

A comparison of pitfall traps with different liquids for studying ground-dwelling ants (Hymenoptera: Formicidae)

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Abstract

The ability of different liquids in pitfall traps to provide a relatively unbiased representation of ant species foraging activity is poorly understood. We examined the effectiveness of a sugar / vinegar solution, commonly used in ant studies in China, by comparing its catches with those using ethylene glycol in lac agroecosystems in Yunnan Province, China. A total of 11052 individual ants were collected in the traps with sugar / vinegar mixture, representing 82 species, while 6102 individuals were captured in traps with ethylene glycol, representing 75 species. Individual-based curves as well as sample-based curves were so close that the two solutions showed no significant difference in species capture efficiency. Ordination analysis revealed that ant community composition was similar for captures by the two liquids. Liquid type had a significant effect on two of the six most abundant species, but this was confounded by traps with different liquids being placed in different transects. We conclude that the use of sugar / vinegar in traps provides a relatively unbiased representation of ant foraging activity, and is a valid option if other commonly used liquids are unavailable.

Key words: Capture effect, ethylene glycol, pitfall trapping, sugar / vinegar mixture, trap liquid.

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Introduction

Pitfall trapping is a good method of sampling because of its simplicity and ease of operation (GREENSLADE & GREENSLADE 1971), as well as its convenience and effectiveness (ANDERSEN 1991, WANG & al. 2001). It is an effective and low-cost method of qualitatively surveying ground surface-active arthropods, and allows for comparison of assemblages in different habitats (SCUDDER 1996). Furthermore, it is important in the ALL Protocol for assessing ant diversity (AGOSTI & ALONSO 2000).

There are two main forms of pitfall traps: wet pitfall traps, which contain a solution, and dry pitfall traps, which do not contain a solution. Traps with preservative have been found to obtain the highest accumulation of species when compared to dry pitfall traps or pitfall traps containing water (SANTOS & al. 2007). Non-attractant solutions are typically used, to ensure that catches provide a relatively unbiased representation of arthropod foraging activity. Solutions used in wet pitfall traps include: formalin (5 - 10% formaldehyde), ethylene glycol, methylated spirits (methyl plus ethyl alcohol), ethyl alcohol, trisodium phosphate, and picric acid (GULLAN & CRANSTON 2005). Among these solutions, ethylene glycol is widely used to sample ants and other ground-dwelling arthropods (HOLOPAINEN 1992, EYRE & LUFF 2005, SCHMIDT & al. 2006, NAKAMURA & al. 2007).

However, using a single liquid in traps is unlikely to provide a relatively unbiased representation of foraging activity of all arthropods present on the ground. To address this problem, researchers often use a combination of complementary sampling procedures (DELABIE & al. 2000). SCHMIDT & al. (2006) compared capture efficiency (the number of individuals and species) and preservation attributes between five liquids in a field experiment concerned with spiders and ground beetles. They found a mixture of ethylene glycol and water to be the preferred combination. CHEN & al. (2010) compared trapping efficiency of ground-dwelling beetles using ethylene glycol and sugar / vinegar mixture. Their results revealed that some diversity information about ground-dwelling beetles was lost when sampling only with sugar / vinegar mixture or ethylene glycol.

Ants have been widely used as bioindicators for measuring biodiversity and detecting environmental changes (MAJER 1983, GREENSLADE & GREENSLADE 1984, ANDERSEN 1990, ALONSO 2000, KASPARI & MAJER 2000). Ants merit monitoring due to their inherent ecological qualities, independent of any "indicator" attributes they might have (UNDERWOOD & FISHER 2006). To put individual studies into a larger, global context, AGOSTI & ALONSO (2000) presented a standard protocol for the collection of ground-dwelling ants. This is known as the Ants of the

Leaf Litter (ALL) Protocol, in which pitfall trapping is a necessary sampling method. However, very few studies have addressed the question of how to combine different pitfall trap liquids in order to obtain an ideal and reasonably complete census of an ant community.

