[SHORT COMMUNICATION]

Efficiency of Sampling Oribatid Mites (Acari: Oribatida) from the Canopies of Broad-Leaved Trees in Japan: a Comparison between the Washing and Direct Methods

Shigenori KARASAWA* and Naoki HIJII

*Laboratory of Forest Protection, Graduate School of Bioagricultural Sciences, Nagoya University,
Nagoya 464–8601, Japan*

(Received 19 November 2004; Accepted 16 March 2005)

Key words: canopy, direct method, oribatid mites, washing method

INTRODUCTION

Acari, especially oribatid mites, have been considered to be the most diverse and abundant of the various canopy arthropods (Walter and O'Dowd, 1995), and clarifying the diversity and patterns of their microhabitat-use in canopies would thus contribute to a better understanding of the ecology of canopy-dwelling arthropod communities.

In several studies that investigated oribatid mites in tree canopies in Japan, researchers have used a washing method to collect mites, in which the mites were washed off leaves and branches using a solution of water mixed with soap or ethanol, after which they were collected by filtering (Ichisawa et al., 1999; Ichisawa, 2001; Ito, 1986; Karasawa and Hijii, 2004a, b; Kurimoto, 1978; Yoshida and Hijii, 2005). Alternatively, a method has been adopted in which the mites were directly collected from leaves and branches by brushing the mites from these surfaces under a microscope, though this method has mostly been used in other countries (Spain and Harrison, 1968; Walter and Behan-Pelletier, 1993; Walter et al., 1994; Walter and O'Dowd, 1995). The difference in sampling methods may derive from the fact that most studies of oribatid mites in tree canopies in Japan have been carried out on conifers, for which such a direct method would be difficult to apply because of the complex leaf forms.

Washing is an effective method for collecting small animals from foliage in terms of both its accuracy and its cost (Southwood and Henderson, 2000). For example, Ichisawa (2001) concluded that the washing method was more effective in sampling of oribatid mites from shoots than the beating method, because more species and individuals could be collected. In addition, the washing method has an advantage in that many samples can be dealt with simultaneously. The sampling protocol and efficiency for collecting oribatid mites in soil

日本の広葉樹における樹冠生息性ササラダニの採取効率：洗浄法と直接法の比較

唐沢重考*・肘井直樹（名古屋大学大学院生命農学研究科森林保護学研究室，〒464-8601 名古屋市千種区不老町）

*Corresponding author: e-mail: i031003d@mbox.nagoya-u.ac.jp; fax: +81-52-789-5518

habitats have been examined by comparing several methods (André et al., 2002); for oribatid mites in tree canopies, however, there have been no quantitative studies on the sampling efficiency of these methods.

The present paper reports the results of a study that aimed to quantify the sampling efficiency for collecting oribatid mites from leaves and branches in the canopies of broad-leaved trees by comparing the washing and direct methods.

MATERIALS AND METHODS

Study area

Samples were collected at seven sites on main Okinawa Island, in the Ryukyu archipelago, southwest of Japan. Two sites were situated on the banks of the Okukubi (Okm) and Gesashi (Ge) Rivers. The vegetation at both sites was a typical mangrove forest of the Ryukyu archipelago, consisting of *Bruguiera gymnorrhiza* and *Rhizophora stylosa*. Another site (Okb) was located about 100 m southeast of Okm. The vegetation was a typical river-bank forest, in which *Pinus luchuensis* and *Casuarina equisetifolia* were dominant. Two other sites were situated on the campus of the University of the Ryukyus (Ryu) and at Sueyoshi Park (Su) in the southern part of Okinawa Island, both of which were geologically characterized by limestone topography and dominated by *Ficus microcarpa* and *Ficus virgata*. Sampling was also conducted at a site dominated by *Castanopsis sieboldii*, in the northern part of Okinawa Island (Yam) and another dominated by *Machilus thunbergii* on the grounds of Chibana castle in the central part of Okinawa Island (Chi).

