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EFFECTS OF PITFALL TRAP PRESERVATIVE ON COLLECTIONS OF CARABID BEETLES (COLEOPTERA: CARABIDAE)

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ABSTRACT

Effects of six pitfall trap preservatives (5% acetic acid solution, distilled water, 70% ethanol, 50% ethylene glycol solution, 50% propylene glycol solution, and 10% saline solution) on collections of carabid beetles (Coleoptera: Carabidae) were studied in a west-central Illinois deciduous forest from May to October 2005. A total of 819 carabids, representing 33 species and 19 genera, were collected. Saline produced significantly fewer captures than did acetic acid, ethanol, ethylene glycol, and propylene glycol, while distilled water produced significantly fewer captures than did acetic acid. Significant associations between numbers of captures and treatment were seen in four species: *Amphasia interstitialis* (Say), *Calathus opaculus* LeConte, *Chlaenius nemoralis* Say, and *Cyclotrachelus sodalis* (LeConte). Results of this study suggest that type of preservative used can have substantial effects on abundance and species composition of carabids collected in pitfall traps.

Pitfall trapping is a commonly used method of sampling surface-active soil and litter arthropods such as carabid beetles (Greenslade 1964, Holopainen 1992, Lemieux and Lindgren 1999), rove beetles, Staphylinidae (Honêk 1988), ants, Formicidae (Greenslade 1973), and wandering spiders such as wolf spiders, Lycosidae (Curtis 1980, Honêk 1988). Pitfall trap collections reflect an interaction between arthropod activity and abundance (Thiele 1977), however, there is evidence that different arthropod species perceive and respond to pitfall traps differently and that trap characteristics can affect capture rates (Halsall and Wratten 1988, Digweed et al. 1995, Work et al. 2002). Pitfall traps are often used with a preservative/killing agent to maintain the condition of the trapped specimens and to reduce escape and within-trap predation. One factor that can affect arthropod response to pitfall traps is the type of preservative used. A wide variety of preservatives have been used in pitfall traps, including ethylene glycol, propylene glycol, water, formalin, kerosene, brine, alcohol, acetic acid, chloral hydrate, and benzoic/acetic acid (Woodcock 2005); however, the type of preservative used can affect the number, species, or even sex ratio of arthropod captures. For carabids, effects on pitfall trap collections have been found for ethylene glycol (Holopainen 1990, 1992), propylene glycol (Hammond 1990), benzoic/acetic acid (Scheller 1984), and formalin (Luff 1968, Scheller 1984; Holopainen and Varis 1986), although Waage (1985) found no evidence of formalin influencing collections.

Carabidae is one of the most diverse insect families, with over 40,000 described species (Lövei and Sunderland 1996). Carabids are important predators in many terrestrial ecosystems, and can be important biological control agents (Lövei and Sunderland 1996). The ecology and behavior of carabids are often closely associated with factors such as soil type, vegetation cover and microclimate, making them potentially important bioindicators (Thiele 1977,

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Niemelä et al. 1992, Ings and Hartley 1999, Villa-Castillo and Wagner 2002, McCravy and Willand 2005, Willand and McCravy 2006). Knowledge of the potential effects of type of preservative used could be important in interpreting results of research on carabids using pitfall traps. In this study we compared the effects of six pitfall trap preservatives on collections of midwestern forest ground beetles.

