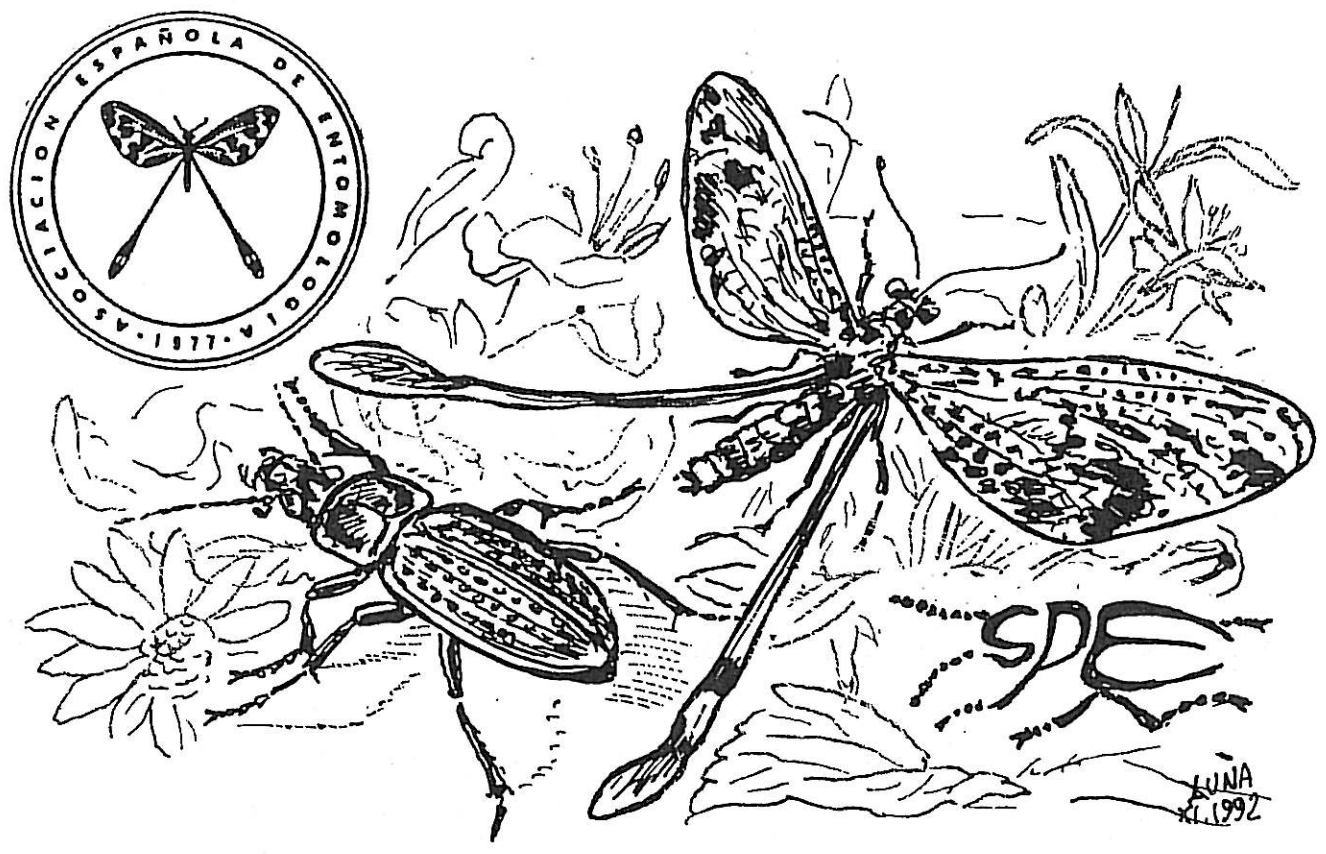


V CONGRESSO IBÉRICO DE ENTOMOLOGIA



LISBOA - 9/13 NOVEMBRO 1992

Suplemento n.º 3
ao Boletim da
SOCIEDADE PORTUGUESA DE ENTOMOLOGIA

THE RELATIVE EFFICIENCY OF FORMALIN, VINEGAR AND TURQUIN IN PITFALL TRAPS ON AN AZOREAN PINE WOODLAND AREA

By P. A. V. Borges (*)

(*) Universidade dos Açores, Departamento de Ciências Agrárias, Terra-Chã. 9702 Angra do Heroísmo Codex. Terceira, Açores, Portugal.