Sugar / vinegar mixture, a mixture of sugar, vinegar, alcohol, and water, has been widely used in China, to sample ants and ground-dwelling beetles (YU & al. 2004, 2006a, b, LI & al. 2007), as well as to control some pest insects such as fruit flies and flower chafers (TAI & al. 2009). The specific reason for using this mixture is not clear; perhaps it is due to its unbiased representation of arthropods.

This paper compares catches from sugar / vinegar mixture with catches from the more widely used ethylene glycol by describing composition, abundance and species richness of the ant communities inhabiting lac agroecosystems in China. (Lac is a layer of red resin on branches of host-trees on which lac insects settle. Lac resin is natural, biodegradable and non-toxic, and thus widely used in food, textiles and pharmaceutical industries in addition to surface coating, electrical component manufacturing, and other fields.)

Material and methods

Site description: We set up 12 sampling plots at three sites in the Niukong region of Lüchun County, China (22° 53' N, 101° 56' E). All plots were between 1000 - 1300 m above sea level, and receive approximately 1500 mm of rain per year. The sites represent different patterns of agroforestry land, including natural lac forest (plots 1 - 5), lac plantation (plots 6 - 8), and dry land (plots 9 - 12). The trees in natural lac forest were mostly lac-insect host plants (local trees), such as *Ficus semicordata*, *Dalbergia obtusifolia* and *D. szemaoensis*. Lac insects (*Kerria yunnanensis* OU & HONG, 1990) were raised on trees from the fifth year onwards. The density of trees was limited to 150 - 200 per hectare in upland fields in order to preserve grain yield. The lac plantation consisted of few or only one tree species. Half of these tree species were introduced from other provinces and the density of trees was up to 900 - 1500 per hectare. Farmers cultivated crops next to these lac-insect host trees when the trees were young, but ceased cultivation completely when the trees were mature as the thick crown desired for lac-insect host trees intercepted most sunlight the crops required. The dry land was next to natural lac forest or lac plantation, and was used to cultivate corn from May to October.

Ant sampling: At each site, two 50 m transects were established for ant collection. Five pitfall traps with sugar / vinegar mixture (a mixture of sugar, vinegar, alcohol and water, in the ratio of 1 : 2 : 2 : 20 by mass) were set along the first transect at 10 m intervals at ground level, while another five pitfall traps with ethylene glycol were set along the second transect 20 m away from the first one. Pitfall traps were made from plastic containers, 8 cm diameter and 15 cm deep, covered by a stone plate to protect the trap from rain. Traps were opened for 5 days and nights. Sampling was carried out semi-monthly from May 2006 to April 2007 for all plots; for every pitfall trap, 24 samplings were carried out in total. The contents of each transect were placed separately in plastic bottles and stored in 75% alcohol. Specimens were manually sorted and identified to

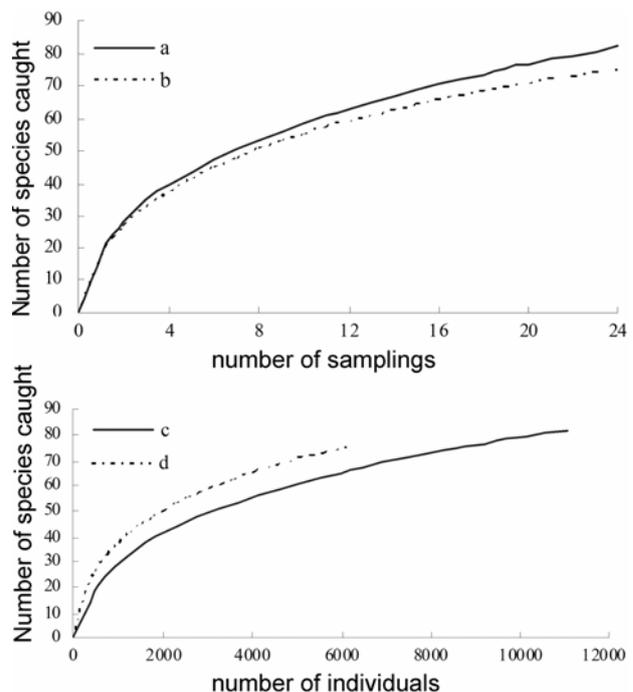


Fig. 1: Sample-based accumulation curves (a, b) and individual-based accumulation curves (c, d), represented by the means of 50 randomizations of sample accumulation order pooled from 12 plots. "a" and "c" were based on pitfall traps with sugar / vinegar mixture (a mixture of sugar, vinegar, alcohol and water, in the ratio of 1 : 2 : 2 : 20), "b" and "d" were based on pitfall traps with ethylene glycol.

species when possible, or otherwise to morphospecies (OLIVER & BEATTIE 1993, BURGER & al. 2003, SCHNELL & al. 2003), and enumerated.

