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## **Tool selection during foraging in two species of funnel ants**

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## **Tool selection during foraging in two species of funnel ants**

### **Highlights**

- Some ant species use debris as tools to transport liquid food to the nest.
- When given the choice between different kinds of potential tools ants are selective.
- They show preference for materials with optimal handling and/or soaking properties.
- They can employ artificial materials novel to them, thus tool use is flexible in ants.

# 1 **Tool selection during foraging in two species of funnel ants**

## 2 **ABSTRACT**

3 Tool use by non-human animals has received much research attention in the last couple of  
4 decades. Nonetheless, research has focused mostly on vertebrates, particularly primates and  
5 corvids, even though tool use has also been documented in insects. One of the best-  
6 documented examples involves ants using debris (e.g., sand grains, mud, leaf fragments) to  
7 collect and transport liquid food to their nest. However, little is known about the factors that  
8 determine the selection of materials to be used as tool. We investigated tool selection in two  
9 species of *Aphaenogaster* ants by giving them the choice between different kinds of potential  
10 tools (natural and artificial objects). Ant workers showed a clear preference for certain  
11 materials to be used as tool objects. Tool selection was also shaped by familiarity with the  
12 material as ants developed a preference for artificial tools with a good soaking capacity that  
13 cannot be found in their natural environment. Our results indicate that ants of this genus  
14 evolved unique foraging strategies and show plasticity in their behaviour.

15 **Keywords:** ants, *Aphaenogaster*, foraging, food transport, tool use

## 16 INTRODUCTION

17 Once considered unique to humans, tool use is now known to be widespread in the animal  
18 kingdom (Bentley-Condit & Smith, 2010; Shumaker, Walkup, & Beck, 2011). Moreover, in  
19 the last two decades our general understanding of the mechanisms underlying flexible tool use  
20 has significantly increased (Sanz, Call, & Boesch, 2013). Some species can make tools to  
21 meet specific task demands (e.g., Auersperg, Szabo, von Bayern, & Kacelnik, 2012; Bird &  
22 Emery, 2009; Sanz, Call, & Morgan, 2009), use multiple tools in succession to fulfil the sub-  
23 goals required to complete a task (Martin-Ordas, Schumacher, & Call, 2012; Mulcahy, Call,  
24 & Dunbar, 2005; Wimpenny, Weir, Clayton, Rutz, & Kacelnik, 2009) or select appropriate  
25 tools depending on their physical attributes or functional properties (Bird & Emery, 2009;  
26 Chappell & Kacelnik, 2002; Manrique, Gross, & Call, 2010; Visalberghi et al., 2009).

27 Although these findings are concerned with vertebrates, mostly primates  
28 (chimpanzees, orangutans and capuchin monkeys) and passerine birds (New Caledonian  
29 crows, rooks, woodpecker finches) (Sanz et al., 2013), tool use also occurs in invertebrates.  
30 For instance, a recently compiled catalogue reports about 50 cases of tool use in insects,  
31 involving 30 different genera (Bentley-Condit & Smith, 2010). However, little is known about  
32 the occurrence of flexible tool use in invertebrates. For instance, weaver ants use the silk  
33 produced by their larvae in nest building but this is not considered “true” tool use because  
34 ants use an animate object (Pierce, 1986). Antlions and wormlions throwing out sand to make  
35 small prey fall inside their conical pits does qualifies as tool use. Although it is a fairly  
36 stereotyped behaviour, it is not completely fixed: antlions modify the characteristics of their  
37 trap according to hunger level, prey availability, predation threat and other environmental  
38 conditions such as light and temperature; however, the influence of experience upon this  
39 flexibility is unclear (Scharf, Lubin & Ovadia, 2011). Antlions and wormlions also prefer  
40 specific substrates for pit building or ambushing prey (Devetak & Arnett, 2015). The use of  
41 pebbles to close burrows containing eggs and prey in some apoid wasps (*Ammophila*, *Sphex*)  
42 is flexible because it requires the selection of suitable pebbles (Evans & Eberhard, 1970).

43 One of the best documented examples of tool use by insects involves using debris to  
44 transport food by some species of ants including the harvester ant *Pogonomyrmex badius*  
45 (Morrill, 1972), *Solenopsis invicta* (Barber, Ellgaard, Thien, & Stack, 1989), and several

46 species of *Aphaenogaster* (Agbogba, 1985; Fellers & Fellers, 1976; McDonald, 1984; Tanaka  
47 & Ono, 1978), all belonging to the subfamily *Myrmicinae*. Many of the species from this  
48 subfamily are characterised by a very chitinous gaster (abdomen) and by the lack of a  
49 distensible crop, which prevents the transportation of large amounts of liquid food inside their  
50 bodies, a feature very common in other subfamilies, such as *Formicinae* or *Dolichoderinae*  
51 (Davidson, Cook, & Snelling, 2004; Hölldobler & Wilson, 1990). Furthermore, some  
52 myrmicine genera, namely *Aphaenogaster*, do not perform trophallaxis (Delage & Jaisson,  
53 1969) (i.e., mouth-to-mouth exchange of liquid food, also common in other subfamilies) and  
54 as a consequence the foragers of these species cannot exchange liquids stored in their crops  
55 with in-nest workers performing other tasks, such as feeding the larvae. The characteristic  
56 anatomy of the digestive tract in myrmicine ants, in particular the absence of an expandable  
57 crop, may have favoured the evolution of tool using behaviour, which allows for an efficient  
58 gathering, transport and sharing of liquid food (Fellers & Fellers, 1976; Tanaka & Ono,  
59 1978).

60         When foragers of these myrmicine species encounter liquid food sources (e.g., fruit  
61 pulp, body fluids of dead arthropods) they drop debris of various kinds (e.g., sand grains, soil  
62 particles, leaf fragments) into the food source and then transport the food-soaked debris back  
63 into the nest. Furthermore, some evidence suggests that these ants do not drop debris into non-  
64 food substances (Agbogba, 1985; Banschbach, Brunelle, Bartlett, Grivetti, & Yeaman 2006).  
65 Tool-assisted food transportation has been observed in both field and laboratory experiments  
66 with artificial baits (Agbogba, 1985; Banschbach et al., 2006; Barber et al., 1989; Fellers &  
67 Fellers, 1976; Fowler, 1982; Lőrinczi, 2014; McDonald, 1984; Morrill, 1972; Tanaka & Ono,  
68 1978). Ants use as tools different objects found near the food source including mud clods, leaf  
69 fragments, pine needles, sand grains, or any particles of a suitable size (Banschbach et al.,  
70 2006; Fellers & Fellers, 1976; Lőrinczi, 2014; Morrill, 1972; Tanaka & Ono, 1978). So far,  
71 however, only one comprehensive study has been carried out on tool selectivity in these ants  
72 (Tanaka and Ono, 1978). Other studies have reported limited observations that might indicate  
73 selectivity in use of tools in *Aphaenogaster* species (e.g., Banschbach et al., 2006; Fellers &  
74 Fellers, 1976; Lőrinczi, 2014; Morrill, 1972).

75           This putative material selectivity is important because it may indicate that ants choose  
76 materials flexibly, something that has been mainly documented in vertebrates. However, little  
77 is known about the factors that determine ants' preference for various materials as suitable  
78 tools for liquid transportation. The aim of this study was to comprehensively investigate  
79 material selectivity in liquid food transportation in two *Aphaenogaster* ant species in the  
80 laboratory to assess their flexibility and establish a possible link with the literature on tool use  
81 in vertebrates. We adopted Bentley-Condit and Smith's (2010) definition of tool use, which in  
82 turn closely followed that of St Amant and Horton's (2008): "the exertion of control over a  
83 freely manipulable external object (the tool) with the goal of (1) altering the physical  
84 properties of another object, substance, surface or medium (the target, which may be the tool  
85 user or another organism) via a dynamic mechanical interaction, or (2) mediating the flow of  
86 information between the tool user and the environment or other organisms in the  
87 environment" p.1203. We chose this definition instead of Beck's (1980) or Pierce's (1986)  
88 because it provided a good balance between specificity and generality and crucially, it fully  
89 captured the behaviour that we investigated here.

90           We confronted ants with a liquid food source away from the nest and a set of natural  
91 or artificial (novel) objects with different weight/soaking properties. One might expect that  
92 tools with more efficient soaking properties would be preferred over alternative choices.  
93 Additionally, we expected that ants would preferentially drop debris in nutritious baits. The  
94 presentation of natural objects allowed us to link this study with previous ones while the  
95 inclusion of the artificial objects allowed us to explore the ants' flexibility in learning to use  
96 the most efficient novel materials.

## 97 **METHODS**

### 98 *Study species and housing*

99 We studied two monogynous Mediterranean ant species belonging to the subfamily  
100 *Myrmicinae*, *Aphaenogaster subterranea* and *Aphaenogaster senilis* (Czechowski,  
101 Radchenko, Czechowska, & Vepsäläinen, 2012; Stukalyuk & Radchenko, 2011).  
102 *A. subterranea* (Latreille, 1798) is a highly thermophilous species distributed in southern and  
103 central Europe (Czechowski et al., 2012; Seifert, 2007). It lives in moderately wet and warm

104 deciduous and pine forests, and builds nests under stones, in the soil, litter or occasionally in  
105 fallen branches. Colony size can vary from several hundred up to 2000 workers (Czechowski  
106 et al., 2012; Seifert, 2007; Stukalyuk & Radchenko, 2011). *A. senilis* (Mayr, 1853) inhabits  
107 open, sunny locations such as forest edges, lawns, fields and sand dunes. The nests are built  
108 into the soil, often sheltered by stones. Workers forage individually mostly at the ground  
109 level, but they can occur on shrubs and trees. Since these habitats have scarce food sources,  
110 workers can cover large areas with the help of their long legs. Colony size can vary between a  
111 few hundred to a few thousand workers (Boulay et al., 2007).

112         Eight medium sized colonies of *A. subterranea* (two queenright and 6 queenless,  
113 between 500-1500 workers) were collected in a black pine forest near the village of Litér  
114 (Hungary) and kept under standard conditions (temperature  $24 \pm 4$  °C; relative humidity 42-  
115 43%; 12 L: 12 D cycle) in the laboratory. Together with some material coming from the  
116 original habitat (soil, dried pine needles and leaf fragments), the colonies were kept in plastic  
117 boxes (44 cm × 31 cm × 23 cm) with their cover cut in a circular shape (diameter 15 cm) and  
118 covered with a fine-wired metal mesh for ventilation and easy moistening of the nest. Every  
119 box containing a colony was connected with a 10 cm long plastic tube to an arena (60 cm ×  
120 30 cm × 15 cm). They were watered daily, and fed every second day with a commonly used  
121 artificial diet (Bhatkar & Whitcomb, 1970) in a distant location of the foraging arena. During  
122 the experimental period the colonies were not fed, to increase motivation for food found on  
123 the baits. Water was always provided *ad libitum*.

