by hydronic radiators,

127   while the three control chambers blow air at ambient temperatures into the plots (see Pelini et al.

128   (2011b) for a detailed description of the chambers). Warming treatments have been maintained

129   continuously since January of 2010 and have been successful at maintaining the targeted

130   temperature increases. For 2011, a significantly positive relationship between the target

131   temperature increase and actual temperature increase was maintained ($p < 0.01$, $R^2 = 0.99$).

132   Significant shifts in levels of ant foraging activity at food baits have been documented in the

133   chambers across the temperature treatments, with more thermally tolerant species exhibiting

134   higher levels of foraging activity in warmer chambers than species with lower thermal tolerances

135   (Stuble et al. 2013a). Those results suggest that temperature does mediate foraging behavior.

136   Such a result, that environmental context can mediate foraging behavior, is in line with previous

137   work in this system (Pelini et al. 2011) and others (Cerdá et al. 1997, Sanders and Gordon 2000,

138   2003, Gibb and Parr 2010).

139

140   **Seed removal**

141   To assess the impact of temperature on rates of seed dispersal, we haphazardly positioned

142   one seed cache in each of the 12 chambers at Duke Forest and Harvard Forest. Each cache

143   contained 20 seeds of the myrmecochorous species *Asarum canadense.* The range of *Asarum*

144   *canadense* extends from New Brunswick, Canada to North Carolina in the southern US (Cain

145   and Damman 1997). Seeds of *A. canadense* are similar in mass to many other myrmecochorous

146   plant species (Michaels et al. 1988). Seeds used in the trials at Duke Forest were collected at

147   North Carolina State University's Schenck Forest in Raleigh, North Carolina on May 11, 2011

148   and those used in the Harvard Forest trials were collected from Mt. Toby in Massachusetts on

149   June 8, 2011. We covered each seed cache with a mesh cage to allow ants to access the seeds

150   while preventing access by rodents. Caches were left out for one hour, after which time the

151   number of seeds remaining in the cache was counted and any remaining seeds were removed

152   from the chamber. Though observing seed removal for an hour limits our ability to account for

153   the fate of all seeds, using this standard timeframe allowed us to compare relative rates of seed

154   removal across treatments. A total of ten trials were conducted at Duke Forest between May 12

155   and May 25, 2011, with five trials conducted during the day (between 0900 and 1900) and five

156   during the night (between 2100 and 0500). Another five trials were conducted at Harvard Forest

157   between June 16 and June 30, 2011; three during the day and two at night. These dates

158   corresponded with the time periods during which the seeds were naturally released at each site,

159   as opposed to conducting this experiment in the hottest part of the year when the impacts of

160   warming might be expected to be greater, but when any results might be less ecologically

161   relevant.

162   We calculated the average seed dispersal rate (number of seeds removed in an hour) for

163   each chamber at each site. We used ANCOVA to examine differences in seed dispersal rates as a

164   function of temperature treatment (which we refer to as Δ°C, included as a continuous variable)

165   and site. The number of seeds removed per hour was square root transformed to meet

166   assumptions of normality. All statistics were performed in SAS, version 9.2.

167   To determine the ant species responsible for removing the seeds, we continuously

168   observed caches of 10 *A. canadense* seeds within the chambers at Duke Forest for one hour, or

169   until all seeds were removed. Four seed removal observations were conducted in each chamber:

170   two during the day and two during the night. Nighttime observations were conducted using red

171   lights, which is typical in studies of ant behavior at night (Hodgson 1955, Narendra et al. 2010).

172   We recorded the identity of the ant species removing the seeds. When possible, we also followed

173   the seed back to the nest (or under leaf litter in some cases) and noted the distance it had been

174   moved. At the beginning of each observation, we took four ground surface temperature

175   measurements using a handheld infrared thermometer (Raytek® Raynger ST, +/- 1 °C), one at

176   each corner of the seed cache, which were averaged together. These temperature readings

177   provided us with estimates of ground-surface temperature conditions in the immediate vicinity of

178   the seeds. Ground-surface temperature has been shown to be an important driver of foraging

179   activity in ants (Whitford and Ettershank 1975, Crist and MacMahon 1991). We calculated the

180   percentage of seeds removed by each species overall, as well as separately for day and night. We

181   also calculated the mean number of seeds removed by each species in each chamber across all

182   trials.

