2007

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Abstract
For their simplicity and effectiveness, pitfall traps have become a standard technique to measure the activity and relative abundance of ground-dwelling arthropods. Permeable screen or mesh bags filled with plant material, referred to as litter bags, have also recently been employed as a complementary sampling technique for epigeal taxa. The anticipated need for increased field research on arthropod populations, particularly in transgenic crops with insecticidal properties, suggests that a relative assessment of both sampling methods could contribute to the design of future studies. Comparisons among pitfall traps, and litter bags placed above- or below-ground indicated that aboveground litter bags most frequently succeeded in collecting certain groups of arthropods associated with moisture and sheltered areas, including centipedes (Chilopoda) and beetle larvae (Carabidae, Staphylinidae). Conversely, pitfall traps most often captured taxa considered active at ground level, such as adult carabids or harvestmen (Opiliones). For taxa collected in >40% of all three trap types, bootstrap confidence intervals for the coefficient of variation (CV; used to assess precision or sampling efficiency) showed that above-ground litter bags were significantly more precise than pitfall traps for sampling elongate springtails (Collembola) and adult rove beetles (Staphylinidae), but only during the first year of sampling. While below-ground litter bags often appeared similar to one or both of the other trap types, in no case were below-ground litter bags best based on frequency of collection or CV. Though differences were not always consistent between years, results suggest that the additional effort required to sample using litter bags may be justified for the collection of some ground-dwelling taxa.

Keywords
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Disciplines
Entomology

Comments
This article is from *Journal of Applied Entomology*; 131 (2007); 115-120; doi: 10.1111/j.1439-0418.2006.01141.x

J. Appl. Entomol. **131(2)**, 115–120 (2007) doi: 10.1111/j.1439-0418.2006.01141.x

Comparison of pitfall traps and litter bags for sampling ground-dwelling arthropods

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Ms. received: July 13, 2006; accepted: December 5, 2006

Abstract: For their simplicity and effectiveness, pitfall traps have become a standard technique to measure the activity and relative abundance of ground-dwelling arthropods. Permeable screen or mesh bags filled with plant material, referred to as litter bags, have also recently been employed as a complementary sampling technique for epigeal taxa. The anticipated need for increased field research on arthropod populations, particularly in transgenic crops with insecticidal properties, suggests that a relative assessment of both sampling methods could contribute to the design of future studies. Comparisons among pitfall traps, and litter bags placed above- or below-ground indicated that above-ground litter bags most frequently succeeded in collecting certain groups of arthropods associated with moisture and sheltered areas, including centipedes (Chilopoda) and beetle larvae (Carabidae, Staphylinidae). Conversely, pitfall traps most often captured taxa considered active at ground level, such as adult carabids or harvestmen (Opiliones). For taxa collected in >40% of all three trap types, bootstrap confidence intervals for the coefficient of variation (CV; used to assess precision or sampling efficiency) showed that above-ground litter bags were significantly more precise than pitfall traps for sampling elongate springtails (Collembola) and adult rove beetles (Staphylinidae), but only during the first year of sampling. While below-ground litter bags often appeared similar to one or both of the other trap types, in no case were below-ground litter bags best based on frequency of collection or CV. Though differences were not always consistent between years, results suggest that the additional effort required to sample using litter bags may be justified for the collection of some ground-dwelling taxa.

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1 Introduction

Pitfall trapping is a standard method for sampling epigeal arthropods because of its simplicity, efficacy and low cost (Southwood 1978). In agriculture and forestry, collections of arthropods from pitfall traps have often been used to assess the effects of insecticides, including those based on the bacterium *Bacillus thuringiensis* Berliner (Duffield and Aebischer 1994; Cárcamo et al. 1995; Wang et al. 2000; Rieske and Buss 2001). More recently, research on the impacts of transgenic crops with insecticidal properties (usually derived from *B. thuringiensis*) has routinely employed pitfall trapping to test for unintended effects on epigeal taxa (Riddick et al. 1998; French et al. 2004; de la Poza et al. 2005).