Statistical analysis: Individual-based accumulation curves and sample-based accumulation curves were generated by using EstimateS (COLWELL 2005). These represented the means of 50 randomizations of sample accumulation order pooled during 24 samplings from 12 plots, in order to gauge the completeness of sampling (MORENO & HALFFTER 2001). Expected species richness was calculated by the estimators of ACE with EstimateS (HORTAL & al. 2006). Specimens of the 24 samplings from each plot were pooled for data analysis. Abundance and richness data were log-transformed to stabilize variance (HETTERICK & al. 2000, GOVE & al. 2005).

Principal Coordinate Analysis (PCoA) for ant species was conducted after applying the Hellinger transformation to the abundance data and followed by calculation of Euclidean distance, using the "decostand", "dist", and "cmdscale" functions of the R language package "vegan" (OKSANEN & al. 2009, R DEVELOPMENT CORE TEAM 2009). Jaccard's index of similarity matrix was generated by using EstimateS (COLWELL 2005). We analyzed the effects of liquid type and habitat on the difference of richness and abundance of ants among the plots with a general linear model (Univariate) followed by Tukey's multiple comparisons (SPSS 16.0).

Results

Species accumulation curve: The individual-based and sample-based accumulation curves are shown in Figure 1.

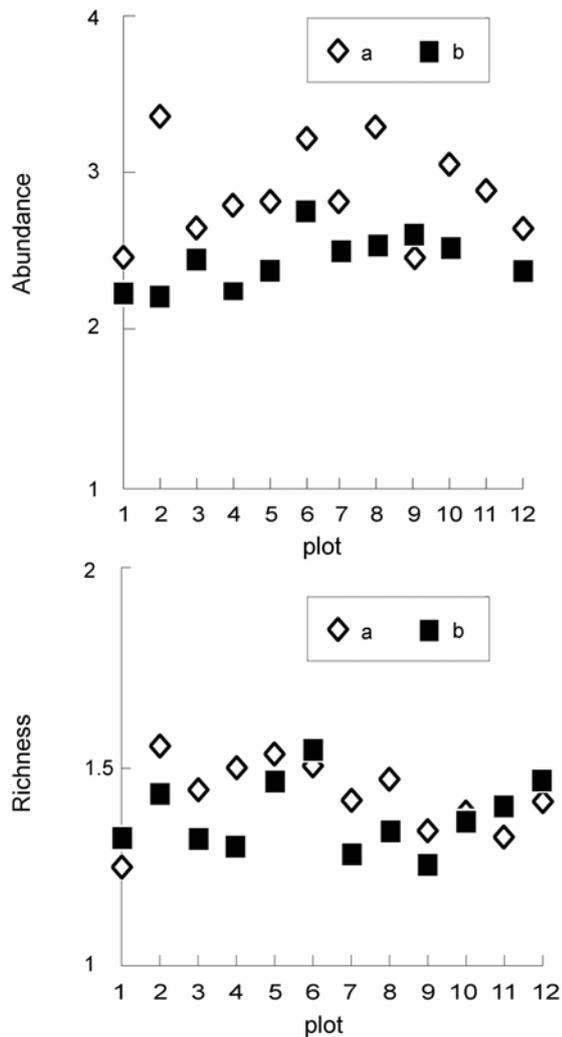


Fig. 2: Ant individuals and species captured in 12 plots. Among 12 plots, plots 1 - 5 were from natural lac forest, plots 6 - 8 were from lac plantation, and plots 9 - 12 were from dry land. "a" and "b" were based on pitfall traps with sugar / vinegar mixture and pitfall traps with ethylene glycol, respectively. Data were log transformed.

