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Multivariate Analysis on Invertebrate Communities in Litter and Soils of Japanese Red Pine Forests treated by *Beauveria bassiana*[1]

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白殭菌을 處理한 소나무림의 落葉과 土壤에 棲息하는 無脊椎動物 群集에 대한 多變量分析[1]

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ABSTRACT

We tested if the treatment of *Beauveria bassiana* would influence invertebrate communities in litter and soils by multivariate analysis. The PCA (principal components analysis) was used for the analysis. Using the distances between communities in the ordination space, we carried out statistical tests whether any factors would influence structures of the communities. We did not found any significant effects of the *Beauveria* treatment on invertebrate communities in both litter and soils.

Key words : principal components analysis, biological control, environmental effect, Euclidean diatance, clustering, fungus, biological pesticide, soil, litter, ordination, arthropoda, insect

要 約

다변량분석법(주성분분석법, PCA)을 이용하여 무척추동물 군집에 백강균 처리가 미치는 영향을 검정하였다. 좌표공간내의 군집들간의 거리를 이용하여, 군집구조에 미치는 요인들의 영향을 통계 검정하였다. 백강균 처리는 낙엽과 토양의 무척추동물 군집에 유의한 영향을 주지 않았다.

INTRODUCTION

Forests are the major storags for the biological resources and carbons (Jeong et al, 1998; Vitousek, 1991), the main buffer zones for stabilizing ecosystem, and filtering pollutants (Chang and Lee, 1995). In Korea, insect pests have been thought as the most damaging agents to forests (Forest Administration, 1999). The Japanese red pine forests, occupying 45% of total forests in Korea (Lim, 1993), have been severely damaged by the pine needle gall midge, *Thecodiplosis japonensis,* since the early 1970s. For the control of this pest, the trunk injection of pesticide and the translocation of the hymenopterous parasites are now only applicable. Such control methods are so expensive for the application that treated area is restricted. The development of inexpensive, widely applicable, and non-poisonous biological pesticide is required for an effective control of the injurious pest.

The entomogenuous fungi, *Beauveria bassiana,* being isolated from the dead body of *Acantholyda*

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parki showed an effective pathogenicity to *T. japonensis* (Forestry Research Institute, 1997). *B. bassiana* is expected to be easily applicable economically to an wide area, along with the possibility of the mass culture of pathogens (Seo et al., 1995; Seo et al., 1996). However, the general pathogenicity of *B. bassiana* to diverse insects (Li, 1988; McCoy et al., 1988) might disturb invertebrate communities by attacking non-target organisms. To study effects of *B. bassiana* on the invertebrate communities, we had monitored the invertebrate communities in litter and soils of the pine forests treated with *B. bassiana* (Kwon et al., 1999).

In the previous study, diversities of communities and abundances of the major groups of invertebrates in litter and soils were not affected by the treatment of *B. bassiana* (Kwon et al., 1999). Despite the insignificant effects on each selected taxon, it is expected that the *Beauveria* treatment could change invertebrate communities (composition of components) in the treated forests. In this study, we tried to find out whether the fungus treatment might change invertebrate communities.

MATERIALS AND METHODS

1. Study area and invertebrate sampling

To study the control effects of *B. bassiana* on the pine needle gall midge, spores or conidia of *B. bassiana* F 101 strain cultured in laboratory were treated in several damaged pine forests in Kwangwon-do from 1992 to 1997. The strain was selected from the bodies of the infected black-tipped sawfly, *Acanthodyla parki*. The treatment decreased larval density of *Thecodiplosis japonensis*. The larval decreasing ratio was 13-15% higher in treated stands than in control stands (Shin, 2001). From an half or one year after treatment of *B. bassiana*, the invertebrates in litters and soils were seasonally sampled three to five times in the four forests located at Hoengsung and Pyongchang- guns in Kwangwon-do. Each experimental stand had three treated plots with one control plot. Details for the study areas and the sampling methods were previously reported (Kwon et al., 1999). Invertebrates were identified to the higher taxa levels such as family, order or class.

2. Ordination of invertebrate community

The pooled results of five replicated samples were used for the ordinational analysis of the invertebrate communities. The data matrix used in this study came from the previous study of Forestry Research Institute (1997). PCA (principal components analysis) was used for the ordination through SPSS (SPSS, 1997). The aim of PCA is to resolve the total variation of a set of variables into linearly independent composite variables (principal components) which successively account for the maximal variability (Shinha, 1977). A few major components can represent a numerous variables. This makes communities with diverse components be handled more easily. The dominant invertebrate groups found in more than 40% of the total plots were used for PCA. Densities were log-transformed to be normalized prior to analysis with a following equation : $x = \log_{10}(\text{count} + 1)$.