MATERIALS AND METHODS

The study was conducted in a deciduous forest in McDonough Co., Illinois from May to October 2005. The site was located at 40.4973° N and 90.5993° W. Dominant tree species consisted of white oak (*Quercus alba* L.), northern red oak (*Quercus rubra* L.), black oak (*Quercus velutina* Lam.), shagbark hickory (*Carya ovata* (Miller) K. Koch.), and red maple (*Acer rubrum* L.). Plant nomenclature follows that of Gleason and Cronquist (1991). Sixty pitfall traps were deployed in ten rows of six traps each. Each trap consisted of two 473 ml plastic cups (Solo®, Urbana, IL) one nested inside the other so that the inner cup could be removed during collections and replaced with a fresh one with minimal disturbance to the trap site. The diameter of the cup opening was 9.3 cm. Traps were placed so the trap rim was flush with the ground, and efforts were made to return surrounding soil and litter to former conditions. Traps within rows were five meters apart and rows were six meters apart. In each row, each trap was filled with approximately 150 ml of one of six preservatives: 1) 5% acetic acid solution, 2) distilled water, 3) 70% ethanol, 4) 50% ethylene glycol solution (using Prestone® antifreeze), 5) 50% propylene glycol solution (using Sierra® antifreeze), and 6) 10% saline solution (10 g rock salt per 90 ml water). Distilled water was used as the solvent/diluent for all solutions. Traps were operated for eight 5-day trapping periods: 22 – 27 May, 12 – 17 June, 1 – 6 July, 22 – 27 July, 8 – 13 August, 23 – 28 August, 10 – 15 September, and 30 September – 5 October. For each trapping period, fresh preservative was used, and positions of the six treatments were randomly assigned within each row, with the caveat that each treatment was assigned to each position at least once and not more than twice over the course of the study. This was done to control for possible trap location effects. A drop of unscented detergent was placed in each trap to reduce surface tension. Traps were collected at the end of each trapping period, and carabids were collected, pinned, and identified using a synoptic reference collection of local ground beetle species. Instances of trap disturbance (trap pulled out of the ground and/or mutilated) were noted.

Species richness and Simpson's diversity indices were calculated for each preservative. Species richness is associated with sample size, so rarefaction was used in comparing species richness of different preservatives. Rarefaction provides an estimate of the expected number of species for a given sample size (Krebs 1999). The University of Alberta Department of Biology online rarefaction calculator (U of A 2007) was used in these analyses. Differences in numbers of beetles collected among treatments were analyzed using permutational multivariate analysis of variance (PERMANOVA – Anderson 2001, McArdle and Anderson 2001). A one-way design was used, with beetle numbers summed across dates, and rows serving as replicates. Beetle numbers were expressed as numbers per trap to compensate for instances of trap disturbance. Analyses were done for all species collectively and for each of eleven species that produced the greatest number of captures. Results of paired comparisons among treatments were evaluated using the Bonferroni method to control for Type I error associated with multiple tests (Sokal and Rohlf 1995). This produced a threshold *P*-value of 0.0033 (0.05 divided by 15 – the number of paired comparisons among 6 treatments) for evaluating significance of paired comparisons.

RESULTS

A total of 819 carabids, representing 33 species and 19 genera, were collected over the course of the study (Table 1), resulting in a capture rate of 0.35 beetles/trap/day. Mean number collected per trapping period (± SE) was 102.38 ± 22.59, with a low of 29 for the 13 August collection and a high of 217 for the 6 July collection. Thirteen instances of trap disturbance occurred over the course of the study, for a disturbance rate of 2.7%. There was no apparent association between trap disturbance and treatment. Each treatment had at least one disturbance, and none more than three disturbances. Species richness per treatment ranged from a low of 17 for saline to a high of 23 for ethylene glycol (Table 1). Rarefaction estimates (Table 1) indicated that observed species richness values did not differ from expected for any of the preservatives, based on 95% confidence intervals. Simpson's diversity indices ranged from a low of 0.86 for acetic acid and distilled water to a high of 0.91 for ethylene glycol (Table 1). Mean number of carabids collected per treatment (± SE) was 136.50 ± 14.39, with a low of 83 total carabids collected in saline and a high of 175 in acetic acid (Table 1). Numbers of carabids collected differed significantly among treatments (Table 2; $F = 5.632$; df = 5, 54; $P = 0.0002$). Saline produced significantly fewer captures than did all other preservatives except distilled water, and distilled water produced significantly fewer captures than did acetic acid (Fig. 1; $P < 0.0033$, each comparison).

Eleven species of carabids each produced at least 22 captures (Table 1), and these species comprised 90.7% of total captures. Significant associations between numbers of captures and treatment were seen in four species: *Amphasia interstitialis* (Say) ($F = 5.173$; df = 5, 54; $P = 0.0007$), *Calathus opaculus* LeConte ($F = 3.754$; df = 5, 54; $P = 0.0044$), *Chlaenius nemoralis* Say ($F = 3.591$; df = 5, 54; $P = 0.005$), and *Cyclotrachelus sodalis* (LeConte) ($F = 2.217$; df = 5, 54; $P = 0.0494$) (Table 2; Fig. 2). *Amphasia interstitialis* was significantly more abundant in acetic acid and saline than in ethanol, which collected none of this species ($P < 0.0033$, each comparison). *Calathus opaculus* was significantly more abundant in ethylene glycol than in either distilled water or saline ($P < 0.0033$, each comparison). Neither *C. nemoralis* nor *C. sodalis* produced significant pairwise comparisons.