ABSTRACT. Using three types of killing-preserving agents (formalin solution, vinegar solution and Turquin's liquid), soil surface arthropods, caught by pitfall traps, were studied in an area of pine woodland in the eastern, geologically oldest, part of Terceira island (Azores), between November 1989 and November 1990. There are great differences in the distribution of the *taxa* in the three solutions. Some evidence was found which supports that formalin may act as an attractant. However, the main difference was between the formalin, considered as a more neutral preservative, and the other two solutions acting as true baits (both attract and preserve arthropods). Turquin's liquid (a mixture of beer and preservatives) can currently be used to catch a large set of arthropods, being the best attractant. Finally, the results show that none of the three solutions influenced the sampling of the seasonal diversity of arthropods in the studied habitat, being ecologically neutral.

RESUMO. Neste trabalho estudam-se amostras de artrópodes epígeos do solo, capturados usando três tipos de fixadores-engodos (solução de formalina, solução de vinagre, e líquido de Turquin) em armadilhas de Barber (Pitfall). O estudo realizou-se na parte oriental, geologicamente mais antiga, da ilha Terceira (Açores), entre Novembro de 1989 e Novembro de 1990. Foram encontradas grandes diferenças na distribuição relativa dos *taxa* pelos três tipos de fixadores-engodos. Foram igualmente observadas evidências que comprovam o efeito atractivo da formalina. No entanto, a principal diferença ocorre entre a formalina, considerado como mais neutral, logo menos atractivo, e as outras duas soluções, que actuam como verdadeiros engodos (atraindo e conservando os artrópodes). Por outro lado o líquido de Turquin (uma mistura de cerveja e fixadores), possui um largo espectro de acção atraindo grande variedade de artrópodes, sendo assim o melhor engodo. Finalmente, os resultados mostram que os três fixadores-engodos não influenciaram a amostragem da variação sazonal da diversidade de artrópodes do habitat estudado, demonstrando ser neutrais em termos ecológicos.

INTRODUCTION

In a long ecological study, on natural insect communities, it is essential to use a sample methodology that enable us to joint a high level of accuracy with low operation costs. Pitfall traps (BARBER, 1931), are the most common and convenient instrument to collect soil surface invertebrates (GREENSLADE, 1964; BORROR *et al.*, 1976; THIELE, 1977; SOUTHWOOD, 1978; ADIS, 1979; SCHELLER, 1984; BENEST, 1989a and 1989b).

The use of the pitfall trapping in annual cycles of activity studies (HAGVAR *et al.*, 1987; KEER & MAELFAIT, 1987; NIEMELÄ *et al.*, 1989) and in biodiversity studies (OBRTTEL, 1971; ASHMOLE & ASHMOLE, 1987; HAGVAR *et al.*, *op. cit.*; MOLINÉ *et al.*, 1988) has been accepted as of great relevance. Furthermore, with this method it is possible to collect a great variety of arthropods that rarely are collected by other means (ASHMOLE & ASHMOLE, *op. cit.*; FRANKLIN, 1988; BORGES, 1990, 1991a and in prep.).

Many factors may influence the sampling effectiveness of the pitfall traps, causing some bias in the sample results (thorough revision in ADIS, 1979). For instance several studies show that the pitfall samples are more influenced by the intensity of activity of the populations than by their density (FICHTER, 1941; GREENSLADE, 1964; THIELE, 1977; SOUTHWOOD, 1978; CRAWFORD & EDWARDS, 1989). Nevertheless, the sampling by pitfall traps is sensitive enough to show up real differences of ecological significance (WILLIAMS, 1958; SOUTHWOOD, *op. cit.*).

Moreover, the utilization of killing-preserving agents, may also cause some distortion on the interpretation of the pitfall results (LUFF, 1968; ADIS, 1979). Therefore, to achieve a better inventory of the community species, various killing-preserving agents should be used (ADIS, *op. cit.*).

In the work reported on this paper, three types of killing-preserving agents (formalin solution, vinegar solution and Turquin's liquid) were used. The goal of the present paper is to study the relative efficiency of these three liquids, and to estimate which of them best describes the diversity of an epigeal arthropod community, in a Azorean Pine woodland. Other purpose, was to investigate if any of the three killing-preserving agents caused distortion on the interpretation of the seasonal variation of the soil epigeal arthropod diversity.

MATERIAL AND METHODS

A - STUDY AREA

The experimental work was performed in Fontinhas, in the geologically oldest part of Terceira island (Azores, Portugal), with U.T.M. 489,2 / 4287,8. The field site was an old and small population of *Pinus pinaster* with approximately 800 m² of area. This small Pine woodland is an "island" surrounded by pasture and abandoned fields of *Ulex europaea*. The altitude is about 300 m.