Smooth accumulation curves were generated, indicating that the sampling effort in the current investigation was sufficient by both liquids in the traps.

Sample-based rarefaction curves for pooled ecosystem data showed that the captured ant species by traps with sugar / vinegar mixture were slightly more than those by traps with ethylene glycol. The shape of the curves also revealed that the accumulated ant species sampled by traps with sugar / vinegar mixture were similar to those by traps with ethylene glycol. Individual-based rarefaction curves for pooled ecosystem data showed that the individual ants captured by traps with sugar / vinegar mixture were nominally more than those by traps with ethylene glycol. However, both individual-based curves and sample-based curves were so close that there was no significant difference in species accumulation by the two liquids in the traps.

According to the ACE estimators, the expected species richness in traps with sugar / vinegar and ethylene glycol was 92 and 93 respectively; the captured species by traps

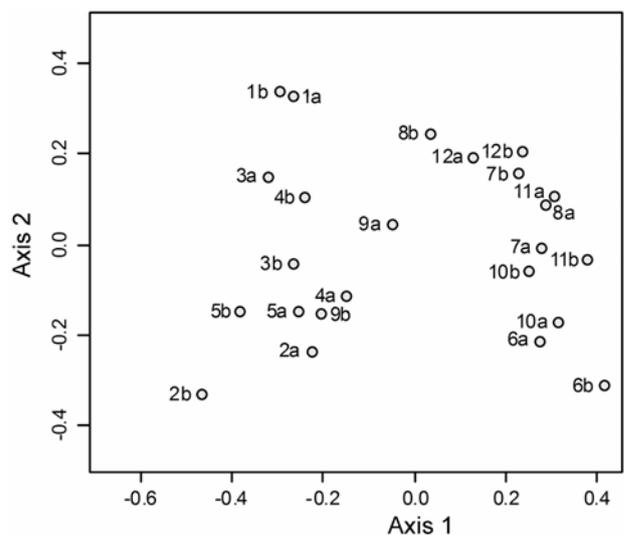


Fig. 3: Principal coordinate analysis for ant species among the 12 plots. Among 12 plots, plots 1 - 5 were from natural lac forest, plots 6 - 8 were from lac plantation, and plots 9 - 12 were from dry land. Hellinger transformation was applied for the abundance data and followed by calculation of Euclidean distance. "a" and "b" were based on pitfall traps with sugar / vinegar mixture and pitfall traps with ethylene glycol, respectively.

with sugar / vinegar mixture were 89.3% of the estimated value of species richness, and for traps with ethylene glycol they were 80.6%. This suggests that traps with sugar / vinegar mixture are likely to capture enough species for species richness estimates as compared to traps with ethylene glycol.

Ant abundances and species richness: The total number of ant individuals and species captured by pitfall traps with sugar / vinegar mixture was more than that by traps with ethylene glycol. A total of 17154 individuals were collected by all of the traps, representing 95 species of Formicidae. Among them, 11052 individuals were collected by the traps with sugar / vinegar mixture, representing 82 species, while 6102 individuals were captured by traps with ethylene glycol, representing 75 species.

Except for plots 9 and 11 (Fig. 2), the number of individuals captured by traps with sugar / vinegar mixture was higher than that captured by traps with ethylene glycol. The difference was mostly due to a few species during several of the sampling periods. In plot 2, the number of *Pseudolasius familiaris* (SMITH, 1860) captured by sugar / vinegar mixture in the third sampling period was more than 900. In plot 8, the number of *Camponotus nicobarensis* (MAYR, 1865) captured by sugar / vinegar mixture in the last two sampling periods was more than 1300. In plot 11, the number of *Pheidole noda* (SMITH, 1874) captured during the last three sampling periods in traps with ethylene glycol was more than 1800.

The number of ant species captured by pitfall traps with sugar / vinegar mixture was not always more than that by traps with ethylene glycol in all plots. In plots 2 and 4, the number of ant species captured by pitfall traps with sugar / vinegar mixture was more than that by traps with ethylene glycol, while the ant species captured by pitfall traps with ethylene glycol were nearly equal to or slightly more than those by traps with sugar / vinegar mixture (plots 1, 11) (Fig. 2).

