

The community structures of fungivorous insects on *Amanita muscaria* in New Zealand

N. OSAWA¹, R. TOFT², N. TUNO³, K. KADOWAKI⁴, T. FUKIHARU⁵, P.K. BUCHANAN⁶, C. TANAKA⁷

¹Laboratory of Forest Ecology, Graduate School of Agriculture, Kyoto University, 606-8502 Japan. E-Mail: osawa@kais.kyoto-u.ac.jp

²Entecol Ltd, PO Box 142, Nelson, New Zealand

³Laboratory of Ecology, Graduate School of Sciences, Kanazawa University, 920-1192, Japan

⁴School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand

⁵Natural History Museum and Institute, Chiba, 260-8682, Japan

⁶Landcare Research, Private Bag 92170, Auckland, New Zealand

⁷Laboratory of Environmental Mycoscience, Graduate School of Agriculture, Kyoto University, 606-8502 Japan

ABSTRACT

The toxic ectomycorrhizal fungi *Amanita muscaria* (Agaricales: Amanitaceae) was first recorded in New Zealand in 1937, and it is now widespread throughout the country. We collected decaying fruiting bodies of the mushroom in 2005 and 2006 and placed them in enclosed emergence traps to determine use by fungivorous insects. This study clarified that the endemic species *Mycetophila fagi*, *M. filicornis* (Diptera: Mycetophilidae) and *Zedura curtisi* (Diptera: Trichoceridae), as well as exotic *Drosophila busckii* (Diptera: Drosophilidae), utilised the exotic *A. muscaria* in various vegetation types in the North and South Islands of New Zealand. A significant difference was observed in the number of fungivorous insects found on the mushrooms between North and South Islands; the endemic *M. fagi* was dominant in South Island, while Psychodidae sp. dominated North Island. A significant difference was observed in the number of fungivorous insects between the exotic and endemic vegetation inhabited by *A. muscaria*. Furthermore, the biodiversity of fungivorous insects on *A. muscaria* within endemic *Nothofagus* vegetation was more than three times greater than that within the exotic *Betula*, *Pinus*, *Pseudotsuga* and *Tsuga* vegetation. These observations suggest that the greater diversity of fungivorous insects on *A. muscaria* in natural *Nothofagus* forests may reflect the higher diversity found in natural forests compared with plantation forests.

Keywords. *Amanita muscaria*, Drosophilidae, fungivorous insect, Mycetophilidae, Trichoceridae.

INTRODUCTION

Mycophagous insects are generally recognised as being polyphagous (Hackman & Meinader 1979; Hanski 1989). To explain this, Hanski (1989) proposed the quantitative and qualitative hypotheses: the quantitative hypothesis proposes that polyphagy is caused by the low predictability of the occurrence of fungal fruiting bodies (Jaenike 1978), whereas the qualitative hypothesis proposes that differences in chemical traits between host species are not a major barrier to wider host use. As an example in support of the qualitative hypothesis, agaric fruiting bodies contain insecticidal compounds that affect non-mycophagous insects (Jaenike *et al.* 1983), and the toxicity differs among fungal species (Mier *et al.* 1996). This suggests that these fungal insecticides do not affect mycophagous insects

that are adapted to them (Jaenike *et al.* 1983; Hanski 1989), and that fungivorous insects have developed a common tolerance of a range of fungal insecticides.

The genus *Amanita* (Agaricales: Amanitaceae) is one of the most well known macrofungal genera (e.g., Oda 2005). It is mostly found in forests, and at present, ca. 500 species have been described (Kirk *et al.* 2001). Most *Amanita* species are ectomycorrhizal (i.e., with a thick network of cells that form a sheath around the root hairs of the associated plant; e.g., Oda 2005) and have strong relationships with birch (*Betula*), pine (*Pinus*), fir (*Abies*) and larch (*Larix*) (Gelm *et al.* 2006). At least 16 species of the genus *Amanita* have been recorded in New Zealand, with 10 species (*Amanita australis*, *A. karea*, *A. mumura*, *A. nehuta*, *A. nigrescens*, *A. nothofagi*, *A. pareparina*, *A. pekeoides*, *A. pumatona* and *A. taipea*) considered native and six (*A. inopinata*, *A. muscaria*, *A. nauseosa*, *A. pantherina*, *A. phalloides* and *A. rubescens*) introduced (McKenzie *et al.* 2004). The introduced *A. muscaria* (L.:Fr.) Lam. was first recorded in New Zealand in 1937 (e.g., McKenzie *et al.* 2004). The major expansion in its range occurred from 1970 to 1980, and *A. muscaria* is now distributed throughout the country (e.g., McKenzie *et al.* 2004, Johnston 2010). In New Zealand, *A. muscaria* has a strong relationship with native beech species (genus *Nothofagus*) as well as a range of exotic trees (e.g., *Betula*, *Tsuga*, *Pinus* and *Pseudotsuga*) (Bagley & Orlovich 2004; Johnston 2010). The latter association probably assisted the range expansion via human activities. In addition, fungivorous insects are believed to be involved in spore dispersal (e.g., Pirozynski & Malloch 1988; Tuno 1999), and this may also be the case in New Zealand. However, the community structure of fungivorous insects on *A. muscaria* in New Zealand has not previously been investigated.