B - SAMPLING METHODS

A set of nine pitfall traps sampled the area continuously, during one year (from 10th November 1989 to 9th November 1990), except between the 9th of December 1989 and the 11th of January 1990 as well as the 18th August and 29th of September 1990. The pitfall traps used had a radius of 22mm and a depth of 80mm. They were dug into the ground, lined up at approximately 5 meter distance from each other, and were emptied fortnightly. All arthropod specimens were identified to the Order (see Table I in appendix). More details on the sampling method used are given on BORGES (1991b and in prep.). The killing-preserving agents used were: a 5% FORMALIN solution with 1 ml of detergent added in one liter; TURQUIN liquid - an ASHMOLE & ASHMOLE (1987) modified formula of the original one (TURQUIN, 1973) [10 g chloral hydrate, 5 ml formalin, 5 ml glacial acetic acid, 1 ml detergent and dark beer to 1 litre]; VINEGAR solution (modified from IACOVONE, 1985) [50% of commercial vinegar and 50% of water, and 10 ml of formalin and 1 ml of detergent for each litre of solution].

C - DATA ANALYSIS

Two multivariate techniques were applied to analyse: a) differences in *taxa* composition in the three killing-preserving agent samples (correspondence analysis - CA); b) the answers of the killing-preserving agents working like ecological parameters (canonical correspondence analysis - CCA). We used the computer program CANOCO, an advanced version of the frequently used program DECORANA (HILL, 1979). These techniques were mostly developed for vegetational data, but are being used also to analyse animal data (*vide* references in ALDERWEIRELDT, 1989; BAUER, 1989; and also BERBIERS *et al.*, 1989).

RESULTS AND DISCUSSION

A - ANALYSIS OF THE EFFICIENCY OF FORMALIN, VINEGAR AND TURQUIN

Nine pitfall traps with the three killing-preserving agents (formalin solution, vinegar solution and Turquin's liquid), caught a total of 11,805 specimen of arthropods (Table I), in a Pine woodland area in Fontinhas (Terceira, Azores), during 12 months.

Turquin's liquid captured more than 50% of the total, being also the killing-preserving agent that attracted more *taxa* (see Table I).

Figure 1 shows the ordination of the 14 *taxa* in the two first axes, obtained by Correspondence Analysis (CA). CA resulted in the distinction of 4 ordination axes of which the first two explain almost all the observed variation (52% and 20%, respectively). The ordination of the 14 *taxa* (Fig. 1) results in two evident groups: one formed by the Pseudoscorpiones and by the Orders of the Insects (Collembola, Heteroptera, Homoptera, Diptera, Lepidoptera, Hymenoptera and Coleoptera); and a second group with *taxa* other than Insecta.

Also noticeable is a clear detachment in the axis I between the Chilopoda, Crustacea (Isopoda and Amphipoda) and the remaining *taxa*. Axis II separates the Insecta from the Diplopoda and Arachnida (Araneae and Opiliones). This means that there is a clear difference in the *taxa* composition among the three types of samples.

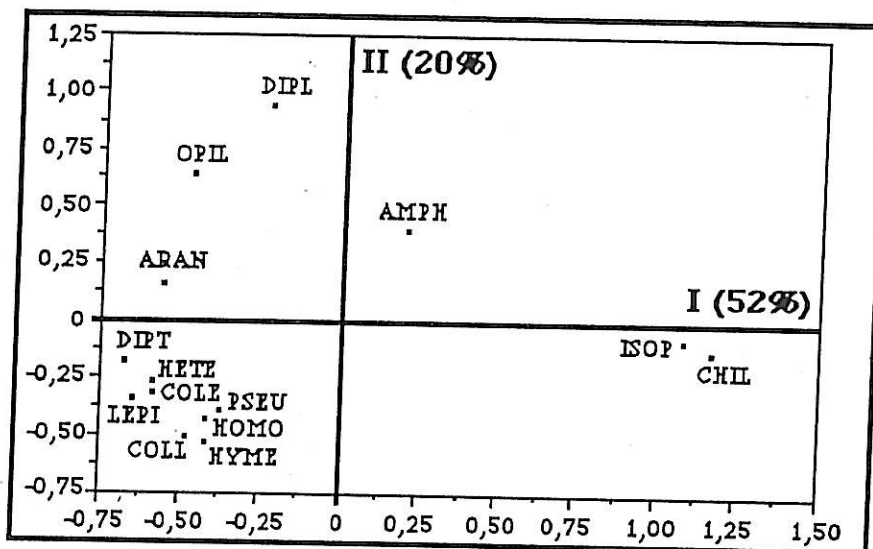


Figure 1.- Correspondence analysis (CA) on the proportional captures of arthropods (excl. *Acari*) with Pitfall, using three types of killing-preserving agents (formalin solution, vinegar solution and Turquin's liquid) (see Table I). Abbreviations: PSEU= Pseudoscorpiones; ARAN= Araneae; OPIL= Opiliones; ISOP= Isopoda; AMPH= Amphipoda; DIPL= Diplopoda; CHIL= Chilopoda; COLL= Collembola; HETE= Heteroptera; HOMO= Homoptera; DIPT= Diptera; LEPI= Lepidoptera; HYME= Hymenoptera; COLE= Coleoptera.