124         Three queenright colonies of *A. senilis* (colony size 500-1500 workers) were collected  
125 at Banyuls-sur-Mer (France) in a sandy area and kept in the laboratory under standard  
126 conditions (temperature  $24 \pm 4$  °C; relative humidity 50–60%; 12 L: 12 D cycle). They were  
127 housed in artificial nests, each consisting of a cylindrical plastic box (diameter 12.5 cm) with  
128 regularly moistened plaster floor, and a hole giving access to the foraging area, which was  
129 represented by the space left in a larger plastic box (18 cm × 25.5 cm × 7.7 cm) containing the  
130 circular nest. The standard diet for each colony consisted of five dead crickets (*Acheta*  
131 *domestica*) and about 5 g of apple/honey mix two times per week. During the experimental  
132 period, to increase motivation for food, colonies were fed with only three crickets and 2 g of  
133 apple/honey mix (twice a week); water was always provided *ad libitum*.



134 *Experimental setup*

135 Ant colonies were presented with food baits and different types of potential tools in the  
136 foraging arena (Fig. 1, 2). The methodology used in *A. subterranea* and *A. senilis* and  
137 described below is slightly different because this study is the combined output of two initially  
138 separate studies conducted independently in two different laboratories.

139 *Aphaenogaster subterranea*

140 The duration of the experiments was 3 hours, which was enough time to observe the transport  
141 of tools into the nests. Each observation period lasted one minute, repeated every four minutes  
142 until the end of the experiment. During this time we noted the number of tools transported to  
143 the bait and from the bait into the nests. Tool preference was tested for tools found and used  
144 in nature: small soil grains (diameter 1 mm), large soil grains (diameter 2-3 mm), and  
145 fragments of pine needles and leaves. Additionally, we also used a fifth tool type of  
146 anthropogenic origin with good soaking/weight ratio: small pieces of sponges (diameter 5  
147 mm) (Fig. 1 and Fig. A1, appendix). Tools were placed at a distance of 12 cm from the baits  
148 in a random order. To estimate the soaking properties, the weight of 10 tools of each type was  
149 measured with a precision analytical balance (10 mg accuracy) before and after soaking them  
150 in the different types of baits (Table 1). The objects were placed on the surface of the baits  
151 and left there for 10 minutes. Three different baits were tested: water, honey diluted in water  
152 (1:3 ratio; further honey-water) and honey (condensed with sugar powder; further honey)  
153 placed in plastic plates (diameter 4 cm, Fig. 1 and A1). The baits were given to the colonies in  
154 a random order.

155

156 *Aphaenogaster senilis*

157 The experiments lasted 3 hours, during which the activity of the colonies was videotaped. The  
158 tapes were analysed as for *A. subterranea*. The experiments with the same setup were  
159 repeated 10 times per each colony.

160 Tool use preference was tested for a total of 6 different, novel potential tools, such as pieces  
161 of paper, sponges, artificial foam, twigs, string and parafilm (Fig. 2). Groups of 10 tools of

162 each type (average weight for each tool: 2.5 mg) were placed 12 cm from the food source  
163 (diluted honey on a piece of aluminium foil). The soaking properties were estimated by  
164 measuring the weight of 10 tools of each type with a precision analytical balance (10 mg  
165 accuracy) before and after soaking them in diluted honey (Table 2).

#### 166 *Ethical Note.*

167 Ant colonies were collected with care in the field and maintained in nearly natural conditions  
168 in the laboratory. Ants were provided with suitable nesting sites, food and water thus  
169 minimizing any adverse impact on their welfare. Our experimental designs include only  
170 behavioural observations, no insect was harmed or stressed during the experimental procedure  
171 and all ant colonies were healthy at the end of the experiments.

#### 172 *Statistical analysis*

173 In *A. subterranea*, we did not observe any transport of tools into the nests in the case of water  
174 baits; furthermore, the transport rate towards the water baits was much lower than towards the  
175 other baits, so we focused on the analysis of tool use in honey-water and honey baits. The  
176 effect of the bait type on the total number of tools dropped into the baits was analysed using a  
177 GLMM model (Poisson errors, maximum likelihood fit, log link). In the full model, bait type  
178 was included as a factor and colony ID as a random factor ( $N = 12$ ) to account for within  
179 colony similarities. Differences in the number of tools dropped into baits by different colonies  
180 according to different tool types were analysed with GLMM models (Poisson errors,  
181 maximum likelihood fit, log link). In the full model, tool type was included as a fixed factor  
182 and colony ID as a random factor ( $N_{A.su} = 30$ ,  $N_{A.se} = 18$ ).

183 The transport rate of tools towards the baits for the colonies of both species was analysed with  
184 the help of Log-rank test with Montecarlo (100.000) simulations ( $N_{A.su} = 60$  corpses,  $N_{A.se} =$   
185 30). The removal rate was tested until at most the 10<sup>th</sup> piece of tools was removed. The  
186 different tool types were included as dummy variables, while colony ID was included as a  
187 random factor. The transport rate of the different tools into the nests was analysed with the  
188 same model construction in both species.

189 In *A. subterranea*, the transport of tools to the different baits was analysed in separate  
190 models, and because 2 colonies did not transport anything to the baits, only 6 colonies were  
191 used for the analysis. In *A. senilis*, the same model constructions were used for the analysis of  
192 the 1<sup>st</sup>, the 5<sup>th</sup> and 10<sup>th</sup> experiment. The three-hour experimental period was enough in the  
193 case of both species to observe the transport of tools into the nests. In *A. subterranea*, 3  
194 colonies in the case of honey-water, and 6 colonies in the case of honey baits were actively  
195 transporting tools into their nests, so only these colonies were used in the analysis with the  
196 same model constructions as described before ( $N_{\text{honey-water}}= 15$ ,  $N_{\text{honey}}= 30$ ). All statistical  
197 analyses were carried out in R (version 3.0.2, The R Foundation for Statistical Computing,  
198 Vienna, Austria, <http://www.r-project.org>). GLMMs were performed using *glmer* function in  
199 *lme4* package (Bates, Maechler, Bolker, & Walker, 2013). In the analyses, all tools used were  
200 included. Log Rank Test was carried out with the *surv\_test* function in *coin* package  
201 (Hothorn, Hornik, van de Wiel, & Zeileis, 2008). Pairwise comparisons were performed with  
202 separate Log Rank Tests and the *Relevel* function was used to carry out post-hoc sequential  
203 comparisons among factor levels when performing GLMM analyses. We applied table-wide  
204 sequential Bonferroni-Holm correction to reveal the exact significance levels in these cases.

## 205 RESULTS

### 206 *Aphaenogaster subterranea*: transport to the baits

207 Workers of *A. subterranea* dropped significantly more tools into honey-water (GLMM:  $z =$   
208  $12.5$ ,  $N = 24$ ,  $P < 0.001$ ) and honey ( $z = 11.97$ ,  $P < 0.001$ ) than into water, but no difference  
209 was observed between the honey-water and honey ( $z = -0.96$ , NS). We therefore concentrate  
210 on honey-water and honey baits in the following analyses.

211 Overall, small soil grains were the most frequently transported tool to the honey-water,  
212 and their number was significantly different from the number of sponges ( $z = -3.33$ ,  $P <$

213 0.001) and leaf fragments (Fig. 3), with the latter being the least frequently dropped tools  
214 compared to every other tool type ( $z < -3.22$ ,  $P < 0.01$ ). Furthermore, leaf fragments were  
215 transported at the lowest rate (Log Rank Test:  $13 < \chi^2 < 29.76$ ,  $P < 0.001$ ) while sponges were  
216 transported at a slower rate than small soil grains ( $\chi^2 = 29.76$ ,  $P < 0.01$ ) (Fig A2).

217 In the case of honey baits, the preferred tools were the small soil grains and pine  
218 needles, which were transported in significantly higher numbers than leaf fragments ( $z < -$   
219  $3.07$ ,  $P < 0.001$ ) and large soil grains ( $z < -2.65$ ,  $P < 0.05$ ); sponges were also used more  
220 frequently than leaf fragments ( $z = -2.83$ ,  $P = 0.03$ ) (Fig. 3). Every tool type was transported  
221 at a faster rate than leaf fragments ( $11.5 < \chi^2 < 40.29$ ,  $P < 0.003$ ) (Fig. A2).

222 *Aphaenogaster subterranea*: transport into the nests

223 In the case of honey-water baits, small soil grains were transported into the nest in  
224 higher numbers than pine needles ( $z = 3.43$ ,  $N = 24$ ,  $P < 0.001$ ), leaf fragments ( $z = 3.56$ ,  $P <$   
225  $0.001$ ) and sponges ( $z = 2.91$ ,  $P < 0.001$ ) (Fig 4). Small soil grains were transported also at a  
226 faster rate than every other tool type ( $11.43 < \chi^2 < 29.26$ ,  $P < 0.003$ ) (Fig. A3).

227 In the case of honey baits, sponges were transported into the nest in highest numbers,  
228 which differed significantly from every other tool type ( $z < -4.02$ ,  $P < 0.001$ ). The second  
229 most preferred tools were small soil grains, which were transported significantly more often  
230 than leaf fragments ( $z = -2.79$ ,  $P = 0.03$ ) (Fig 4). Sponges were transported at a faster rate than  
231 every other tool type ( $8.56 < \chi^2 < 37.39$ ,  $P < 0.01$ ). Leaf fragments were transported at the  
232 slowest rate, which significantly differed from the small ( $\chi^2 = 22.31$ ,  $P < 0.001$ ) and large soil  
233 grains ( $\chi^2 = 7.07$ ,  $P < 0.05$ ) (Fig A3).

234 *Aphaenogaster senilis*: transport to the baits

235 Overall, the number of tools used by *A. senilis* workers significantly changed across  
236 trials (from the 1<sup>st</sup>, to the 5<sup>th</sup> and the 10<sup>th</sup> trials: GLMM:  $z = 3.09$ ,  $N = 36$ ,  $P < 0.01$ ), and there  
237 was no significant difference between the 5<sup>th</sup> and the 10<sup>th</sup> trial ( $z = 0.95$ , NS), therefore the use  
238 of potential tools was analysed separately for each trail (Fig. A4).