Tab. 1: The number of ant individuals and species captured by pitfall traps with different liquids in three land-use habitats (arithmetic mean \pm 1 standard error). Data were log transformed.

Solution	Abundance			Richness		
	Natural lac forest	Lac plantation	Dry land	Natural lac forest	Lac plantation	Dry land
sugar-vinegar mixture	2.8 \pm 0.1	3.1 \pm 0.1	2.8 \pm 0.1	1.5 \pm 0.1	1.5 \pm 0.0	1.4 \pm 0.0
ethylene glycol	2.3 \pm 0.0	2.6 \pm 0.1	2.7 \pm 0.2	1.4 \pm 0.0	1.4 \pm 0.1	1.4 \pm 0.0

Tab. 2: The comparisons of the most common ants captured by each liquid. "-" indicate species that were not among the six most common species.

Species	By two liquids		By sugar / vinegar mixture		By ethylene glycol	
	Percentage of total ants	Ranking	Percentage of total ants	Ranking	Percentage of total ants	Ranking
<i>Pheidole noda</i> SMITH, 1874	23.6	1	15.1	2	39.0	1
<i>Camponotus nicobarensis</i> MAYR, 1865	12.2	2	18.7	1	< 0.6	-
<i>Camponotus mitis</i> (SMITH, 1858)	8.7	3	8.6	5	8.9	2
<i>Pseudolasius familiaris</i> (SMITH, 1860)	8.0	4	11.9	3	< 1.0	-
<i>Lepisiota rothneyi</i> (FOREL, 1894)	7.1	5	9.0	4	3.7	-
<i>Polyrhachis halidayi</i> EMERY, 1889	4.3	6	5.5	6	< 2.1	-

An ordination of log-transformed numerical ant data is presented in Figure 3. Ordination analysis revealed that natural lac forest had very different community profiles, compared with lac plantation and dry land. Moreover, regardless of what the habitat type was, the ant community composition captured by two liquids was similar.

When the data of all plots from the same type of land utilization were combined, the liquid type, habitat type, and the interactions of liquid type and habitat type had no significant effect on species richness; only liquid type had a significant effect on abundance ($F_{1,18} = 7.978$, $P = 0.011$, $n = 24$) (Tab. 1). In summary, these results indicate that traps with sugar / vinegar mixture are as effective at sampling ant species as ethylene glycol, and collected more individuals of some ant species.

Shared species captured by two liquids: A total of 62 species were co-captured by traps with two kinds of liquid in 12 plots, representing 65.3% of the total ant species. In each plot, the percentage of shared species co-captured by both liquids was 40.0 - 62.5% of the total (Fig. 4). The Jaccard's index of similarity matrix ranged from 0.4 to 0.625, which again suggests high similarity of species composition between ants captured by two liquids.

Most common species: Composition and number of individuals of the most common species captured by traps with two liquids were similar (Tab. 2). Among the six most abundant ants (Tab. 3), liquid type only had a significant effect on *Camponotus nicobarensis* and *Lepisiota rothneyi* (FOREL, 1894) ($F_{1,18} = 4.497$, $P = 0.048$, $n = 24$; $F_{1,18} = 8.468$, $P = 0.009$, $n = 24$). Habitat type had a significant

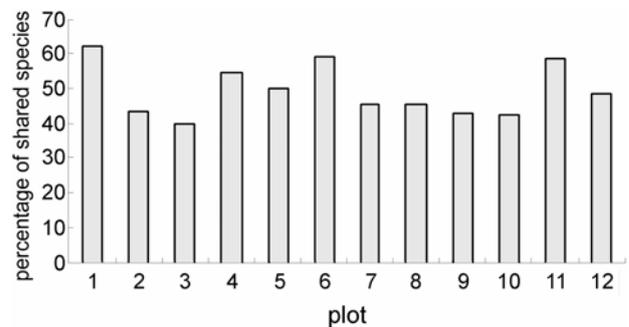


Fig. 4: Percentage of shared ant species co-captured in each plot by traps with sugar / vinegar mixture and traps with ethylene glycol in 12 plots. Among 12 plots, plots 1 - 5 were from natural lac forest, plots 6 - 8 were from lac plantation, and plots 9 - 12 were from dry land.

effect on *Polyrhachis halidayi* (EMERY, 1889) and *Camponotus mitis* (SMITH, 1858) ($F_{2,18} = 10.265$, $P < 0.000$, $n = 24$; $F_{2,18} = 42.492$, $P < 0.000$, $n = 24$); the differences were between natural lac forest and other two habitats. However, there was no interaction of liquid type and habitat. The results suggest that traps with sugar / vinegar contributed more to the most common species than traps with ethylene glycol.