Using the same method, Fig. 2 shows the ordination of the samples in the first two axes. The ordination resulted in a clear separation of three groups: one only with vinegar samples; a second one with almost only formalin samples and a third one with a majority of Turquin's samples.

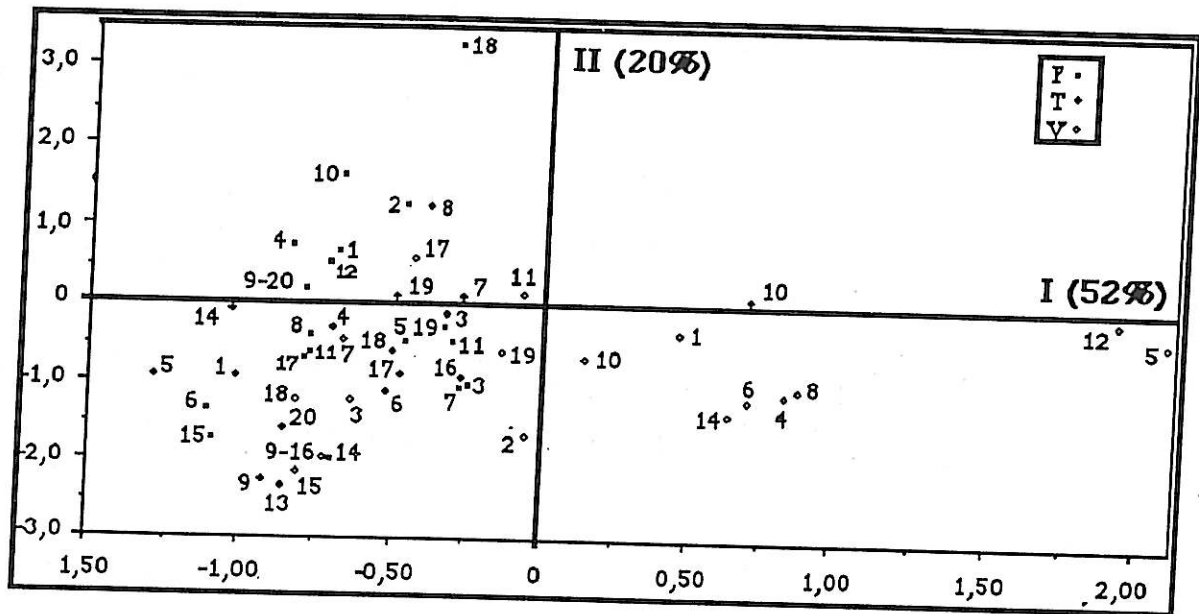


Figure 2.- Correspondence analysis (CA) on the samples obtained during one year with the following methods: F= pitfall with a 5% formalin solution; T= pitfall with Turquin's liquid; V= pitfall with a 50% vinegar solution. The numbers are the same of Table I.

The first axis detaches the samples of Turquin and formalin from those of vinegar, and the second axis separates, although less clearly, the samples of formalin from those of Turquin and vinegar. There is a tendency in the axis I for a separation of the samples by the number of *taxa* collected, occurring a variation among the 9 *taxa* sampled in the T5 and T1 and the only 5-6 *taxa* collected in V12 and V5 (*Vide* Table I). The ordination in the axis II probably signifies differences in the relative porportion of sampling of each *taxa* by each of the three killing-preserving agents.

If we analyse simultaneously the two CA ordination figures (Figs.1 and 2) we may conclude that: a) all the Orders of the Insecta seem to be attracted, preferly by the Turquin and in a less scale by the vinegar and the formalin; b) the Turquin and the formalin could be the best baits for the Opiliones and Araneae; c) the Diplopoda, Amphipoda, Isopoda and Chilopoda seem to be adequately sampled by the three killing-preserving agents.

B - ARE FORMALIN, TURQUIN AND VINEGAR ECOLOGICALLY NEUTRAL?

The second goal of the present work was to investigate if the three killing-preserving agents used worked as ecologically neutral samplers. So a Canonical Correspondence Analysis (CCA), was computed (Figs. 3 and 4), using the data of Table I. The ecological parameters (ecological vectors) used were the three killing-preserving agents. So we created a matrix of presence / absence for every sample.