239 In the 1<sup>st</sup> and 5<sup>th</sup> trials, there was no significant difference in the number of different  
240 tools transported to the bait (between every tool type 1<sup>st</sup>  $z > 0.2$ ,  $N = 18$ , NS; 5<sup>th</sup>  $z > -2.2$ ,  $N =$   
241 18, NS) (Fig. 5), although in the 1<sup>st</sup> trial paper was transported at a significantly faster rate  
242 than the artificial foam (Log Rank Test:  $\chi^2 = 12.72$ ,  $P < 0.01$ ; Fig. A5). In the 5<sup>th</sup> trial, the  
243 sponges (Log Rank Test:  $\chi^2 = -4.33$ ,  $P < 0.05$ ; Fig. A5) and the pieces of paper (Log Rank  
244 Test:  $\chi^2 = -3.82$ ,  $P < 0.05$ ) were transported significantly faster than every other tool types, but  
245 there was no significant difference between these two tool types (Log Rank Test:  $\chi^2 = -0.28$ ,  
246 NS), and paper did not differ significantly from string (Log Rank Test:  $\chi^2 = -2.33$ , NS; Fig.  
247 S5).

248 In the 10th trial, the preferred tools were paper and sponges ( $\chi^2 = 0.89$ , NS), which  
249 were used more frequently than artificial foam ( $z > 3.24$ ,  $N = 18$ ,  $P < 0.01$ , Fig. 5) and  
250 transported at a significantly faster rate than parafilm and twigs (respectively:  $\chi^2 > 8.66$ ,  $P <$   
251 0.05 and  $\chi^2 > 8.22$ ,  $P < 0.05$ , Fig. A5). Artificial foam was transported at a significantly  
252 slower rate than every other tool type ( $\chi^2 = 9.3$ ,  $P < 0.05$ , Fig. A5).

### 253 *Aphaenogaster senilis*: transport into the nest

254 Overall, the number of tools transported into the nest by *A. senilis* workers  
255 significantly changed across trials (GLMM:  $z = 2.79$ ,  $N = 36$ ,  $P < 0.01$ ), and there was no  
256 significant difference between trial 5<sup>th</sup> and 10<sup>th</sup> ( $z = 1.01$ , NS), therefore the use of artificial  
257 tools was analysed separately for the 1<sup>st</sup>, 5<sup>th</sup> and 10<sup>th</sup> trials (Fig. A4).

258 In the first trial, there was no significant difference in the number (GLMM:  $1.82 > z$   
259  $> 0.001$ , NS) and transport speed (Log Rank Test:  $0 > \chi^2 > 1$ , NS) of different tools brought  
260 into the nest (Fig. 6 and Fig. A5). In contrast, in the 5<sup>th</sup> trial the pieces of papers and sponges  
261 were transported in a significantly higher number than the foam ( $z > -3.16$ ,  $P < 0.05$ ) and  
262 parafilm tools ( $z > -3.38$ ,  $P < 0.01$ ; Fig. 6). Moreover, paper and sponge (Log Rank Test:  $\chi^2 =$   
263 0.29, NS) were transported at a higher rate than every other tool type ( $\chi^2 > 2.85$ ,  $P < 0.05$ ;  
264 Fig. S5). Strings and twigs ( $\chi^2 = -0.91$ , NS) were transported at a higher rate than foam ( $\chi^2 >$   
265 6.24,  $P < 0.01$ ) and parafilm ( $\chi^2 > 2.48$ ,  $P < 0.05$ ; Fig. A5).

266 In the 10<sup>th</sup> trial, paper was the most frequently transported tool to the nest, differing  
267 significantly from the number of artificial foam ( $z = 2.73$ ,  $P < 0.05$ ) and parafilm tools ( $z =$   
268  $2.73$ ,  $P < 0.05$ ) (Fig. 6). The fastest transport rate was observed for paper tools, which differed  
269 significantly from every other tool type ( $\chi^2 > 8.2$ ,  $P < 0.05$ , Fig. A5) except for sponges and  
270 strings ( $\chi^2 > 6.78$ , NS, Fig. A5). Parafilm and artificial foam ( $\chi^2 = 3.35$ , NS) were transported  
271 at a significantly slower rate than every other tool type ( $\chi^2 > 8.66$ ,  $P < 0.05$ ).

## 272 DISCUSSION

273 We observed the occurrence of tool use to transport liquid food in two ant species of the  
274 genus *Aphaenogaster* thus confirming previous observations (Agbogba, 1985). More  
275 importantly, ant workers were selective in both the materials they chose and the baits that they  
276 exploited. *A. subterranea* was mainly tested with natural materials. Small soil grains were the  
277 most preferred item and leaf fragments, despite having a superior soaking power than soil  
278 grains, the least preferred item. Ants showed a remarkable preference for sponges despite  
279 being novel (they were the only artificial material tested in this species). Interestingly, ant  
280 workers in more than 80% of the trials broke the sponges into smaller fragments, presumably  
281 to facilitate handling. Once tools were dropped into the baits, ants behaved differently  
282 depending on the bait's viscosity (honey-water or pure honey). Imbibed small soil grains were  
283 transported to the nest in the highest numbers and faster than other tool types from honey-  
284 water baits, while sponges were the preferred transported tools from the honey baits (small  
285 soil grains were the second preferred tool to be brought to the nest). Additionally, ants threw  
286 much fewer items into water baits compared to those baits that contained honey.

287 *A. senilis* could choose among six materials that differed in terms of soaking power  
288 (paper, sponges, artificial foam, twigs, sting and parafilm). It is likely that ants of this species,  
289 which live in sandy areas, were unfamiliar with most of these materials. Initially, *A. senilis*  
290 showed no preference for any of the tools for dropping them into the baits, although paper  
291 was transported faster to the bait compared to the artificial foam. However, along trials, ants  
292 significantly preferred dropping paper and sponges into the baits, thus choosing in accordance  
293 with the tools' soaking properties. A similar pattern was observed for tool transportation to

294 the nest: ants developed a preference for paper, followed by sponges, thus optimizing their  
295 foraging effort by using the tools with the best soaking properties and ease of grasping  
296 compared to other tool types.

297 Our results suggest that tool use to transport liquid to the nest in the two studied ant  
298 species is not behaviourally fixed. Ants incorporated novel tools, many of them made of  
299 artificial materials, into their foraging activities. Moreover, *A. senilis* learned within 10 trials  
300 to select the best tool options available based on their soaking properties and *A. subterranea*  
301 chose tools in relation to the characteristic of the food itself, e.g., food viscosity. This means  
302 that ants can select tools according to both food and tool properties. Nevertheless, other  
303 factors (besides a tool's soaking properties) may have played a role in determining ants'  
304 preferences. One possibility is that the low density of some materials in the natural habitat  
305 (e.g., leaf fragments are not abundant in the pine forest inhabited by these *A. subterranea*  
306 colonies) may have been the reason for largely ignoring this item. However, a lack of  
307 familiarity with potential tools cannot explain why *A. subterranea* used sponge pieces, which  
308 were totally novel objects. Even more compelling are the data on *A. senilis*, which developed  
309 a preference for unfamiliar materials during testing.

310

311 Handling effort is another factor that could have played an important role in tool  
312 selection, perhaps in combination with the tool's soaking properties. During our observations  
313 we noticed that *A. subterranea* workers had problems with grasping, handling and orienting  
314 the leaf fragments, while the pieces of sponges could be grasped more easily. In fact, ants may  
315 have torn apart sponges to reduce their size and facilitate handling. Although the soaking  
316 power of sponges was lower when dropped into honey than into honey-water, their relatively  
317 low weight made them more buoyant than other tool types and their irregular texture  
318 increased their potential for being grasped. We found that the preference for particular items  
319 often depended on the type of task that ants carried out. In particular, sponges may have been  
320 preferentially transported to the nest, but small soil grains were the most dropped tools into  
321 the baits. This suggests that selection among different tool types occurred both when first  
322 encountered and also at the food source after the tools had been dropped into the baits. This  
323 two-stage selection process might be especially important in the case of novel tools whose  
324 properties are still unknown. Indeed, *A. senilis* ants transported very few foam fragments to

325 the bait in the first trial but subsequently increased their transportation during the 5<sup>th</sup> trial.  
326 Nevertheless, foam fragments were rarely transported from the bait to the nest and by the 10<sup>th</sup>  
327 trial ant workers even reduced their transportation to the baits. This suggests that tool  
328 selection, after familiarization with novel material, may have also occurred at the baits. Other  
329 authors observed that not all the tools dropped into the baits were transported into the nest  
330 (Fowler, 1982; McDonald, 1984), corroborating our interpretation that further tool selection  
331 can occur once the tools have been dropped into the liquid food source.

332 Availability, weight, soaking properties, easy handling and the possibility of shaping  
333 the material to a desired form are therefore important factors in tool selection. The assessment  
334 of these characteristics appears to be the result of a familiarization and learning process, at  
335 least for the novel objects offered to the ants in our experiments. As the natural availability of  
336 certain kinds of tools varies both spatially and seasonally, plasticity in tool selection is likely  
337 to be adaptive. Tool use behaviour in ants may have evolved from the tendency shown by  
338 many species to cover with debris liquid or viscous substances, which may otherwise cause  
339 drowning or entanglement of workers near their nest (Fellers & Fellers, 1976; McDonald,  
340 1984). Note, however, that it has been experimentally shown that *Aphaenogaster* ants  
341 typically drop debris in food substances (Agbogba 1985; Banschbach et al., 2006) and our  
342 experiments confirm this. The adaptive advantage of tool use in *Aphaenogaster* ants is that,  
343 by using tools, foragers are capable of efficiently exploiting ephemeral food sources by  
344 transporting much larger quantities of liquid nutrients than they could do by internal transport  
345 (Fellers & Fellers, 1976; Tanaka & Ono, 1978), given the non-expandable crop characteristic  
346 of myrmicine ants. Tool use might help these ants to compete with more dominant ant species  
347 which are able to monopolize food sources by numbers: if the food is quickly covered by  
348 debris, other ant species cannot exploit it and tool users can take the necessary time to bring  
349 the soaked debris back to their nest (Banschbach et al., 2006; Fellers & Fellers, 1976).