As an alternative or complementary sampling method to pitfall traps, screen or mesh bags filled with plant material have been used to sample arthropods at or below the soil surface (Donegan et al. 1997; Perry et al. 1997). Such traps, referred to as litter bags, have been used in both terrestrial and aquatic ecosystems to study decomposition of organic matter (Donegan et al. 1997; Whiles and Wallace 1997; Lachnicht et al. 2004). However, gradual movement of arthropods into the decaying plant material effectively makes each litter bag a separate trap, from which living arthropods may be extracted using heat and light (using Berlese or Tullgren funnels; Edwards 1991).

A comparison of pitfall traps and litter bags as sampling methods for epigeal arthropods is appropriate for several reasons. First, the results of many field experiments, particularly those with transgenic crops, suggest that sampling methods and experimental designs utilized sometimes have insufficient statistical power to detect possible impacts on arthropods (Bourguet et al. 2002; Lopez et al. 2005). Secondly, the expansion of commercially-available transgenic crops to include toxins active against coleopteran pests (USEPA [United States Environmental Protection Agency] 2003) has increased emphasis on other beetles near the soil surface, particularly ground (Carabidae) and rove (Staphylinidae) beetles. Finally, increased monitoring of arthropod populations may be required as more transgenic insecticidal crops (some incorporating toxins less well-known than the *B. thuringiensis* Cry proteins) are likely to be commercialized in the next few years. To examine whether litter bags are a useful complement to pitfall trapping, arthropod collections from pitfall traps and litter bags were compared for several taxa with regard to: (i) the frequency of capture and (ii) the precision of sampling.

2 Materials and Methods

As part of a study evaluating the effects of plot size and isolation on assessments of arthropod abundance (Prasifka et al. 2005), pitfall traps were used to sample ground-dwelling arthropods in plots of non-transgenic field corn (maize). To provide a comparison, litter bags were placed adjacent to pitfall traps in four blocks (groups of plots). Plots containing the litter bags varied according to plot size, isolation or insecticide treatment. However, because a relative assessment of both trapping techniques can be performed without comparing the treatments evaluated by Prasifka et al. (2005), plot-level effects were excluded and treated as nuisance variation unrelated to the effects of trap type.

Pitfall traps were made from nested clear plastic cups (9 cm diameter, TP12, Solo Cup Co., Urbana, IL) and placed beneath a rain cover made from two plastic plates (26 cm diameter, PS15W, Solo Cup Co.) held in place by bolts. When traps were not in use, an inner cup filled with soil helped retain trap shape. Pitfall traps were activated by exchanging the soil-filled cup for a sample cup partially filled with ethylene glycol-based antifreeze. Sample cups were collected after 24 h (2003) or 72 h (2004). Litter bags consisted of mesh onion bags (0.9 kg capacity, General Bag Corporation, Cleveland, OH) filled with 100 g of wheat straw and closed with duct tape. After filling, openings in the flexible mesh were approximately square with 0.9 cm sides. To ensure that litter bags were free of any living arthropods when placed into the plots, all straw was sterilized in an autoclave for 20 min. Within 1 m of each pitfall trap (2003, $n = 76$; 2004, $n = 60$), two litter bags were placed, with one located approximately 8 cm beneath the soil surface and another resting on top at ground level, anchored in place by a single landscaping flag (fig. 1).