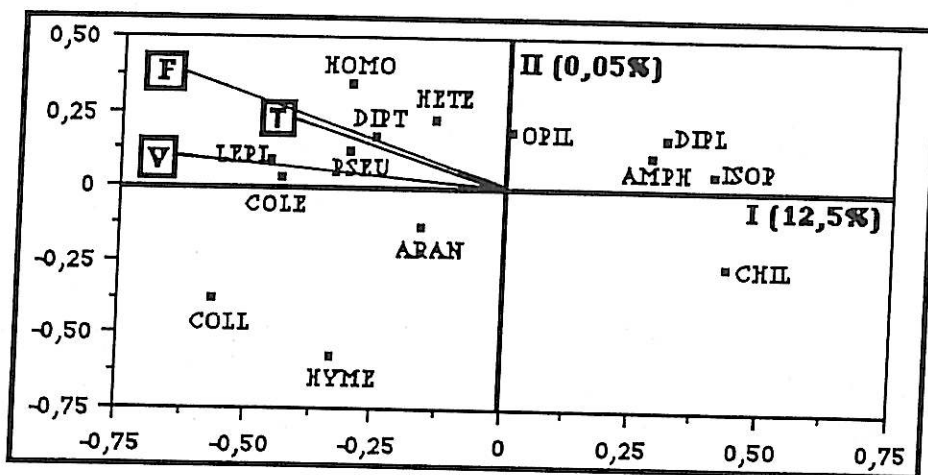


Figure 3.- Canonical correspondence analysis (CCA) on the proportional captures of arthropods (excl. *Acari*) with Pitfall, using as ecological vectors the three types of killing-preserving agents (formalin solution, vinegar solution and Turquin's liquid) (see text for further explanations). Abbreviations: PSEU= Pseudoscorpiones; ARAN= Araneae; OPIL= Opiliones; ISOP= Isopoda; AMPH= Amphipoda; DIPL= Diplopoda; CHIL= Chilopoda; COLL= Collembola; HETE= Heteroptera; HOMO= Homoptera; DIPT= Diptera; LEPI= Lepidoptera; HYME= Hymenoptera; COLE= Coleoptera.

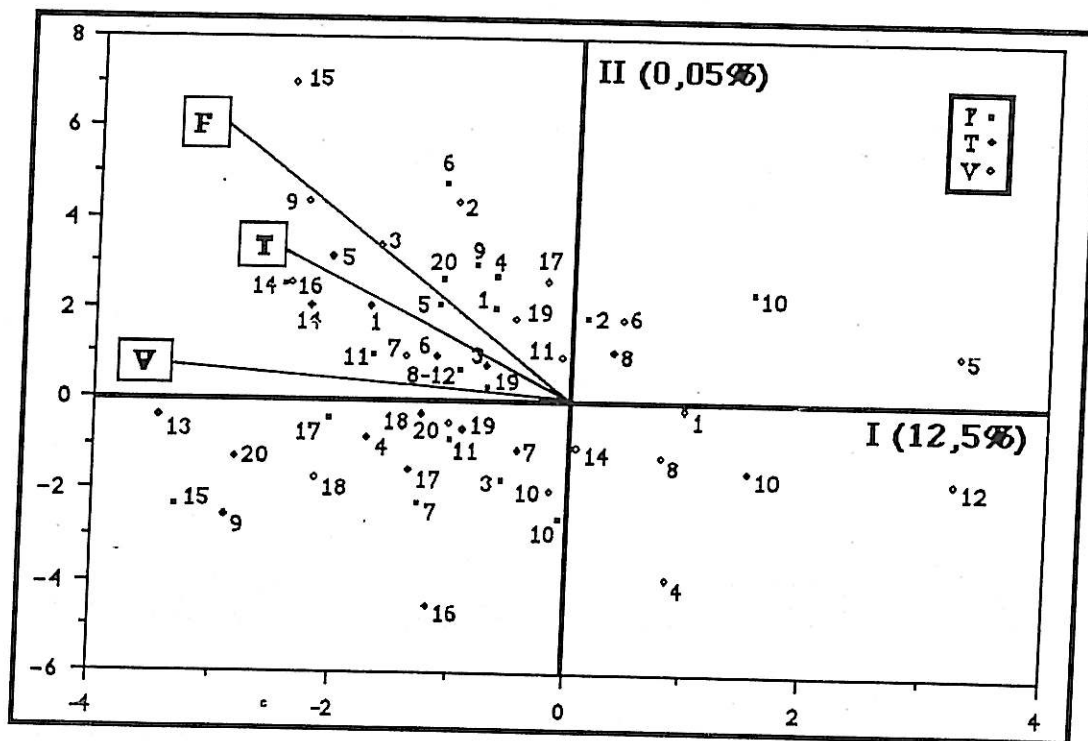


Figure 4.- Canonical correspondence analysis (CCA) using as ecological vectors the three types of killing-preserving agents (formalin solution, vinegar solution and Turquin's liquid) (see text for further explanations), on the samples obtained during one year with the following methods: F= pitfall with a 5% formalin solution; T= pitfall with Turquin's liquid; V= pitfall with a 50% vinegar solution. The numbers are the same of Table I.