183   We used linear regressions to examine differences in seed dispersal rates across

184   temperature treatments for each ant species. (We examined several polynomial regressions, but

185   found none of them to be a better fit than simple linear regressions.) Mean numbers of seeds

186   removed were log transformed to meet assumptions of normality for *A. rudis* and *C. lineolata.*

187   Finally, we calculated the average ground surface temperature (based on temperatures

188   collected with the infrared thermometer) at which each species removed seeds across all

189   treatments and times. We then examined the relationship between the average temperature at

190   which a species removed seeds and the CT_{max} of that species (as calculated by Diamond et al.,

191   2012) across all species observed removing seeds in the system. *Aphaneogaster lamellidens* was

192   excluded from this analysis as it was only observed removing seeds from two seed caches and

193   was an outlier (as indicated by a plot of residuals by predicted values).

194

Results and Discussion

196   Seed removal rate did not depend on temperature treatment and did not vary between

197   sites ($F_{2,21} = 0.93$, $p = 0.41$; Fig. 2). This is despite the fact that most of the foragers observed in

198   this study were from colonies within the experimental chambers. At the southern site, where seed

199   dispersal observations were conducted, the mean seed removal distance was 51 cm, and only 2%

200   of observed seeds were removed more than 2 m. The lack of response to experimental warming

201 contrasts with the prediction that, based on the thermal limits of *A. rudis* and its disproportionate

202 role in seed dispersal, seed dispersal rate should decline with increasing temperatures. Regardless

203 of temperature treatment or site, ants removed ~ 23% of seeds per hour (an average of 4.6 seeds

204 out of 20). At the southern site, we observed seven ant species removing seeds across a range of

205 ground surface temperatures from 17ºC to 30ºC (Table 1). *Aphaenogaster rudis* was the most

206 common seed disperser, removing approximately 45.5% of seeds (Table 2). However, there was

207 no relationship between the rate of seed dispersal by *A. rudis* and temperature treatment (Table

208 1). With the exception of *C. lineolata*, which showed a marginally significant increase of

209 approximately 0.1 seeds removed per degree of warming, seed removal did not vary

210 systematically with temperature treatment for any ant species (Table 1). This is despite

211 previously observed shifts in foraging under experimentally warmed conditions (Pelini et al.

212 2011a, Stuble et al. 2013a). Pelini et al. (2011) found an approximately 50% decrease in several

213 types of foraging, including seed removal, as a result of 1°C of warming at the southern site,

214 though no change was observed at the northern site. Using the same warming chambers as in this

215 study, Stuble et al. (2013) found species-specific shifts in foraging activity as a result of

216 experimental warming consistent with the thermal tolerances of the foraging species. Further, ant

217 community composition shifts in response to experimental warming, demonstrating the

218 importance of temperature in regulating the ant community (Diamond et al. 2012a). Despite this,

219 experimental warming apparently does not affect the aspects of the seed-dispersal mutualisms we

220 studied in this system. This begs the question - why isn't this seed-dispersal mutualism disrupted

221 by experimental warming?

222 　　　　One possible answer is that foraging behavior by *Aphaenogaster rudis* may be more

223 tolerant to experimental warming than previously thought. *A. rudis* is a keystone mutualist in this

224 and other systems, responsible for the majority of ant-mediated seed dispersal (Zelikova et al.

225 2008, Ness et al. 2009, Canner et al. 2012). The abundance and activity of *A. rudis* declines with

226 elevation (i.e., lower temperatures) at biogeographic scales (Zelikova et al. 2008). Additionally,

227 the relatively low thermal tolerance of this species accurately predicts its activity relative to other

228 species (Stuble et al. in press). However, when exposed to experimental warming, the abundance

229 (Pelini et al. 2011a) and foraging activity (Stuble et al. 2013) of *A. rudis* apparently do not

230 decline at either study site. Importantly, the average foraging distance of *Aphaenogaster* spp. was

231 ~70cm at the study site (L. Nichols, unpublished data). Further, based on the proportion of these

232 ants observed foraging into the chambers from outside in observations, we'd predict that only

233 about one of the eighty seeds observed being removed by *A. rudis* was likely to have been

234 removed by a worker originating outside of the chambers. Thus, it is not likely the case that ants

235 are coming to baits from nests that are outside the chambers. However, even in cases in which

236 individual workers do forage at the experimental baits from colonies outside of the chambers,

237 these individuals are still exposed to the experimental temperature conditions while discovering,

238 foraging at, and recruiting to the seed caches. These results suggest that temperature does not

239 substantially alter this foraging behavior. The apparent tolerance of the foraging activity of this

240 important seed dispersing species to warming may play a major role in promoting the stability of

241 ant-plant seed dispersal in light of global change.