After sample cups were collected from pitfall traps, arthropods were separated from antifreeze using filter paper and a vacuum system. Samples on filter paper were then identified with the aid of a dissecting microscope. Eight taxonomic groups were counted in samples including: wolf spiders (Lycosidae), non-lycosid spiders, harvestmen (Opiliones), centipedes (Chilopoda), globular or oval springtails (Collembola: Sminthuridae), elongate springtails (e.g., Entomobryidae, Isotomidae), ground beetles (adults and larvae separately) and rove beetles (adults and larvae separately). Absolute counts of all taxa were made except for springtails, which (when very abundant) were estimated via extrapolation from counts covering 10% of the area of the filter paper. Litter bags were removed from the plots and placed into labelled plastic bags for transport to Tullgren funnels. The mesh bags were then opened and the contents of each mesh and surrounding plastic bag were emptied into a screen-bottomed cylinder located beneath a 52 W light bulb. Beneath each sample, a funnel was positioned to direct any arthropods moving away from the light bulb (downward) into a cup of 70% ethanol. After 72 h, cups were removed and the contents of each sample were separated from the alcohol and identified as described above for the pitfall traps. Because of the large number of litter bag samples and a limited number (40) of Tullgren funnels, litter bags were removed one block at a time at 72 h intervals.

The sampling use of pitfall traps and litter bags differed, but in ways appropriate to each technique. Pitfalls were activated briefly nine times in 2003 and 2004, which helped to avoid the local depletion of ground-dwelling arthropods that continuous pitfall trapping can cause (Digweed et al. 1995). Conversely, litter bags were placed into plots once each year and left in the field for several weeks, allowing time for partial decomposition of the vegetation inside the mesh bags and colonization by epigeal arthropods (Donegan et al. 1997; Lachnicht et al. 2004). However, because active movement (avoidance) was used to remove arthropods from the litter bag samples (dynamic extraction, *sensu* Edwards 1991), only those arthropods alive when the mesh bags were removed from the plots were extracted, identified and counted. Accordingly, to make the most appropriate comparisons among trap types, only pitfall trap samples from the period (dates) corresponding most closely with the removal of the litter bags were used. The timing of pitfall and litter bag sampling is shown for 2003–2004 in table 1.

Sample-to-sample variability was influenced by at least two non-random components. Variation was increased among samples from plots receiving distinct treatments (plot size, isolation or insecticide use; Prasifka et al. 2005). Conversely, some traps within plots were placed too close to each other to be considered independent (< 10 m apart), which reduced variability. Accordingly, samples for each trap type and year combination were pooled, acknowledging that analyses would provide relative assessments of the trap types. Analysis of data did not include testing for differences in the

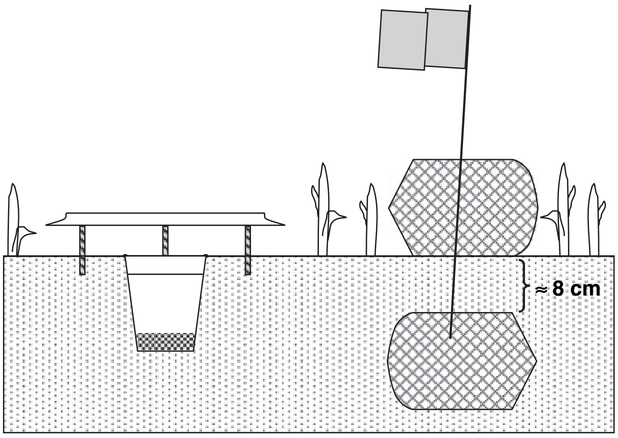

Fig. 1. *Cross-section representation of pitfall trap and litter bags in row of field corn (maize). Diagram is not to scale*

Table 1. *Timing of pitfall and litter bag samples relative to planting date*

Year	Sampling activity	Date	Days after planting
2003	Corn (maize) planted	27 May	0
	Litter bags introduced	1 July	35
	Litter bags removed	8–19 August	73–84
	Pitfall traps activated[1]	11–12 August	76–77
2004	Corn (maize) planted	11 May	0
	Litter bags introduced	28 May	17
	Litter bags removed	14–29 July	64–79
	Pitfall traps activated[1]	16–19 July	66–69

[1]Only dates of pitfall trap samples compared with litter bag samples shown.

mean number of arthropod types because: (i) to determine if one trap type is more effective or powerful for comparing experimental and control treatments, the variance-to-mean relationship is more important than considering means alone and (ii) significant differences in the frequency of collection among trap types (i.e., large numbers of zeros in some groups of data) made comparisons using common parametric statistics inappropriate (for some taxa means were clearly related to the number of traps with no individuals collected). However, to simply summarize arthropod captures by trap type and year, means and standard errors derived from individual traps were calculated for each arthropod group, but not tested for differences among means based on trap type.