An intriguing question is whether New Zealand's endemic fungivorous insects are able to expand their host range to include *A. muscaria* and/or whether introduced insects are utilising it. Furthermore, the distribution of endemics is remarkably uneven in New Zealand, especially between the North and South Islands (Gibbs 2006), and the community structure of fungivorous insects on *A. muscaria* may differ between islands. Moreover, the community structure on *A. muscaria* may also change between exotic plantation forests and native forests.

In this study, we conducted field research to determine the community structure of the fungivorous insects on *A. muscaria* in New Zealand at different geographic locations (North vs. South Island) and on different hosts (endemic vs. exotic tree hosts).

MATERIALS AND METHODS

Decaying fruit bodies of *Amanita muscaria*, *A. phalloides* (Fr.: Fr.), *Amanita* sp., *Thaxterogaster porphyreum* (Cunn.) Hk. (Agaricales: Cortinariaceae) and *Tremella fuciformis* Berkeley (Tremellales: Tremellaceae) were collected at 19 sites from late

April to mid-May in 2005 and 2006 (Table 1). The nearest tree from the fruit body was regarded as the likely host of the fungi, and the host also reflected the vegetation types (i.e., *Nothofagus*, *Betula*, *Pinus*, *Pseudotsuga* and *Tsuga*) of the site; *Nothofagus*, *Pinus* and *Pseudotsuga* were dominant species of the forest, whereas *Betula* and *Tsuga* were consistent with vegetation found in small areas. The fruiting bodies (one to three combined) were kept in a cooler box throughout the field collection period. In mid-May, they were placed in enclosed emergence traps at Landcare Research, Nelson, and kept humid and at natural temperature

Table 1. List of collection sites, sampled fungi and their related hosts.

Year	Island	Site	Host Plant (Common name)	Vegetation type (endemic vs. exotic)	Species of fungi
2005	South	Nelson Lakes National Park+	<i>Nothofagus</i> sp. (<i>Nothofagus</i>)	Nothofagus (endemic)	<i>Amanita muscaria</i> *
2005	South	Nelson Lakes National Park+	<i>Pseudotsuga menziesii</i> (Douglas Fir)	Pseudotsuga (exotic)	<i>Amanita muscaria</i> *
2005	South	Nelson Lakes National Park+	<i>Pinus radiata</i> (Radiata Pine)	Pinus (exotic)	<i>Amanita muscaria</i> *
2005	South	Nelson Lakes National Park+	<i>Nothofagus</i> sp. (<i>Nothofagus</i>)	Nothofagus (endemic)	<i>Amanita muscaria</i> *
2006	North	Ohakune+	<i>Nothofagus</i> sp. (<i>Nothofagus</i>)	Nothofagus (endemic)	<i>Amanita muscaria</i> *
2006	North	Ohakune	<i>Nothofagus</i> sp. (<i>Nothofagus</i>)	Nothofagus (endemic)	<i>Amanita muscaria</i> *
2006	North	Piha			<i>Amanita</i> sp.:
2006	North	Piha			<i>Amanita phalloides</i> *
2006	North	Rotorua (town)			<i>Amanita muscaria</i> *
2006	North	Turangi (town)+	<i>Betula</i> sp. (Birch)	Betula (exotic)	<i>Amanita muscaria</i> *
2006	North	Turangi (forest)+	<i>Nothofagus solandri</i> (Black Beech)	Nothofagus (endemic)	<i>Amanita muscaria</i> *
2006	South	Spring Junction+	<i>Pseudotsuga menziesii</i> (Douglas Fir)	Pseudotsuga (exotic)	<i>Amanita muscaria</i> *
2006	South	Nelson Lakes National Park+	<i>Tsuga</i> sp. (Tsuga)	Tsuga (exotic)	<i>Amanita muscaria</i> *
2006	North	Barly Spruce+	<i>Nothofagus solandri</i> (Mountain Beech)	Nothofagus (endemic)	<i>Amanita muscaria</i> *
2006	South	Charm Creek Track			<i>Thaxterogaster porphyreum</i>
2006	South	Rotoiti, Nelson Lakes	<i>Nothofagus</i> sp. (<i>Nothofagus</i>)	Nothofagus (endemic)	<i>Tremella fuciformis</i> *
2006	South	Waitafu+	<i>Pinus radiata</i> (Radiata Pine)	Pinus (exotic)	<i>Amanita muscaria</i> *
2006	South	Atapo+	<i>Pinus</i> sp. (Pine)	Pinus (exotic)	<i>Amanita muscaria</i> *
2006	South	Westport+	<i>Nothofagus</i> sp. (<i>Nothofagus</i>)	Nothofagus (endemic)	<i>Amanita muscaria</i> *