242 It is important to note that six ant species other than *A. rudis* were observed removing

243 seeds in this study, and they removed > 50% of the seeds. This runs counter to several studies

244 suggesting seed dispersal mutualisms may be highly specialized (Gove et al. 2007, Ness et al.

245 2009). Both the foraging activity and abundances of several of these species, including *C.*

246 *lineolata* and *Formica pallidefulva*, shift with warming (Pelini et al. 2011a, Diamond et al.

247 2012a, Stuble et al. 2013a), resulting in an altered community of foragers (Diamond et al.

248 2012a). By having multiple ant species interacting with myrmecochorous plants, this ant-plant

249 seed dispersal mutualism may be relatively resistant to the effects of warming as some ant

250 species increase in activity and abundance while others decline in abundance with temperature.

251 Previous work on ant foraging and community composition as a result of the experimental

252 warming at these sites suggests quite a bit of variability in species-specific responses to warming

253 which may, in fact, play a role in moderating the effects of climatic warming (Stuble et al.

254 2013a).

255   In addition to the apparent (and of course relative) resistance of the foraging of *A. rudis*

256 to warming, along with the diversity of ants engaging in this mutualism, another factor possibly

257 strengthening the resistance of the ant-plant seed dispersal mutualism may be the timing of ant-

258 mediated seed dispersal within deciduous forests of the eastern US. Ant-dispersed seeds in these

259 forests, including those of *Asarum canadense*, are primarily dispersed in the spring (Thompson

260 1981). Temperatures in May in North Carolina and June in Massachusetts at the study sites are

261 far from the critical thermal maxima of ant species in the system. For example, the critical

262 thermal maximum for *A. rudis* is 38ºC and 40ºC for populations at the northern and southern

263 sites, respectively (Diamond et al. 2012b), as opposed to the mean environmental temperatures

264 during the sampling period, which were 20ºC at the northern site and 22ºC at the southern site.

265 The thermal buffer between CT_{max} and the environmental temperature during the time of year

266 when seeds are dispersed may confer some degree of tolerance on this mutualism. Pelini (2011a)

267 found that rates of seed removal decreased in a passive experimental warming at the same two

268 sites, despite achieving warming of only 0.3˚C above ambient. However, the seed removal trials

269 in Pelini et al.'s experiment were conducted mostly in August when ambient environmental

270   temperatures are hotter than those experienced in the present study. We suggest that the

271   proximity of ants to their upper thermal limits in August may have driven the effects of warming

272   observed in the Pelini et al. (2011a) study while seed dispersal occurring in the spring when our

273   study was conducted may be less likely to be detrimentally impacted by warming. However, this

274   protection assumes that the peak of *A. rudis* activity and seed set coincide. Phenological shifts in

275   plant reproduction caused by ongoing warming (Price and Waser 1998, Dahlgren et al. 2007,

276   Inouye 2008, Liu et al. 2011, Wolkovich et al. 2012) have the potential to result in seeds

277   appearing before ants become active (Warren et al. 2011). Warren et al. (2011) suggest that

278   while both seed release by plants and onset of foraging in ants seem to be driven by temperature,

279   variability in activation temperatures among ant species may result in situations in which early

280   seeding plant species may become decoupled from their foragers in some areas.

281   Despite the apparent tolerance of the ant-plant seed dispersal mutualism to experimental

282   warming in this study, there was a significant relationship between the ground-surface

283   temperature at which a species removed seeds and the critical thermal maximum of that species

284   ($F_{1,4} = 7.35$, $p = 0.05$, $R^2 = 0.65$, Fig. 3). That is, those species with high thermal tolerances were

285   most active under the warmest temperatures. The positive relationship between CT_{max} and seed

286   removal temperature suggests that while chronic experimental warming may not affect rates of

287   seed dispersal, temperature does relate to rates of seed removal. This finding incorporates both

288   temperature variability associated with the temperature treatments as well as daily temperature

289   variability and complements other studies that have shown physiological tolerance to be an

290   important predictor of ant activity (Diamond et al, in press).