Discussion

Ants are among the most abundant and easily collected of all animals. However, a single liquid in traps has not been

Tab. 3: Abundance of most common species captured in three habitats by traps with different liquids (mean \pm 1 standard error). The species in the first column were the six most abundant species based on all data; data in the third and fourth column were mean numbers of these six species captured by each of the two liquids. Data were log transformed.

Species	Habitat	Sugar-vinegar mixture	Ethylene glycol
<i>Pheidole noda</i> SMITH, 1874	Natural lac forest	1.5 \pm 0.2	1.0 \pm 0.2
	Lac plantation	2.1 \pm 0.4	1.6 \pm 0.5
	Dry land	1.9 \pm 0.3	2.2 \pm 0.4
<i>Camponotus nicobarensis</i> MAYR, 1865	Natural lac forest	0.4 \pm 0.4	0.0 \pm 0.0
	Lac plantation	1.9 \pm 1.0	0.7 \pm 0.4
	Dry land	1.1 \pm 0.6	0.3 \pm 0.3
<i>Camponotus mitis</i> (SMITH, 1858)	Natural lac forest	1.2 \pm 0.1	1.1 \pm 0.1
	Lac plantation	2.1 \pm 0.3	1.9 \pm 0.1
	Dry land	1.9 \pm 0.0	1.8 \pm 0.0
<i>Pseudolasius familiaris</i> (SMITH, 1860)	Natural lac forest	1.1 \pm 0.6	0.2 \pm 0.2
	Lac plantation	0.5 \pm 0.4	0.0 \pm 0.0
	Dry land	0.0 \pm 0.0	0.4 \pm 0.4
<i>Lepisiota rothneyi</i> (FOREL, 1894)	Natural lac forest	1.3 \pm 0.3	0.1 \pm 0.1
	Lac plantation	0.7 \pm 0.4	0.8 \pm 0.1
	Dry land	2.0 \pm 0.3	0.8 \pm 0.5
<i>Polyrhachis halidayi</i> EMERY, 1889	Natural lac forest	1.9 \pm 0.1	1.2 \pm 0.1
	Lac plantation	0.7 \pm 0.7	0.6 \pm 0.5
	Dry land	0.2 \pm 0.2	0.1 \pm 0.1

proven adequate to sample ground-dwelling ants. Neither of the liquids used in traps could collect all expected ant species in the selected areas. Annual sampling captured less than 90% of the expected ant species; this supports KING & PORTER (2005), who stated that a large sampling effort should include multiple sampling methods. This is the most effective way of thoroughly sampling ant assemblages even in relatively species-poor (compared to the tropics) temperate and subtropical regions.

A wide variety of methods and a greater effort is needed to obtain a thorough inventory of the ants of an area and to collect as many species as possible (AGOSTI & ALONSO 2000). When pitfall trapping is used to investigate the ant fauna, it is suggested that more than one liquid should be used. If the purpose of the research is to study ant diversity, ethylene glycol is preferable to sugar / vinegar mixture, as it can provide adequate species information with fewer individuals, and sugar / vinegar mixture could be recommended if ethylene glycol is unavailable.