350 We began our paper by referring to the research on flexible tool use in vertebrates  
351 hoping to establish a link with the existing observations on invertebrate tool use. Our  
352 experiments showed some indication that ants preferred materials with good soaking power  
353 although other aspects such as ease of grasp may have also contributed to their choices.  
354 Chimpanzees create a vegetable mass with good absorbent properties by chewing leaves that



355 they use to extract liquid from crevices (Goodall, 1986). It is unknown, however, whether  
356 chimpanzees select certain plant species for their high soaking power. In a liquid extraction  
357 task in the laboratory, Lehner, Burkart and van Schaik (2011) reported that captive orangutans  
358 developed liquid extraction techniques based on materials with high soaking power (e.g.,  
359 wood wool, paper) over those with low soaking power (e.g., leafless branch). Although this  
360 may indicate that orangutans, like ants, were sensitive to the properties of such materials, the  
361 lack of a direct and systematic comparison between different kinds of materials considerably  
362 reduces the conclusions that one may be able to draw from that study. Nevertheless, other  
363 studies with vertebrates in non-liquid extractive foraging tasks have systematically varied the  
364 materials presented and have found evidence of selectivity in terms of hardness and weight  
365 (Visalberghi et al., 2009), weight (Bird & Emery, 2009) or rigidity (Manrique et al., 2010).  
366 Our findings with ants are in some ways comparable to those studies although admittedly, our  
367 results are less clear than those reported in vertebrates. This is partly understandable because  
368 our study is only the second systematic study on material selectivity in ants (besides Tanaka  
369 and Ono, 1978) and we may have not fully considered a number of factors. For instance,  
370 dropping a substantial number of tools (e.g., pine needles) inside the liquid and abandoning  
371 them there may seem puzzling from the point of view of efficiency. Note, however, that  
372 dropping those materials on the liquid may have accomplished another function (e.g.,  
373 preserving the liquid food against exploitation from other species). Also, the absorbed liquid  
374 may have altered the potential for grasping the potential tool object or increased its weight in  
375 excess. Another possibility is that ants can only identify suitable materials *after* observing its  
376 effect on the substance to be collected instead of selecting them *prior* to their use (e.g.,  
377 artificial foam). Although selecting materials to collect liquid has not been described in  
378 corvids or primates, selecting and manufacturing tools prior to their use is well-documented in  
379 those taxa (e.g., Manrique et al., 2010; Sanz, Call & Morgan, 2009; Visalberghi et al., 2009;  
380 Wimpeny et al., 2009). Whether ants (or any other species) can also select new materials prior  
381 to experiencing its absorbent properties is an open question that future studies should address.

382         Some authors may question our use of the term tool use to describe the behaviour of  
383 the ants. Tool use is after all a contentious term in the literature typically characterized by  
384 rather long definitions, a number of clear examples, and some areas of substantial

385 disagreement (see Bentley-Condit & Smith, 2010; Shumaker et al., 2011). Although we  
386 would be ready to accept a less contentious terminology such as ‘material use’, it is difficult  
387 to do so when chimpanzees using leaves to extract liquid from crevices is considered a  
388 classical example of tool use (Goodall, 1986). More importantly, we think that the behaviour  
389 of the ants meets one of the most important criteria that define tool use in foraging contexts  
390 (Beck, 1980; Bentley-Condit & Smith, 2010; St Amant & Horton, 2008), i.e., the use of an  
391 external object to affect the position or location of another object or substance. Obviously  
392 one could argue that nest building materials affect the position of other nesting materials,  
393 which is why some authors object to the idea of tool use and would perhaps like to see either a  
394 more restricted use of the term, or its complete abandonment for a wider term such as  
395 construction behaviour (Hansell & Ruxton, 2008). Although we understand this position and  
396 the reasons for it, given the above considerations, we still prefer to refer to the behaviour of  
397 the ants as tool use, or the similar denomination of object use.

398 In conclusion, we observed *A. senilis* and *A. subterranea* using natural and artificial  
399 debris to collect and transport liquid food to their nests. We also documented the development  
400 of a preference for materials with optimal soaking properties in *A. senilis* although other  
401 factors such as familiarity and ease of grasping may have played a role in determining ants’  
402 choices. Additional studies are needed to better characterize the extent and limits of this form  
403 of flexible tool use particularly in relation to the existing forms of flexible tool use displayed  
404 by vertebrates.

405

406

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- 505

506 **Table 1.** Soaking properties of the different tools used by *Aphaenogaster subterranea*.

Tool type	Imbibed /Initial weight (weight ratio)	
	Honey-water	Honey
Sponges	7.45	1.29
Leaf fragments	1.94	11.9
Pine needles	1.33	1.85
Small soil grains	1.1	1.48
Large soil grains	0.7	0.64

507 **Table 2.** Soaking properties of the different tools used by *Aphaenogaster senilis*.

Tool type	Imbibed /Initial weight (weight ratio)	
	Honey-water	
Paper	28.37	
Sponges	23.31	
Artificial foam	6.37	
Twigs	3.06	
String	2.00	
Parafilm	1.47	

508

509 **Figure legends**

510 **Figure 1.** Experimental setup and tool types offered to *Aphaenogaster subterranea*.

511 **Figure 2.** Experimental setup and tool types offered to *Aphaenogaster senilis*.

512 **Figure 3.** Number of different tools transported to honey-water (a) and honey (b) baits by  
513 *Aphaenogaster subterranea* workers. Box plots show the median (internal line), quartiles and  
514 range (whiskers). Different letters above boxes represent groups that differ significantly from  
515 each other.

516 **Fig. 4.** Number of different tools transported into the nest from the honey-water (a) and honey  
517 (b) baits by *Aphaenogaster subterranea* workers. Box plots show the median (internal line),  
518 quartiles and range (whiskers). Different letters above boxes represent groups that differ  
519 significantly from each other.

520 **Figure 5.** Number of different tools transported to the baits in the 1<sup>st</sup>, 5<sup>th</sup> and 10<sup>th</sup> trials by  
521 *Aphaenogaster senilis* workers. Box plots show the median (internal line), quartiles and range  
522 (whiskers).

523 **Figure 6.** Number of different tools transported into the nest in the 1<sup>st</sup>, 5<sup>th</sup> and 10<sup>th</sup> experiment  
524 by *Aphaenogaster senilis* workers. Box plots show the median (internal line), quartiles and  
525 range (whiskers).

526 **Appendix - Figure legends**

527 **Figure A1.** Different tool types used for experiments with *Aphaenogaster subterranea* (a)  
528 from left to right: small soil grains, sponges, pine needles, large soil grains, leaf fragments; (b)  
529 the dynamics of bait covering with tools.

530 **Figure A2.** Estimated functions of tool transport time to honey-water (a) and honey (b) by  
531 workers of *Aphaenogaster subterranea* (Log-rank test). Different tool types are depicted by  
532 different colours.



533 **Figure A3.** Estimated functions of tool transport time into the nest from the honey-water (a)  
534 and honey (b) baits by *Aphaenogaster subterranea* workers (Log-rank test). Different tool  
535 types are depicted by different colours.

536 **Figure A4.** Summed number of tools transported on the baits and into the nest during the  
537 three trials (box plots show: median, quartiles and range). Different letters above boxes  
538 represent groups that differ significantly from each other.

539 **Figure A5.** Estimated functions of tools transport time on the baits (a; c; e) and into the nests  
540 (b, d, f) in the 1<sup>st</sup> (a, b), 5<sup>th</sup> (c, d) and 10<sup>th</sup> (e, f) experiment by *Aphaenogaster senilis* workers  
541 (Log-rank test). Different tool types are depicted by different colours.

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Figure

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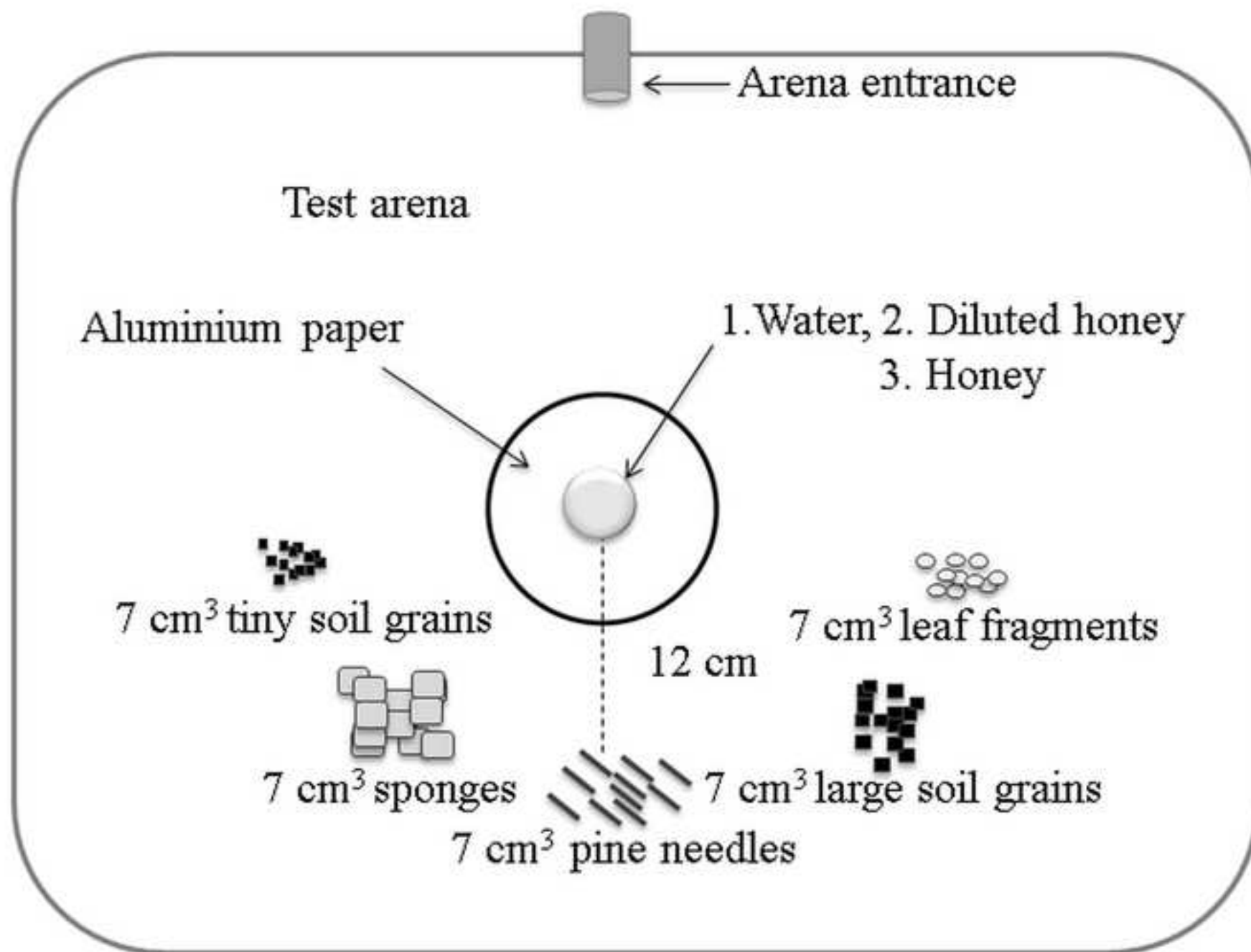