291   One important caveat to our study (and to most studies of ant-seed dispersal mutualisms)

292   is that we do not know the ultimate fate of the seeds once they were removed by the ants. It is

293   possible that warming could still alter the dynamics of plant populations by altering rates of

294   germination and seedling survival post-germination (De Frenne et al. 2012), even in cases in

295   which seed dispersal remains unaffected as temperatures increases. Additionally, some species,

296   including *C. lineolata*, dispersed seeds very short distances (only a few centimeters) while other

297   species, such as *C. castaneus*, often carried seeds several meters. Even slight shifts in dispersal

298   rates among these species may alter plant population dynamics if dispersal distances differ

299   substantially among species. Finally, by observing seed caches for an hour, we fail to collect data

300   on the fate of seeds not removed in that time frame. However, a considerable proportion of the

301   seeds that will be removed by ants are, in fact, removed soon after release from the parent plant

302   (Turnbill and Culver 1983, Beaumont et al. 2013) and seeds not dispersed by ants are at risk of

303   predation by rodents (Heithaus 1981). Additionally, as in most studies of ant-seed interactions,

304   we focus on seeds of only a single plant species. Including more seeds from more species

305   would be ideal, but would have been beyond what was possible in this (or in most) studies.

306

307   It has become axiomatic that interactions among species are being affected by ongoing

308   climatic change. However, in this study, we found no reduction in overall rates of seed removal

309   as a result of experimental warming. Moreover, there were no differences in species-specific

310   seed removal rates at the southern site. We suggest that the ant-plant seed dispersal mutualism

311   may be resistant, at least in part, to climatic warming as a result of the diversity of ants active in

312   this mutualism as well as the seasonal timing of this mutualism in the spring when most ant

313   species are far from their upper thermal limits. Importantly, however, if phenological

314   mismatches arise, or if the fate of seeds after dispersal is altered, the consequences of warming

315   on plant populations and communities could emerge in unexpected ways.

316

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462   162.

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465 **Tables**

466 Table 1. Test statistics are from linear regressions examining the influence of temperature

467 treatment on the number of seeds removed by each ant species (d.f. = 11 for all species).

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Species	F	p
Aphaenogaster lamellidens	2.58	0.58
Aphaenogaster rudis	2.45	0.15
Camponotus castaneus	0.10	0.75
Camponotus pennsylvanicus	0.24	0.64
Crematogaster lineolata	4.14	0.07
Formica pallidefulva	0.34	0.58
Formica subsericea	0.02	0.90

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471   Table 2. Percentage of seeds removed by each species overall, during the day, and the night.

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Species	Overall percent	Day percent	Night percent
Aphaenogaster lamellidens	8.5	17.2	0.0
Aphaenogaster rudis	45.5	48.3	42.7
Camponotus castaneus	26.7	0.0	52.8
Camponotus pennsylvanicus	2.8	2.3	3.4
Crematogaster lineolata	6.8	12.6	1.1
Formica pallidefulva	2.3	4.6	0.0
Formica subsericea	7.4	14.9	0.0

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Figures

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487   Figure 1. Warming chamber at Duke Forest.
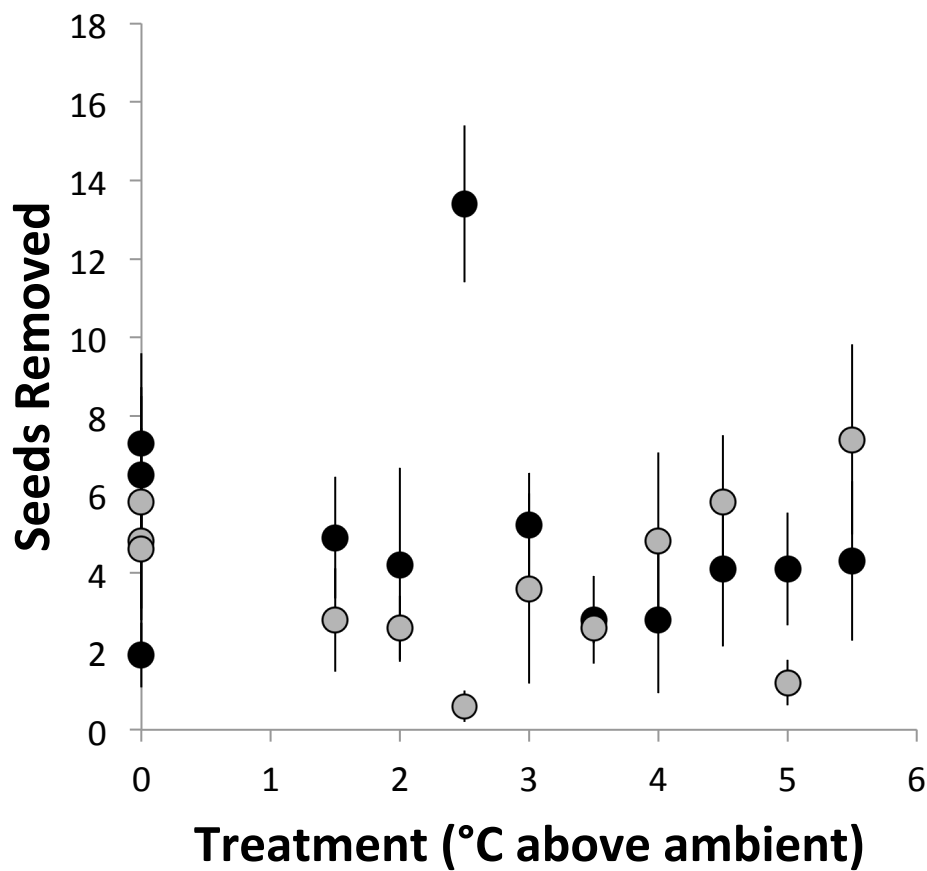
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Figure 2. Number of seeds removed in the course of an hour as a function of temperature