Sampling

Sampling was carried out 18 times from October 2001 to October 2003. On each sampling occasion, some 10- to 50-cm-long shoots were taken from a tree at a height between 1 and 15 m above the ground. At every sampling site, shoot samples were divided into leaves (200 or 400 leaves) and branches (20 to 100 branches), each of which was then randomly separated into two groups to permit a comparison of the sampling efficiency between the two methods. The numbers of the leaves and branches were roughly based on these sizes. All the samples were placed in separate plastic bags and brought to the laboratory at the University of the Ryukyus within 3 h. All the tree species sampled are listed in Tables 1 and 2.

Protocol for mite collection

Washing method: Each sample was immersed in a bucket with water (ca. 9 L) that included about 3 cm³ of detergent (Power plus Joy, Proctor and Gamble Co.; Fig. a). The bucket was then covered with a cloth to prevent invasion by animals and kept for one night (8 to 12 h) indoors. Leaves and branches were then washed in the bucket (Fig. b), and the solution was filtered through a 35- μ m nylon mesh (PP-35n, Kyôshin-Rikô Co.; Fig. c, d). When there were too many soap bubbles, we added a small quantity of ethanol to the solution to make them disappear. Finally, under a microscope we collected oribatid mites from the sediments on the nylon mesh.

Direct method: Oribatid mites were directly separated from the leaf and branch-samples using forceps under a microscope with cool-lighting. Most branches were thin and had no cracked bark, except *B. gymnorrhiza* and *R. stylosa*. We investigated oribatid mites in the

Table 1. Numbers of species (*S*) and individuals (*N*) and the similarity indices for the oribatid mites collected from the leaves.

Sample No.	Date	Site	Tree species	Number of leaves	Washing method			Similarity* C_{λ}	Direct method				
					S	N	Total		Adult	Juvenile	S	N	Total
1	2001.10.11	Okm	<i>Bruguiera gymnorrhiza</i>	100	4	78	67	11	0.997	1	52	29	23
2	2001.10.12	Okm	<i>Bruguiera gymnorrhiza</i>	100	1	30	29	1	1.000	1	22	11	11
3	2001.12.26	Okm	<i>Bruguiera gymnorrhiza</i>	100	2	28	20	8	0.994	1	19	18	1
4	2001.12.26	Okm	<i>Bruguiera gymnorrhiza</i>	100	1	8	8	0	—	1	38	37	1
5	2002.4.22	Okm	<i>Bruguiera gymnorrhiza</i>	100	1	10	10	0	1.000	1	47	27	20
6	2002.11.29	Okm	<i>Kandelia candel</i>	100	1	64	62	2	1.000	1	123	69	54
7	2002.12.1	Ryu	<i>Ficus microcarpa</i>	100	1	2	1	1	—	1	1	1	0
8	2003.1.24	Yam	<i>Castanopsis sieboldii</i>	200	2	41	41	0	0.955	2	32	32	0
9	2003.1.24	Yam	<i>Distylium racemosum</i>	200	1	1	1	0	—	0	0	0	0
10	2003.3.16	Yam	<i>Schefflera octophylla</i>	100	1	1	1	0	—	0	0	0	0
11	2003.6.5	Okb	<i>Symplocos lucida</i>	200	2	2	2	0	—	0	0	0	0
12	2003.6.5	Okb	<i>Machilus thunbergii</i>	200	4	4	4	0	—	2	7	3	4
13	2003.6.5	Okb	<i>Elaeocarpus sylvestris</i>	200	1	1	1	0	—	1	3	1	2
14	2003.6.10	Ge	<i>Rhizophora stylosa</i>	100	2	83	80	3	1.000	2	59	59	0
15	2003.6.12	Chi	<i>Neolitsea sericea</i>	200	1	1	1	0	—	1	1	1	0
16	2003.10.10	Ryu	<i>Elaeocarpus sylvestris</i>	200	0	0	0	0	—	0	0	0	0
17	2003.10.10	Su	<i>Ficus erecta f. sieboldii</i>	100	1	2	2	0	—	1	1	1	0
18	2003.10.10	Su	<i>Bischofia javanica</i>	100	0	0	0	0	—	0	0	0	0

*The similarity index was calculated for samples including more than 10 oribatid individuals.