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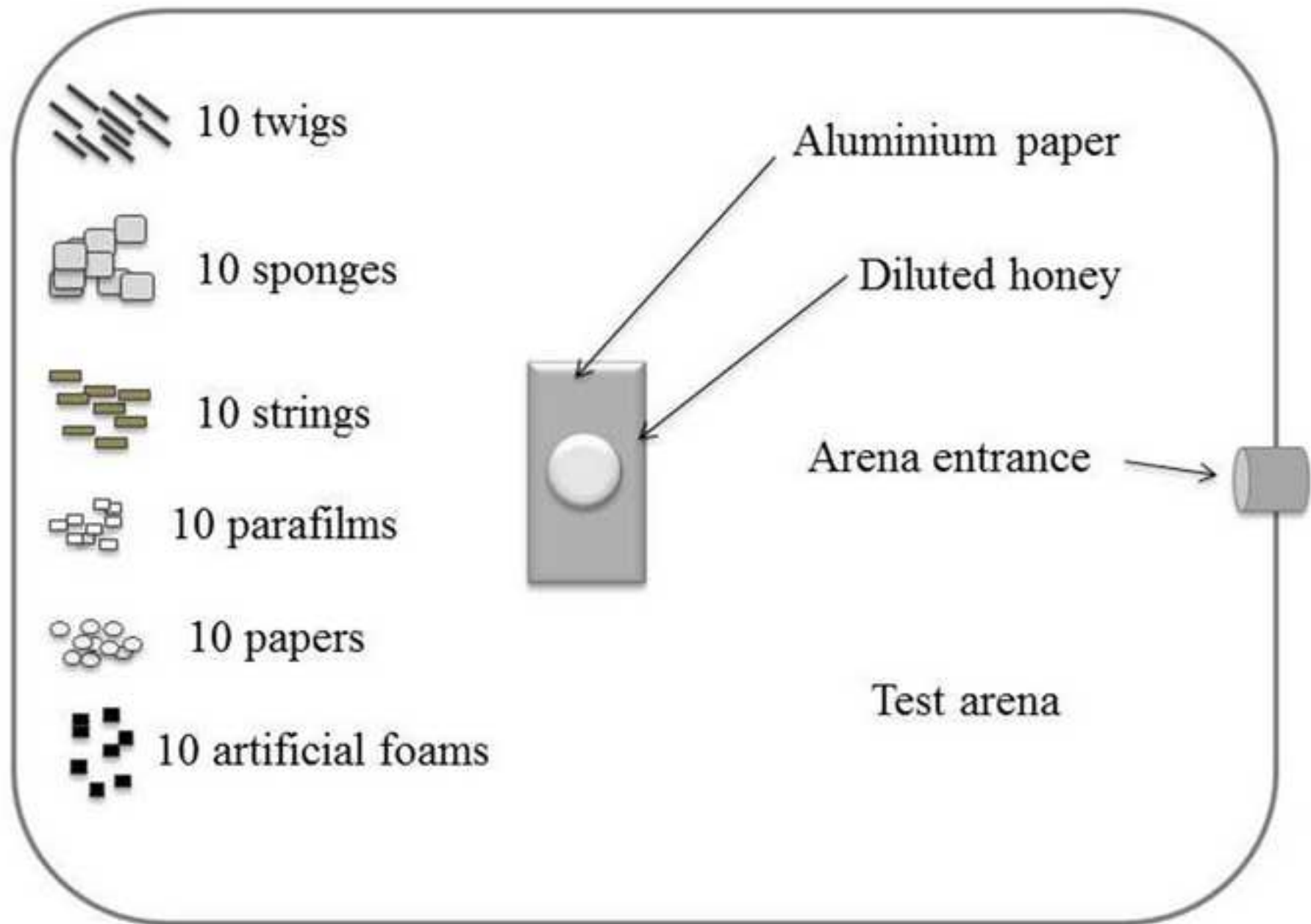


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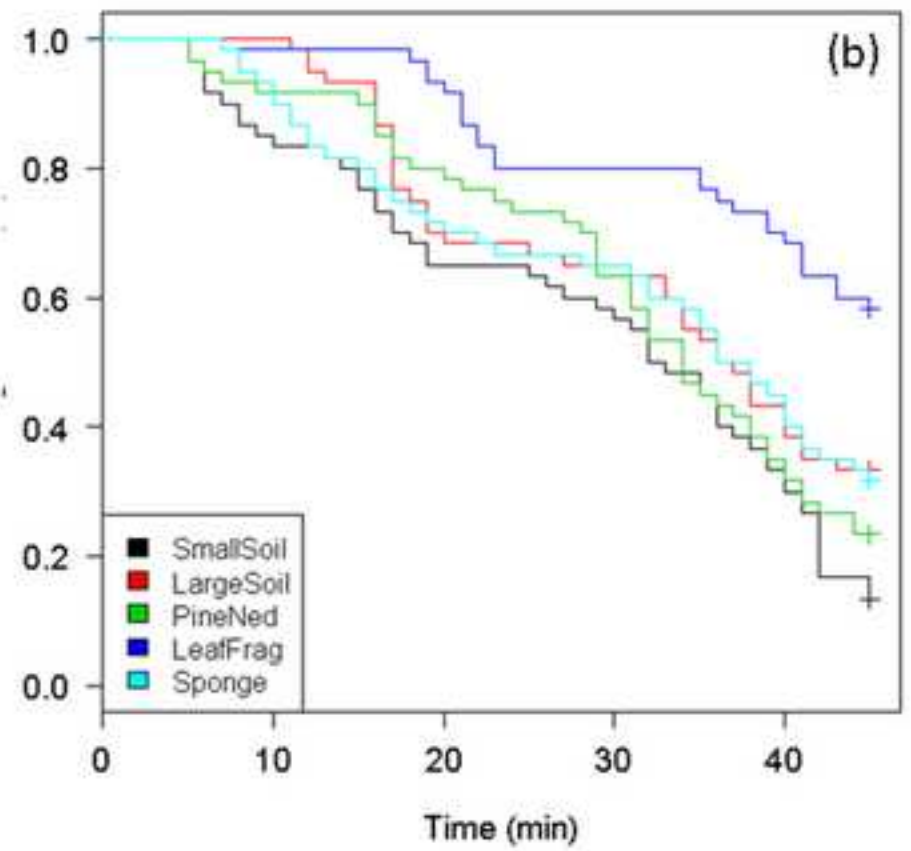
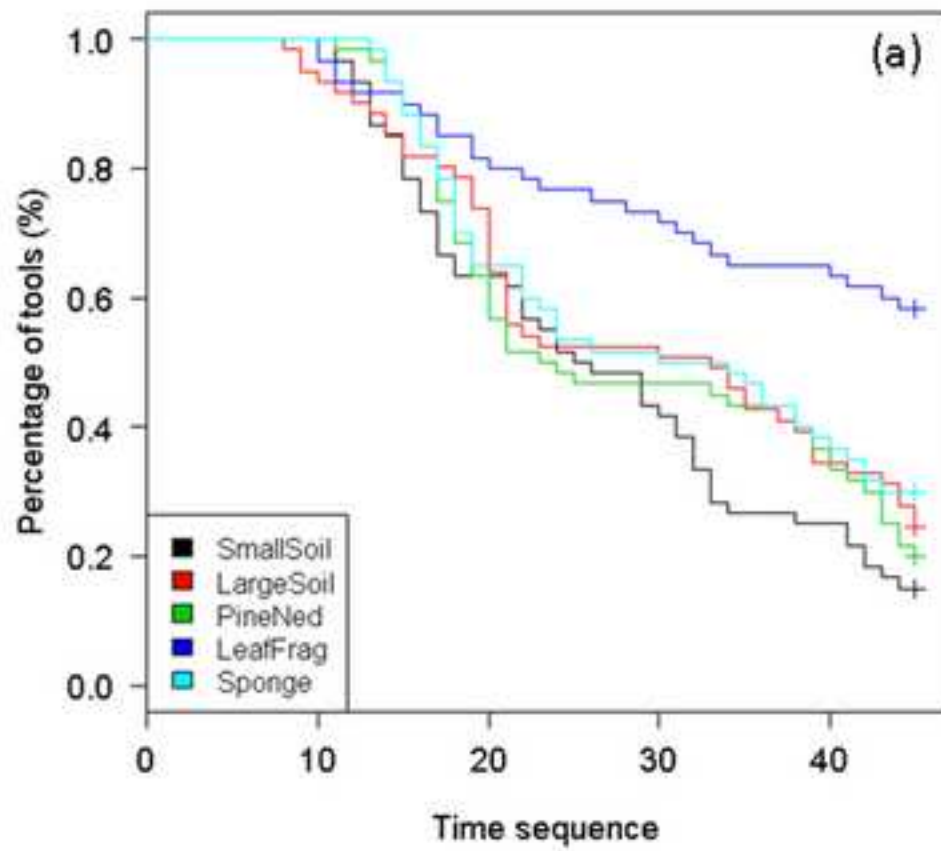