Subsequent analysis used SAS statistical software (SAS Institute 1999), with specific procedures or options indicated in capital lettering. To test for differences in the frequency with which particular arthropod taxa were collected by the three trap types, 2 × 3 contingency tables categorized each trap as successful (one or more individuals collected) or unsuccessful (zero individuals collected), and differences were assessed with chi-squared tests (FREQ procedure, CHISQ option). Significant chi-squared values indicate an effect of trap type on the proportion of samples containing one or more individuals of an arthropod taxon (Conover 1999). For arthropod taxa captured with a relatively high frequency (>40%) using all three trap types, an additional comparison was made to test for differences in precision based upon the coefficient of variation ($CV = 100\ \sigma/\mu$; Sokal and Rohlf 1995). Confidence intervals (CI) for the CV for each sample type were created using 2000 bootstrap resamples from the original data using an estimation method that adjusts for bias and skewness (MEANS procedure, BOOT and BOOTCI macros, HYBRID option). Assuming an equal number of samples for each method, a lack of overlap between the 95% bootstrap CI for a pair of methods suggests the method with the lower CV (i.e., that has less variation relative to the mean) may be better able to detect treatment effects.

3 Results

A summary of arthropod captures of epigeal taxa in the three trap types (mean ± standard error of the mean) is shown in table 2. Though no statistical comparisons of these means were attempted, there appear to be order of magnitude differences between pitfall traps and litter bags for some taxa (e.g., non-lycosid spiders, elongate collembolans). Chi-squared tests for differences show significant effects of trap type on the frequency with which groups were collected (table 3). In particular, pitfall traps more commonly captured lycosid spiders and adult carabid beetles, while above-ground litter bags were successful in trapping non-lycosid spiders, centipedes and beetle larvae. Below-ground litter bags often appeared similar to one or both of the other trapping types, but in no case were below-ground litter bags best based on frequency of collection.

For taxa often collected using all three trap types, there were few differences in precision as measured by the CV (table 4). Bootstrap CI (95%) of CV showed that in 2003, above-ground litter bags were most effective for sampling elongate collembolans and more effective than pitfall traps for sampling rove beetle adults. These differences were not detectable in 2004, though the CV estimates and corresponding CI appeared lower for the above-ground litter bags than the other trap types.

4 Discussion

Pitfall traps and above- or below-ground litter bags differed in both the frequency of capture for ground-dwelling taxa and in the relative variability among

Table 2. *Mean (±SEM) number of ground-dwelling arthropods collected from pitfall traps and litter bags*