DISCUSSION

Traps containing acetic acid and ethylene glycol collected the greatest numbers of carabids; however, traps containing acetic acid collected relatively low diversity, based on Simpson's index, whereas those containing ethylene glycol collected the greatest diversity (Table 1). These results suggest that either preservative would be effective in maximizing numbers of carabids collected, but ethylene glycol may be preferable if high diversity of beetle captures is the goal. Species richness of carabids collected varied among the different preservatives used, but rarefaction results (Table 1) suggested that differences in richness among preservatives can be explained by differences in numbers of individuals collected. Substantial differences among the preservatives were found in total numbers of carabids collected and in numbers of some individual species. Pitfall traps containing acetic acid and ethylene glycol collected 111% and 99% more carabids than did traps containing saline. These results are consistent with those of Scheller (1984) and Holopainen (1992). Scheller (1984) collected 39% more carabids with a 5% acetic acid/2% formaldehyde solution than with water in a study in North Zealand, Denmark. It is difficult to ascertain the relative importance of acetic acid *vs.* formaldehyde on trapping efficiency in Scheller's (1984) study, but the 5% acetic acid/2% formaldehyde solution collected significantly more carabids than did a 0.5% formaldehyde solution, suggesting that acetic acid may

Table 1. Numbers, species richness, rarefaction estimates of species richness (± SD), and species diversity of carabid beetles captured in pitfall traps containing the following preservatives: 1) 5% acetic acid solution (AA), 2) distilled water (DW), 3) 70% ethanol (EA), 4) 50% ethylene glycol solution (EG), 5) 50% propylene glycol solution (PG), and 6) 10% saline solution (SA). Trapping was done for eight 5-day trapping periods from 22 May to 5 October 2005 in McDonough Co., Illinois.

Species	AA	DW	EA	EG	PG	SA	Total
Pterostichus stygicus (Say)	48	35	41	32	33	18	207
Pterostichus permundus (Say)	31	9	24	14	19	9	106
Platynus decentis (Say)	12	13	8	16	12	16	77
Cyclotrachelus sodalis (LeConte)	20	5	17	15	7	9	73
Calathus opaculus LeConte	8	5	9	21	12	3	58
Chlaenius nemoralis Say	3	4	17	11	19	2	56
Cyclotrachelus seximpressus LeConte	13	7	6	12	9	1	48
Synuchus impunctatus (Say)	10	5	5	16	6	4	46
Amphasia interstitialis (Say)	11	4	0	2	3	8	28
Anisodactylus agricola (Say)	1	5	5	2	7	2	22
Poecilus lucublandus (Say)	6	3	2	5	3	3	22
Patrobus longicornis (Say)	3	2	1	2	1	2	11
Chlaenius emarginatus Say	1	0	4	0	1	1	7
Chlaenius tricolor Dejean	1	1	3	0	2	0	7
Harpalus herbivagus Say	1	0	0	2	2	1	6
Notiophilus novemstriatus (LeConte)	1	2	1	2	0	0	6
Galerita janus (Fabricius)	0	0	3	1	1	0	5
Chlaenius platyderus Chaudoir	1	1	0	2	0	0	4
Dicaelus furvus Dejean	1	1	0	1	0	1	4
Harpalus pensylvanicus (DeGeer)	0	1	0	3	0	0	4
Cymindis americanus Dejean	1	0	0	0	0	2	3
Scaphinotus elevatus (Haldeman)	0	0	1	0	2	0	3
Chlaenius pusillus Say	0	1	1	0	0	0	2
Harpalus protractus Casey	1	0	1	0	0	0	2
Pentagonica picticornis Bates	1	1	0	0	0	0	2
Poecilus chalcites (Say)	0	0	0	2	0	0	2
Pterostichus praetermissus Chaudoir	0	0	0	1	0	1	2

Table 1. Continued.