Table 2. Numbers of species (*S*) and individuals (*N*) and the similarity indices for the oribatid mites collected from the branches.

Sample No.	Date	Site	Tree species	Number of branches	Washing method			Similarity* C_{λ}	Direct method				
					S	N	Total		Adult	Juvenile	S	N	Total
19	2001.10.11	Okm	<i>Bruguiera gymnorrhiza</i>	10	3	15	9	6	1.112	2	11	7	4
20	2001.10.12	Okm	<i>Bruguiera gymnorrhiza</i>	10	3	32	31	1	0.284	1	16	15	1
21	2001.12.26	Okm	<i>Bruguiera gymnorrhiza</i>	10	4	26	9	17	—	1	3	2	1
22	2001.12.26	Okm	<i>Bruguiera gymnorrhiza</i>	10	0	4	0	4	—	1	2	2	0
23	2002.4.22	Okm	<i>Bruguiera gymnorrhiza</i>	10	1	6	6	0	—	2	14	2	12
24	2002.11.29	Okm	<i>Kandelia candel</i>	30	3	27	19	8	0.947	3	13	10	3
25	2002.12.1	Ryu	<i>Ficus microcarpa</i>	23	1	1	1	0	—	0	0	0	0
26	2003.1.24	Yam	<i>Castanopsis sieboldii</i>	40	2	7	6	1	—	0	0	0	0
27	2003.1.24	Yam	<i>Distylium racemosum</i>	50	2	21	21	0	—	1	1	1	0
28	2003.3.16	Yam	<i>Schefflera octophylla</i>	20	3	5	5	0	—	0	0	0	0
29	2003.6.5	Okb	<i>Symplocos lucida</i>	30	0	0	0	0	—	0	0	0	0
30	2003.6.5	Okb	<i>Machilus thunbergii</i>	20	3	16	12	4	—	4	6	6	0
31	2003.6.5	Okb	<i>Elaeocarpus sylvestris</i>	20	2	2	2	0	—	1	1	1	0
32	2003.6.10	Ge	<i>Rhizophora stylosa</i>	10	1	6	5	1	—	1	3	3	0
33	2003.6.12	Chi	<i>Neolitsea sericea</i>	20	1	1	1	0	—	1	2	2	0
34	2003.10.10	Ryu	<i>Elaeocarpus sylvestris</i>	15	0	0	0	0	—	0	0	0	0
35	2003.10.10	Su	<i>Ficus erecta f. sieboldii</i>	30	2	6	6	0	—	0	0	0	0
36	2003.10.10	Su	<i>Bischofia javanica</i>	30	0	0	0	0	—	0	0	0	0

*The similarity index was calculated for samples including more than 10 oribatid individuals.

Fig. The procedure used in the washing method: (a) Samples are soaked in water (9 L) in a bucket, and about 3 cm³ of detergent is added to the water. (b) Samples are washed manually in the bucket. (c) A piece of 35-μm nylon mesh is attached to the bottom of a PVC pipe 7.3 cm in diameter and 9.8 cm long. (d) The solution is filtered through the 35-μm nylon mesh.

cracks with peeling bark using forceps as far as possible. This method requires researchers to work considerably faster than is the case with the washing method to prevent escape of the mites.

Analyses

We compared the numbers of oribatid species and of individuals collected from the leaves and branches using the washing and direct methods by means of Wilcoxon's signed-ranks test. We then analyzed the similarity of the species composition by these two methods using the C_λ index (Morisita, 1959), which equals 1 for complete similarity between the two methods and 0 for no similarity.

RESULTS AND DISCUSSION

Using both methods, 761 individuals in 8 species of oribatid mites were collected from the leaves, versus 244 individuals in 13 species from the branches (Tables 3, 4). The numbers of oribatid species collected from leaves and branches using the washing method were significantly larger than those collected using the direct method (Tables 1, 2; $p < 0.05$).

Washing method

Table 4. Species and numbers of oribatid mites collected from the branches using the two methods.