		Mean abundance per trap ± SEM[1,2]		
Year	Taxon	Pitfall traps	Litter bags (above)	Litter bags (below)
2003	Lycosidae (Araneae)	0.6 ± 0.2	0.4 ± 0.1	0.1 ± 0.0
	Other Araneae	0.1 ± 0.1	35.2 ± 3.3	16.8 ± 1.9
	Opiliones	1.1 ± 0.2	0.2 ± 0.1	0.0 ± 0.0
	Chilopoda	0.1 ± 0.0	7.3 ± 0.7	0.2 ± 0.1
	Collembola (elongate)	32.5 ± 3.2	2513.7 ± 155.3	355.8 ± 50.2
	Collembola (globular)	9.7 ± 1.9	34.9 ± 5.0	2.7 ± 0.8
	Carabidae (adults)[3]	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
	Carabidae (larvae)	0.0 ± 0.0	6.0 ± 0.7	1.1 ± 0.2
	Staphylinidae (adults)	0.7 ± 0.1	4.1 ± 0.4	1.1 ± 0.1
	Staphylinidae (larvae)	0.0 ± 0.0	4.2 ± 0.9	0.6 ± 0.2
2004	Lycosidae (Araneae)	0.5 ± 0.1	0.0 ± 0.0	*
	Other Araneae	1.2 ± 0.2	21.9 ± 3.1	3.1 ± 0.8
	Opiliones	0.0 ± 0.0	*	*
	Chilopoda	0.2 ± 0.1	2.2 ± 0.3	0.0 ± 0.0
	Collembola (elongate)	208.0 ± 18.4	2711.5 ± 191.0	3685.5 ± 360.6
	Collembola (globular)	13.1 ± 2.1	43.3 ± 5.4	28.7 ± 7.5
	Carabidae (adults)[3]	2.1 ± 0.2	0.1 ± 0.1	0.0 ± 0.0
	Carabidae (larvae)	0.1 ± 0.0	1.0 ± 0.2	1.1 ± 0.3
	Staphylinidae (adults)	1.1 ± 0.2	1.0 ± 0.1	1.0 ± 0.2
	Staphylinidae (larvae)	*	2.9 ± 0.7	0.5 ± 0.2

[1]Mean and standard error values based on 75 (2003) or 60 (2004) samples per trap type.
[2]Asterisk (*) indicates trap × taxon combinations where no individuals were collected.
[3]Including only the genera *Harpalus*, *Poecilus* and *Pterostichus*.
SEM, standard error of the mean.

Table 3. Frequency of collection for ground-dwelling arthropods using pitfall traps and litter bags

Year	Taxon	Frequency of collection (%)[1]			Chi-squared test[2]	
		Pitfall traps	Litter bags (above)	Litter bags (below)	χ^2	P
2003	Lycosidae (Araneae)	20	23	4	11.8	0.003
	Other Araneae	11	100	91	165.9	< 0.001
	Opiliones	55	7	1	80.4	< 0.001
	Chilopoda	5	96	12	167.7	< 0.001
	Collembola (elongate)	97	100	100	4.0	0.135
	Collembola (globular)	74	91	54	25.7	< 0.001
	Carabidae (adults)	36	16	9	17.5	< 0.001
	Carabidae (larvae)	1	93	47	128.3	< 0.001
	Staphylinidae (adults)	41	87	59	34.2	< 0.001
	Staphylinidae (larvae)	1	53	30	50.7	< 0.001
2004	Lycosidae (Araneae)	45	3	0	55.4	< 0.001
	Other Araneae	62	97	70	21.7	< 0.001
	Opiliones	2	0	0	2.0	0.369
	Chilopoda	13	66	2	71.3	< 0.001
	Collembola (elongate)	100	100	100	0.0	1.000
	Collembola (globular)	92	92	80	5.0	0.083
	Carabidae (adults)	83	12	2	108.3	< 0.001
	Carabidae (larvae)	5	46	47	31.0	< 0.001
	Staphylinidae (adults)	55	63	57	0.8	0.669
	Staphylinidae (larvae)	0	46	13	41.8	< 0.001

[1]Percentage of traps containing one or more individuals from 75 (2003) or 60 (2004) samples.
[2]Chi-squared test, d.f. = 2. Analysis conducted on contingency table counts, but percentages used to summarize collections.