Species	AA	DW	EA	EG	PG	SA	Total
Chlaenius pennsylvanicus Say	0	1	0	0	0	0	1
Cicindela sexguttata Fabricius	0	1	0	0	0	0	1
Dicaelus elongatus Bonelli	0	0	0	1	0	0	1
Dicaelus purpuratus Bonelli	0	0	0	1	0	0	1
Pterostichus femoralis (Kirby)	0	0	0	1	0	0	1
Trichotichnus fulgens (Csiki)	0	0	1	0	0	0	1
Total	175	107	150	165	139	83	819
Species Richness	21	21	19	23	18	17	
Rarefaction Estimate	21.9 ± 2.03	18.6 ± 1.99	20.8 ± 2.03	21.4 ± 2.03	20.3 ± 2.03	17.0 ± 1.93	
Simpson's Diversity	0.86	0.86	0.87	0.91	0.88	0.89	

Table 2. Results of PERMANOVAs of overall and species-specific carabid captures in pitfall traps containing the following preservatives: 1) 5% acetic acid solution (AA), 2) distilled water (DW), 3) 70% ethanol (EA), 4) 50% ethylene glycol solution (EG), 5) 50% propylene glycol solution (PG), and 6) 10% saline solution (SA). For each analysis, Preservative df = 5, Residual df = 54, and Total df = 59. Trapping was done for eight 5-day trapping periods from 22 May to 5 October 2005 in McDonough Co., Illinois.

Group	Source	SS	MS	*F*	*P*
All Beetles	Preservative	8061.40	1612.28	5.63	0.0002
	Residual	15460.02	286.30		
	Total	23521.42			
Amphasia interstitialis (Say)	Preservative	46479.63	9295.93	5.17	0.0007
	Residual	97038.48	1797.01		
	Total	143518.11			
Anisodactylus agricola (Say)	Preservative	11773.73	2354.75	1.10	0.3892
	Residual	115124.23	2131.93		
	Total	126897.95			
Calathus opaculus LeConte	Preservative	37607.98	7521.60	3.75	0.0044
	Residual	108193.87	2003.59		
	Total	145801.85			
Chlaenius nemoralis Say	Preservative	39270.31	7854.06	3.59	0.0050
	Residual	118101.43	2187.06		
	Total	157371.75			
Cyclotrachelus seximpressus (LeConte)	Preservative	23797.06	4759.41	1.98	0.0827
	Residual	130022.19	2407.82		
	Total	153819.25			
Cyclotrachelus sodalis (LeConte)	Preservative	25997.75	5199.55	2.22	0.0494
	Residual	126646.61	2345.31		
	Total	152644.36			
Platynus decentis (Say)	Preservative	11615.12	2323.02	1.09	0.3706
	Residual	115586.73	2140.50		
	Total	127201.85			
Poecilus lucublandus (Say)	Preservative	4018.14	803.63	0.35	0.9113
	Residual	123060.38	2278.90		
	Total	127078.52			

Table 2. Continued.

Group	Source	SS	MS	*F*	*P*
Pterostichus permundus (Say)	Preservative	18561.99	3712.40	1.67	0.1204
	Residual	120015.45	2222.51		
	Total	138577.44			
Pterostichus stygicus (Say)	Preservative	9498.64	1899.73	1.27	0.2366
	Residual	81090.85	1501.68		
	Total	90589.49			
Synuchus impunctatus (Say)	Preservative	7744.54	1548.91	0.59	0.7453
	Residual	142129.50	2632.03		
	Total	149874.04			