+: used for the analysis of fungivorous Diptera on *A. muscaria*

*: exotic species from McKenzie *et al.* 2004; -: unknown.

for approximately 1 month. Arthropods that emerged were collected into ethanol and identified to the lowest taxonomic level we could achieve (mostly species-level). In total, 92.64% of samples (390 individuals of 421) were assigned to Diptera, and the Diptera individuals that emerged from *A. muscaria* were used for subsequent statistical analyses. No insects emerged from fruiting bodies found at two sites of 15 sites where *A. muscaria* was collected (Ohakune & Rotorua in Table 1).

The 390 Diptera individuals that emerged from *A. muscaria* came from a total of 13 localities from the North and South Islands. The 13 sites were not significantly biased toward either island (χ^2 test for no. of sites at North and South Island to equal ratio, d.f.=1, $\chi^2=0.6923$, $p=0.1655$). Furthermore, the geographical distribution in New Zealand (i.e., exotic and endemic) and vegetation type (i.e., *Nothofagus*, *Betula*, *Pinus*, *Pseudotsuga* and *Tsuga*) for the sampled *A. muscaria* at the North and South Island were also not significantly biased (Fisher's exact test for no. of endemism: d.f.=1, $\chi^2=1.935$, $p=0.2657$; extended Fisher's exact test for no. of vegetation types: d.f.=4, $\chi^2=5.9583$, $p=0.3105$, respectively). Therefore, the analysis for the effects of geographical differences on the number of fungivorous insects on the North versus the South Island was performed by extended Fisher's exact test. Nominal logistic regression analysis was also performed for the

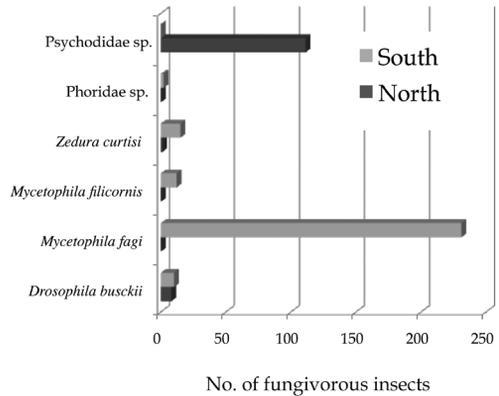


Figure 1. Number of fungivorous insects emerged from *A. muscaria* on the North and South islands.

Table 2. Emerged arthropods from the collected fungi.

Species of fungi	Arthropods on the fungi	No. Individuals
<i>Amanita muscaria</i>	<i>Drosophila busckii</i> Coquillett	18
<i>Amanita muscaria</i>	Hymenoptera sp.	2
<i>Amanita muscaria</i>	<i>Mycetophila fagi</i> Marshall	233
<i>Amanita muscaria</i>	<i>Mycetophila filicornis</i> Coquillett	12
<i>Amanita muscaria</i>	Nematoda sp.	1
<i>Amanita muscaria</i>	<i>Zedura curtisi</i> (Alexander)	16
<i>Amanita muscaria</i>	Phoridae sp.	2
<i>Amanita muscaria</i>	Pseudoscorpiones sp.	1
<i>Amanita muscaria</i>	Psychodidae sp.	118
<i>Amanita phalloides</i>	<i>Drosophila busckii</i>	1
<i>Amanita phalloides</i>	Psychodidae sp.	1
<i>Amanita</i> sp.	<i>Mycetophila fagi</i>	8
<i>Thaxterogaster porphyreum</i>	<i>Drosophila busckii</i>	1
<i>Tremella fuciformis</i>	Psychodidae sp.	7

analysis at vegetation types. An extended Fisher's exact test was used for the analysis of the number of fungivorous insects between endemic and exotic vegetation for *A. muscaria*.