Table 4. Estimated coefficients of variation and confidence intervals for ground-dwelling arthropods collected from > 40% of samples for all trap types

Year	Taxon	Coefficient of variation (100 σ/μ)[1]					
		Pitfall traps		Litter bags (above)		Litter bags (below)	
		CV	Bootstrap CI	CV	Bootstrap CI	CV	Bootstrap CI
2003	Collembola (elongate)	84	71–102	53	44–62	122	91–165
	Collembola (globular)	169	130–225	122	100–144	252	188–340
	Staphylinidae (adults)	152	115–182	77	63–91	109	88–127
2004	Other Araneae	105	82–126	107	90–128	203	156–274
	Collembola (elongate)	68	55–85	54	45–65	75	64–89
	Collembola (globular)	121	95–153	96	77–112	201	148–258
	Staphylinidae (adults)	114	87–136	98	76–118	125	98–148

[1]Coefficients of variation (CV) calculated from mean and standard error of 75 (2003) or 60 (2004) samples. Confidence intervals (95% CI) estimated using bootstrap resamples ($n = 2000$).

samples. In general, pitfall traps appeared better for taxa considered active at ground level, such as ground beetle (carabid) adults or harvestmen (Opiliones), while litter bags were superior for collection of taxa associated with moisture and sheltered areas, including centipedes and beetle larvae. However, no single trap type was effective for all of the groups examined. Also, differences were not always consistent between years, or when relationships among trap types were consistent, differences in relative abundance between years were sometimes apparent. For example, markedly higher levels of beetle larvae were collected from litter bags in 2003 compared with 2004. Research from nearby plots (< 10 km) suggests that the abundance of ground beetle larvae increases late in the season (August–September; Prasifka et al. 2006), indicating that this and other year-to-year differences likely reflect changes in the timing of samples relative to crop phenology or the duration of pitfall trapping [July, 24 h (2003) and August, 72 h (2004)].

Though the use of pitfall traps over relatively brief periods may have biased the results (Sapia et al. 2006), several factors suggest that differences detected among trap types for frequency of capture and precision (and apparent differences in the mean numbers of arthropod taxa collected) reflect real distinctions. First, because the experiment placed pitfall traps adjacent to litter bags, possible interactions between trap types would tend to make them more similar (i.e., reduce the odds of detecting differences). Also, basic differences between the pitfall traps and litter bags are apparent. As noted above, the duration litter bags remained in field plots may enhance chances of catching incidental or colonizing arthropods. Similarly, the shelter or other resources provided by the litter may have increased the number of collembolans, non-lycosid spiders (which frequently included large numbers of spiderlings) and centipedes. Though some differences among the trap types were anticipated, specific information of how trap types differ (tables 2–4) allows more effective and efficient sampling.

Several general attributes favour one trapping method over the other. Pitfall traps have a distinct advantage for ease of use, and effectiveness with taxa active at ground level. However, studies including only a few groups should consider possible differences between types of pitfall traps, which can influence results (Spence and Niemelä 1994; Weeks and McIntyre 1997). Litter bags, which are typically left in the field for several weeks and require additional time for extraction of live arthropods, are more time-consuming, but should be more useful for specific applications; for certain conventional insecticides or transgenic plants targeting coleopteran pests, larval stages more effectively sampled with litter bags are the most likely to be impacted (Kjær et al. 1998; USEPA [United States Environmental Protection Agency] 2002). Also, as evaluation of agriculture's impact moves towards emphasizing ecological processes, litter bags allow contemporaneous collection of both taxonomic data (diversity and relative abundance), and information on ecosystem functions (decomposition and nutrient cycling). Though the decision to use these or other sampling techniques (e.g., soil cores; Edwards 1991) should be made based upon the data required for a study, pitfall traps and litter bags should be considered complementary for taxonomically broad collections of ground-dwelling arthropods.

Acknowledgements

This research was a joint contribution from the USDA Agricultural Research Service and the Iowa Agriculture and Home Economics Experiment Station, Ames (Project 3543). This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by Iowa State University or USDA for its use. We appreciate advice from Laura Higgins on the field use of litter bags. Bob Gunnarson provided field assistance and Marlin Rice allowed use of the Tullgren funnels. Critical comments on earlier versions of this manuscript were provided by Patricia Prasifka and anonymous reviewers. The research was funded by a grant 2004-39454-15193 from the USDA Biotechnology Risk Assessment Research Grants Program.

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