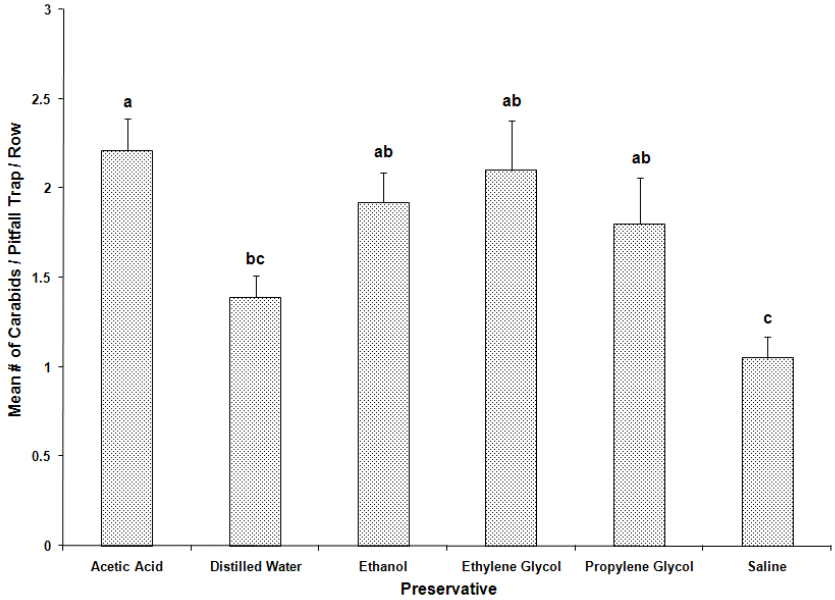

Figure 1. Mean numbers of carabids collected per trap per trap row (± SE) in pitfall traps containing the following preservatives: 1) 5% acetic acid solution, 2) distilled water, 3) 70% ethanol, 4) 50% ethylene glycol solution, 5) 50% propylene glycol solution, and 6) 10% saline solution. Trapping was done for eight 5-day trapping periods from 22 May to 5 October 2005 in McDonough Co., Illinois. Means with the same letter were not significantly different at the 0.0033 level derived using the Bonferroni method.

have contributed to the increased number of carabids collected. In a study of carabids in central Finland, Holopainen (1992) collected 58% more carabids in pitfall traps containing ethylene glycol than in traps containing water; however, Lemieux and Lindgren (1999) found no difference between ethylene glycol and brine trapping efficiency of carabids in British Columbia, Canada.

Because it is relatively inexpensive, has good preservative properties, and is widely available, ethylene glycol is currently a commonly used pitfall trap preservative (Woodcock 2005), however, concerns about its attractiveness and toxicity to mammals, including pets (Beasley 1985, Marshall and Doty 1990), have led some workers to consider the less toxic propylene glycol as an alternative (Hall 1991). In our study, traps containing ethylene glycol accounted for only two of 13 total trap disturbances, suggesting that this preservative, compared with the others, was not unusually attractive to mammals.

Significant differences in captures among preservatives were found for four ground beetle species, and two (*A. interstitialis* and *C. opaculus*) produced significant pairwise comparisons (Fig. 2). Differences among preservatives in numbers and species of carabids captured may result from differential attraction/repellency, differences in escape rates, or some combination of these factors. In our study, pitfall traps containing the four preservatives that appeared to produce the strongest volatiles (acetic acid, ethanol, ethylene glycol, and propylene glycol) collected much greater numbers of carabids than did those containing distilled water or saline. This suggests that chemical attraction could have played a role in these differences, since many insects are known to rely heavily on semiochemical perception; however, the former four preservatives also

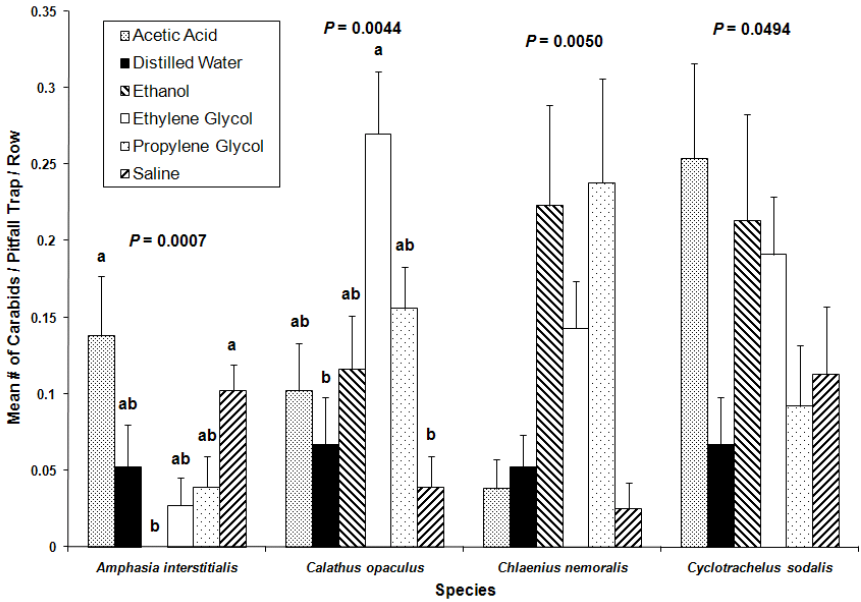

Figure 2. Mean numbers of four species of carabids collected per trap per trap row (± SE) in pitfall traps containing the following preservatives: 1) 5% acetic acid solution, 2) distilled water, 3) 70% ethanol, 4) 50% ethylene glycol solution, 5) 50% propylene glycol solution, and 6) 10% saline solution. Trapping was done for eight 5-day trapping periods from 22 May to 5 October 2005 in McDonough Co., Illinois. For *A. interstitialis* and *C. opaculus*, means with the same letter were not significantly different at the 0.0033 level derived using the Bonferroni method. For *C. nemoralis* and *C. sodalis*, no pairwise comparisons were significant.