Washing method

[illegible]

There was no significant difference in the numbers of total individuals, adults, and juveniles collected from leaves between the two methods (Table 1; $p > 0.05$). On branches, however, the total numbers of individuals and adults collected using the washing method were significantly larger than those collected using the direct method (Table 2; $p < 0.005$). The number of juveniles collected on branches did not differ significantly between the two methods (Table 2; $p = 0.125$).

With respect to the samples from which more than 10 individuals were collected, values of the similarity index were close to 1 for all the samples except branch sample No. 20 (Tables 1, 2). This result indicates that the dominant species of the oribatids did not differ between the two methods. Moreover, in many tree species there were some oribatid species that could only be collected by the washing method: eight species were only collected by the washing method, whereas four species were only collected by the direct method (Tables 3, 4). Thus, the washing method is more likely to be an effective and relatively unbiased method for sampling oribatid mites from the canopies of broad-leaved trees in Japan.

ACKNOWLEDGMENTS

We thank Mr. I. Kawashima in Okinawa for valuable advice on the vegetation of the Ryukyu archipelago.

REFERENCES

- André, H. M., X. Ducarme and P. Lebrun (2002) Soil biodiversity: myth, reality or conning? *Oikos*, 96: 3–24.
- Ichisawa, K., N. Kaneko, V. M. Behan-Pelletier and J. Aoki (1999) Arboreal oribatid fauna of Japanese red cedar (*Cryptomeria japonica*). *Bulletin of the Institute of Environmental Science and Technology, Yokohama National University*, 25: 49–53.
- Ichisawa, K. (2001) A taxonomical and ecological study on arboreal collembolans (Insecta: Collembola) and oribatid mites (Acari: Oribatida). Ph.D. thesis. 121 p., Yokohama Natl. Univ., Kanagawa, Japan. (in Japanese)
- Ito, M. (1986) An ecological survey on arboreal oribatid mites (Acari: Oribatida) in a subalpine coniferous forest of Shiga-Kogen, Central Japan. *Edaphologia*, 35: 19–26.
- Karasawa, S. and N. Hijii (2004a) Effects of microhabitat diversity and geographical isolation on oribatid mite (Acari: Oribatida) communities in mangrove forests. *Pedobiologia*, 48: 245–255.
- Karasawa, S. and N. Hijii (2004b) Morphological modifications among oribatid mites (Acari: Oribatida) in relation to habitat differentiation in mangrove forest. *Pedobiologia*, 48: 383–394.
- Kurimoto, Y. (1978) Arthropods in black pine crowns. *Transactions 26th Management Chubu Branch Japan Forestry Society*, 26: 175–179.
- Morisita, M. (1959) Measuring of interspecific association and similarity between communities. *Memoirs of the Faculty of Science, Kyushu University. Ser. E, Biology*, 3: 65–80.
- Southwood, T. R. E. and P. A. Henderson (2000) *Ecological Methods*. Third ed. 575 p. Blackwell Science Ltd, Oxford.
- Spain, A. V. and R. A. Harrison (1968) Some aspects of the ecology of arboreal Cryptostigmata (Acari) in New Zealand with special reference to the species associated with *Olearia colensoi* Hook.f. *New Zealand Journal of Science*, 11: 452–458.
- Walter, D. E. and V. M. Behan-Pelletier (1993) Systematics and ecology of *Adhaesozetes polyphyllos* sp. nov. (Acari: Oribatida: Licneremaeoidea), a leaf-inhabiting mite from Australian rainforests. *Canadian Journal of Zoology*, 71: 1024–1040.
- Walter, D. E., D. J. O'Dowd and V. M. Barnes (1994) The forgotten arthropods: foliar mites in the forest canopy. *Memoirs of the Queensland Museum*, 36: 221–226.
- Walter, D. E. and D. J. O'Dowd (1995) Beneath biodiversity: factors influencing the diversity and abundance of canopy mites. *Selbyana*, 16: 12–20.
- Yoshida, T. and N. Hijii (2005) The composition and abundance of microarthropod communities on arboreal litter in the canopy of *Cryptomeria japonica* trees. *Journal of Forest Research*, 10: 35–42.