Morisita's β (Morisita 1967) was used to analyse the biodiversity of fungivorous insects, where n_i is the total number of species i , N is the total number of fungivorous insects and β is given by the following equation:

$$\beta = \frac{N(N-1)}{\sum n_i(n_i-1)}$$

The extended Fisher's exact test was performed using R 2.11.1 (R Development Core Team 2010). Nominal logistic regression analysis and Fisher's exact tests were performed using SAS Discovery Software (SAS Institute 2007).

RESULTS

A total of 421 arthropods emerged from the fruiting bodies (Table 2). A diverse range of taxa emerged from *Amanita muscaria*, including three endemic fungivorous flies (*Mycetophila fagi* Marshall, *M. filicornis* Coquillett and *Zedura curtisi* (Alexander)), and an exotic and cosmopolitan fungivorous species (*Drosophila busckii* Coquillett). Other flies of unknown feeding guild, but potentially fungivorous, were species of Phoridae and Psychodidae. Other species collected included one individual of Nematoda, two Hymenoptera and one Pseudoscorpionida. *Drosophila busckii* and Psychodidae also emerged from *A. phalloides*, *M. fagi* from *Amanita* sp., *D. busckii* from *Thaxterogaster prophyreum* and Psychodidae from *Tremella fuciformis*.

Figure 1 shows the number of fungivorous insects to emerge from *A. muscaria* on the North and South Islands. *Mycetophila fagi* was dominant in South Island, while Psychodidae sp. dominated in North Island. A significant difference was observed in the number of fungivorous insects between North and South

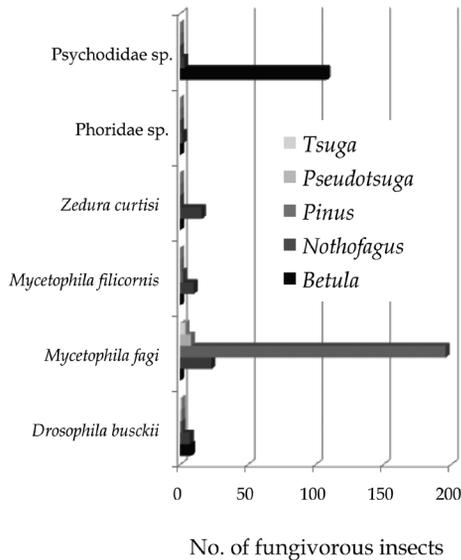


Figure 2. Number of fungivorous insects from *A. muscaria* on different vegetation types.

Islands (extended Fisher's exact test: d.f.=5, $\chi^2=364.735$, $p<0.0001$). Furthermore, the diversity of fungivorous insects in South Island (Morisita's $\beta=1.356$) was slightly higher than that in North Island (1.164).

A significant difference was observed in the number of fungivorous insects from *A. muscaria* on different vegetation types (Nominal logistic regression analysis: d.f.=20, L-R $\chi^2=364.735$, $p<0.0001$): many Psychodidae sp. emerged from exotic *Betula*, and *M. fagi* from exotic *Pinus*, while no clear tendencies were observed for *P. curtisi*, *M. filicornis* and *D. busckii* (Fig. 2). Also, a significant difference was observed in the number of fungivorous insects between exotic and endemic forest types of *A. muscaria* (extended Fisher's exact test: d.f.=5, $\chi^2=165.868$, $p<0.001$; Fig 3). The biodiversity of fungivorous insects on *A. muscaria* at endemic *Nothofagus* was more than three times higher than that on exotic *Betula*, *Pinus*, *Pseudotsuga* and *Tsuga* (Fig. 4).