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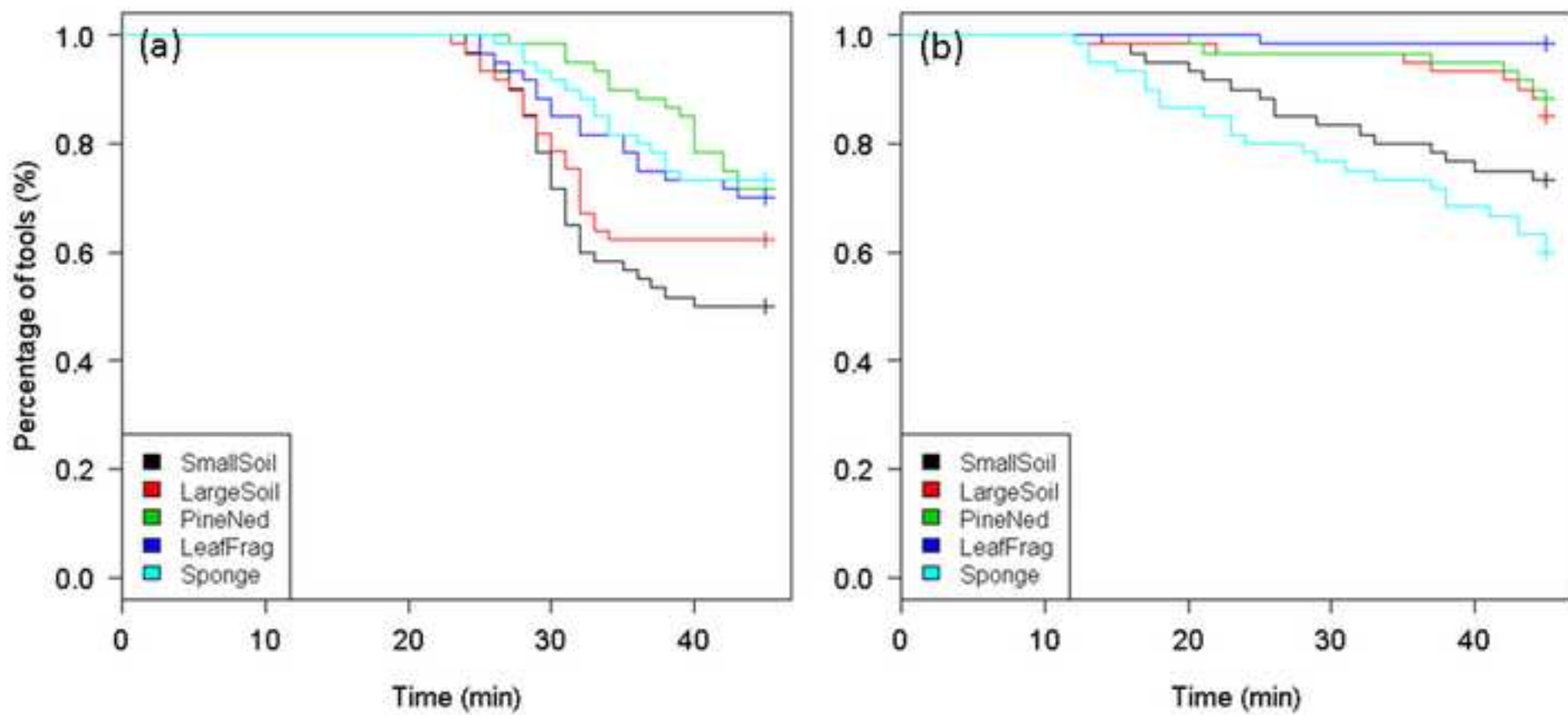


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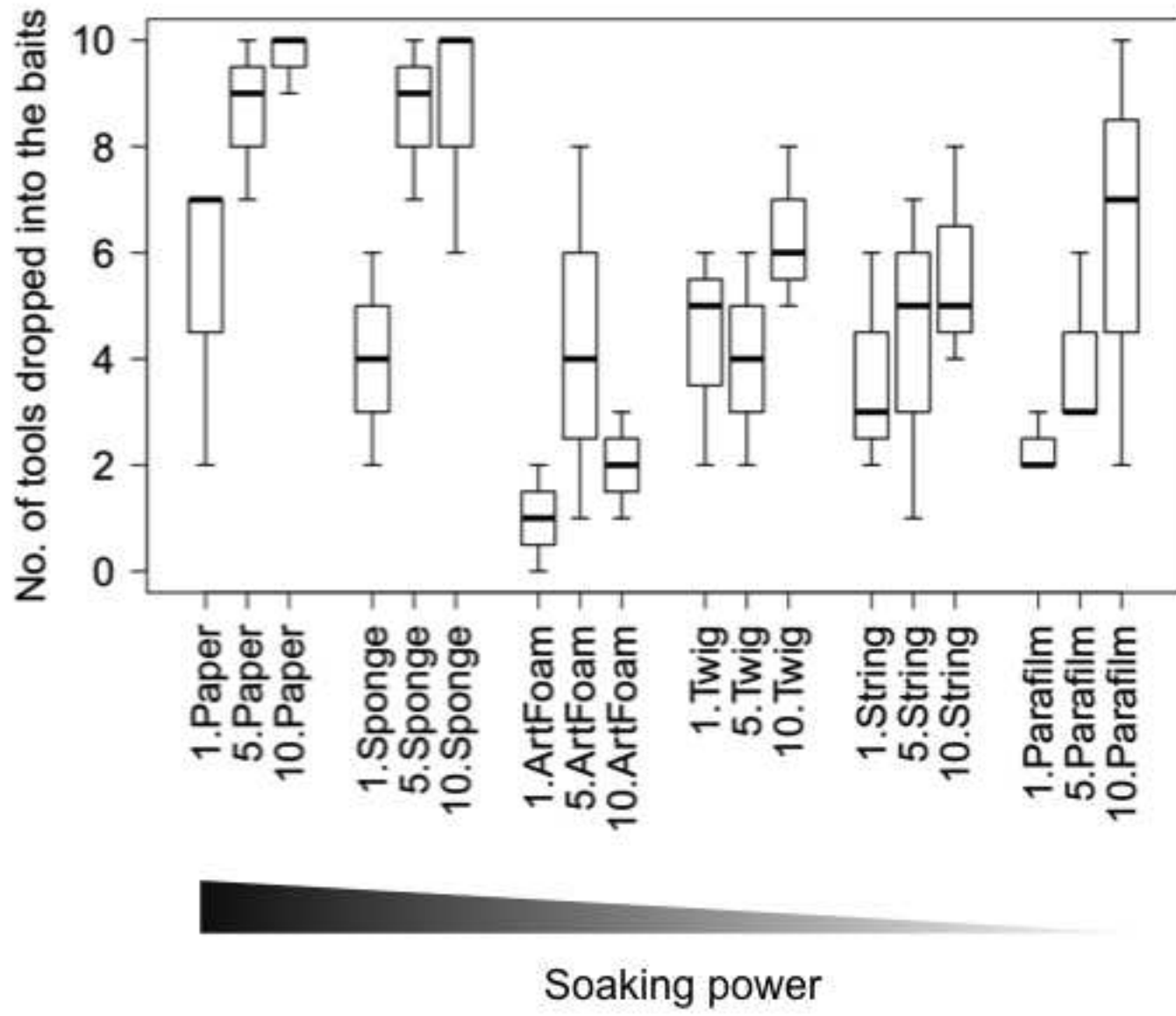


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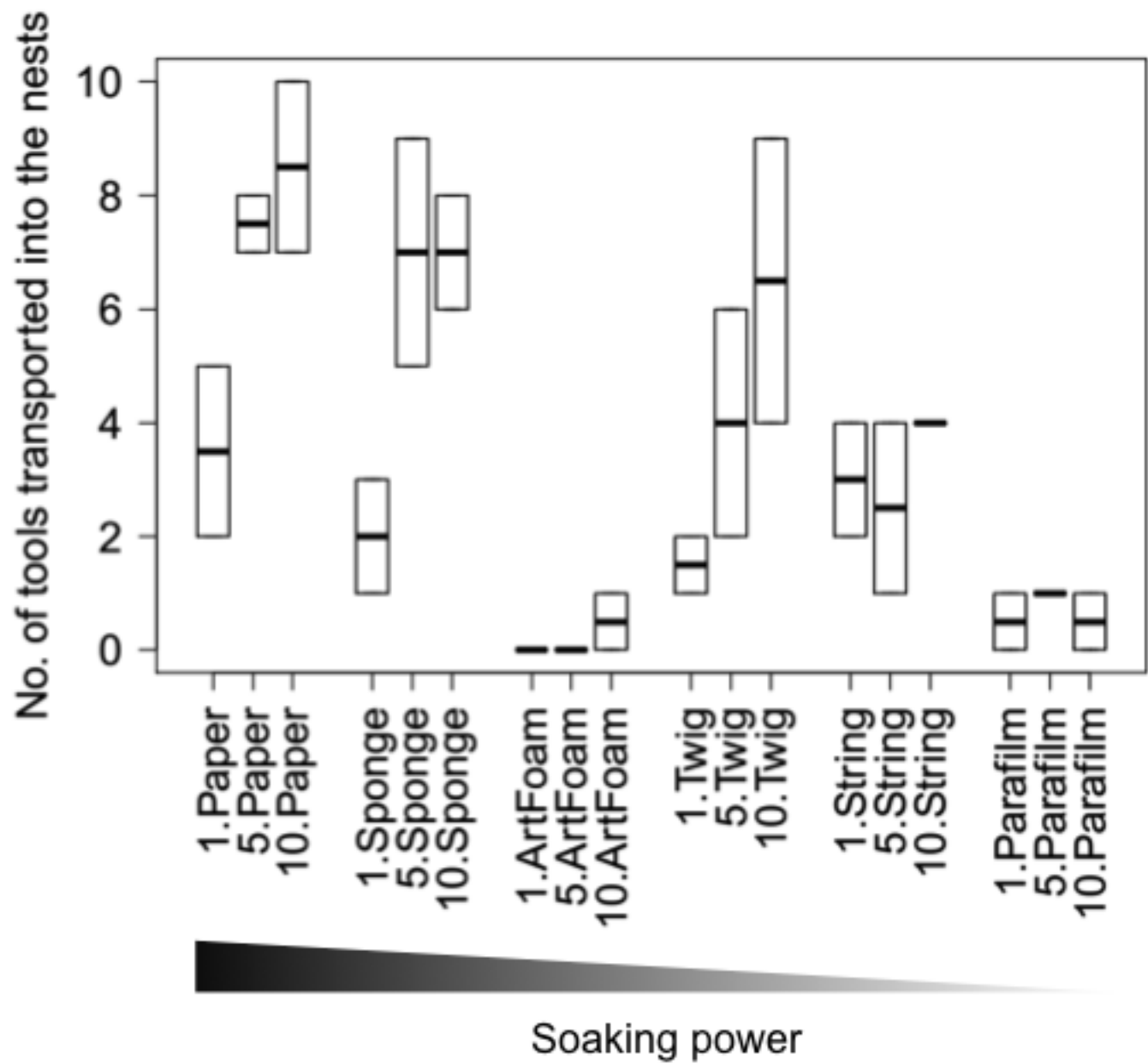