As can be seen in the Figs. 3 and 4, the CCA first two axes explain little of the observed variation (12,5% and 0,05%, respectively). Thus, the three killing-preserving agents (formalin solution, vinegar solution and Turquin's liquid) didn't work as ecological parameters.

Moreover, from the analysis of Fig. 3 we may conclude that the observations made above about the ordination obtained in Fig. 1 for the Isopoda, the Chilopoda, the Amphipoda and the Diplopoda are now strengthened. In fact, these *taxa* are ordinated in an area not influenced by the "ecological vectors" (the killing-preserving agents) (Fig. 3), once they are equally sampled by them.

The Hymenoptera and Araneae are more attracted by the vinegar and lesser by the Turquin, but the latter caught more Lepidoptera, Pseudoscorpiones and Coleoptera (see also Table I).

From Table I, we would expect that the Diptera would be associated with the vector vinegar and the Collembola with the vector Turquin, which was not shown by the CCA ordination. In fact their position in the CCA ordination (Fig. 3) contradicts the data, so, other parameters not tested, apart from the killing-preserving agents, should have influenced the sampling.

The results of the CCA ordination (Fig. 4) also confirmed that the three killing-preserving agents didn't influence the sampling, because their vectors don't explain the obtained ordination.

CONCLUSIONS

We found great differences in the distribution of the arthropods by the three killing-preserving agents used: formalin solution, vinegar solution and Turquin's liquid.

Although it was confirmed that formalin can act as an attractant (LUFF, 1968), the main difference observed was between that agent, a more neutral preservative, and the other two killing-preserving agents, acting as true baits (they both attract and preserve arthropods). Moreover, Turquin's liquid (a mixture of beer and preservatives) can be currently used to catch a large set of arthropods, being the best attractant.

The results also show that the three solutions had no influence on the sampling of the seasonal diversity of arthropods in the studied habitat, being in consequence ecologically neutral. The samples obtained during one year in the studied area were influenced by ecological parameters other than the used killing-preserving agents.

Nevertheless, we should be careful towards the bias that may be caused by factors influencing the sampling effectiveness of the pitfall traps (see revision in ADIS, 1979).

The simultaneous utilization of these three killing-preserving agents is recommended for ecological studies in the Azores, because a more diversified number of soil epigeal arthropods will be sampled.

In other ecological studies, when the aim is only to study a particular *taxa* (at the Order level) the following killing-preserving agents are generally recommended: Pseudoscorpiones= T; Araneae= V or T; Opiliones= V or T; Isopoda= F, V or T; Amphipoda= F, V or T; Diplopoda= F, V or T; Chilopoda= F, V or T; Collembola= T; Heteroptera= T; Homoptera= T; Diptera= V; Lepidoptera= T; Hymenoptera= V or T; Coleoptera= T.

In conclusion, all the three killing-preserving agents used in the present study in the pitfall traps, appear to produce good and accurate results when analyzing the seasonal composition and diversity of the soil surface-active arthropod community.

ACKNOWLEDGEMENTS

We are very thankful to Prof. A.R.M. SERRANO (Fac. Ciências, Univ. Lisboa) and Dr R. GABRIEL (Dep. Ciências Agrárias, Univ of Azores) for their precious comments during several stages of this work. Dr. E. DIAS (Dep. Ciências Agrárias, Univ of Azores) gave us a valuable help with the multivariate techniques.

Thanks are also due to Mr. F. PEREIRA (University of Azores, Terceira) for his help during all the field and laboratoty work.

REFERENCES

- ADIS, J. (1979). Problems of Interpreting Arthropod Sampling with Pitfall Traps. *Zoologischer Anzeiger*, 202 (3-4): 177-184.
- ALDERWEIRELDT, M. (1989). An Ecological Analysis of the Spider Fauna (Araneae) Occurring in Maize Fields, Italian Ryegrass Fields and Their Edge Zones, by Means of Different Multivariate Techniques. *Agriculture, Ecosystems and Environment*, 27: 293-306.
- ASHMOLE, M. J. & N. P. ASHMOLE (1987). Arthropod communities supported by biological fallout on recent lava flows in the Canary Islands. *Entomologica Scandinavica Supplement*, 32: 67-88.