treatment. Black dots represent Duke Forest and gray dots represent Harvard Forest.
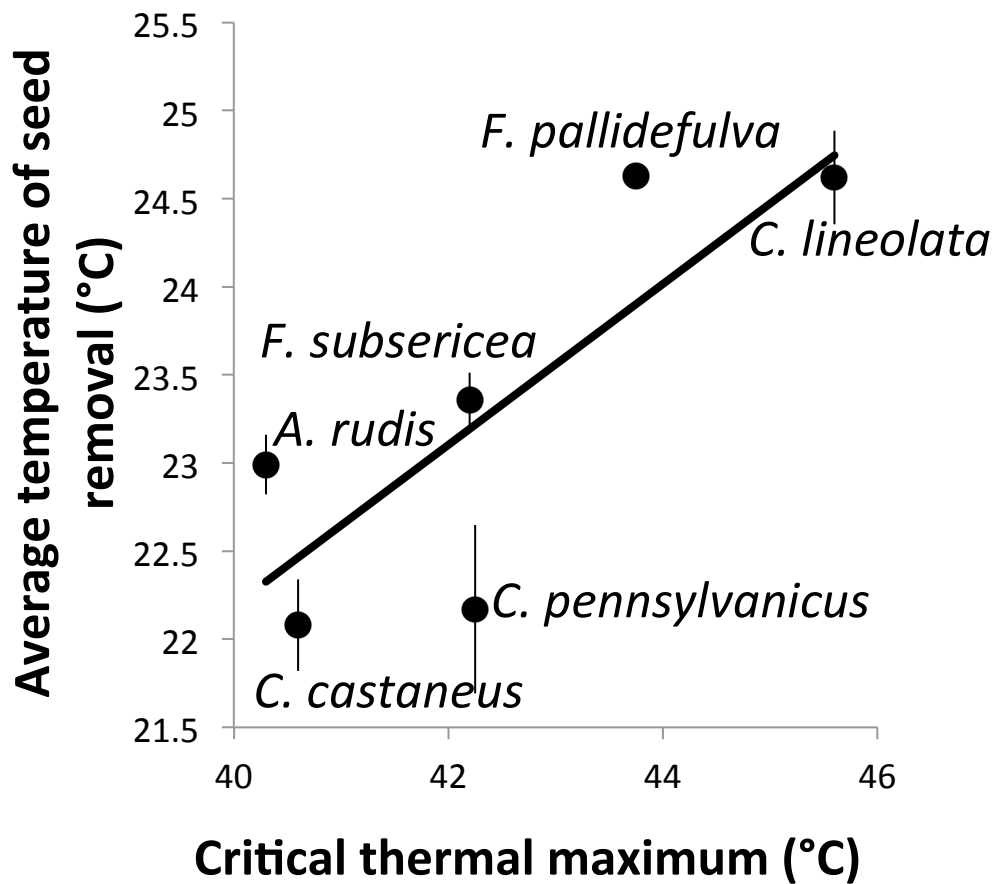
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Figure 3. Temperature at which seeds were removed as a function of a species' critical thermal

497   maximum (CT_{max}).

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