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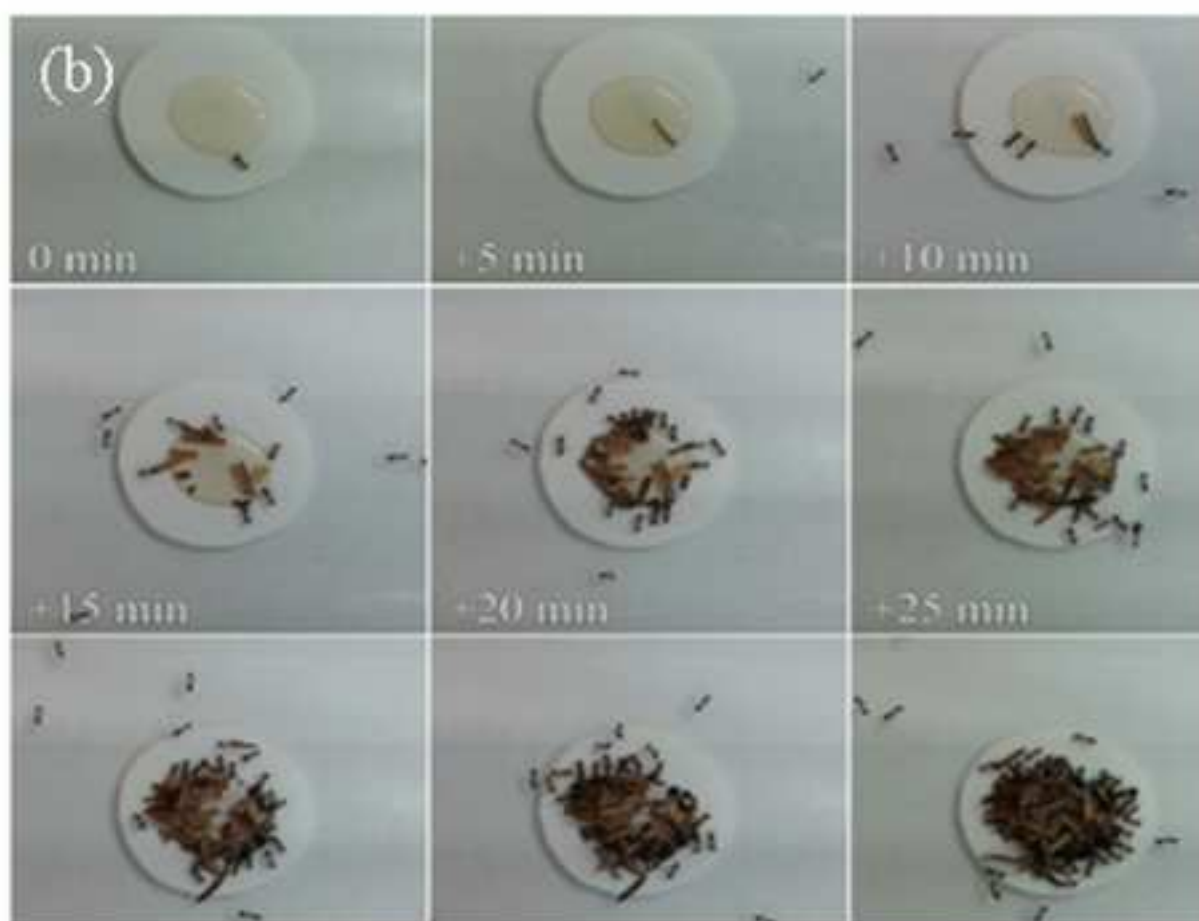


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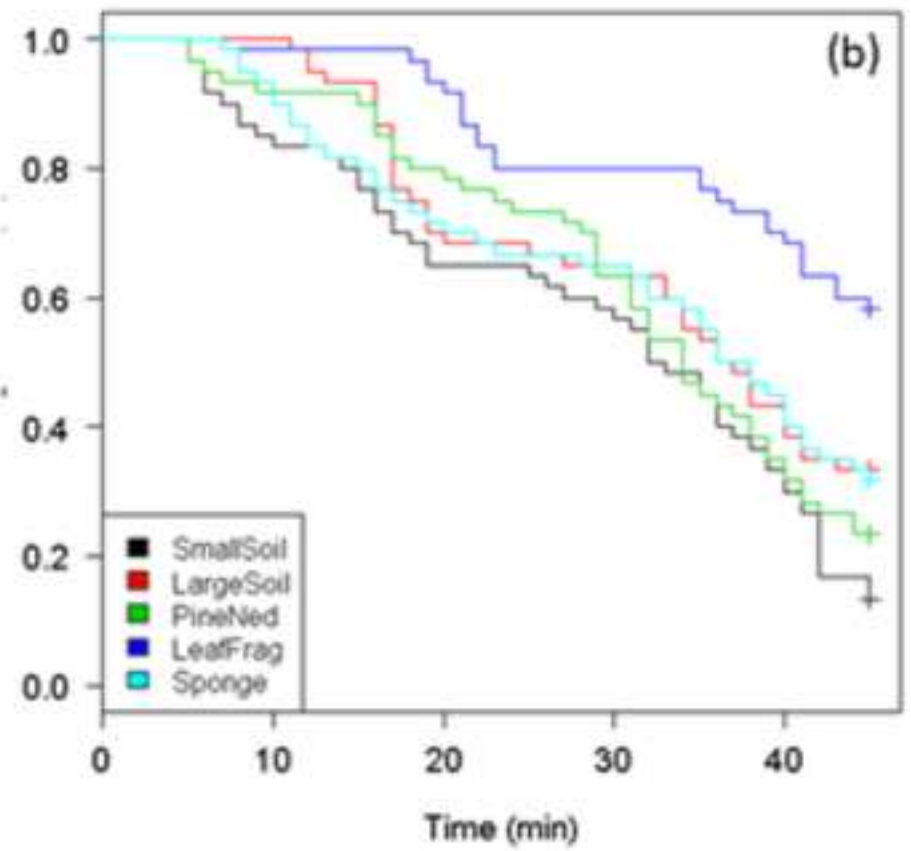
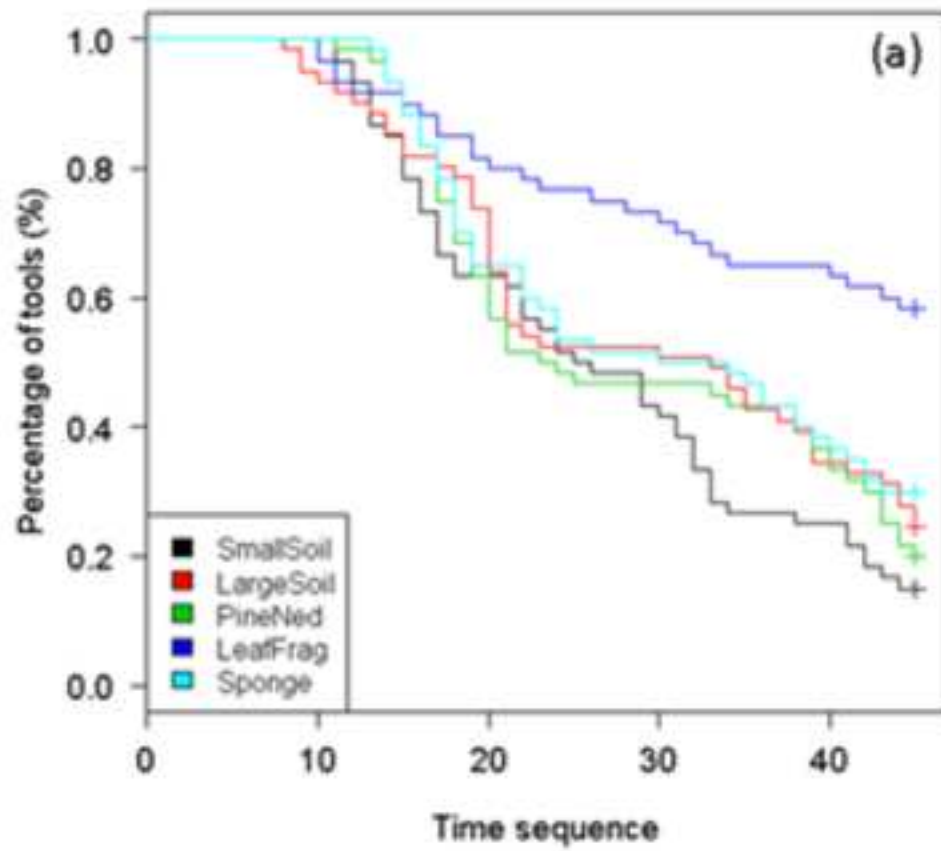