may kill the beetles more quickly, reducing the probability of escape. Saline in particular may also provide enough buoyancy to allow a greater possibility of escape, especially if large-sized or large numbers of beetles create a surface layer on which subsequently captured beetles can crawl. The tendency of arthropods to float in brine may contribute to lower numbers of ground beetle genera and spider individuals captured in that preservative (Schmidt et al. 2006), however, relatively high numbers of *A. interstitialis* were collected in saline in our study (Fig. 2), suggesting that this species may be attracted to saline or may not be as capable of escape as are other species. Trap material may also have an effect. Glass provides fewer abrasions and less traction for carabids to escape than does plastic. This is probably not a problem with fast-killing preservatives (Luff 1975). Waage (1985) found no difference in trapping efficiency between plastic and glass pitfall traps containing preservative, but empty glass traps had higher catches than empty plastic ones. Saline may not be a desirable preservative to use with plastic pitfall traps if beetles have long survival times and float on the surface, thus having greater opportunities to escape.

It is also possible that differences in attraction or repellency may result from secondary volatiles produced by non-target organisms, such as other arthropods, gastropods, or earthworms collected in the traps. This would probably become more of a factor in studies employing longer trapping intervals than our relatively short 5-day trapping periods, particularly for distilled water and saline, which would allow more rapid decomposition to take place. Dilution of

preservative by rainwater would also contribute to rapid decomposition. In our study, greater than trace amounts of rain occurred during two trapping periods, the night of 27-28 August and the night of 13-14 September. In both cases, the rain occurred near the end of the trapping period, and trap liquid levels increased by approximately 1 cm in each case. Under conditions in which preservative dilution was greater or occurred earlier in the trapping period, significant decomposition probably would have taken place; however, decomposing carrion may have little or no effect on ground beetle captures. Greenslade (1964) found that baiting pitfall traps with meat or carrion did not influence collections of carabids.

Carabids collected during this study were generally in good condition, although, compared with the other preservatives, some noticeable softening of specimens collected in distilled water and saline did occur. This indicates that, under environmental conditions similar to those in which this study was done, these two preservatives would probably not be suitable for studies incorporating long trapping periods between collections. Because ethanol tends to evaporate and become diluted relatively rapidly, it would probably be undesirable in such studies as well. Acetic acid is also known to soften ground beetle specimens after several weeks, and would probably produce poor specimens if left for extended periods (anonymous reviewer, pers. comm.), however, beetles collected in these latter two preservatives in our study appeared to be in good condition, probably due to the short trapping periods and primarily shaded conditions.

Type of preservative used is also an important consideration in studies requiring isolation of DNA from trapped arthropods. Gurdebeke and Maelfait (2002) found that 70% ethanol was superior to 4% formaldehyde and a 1:1 mixture of acetic acid:TE buffer (Tris + EDTA) in short term laboratory storage of the amaurobiid spider *Coelotes terrestris* (Wider) for DNA analysis, however, for field collection using pitfall traps collected weekly, they found that 96% ethanol was superior to either 85% or 75% ethanol. In a study of the population genetics of the ground beetle *Chlaenius platyderus* Chaudoir, relatively undegraded DNA has been successfully extracted from beetles captured in pitfall traps containing undiluted propylene glycol antifreeze (M. A. Romano, pers. comm.). Beetles in that study were collected twice weekly and transferred to 95% ethanol after collection. Based on these results, at least two of the preservatives used in this study (ethanol and propylene glycol), if used undiluted or nearly so, are potentially useful in ground beetle population genetics studies requiring DNA extraction and analysis.

Results of this study show that the type of preservative used in pitfall trapping studies can affect collections of carabids and that these effects can be species-specific. This indicates that estimates of ground beetle abundance, species composition, and diversity may not be comparable among studies using different pitfall trap preservatives. Ground beetle researchers should carefully consider the goals of their studies when choosing a pitfall trap preservative.

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