DISCUSSION

The impacts of exotic species on endemic species and ecosystems on New Zealand were recently reviewed (e.g., Brockerhoff et al. 2010; Johnston 2010). However, less information was presented for fungivorous insects on exotic fungi. This is the first study to show that endemic *Mycetophila fagi*, *M. filicornis* and *Zedura (Paracladura) curtisi* and exotic *Drosophila busckii* utilised exotic fungi *Amanita muscaria* in various vegetation types in North and South Island of New Zealand (Table 2). *Mycetophila fagi* and *M. filicornis* are primary fungivores (Burns 1984), which means that their larvae may feed on the fungal tissues. In contrast, *D. busckii* is a secondary fungal species (Burns 1984). Furthermore, *Z. curtisi* belongs to the family Trichoceridae

(Alexander 1981) and in the larval stages of Trichoceridae, insects are generally scavengers in a variety of habitats including mature fungi (Alexander 1981). These data, combined with the toxicity of *A. muscaria*, imply that *D. busckii* and *Z. curtisi* may be regarded as secondary fungivores that feed on nontoxic decaying fungal tissues and/or microbes living on the tissues. Thus, endemic and exotic as well as primary and secondary fungivorous insects have utilised exotic *A. muscaria* throughout New Zealand over 70 years, regardless of the toxicity.

This study identified a significant difference in fungivorous insect communities on *A. muscaria* between the North and South Islands of New Zealand (Fig. 1). Furthermore, the diversity of fungivorous insects in South Island was higher than that on North Island. These geographic differences in the interactions between *A. muscaria* and fungivorous insects are probably due to differences in the mode and history of invasion of *A. muscaria* over the 70 years since it first established.

Hodge *et al.* (2010) reported that different orders of insects associated with commercial mushrooms (*Agaricus* sp.) showed varying patterns of abundance and species richness among the different categories of woodland on the South Island of New Zealand. Our study showed a significant difference in communities of fungivorous insects between exotic and endemic hosts and among vegetation types of *A. muscaria* on the North and South Islands of New Zealand; Psychodidae sp. and *M. fagi* were dominant on exotic vegetation types of *A. muscaria*, whereas no real dominance pattern was apparent on the endemic *Nothofagus* vegetation (Figs 3 & 4). The species-richness of beetles and fungus gnats has been correlated with vascular plant richness (e.g., Toft *et al.* 2001). Therefore, the greater diversity of fungivorous insects on *A. muscaria* in the natural forests in this study (Fig. 4) may reflect the higher plant and insect diversity found in the natural forests in comparison to plantation forests.

ACKNOWLEDGEMENTS

We thank Drs. T. K. Crosby, P. Johnston and E. H. C. McKenzie,

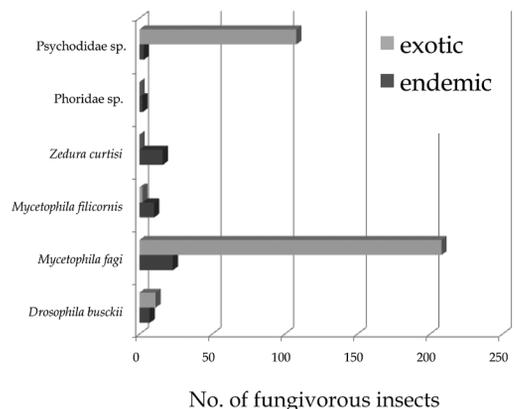


Figure 3. Number of fungivorous insects from endemic and exotic vegetation inhabited by *A. muscaria*.

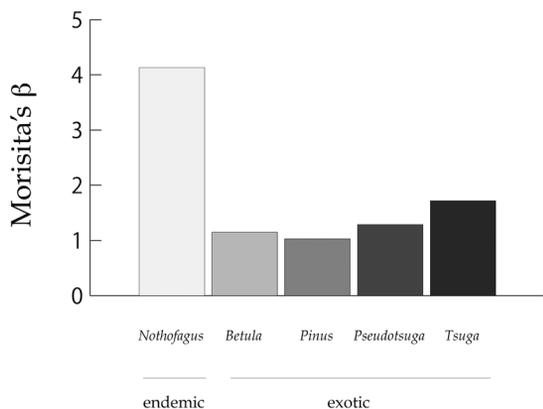


Figure 4. Morisita's β of fungivorous insects from *A. muscaria* on different vegetation types.

Landcare Research, Auckland, for their assistance during this research. This study was supported in part by a Grant-in-Aid for Science Research (No. 20405047 to C. T.) from the Japan Society for the Promotion of Science.