The applied liquids did not have completely unbiased samples of the ant species. For a given ant species, the number of captured individuals is influenced by different liquids. Some researchers believe that ethylene glycol should

not be used because it is attractive and toxic to vertebrates (SCUDDER 1996). Researchers have used propylene glycol for its lower toxicity, and found no significant difference in trap yields (at least as far as ants are concerned) (ABENSPERG-TRAUN & STEVEN 1995, IPSEY & al. 2004). Ideally, the killing agent should not attract or repel ants (or at least not attract or repel species differently), otherwise estimates and comparisons of forager densities may be biased (BESTELMEYER & al. 2000). CALIXTO & al. (2007) found that the mixture of propylene glycol and water affected the behavior of several ant species, including *Solenopsis invicta* BUREN, 1972, *Paratrechina* sp. and *Diplorhoptrum* sp. CHEN & al. (2010) found that sugar / vinegar mixture affected the behavior of Scolytidae beetles. The results from this research showed that the abundance of some ant species was slightly higher when sugar / vinegar mixture or ethylene glycol was used as the preservative. The traps were set semi-monthly in the same transect with each trap in the same location, and opened for five days and nights. Provided there were digging-in effects (GREENSLADE 1973), both transects should be affected. Therefore the results suggest that sugar / vinegar mixture could not have completely unbiased effects on all species.

In our study, enough species were captured by traps with sugar / vinegar mixture, and this liquid, which has widely been used in China, but not reported in other countries, seems to have the same capture effect as that of ethylene glycol and provides similar information on ant communities. There are differences in ant abundance and species richness between the two liquids; however, the captures being compared are actually samples obtained from different locations (transects separated by 20 m). Such transects are likely to yield somewhat different results even if the same solutions were used. It is impossible to determine if differences between the liquids are due to the solutions themselves or to spatial variation. A paired or spatially interspersed design should have been used.

CHEN & al. (2010) reported that sugar / vinegar mixture played a more important role in trapping ground-dwelling beetles, as it sampled different components of the beetle community (species co-captured by sugar / vinegar mixture and ethylene glycol comprised only 27.6% of the total). However, there were obvious disadvantages when the sugar / vinegar mixture was used in ant sampling. One was that a large number of individuals of certain species were being captured, which resulted in heavy sorting and identification work in the laboratory. Moreover, we do not know if so many individuals of certain species being captured would affect the population of ants in the field. The other disadvantage was that the specimens in the sugar / vinegar mixture were not well preserved if the traps were opened in the field for more than one week. More alcohol in the mixture or a shorter sample period could possibly improve this.

Overall, the results showed that sugar / vinegar mixture gives reasonably comparable results to ethylene glycol (there were some differences, but this is confounded by unknown inherent variation between transects), and so the results are reasonably robust. However, sugar / vinegar would be recommended for further use if ethylene glycol (or other commonly used solutions) is unavailable.

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Zusammenfassung

Der Einfluss von verschiedenen Flüssigkeiten auf die Fängigkeit von Barberfallen in Bezug auf die mehr oder weniger unverzerrte Wiedergabe der Fouragieraktivität von Ameisenarten ist schlecht verstanden. Wir haben die Wirksamkeit einer Zucker-Essig-Lösung, wie sie in China häufig in Ameisenstudien verwendet wird, im Vergleich zu jener von Ethylenglykol in Lack-Agroökosystemen in der chinesischen Provinz Yunnan untersucht. In den Fallen mit der Zucker-Essig-Lösung wurden insgesamt 11052 Individuen von 82 Ameisenarten gefangen, während 6102 Individuen von 75 Arten in den Fallen mit Ethylenglykol gefangen wurden. Weder Individuen- noch Proben-basierte Kurven ließen einen signifikanten Unterschied in der Ef-

fizienz erkennen, Arten zu erfassen. Eine Ordinationsanalyse ergab, dass die Zusammensetzung der Ameisenartengemeinschaft bei beiden Fangflüssigkeiten ähnlich war. Die Fangflüssigkeit hatte einen signifikanten Einfluss auf die Erfassung von zwei der sechs häufigsten Arten, aber dieses Ergebnis könnte auch darauf zurückzuführen sein, dass die Fallen mit den unterschiedlichen Flüssigkeiten in unterschiedlichen Transekten aufgestellt waren. Wir kommen zu dem Schluss, dass die Verwendung der Zucker-Essig-Lösung eine relativ unverzerrte Wiedergabe der Ameisenfouragieraktivität ermöglicht und verwendet werden kann, wenn andere häufig verwendete Fangflüssigkeiten nicht zur Verfügung stehen.

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