- BARBER, H.S. (1931). Traps for cave inhabiting insects. *Journal of the Elisha Mitchell Scientific Society*, **46**: 259-266.
- BAUER, L. J. (1989). Moorland Beetle Communities on Limestone "Habitat Islands". I. Isolation, Invasion and Local Species Diversity in Carabids and Staphylinids. *Journal of Animal Ecology*, **58**: 1077-1098.
- BENEST, G. (1989a). The sampling of a carabid community. I. The behaviour of a carabid when facing the trap. *Revue D'Écologie et de Biologie du Sol*, **26** (2): 205-211.
- BENEST, G. (1989b). The sampling of a carabid community: II. Traps and trapping. *Revue D'Écologie et de Biologie du Sol*, **26** (4): 505-514.
- BERBIERS, P., J.-P. MAELFAIT & J. MERTENS (1989). Evaluation of some sampling methods used to study Collembola (Insecta, Apterygota) in a pasture. *Revue D'Écologie et de Biologie du Sol*, **26** (3): 305-320.
- BORGES, P.A.V. (1990). A checklist of the *Coleoptera* from the Azores with some systematic and biogeographic comments. *Boletim do Museu Municipal do Funchal*, **42** (220): 87-136.
- BORGES, P.A.V. (1991a). Two new species of *Tarphiüs* Erichson, 1848 (*Coleoptera*, *Colydiidae*) from the Azores. *Bocagiana*, **144**: 1-9.
- BORGES, P. A. V. (1991b). *A Armadilha de Barber ("Pitfall") como método de estudo da biodiversidade de artrópodes do solo nos Açores*. Universidade dos Açores, Angra do Heroísmo. 60 pp..
- BORGES, P.A.V. (in prep.). A pitfall survey in the Azores: an ecological study.
- BORROR, D. J., D. M. DELONG, C. A. TRIPLEHORN (1976). *An Introduction to the Study of Insects*. Fourth ed. Holt, Rinehart and Winston, New York. 852 pp..
- CRAWFORD, R.L. & J.S. EDWARDS (1989). Alpine spiders and harvestmen of Mount Rainier, Washington, U.S.A.: taxonomy and bionomics. *Canadian Journal of Zoology*, **67**: 430-446.
- FICHTER, E. (1941). Apparatus for the comparison of soil surface arthropod populations. *Ecology*, **22**(3): 338-339.
- FRANKLIN, R. T. (1988). *Cicindela unipunctata* from Pitfall Traps (*Coleoptera*: *Cicindelidae*). *Journal of the Kansas Entomological Society*, **61**(2): 249-250.
- GREENSLADE, P. J. M. (1964). Pitfall trapping as a method for studying populations of Carabidae (*Coleoptera*). *Journal of Animal Ecology*, **33**: 301-310.
- HAGVAR, S., E. ØSTBYE & J. MELAEN (1987). Pit-fall catches of surface-active arthropods in some high mountain habitats at Finse, south Norway. II. General results at group level, with emphasis on Opiliones, Araneida, and *Coleoptera*. *Norwegian Journal of Entomology*, **25**: 195-205.

- HILL, M. O. (1979). *Decorama. A Fortran program for detrended correspondence analysis and reciprocal averaging*. Cornell University, Ithaca, New York.
- IACOVONE, C. (1985). Using vinegar as bait in pitfall traps. *Young Entomologists' Society Quarterly*, 2(2): 9-11.
- KEER, R. de & J.-P. MAELFAIT (1987). Life history of *Oedothorax fuscus* (Blackwall, 1834) (Araneae, Linyphiide) in a heavily grazed pasture. *Revue d'Écologie et de Biologie du Sol*, 24(2): 171-185.
- LUFF, M. L. (1968). Some effects of Formalin on the numbers of Coleoptera caught in pitfall Traps. *The Entomologist's Monthly Magazine*, 104: 115-116.
- MOLINÉ, A.L.G., J.A.T. RANERA & R.G. SÁNCHEZ (1988). Evolución estacional de la entomofauna del suelo en un robledal (*Quercus pyrenaica* Wilde) de Sierra Nevada. *Actas III Congreso Ibérico de Entomología*, -: 557-576.
- NIEMELÄ, J., Y. HAILA, E. HALME, T. PAJUNEN & P. PUNTTILA (1989). The annual activity of carabid beetles in the southern Finnish taiga. *Annales Zoologici Fennici*, 26: 35-41.
- OBRTTEL, R. (1971). Number of pitfall traps in relation to the structure of the catch of soil surface Coleoptera. *Acta Entomologica Bohemoslovaca*, 68: 300-309.
- SHELLER, H.V. (1984). Pitfall trapping as the basis for studying ground beetle (Carabidae) predation in spring barley. *Danish Journal of Plant and Soil Science*, 88: 317-324.
- SOUTHWOOD, T.R.E. (1978). *Ecological Methods with particular reference to the study of Insect Populations*. Second edition, revised. Chapman & Hall, London. 524 pp..
- THIELE, H.-U. (1977). Carabid Beetles in their Environments. *Zoophysiological Ecology*, 10: 1-369.
- TURQUIN, M.-J. (1973). Une biocenose cavernicole originale pour le Bugéy: le puits de Rappe. *Comptes Rendus 96e Congresse Naturel Sociétés Savantes, Toulouse 1971, Sciences*, 3: 235-256.
- WILLIAMS, G. (1958). Mechanical time-sorting pitfall captures. *Journal of Animal Ecology*, 27: 26-35.