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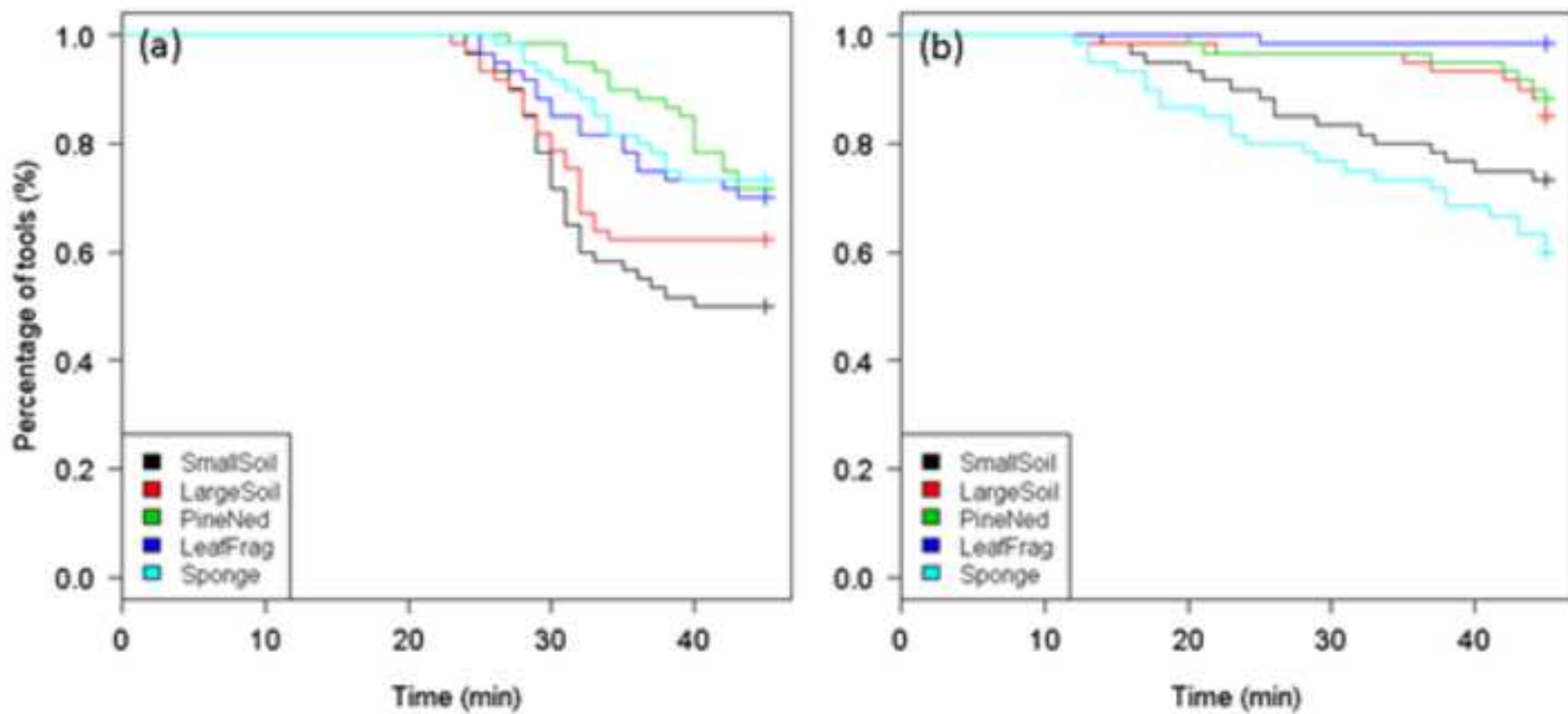


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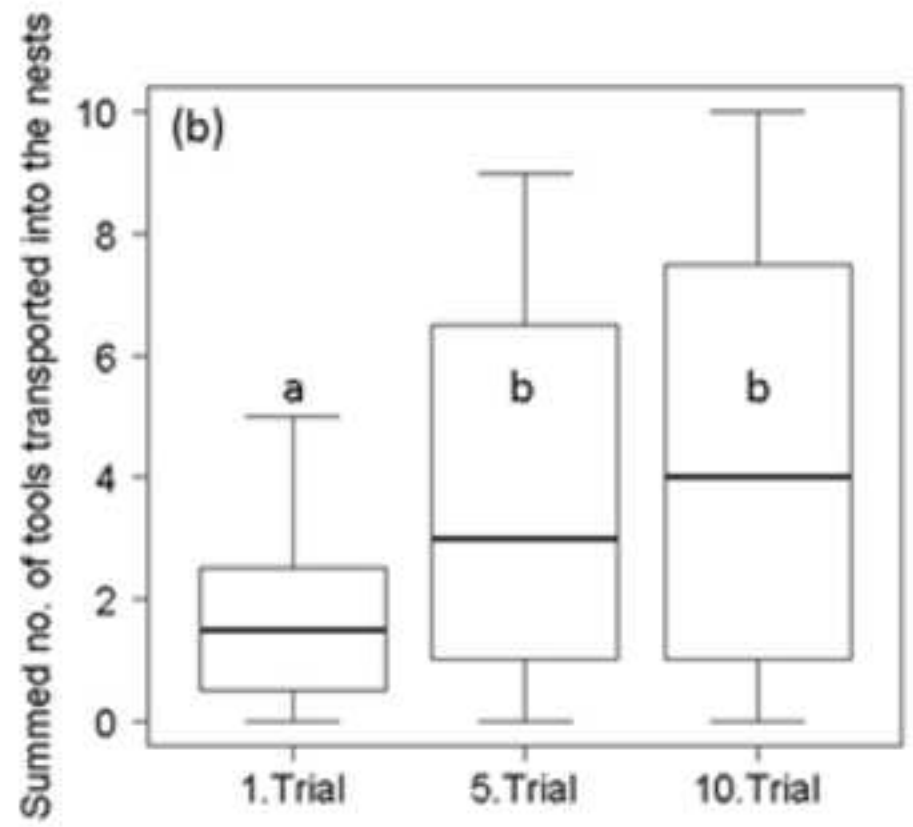
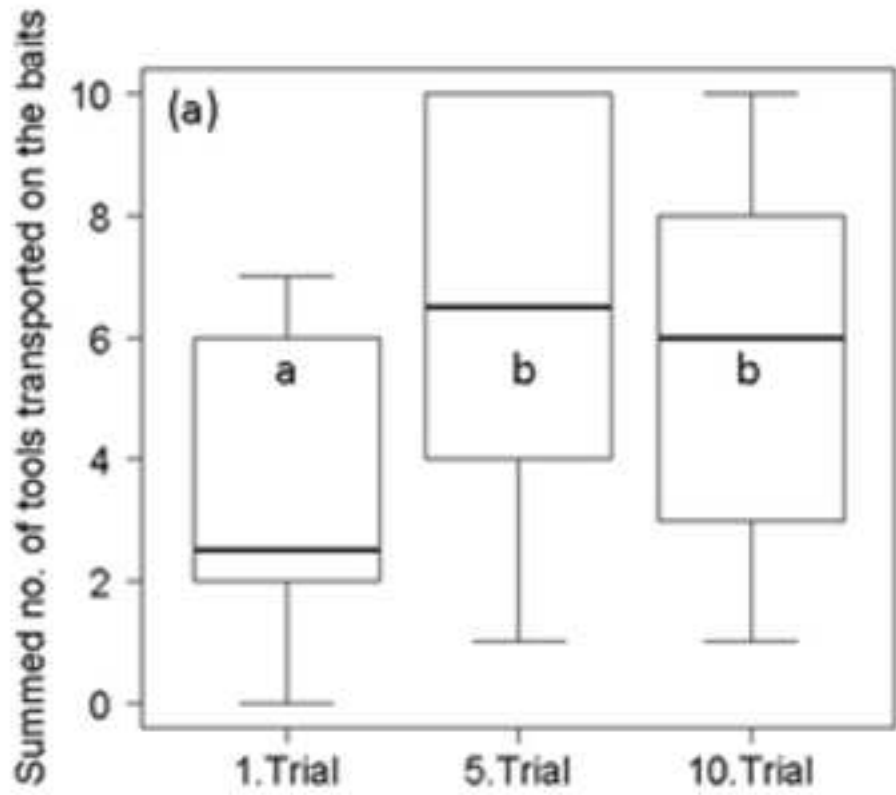
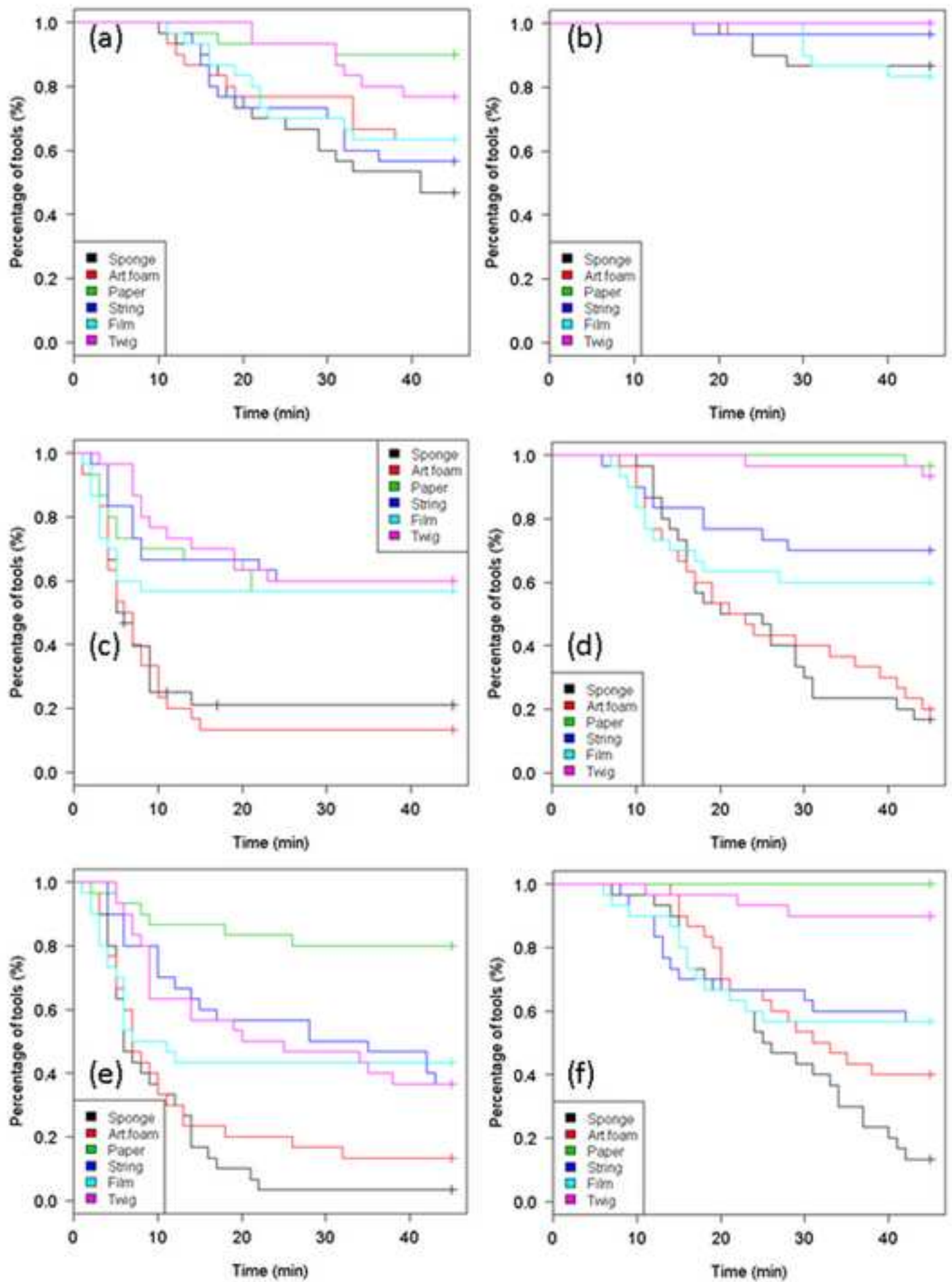


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A



B