REFERENCES

Alexander CP. 1981. Trichoceridae. In: *Manual of Nearctic Diptera Vol. I.* JF McAlpine, BV Peterson, GE Shewell, HJ Teskey, JR Vockeroth, DM Wood). pp 301-304. Canadian Government Publishing Centre, Quebec, Canada.

Bagley SJ, Orlovich DA. 2004. Genet size and distribution of *Amanita muscaria* in a suburban park, Dunedin, New Zealand. *New Zealand Journal of Botany* 42: 939-947.

Brockerhoff EG, Barratt BIP, Beggs JR, Fagan LL, Kay MK(N), Phillips CB, Vink CJ. 2010. Impacts of exotic invertebrates on New Zealand's indigenous species and ecosystems. *New Zealand Journal of Ecology* 34: 158-174.

Burns TD. 1984. Insect mycophagy in Boletales: fungivore diversity and the mushroom habitat. In: *Fungus-Insect Relationships, Perspectives in Ecology and Evolution.* (eds) QD Wheeler, M Blackwell). Pp 91-129. Columbia University Press, New York, NY, USA.

Gelm J, Laursen GA, O'Neill K, Nusbaum C, Taylor DL. 2006. Beringian origins and cryptic speciation events in the flyagaric (*Amanita muscaria*). *Molecular Ecology* 15: 225-239.

Gibbs G. 2006. *Ghosts of Gondwana: the History of Life in New Zealand.* Craig Potton Publishing, Nelson, New Zealand.

Hackman W, Meinader M. 1979. Diptera feeding as larvae on macrofungi in Finland. *Annales Zoologici Fennici* 16: 50-83.

Hanski I. 1989. Fungivory: fungi, insects and ecology. In: *Insect-Fungus Interactions.* N Wilding, NM Collins, PM Hammond, JF Webber). Pp 25-68. Academic Press, London, UK.

Hodge S, Marshall SA, Oliver H, Berry J, Marris J, Andrew

I. 2010. A preliminary survey of the insects collected using mushroom baits in native and exotic New Zealand woodlands. *New Zealand Entomologist* 33: 43-54.

Jaenike J. 1978. Resource predictability and niche breadth in the *Drosophila quinaria* species group. *Evolution* 32: 676-678.

Jaenike J, Grimaldi D, Sluder AE, Greenleaf AL. 1983. Alpha-amanitin tolerance in mycophagous *Drosophila*. *Science* 221: 165-167.

Johnston PR. 2010. Causes and consequences of changes to New Zealand's fungal biota. *New Zealand Journal of Ecology* 34: 175-184.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. *Ainsworth and Bisby's Dictionary of the Fungi, 9th ed.* CAB International, Wallingford, UK.

McKenzie E, Buchanan P, Beever R, Johnston P, Pennycook S. 2004. *Introduction to Fungi of New Zealand.* Fungal Diversity Press, Hong Kong.

Mier N, Canetea S, Klaebe A, Chavanta L, Fournier D. 1996. Insecticidal properties of mushroom and toadstool carpophores. *Ecological Biochemistry* 41: 1293-1299.

Morisita M. 1967. The seasonal variation of butterflies in Kyoto. In: *Nature-Ecological Study* pp 95-132. Cyuoukoronnsa, Tokyo, Japan (In Japanese).

Oda T. 2005. *Molecular Phylogeny of the Genus Amanita, and Its Application to Taxonomy, Biogeography, and Evolution.* Ph.D. dissertation, Kyoto University, Kyoto, Japan.

Pirozynski KA, Malloch DW. 1988. Seeds, spore and stomachs: coevolution in seed dispersal mutualism. In: *Coevolution of Fungi with Plants and Animals* KA Pirozynski & DL Hawksworth). Pp 227-246. Academic Press, London, UK.

R Development Core Team. 2010. *R: A language and Environment for Statistical Computing.* A Foundation for Statistical Computing, Vienna, Austria.

SAS Institute 2007. *JMP User's Guide, Ver. 7.02.* Cary, NC, USA.

Toft RJ, Harris RJ, Williams PA. 2001. Impacts of the weed *Tradescantia fluminensis* on insect communities in fragmented forests in New Zealand. *Biological Conservation* 102: 31-46.

Tuno N. 1999. *The Community Structure and Population Dynamics of Fungivorous Insect.* Ph.D. dissertation, Kyoto University, Kyoto, Japan (In Japanese).