APPENDIX

TAXA	10-24.XI.89	24.XI.-9.XII.89	12-26.I.90	26.I.-9.II.90	9-23.II.90	23.II.-13.III.90	13-24.III.90	24.III.-6.IV.90	6-20.IV.90	20.IV.-5.V.90																				
	F1	V1	T1	F2	V2	T2	F3	V3	T3	F4	V4	T4	F5	V5	T5	F6	V6	T6	F7	V7	T7	F8	V8	T8	F9	V9	T9	F10	V10	T10
ARACHNIDA																														
PSEUDOSCORPIONES																														
ARANEAE																														
OPTILIONES	3	1	8			3	3	3	5	2	1	3	1	1	1	1	6	1	2	4	1	2	3	1	7	4	1	8	7	2
CRUSTACEA																														
ISOPODA	1	4	1	1	1	4	3	3	2	1	3	2	1	4	2	1	4	1	1	1	1	1	4	1	1	2	2	1	4	1
AMPHIPODA	1	2	4	2	4	2	2	4	11	6																				
DIPLOPODA																														
CHILOPODA																														
INSECTA	66	85	69	47	81	148	60	67	130	98	312	265	104	246	218	174	136	57	166	177	43	51	128	17	54	69	117	76	124	
COLLEMBOLA																														
HETEROPTERA																														
HOMOPTERA	1																													
DIPTERA	32	99	57	19	26	69	9	33	33	14	126	18	4	21	1	9	38	14	3	30	9	2	7	4	63	23	4	25	11	
HYMENOPTERA	1	6	5	4	2	5	4	2	2	2	2	1	8	1	8	19	6	3	16	8	2	22	12	6	14	34	29	31	4	
COLEOPTERA	4	4	5	4	2	7	5	2	8	2	2	2	0	2	3	4	6	2	4	2	4	2	15	3	1	3	5	6	18	
TOTAL	106	196	155	74	111	242	80	79	191	119	446	302	121	274	245	89	229	181	68	224	209	47	119	207	37	136	145	171	147	168
TAXA																														
ARACHNIDA																														
PSEUDOSCORPIONES																														
ARANEAE																														
OPTILIONES	2	3	5			8	14	6	6	14	11	4	10	5	9	10	10	9	5	6	3	2	11	1	1	1	1	1	1	6
CRUSTACEA																														
ISOPODA	5	1	1	1	10	38	1	7	28	2	8	2	8	6	5	5	2	2	1	3	3	1	1	1	1	1	3	1	5	
AMPHIPODA	1	2	7		1	2	1	1	1	1	1	4	1	1	3	2	2	2	7	1	2	2	1	1	4	1	1	1	1	
DIPLOPODA																														
CHILOPODA																														
INSECTA	27	57	95	35	49	123	26	27	111	29	45	58	43	74	170	56	337	178	22	31	21	17	177	210	23	143	994	243	1108	
COLLEMBOLA																														
HETEROPTERA																														
HOMOPTERA	6	24	3	3	40	20	5	8	5	1	5	23	19	16	24	4	14	6	3	21	25	17	64	57	22	146	320	22	57	
DIPTERA																														
LEPIDOPTERA																														
HYMENOPTERA	5	23	28	12	37	29	25	17	24	21	31	19	31	23	17	25	14	12	16	12	17	2	6	3	1	5	9	2	6	
COLEOPTERA	4	3	3	5	2	1	6	13	18	100	114	121	103	127	252	97	379	231	50	73	74	46	268	297	58	304	1379	273	149	
TOTAL	51	152	166	55	139	192	108	75	180	100	446	302	121	274	245	89	229	181	68	224	209	47	119	207	37	136	145	171	147	168

TABLE I.- Pitfall captures of epigeic arthropods in a Pine woodland (Fontinhas, Terceira, Azores) during one year. F= formalin a 5%, V= vinegar; T= Turquin.