3. Analysis on the influence of factors in the ordination

To study the treatment effects of *B. bassiana on* invertebrate communities, the factor scores of two major principal components (PC 1 and 2) were compared between the treated and control stands with ANOVA by SPSS (SPSS, 1997). Regional and seasonal effects were also tested in ANOVA for comparison. In addition, we tested influences of these three factors on community clustering by the Euclidean distances between communities in the two-dimensional PCA ordination space. If the *Beuaveria* treatment could change the invertebrate communities, the treated plots would be separated from the control plots in the ordination space. The conformational changes in the ordination space may be detected by comparing distances among communities. We tested the one-tailed hypothesis that the

Euclidean distances between communities in a homogeneous condition (e.g., the same study period for seasonal effect or the same region for regional effect) would be shorter than those in the total. The Student's t-test was used for the test of difference (Zar, 1999). The distances between communities were estimated on the base of the two factor scores (PC 1 and 2) of communities by STATISTICA (StatSoft, 1998). The interaction of two factors were analyzed on the base of the combinational homogeneous condition of the two factors (e.g., the communities in the same study period and the same region for the interaction of region and season).

RESULTS

Details of invertebrate communities in the study sites were reported in another paper (Kwon et al., 1999). Ordination with major invertebrate groups in litter is shown in Figure 1. Eigenvalues of the first and second principal components (PC I and II) were 5.2 and 2.1 which explained 39.7 % and 16.4 % of total variance, respectively. All of invertebrates were positively loaded with PC I. Two groups were recognized on the axis of PC I. Parasitic bees, flies, ants and thrips were lowly loaded with the first component, while other groups were highly loaded. For PC II, invertebrates had a wide variation. In the two dimensional coordinates, invertebrates were clustered into the three groups.

Invertebrate communities were clustered according to region and season, but not to the *Beauveria* treatment (Figure 2). Hence, invertebrate communities in litter might be affected by region and season, but not by the treatments of *B. bassiana.* Season and region influenced significantly the factor scores of PC I and II, while treatment of the fungus did not (Table 1). The analysis of Euclidean distance on the communities coincided with these results (Table 2). Distances between communities within the same region (t-test, $p < 0.1$) and within the same study period (t-test, $p < 0.001$) were shorter than those in the total. However, distances between the *Beauveria* treated plots or between the untreated plots was not shorter than those of total conditions (t-test, $p > 0.05$). In the interaction between *B. bassiana* and season, the distances were

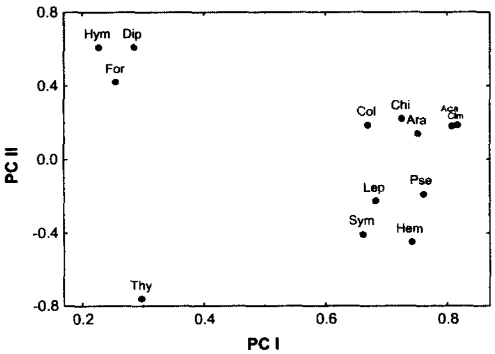


Figure 1. PCA ordination diagram of invertebrate groups in litter by the factor loadings. The symbols in three characters represent the names of taxa as following. Ara; Araneae, Chi; Chilopoda, Col; Coleoptera, Clm : Collembola, Dip : Diptera, Hem : Hemiptera, Hym : Hymenoptera, For : Hymenotera (Formicidae), Lep; Lepidoptera, Aca; Acari, Pse; Pseudoscorpione, and Thy; Thysanoptera.

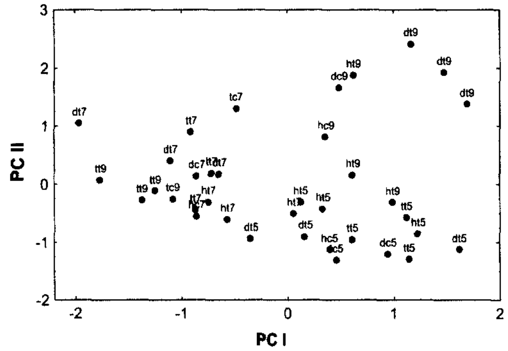


Figure 2. PCA ordination diagram of communities of the study plots in litters. The symbols in three characters represent characteristics of the sites as following. The first character in the legends indicates the region such as d : Dodon, h : Haanmi, t : Dunnae. The second character expresses the site treated with *Beauveria bassiana* as t and the untreated site as c, and the last one represents the sampling date as follows; 5 : May 1997, 7 : July 1997, and 9 : September 1997.

significantly shorter than that of the total (t-test, p < 0.001). However, the mean distance of 1.35 in this interaction was longer than that in a single factor of season (1.27). Therefore, it is likely that the significant effect in the interaction would be caused only by season. In the interaction between region and season, however, it was shorter than any distance in a single factor of season or region. In this case, the synergistic effect of both factors might cause the communities to locate more closely.

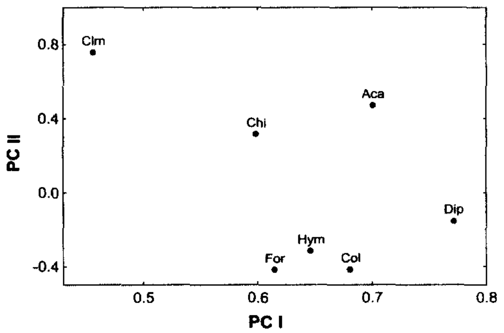
Table 1. Analysis of variance on the effects of environmental factors with the factor scores of the first two principal components.

Habitat	Variable	PC I			PC II		
		F	d.f.	p	F	d.f.	p
Litter	Region	3.944	2	0.038	3.140	2	0.068
	Season	17.840	2	0.000	26.439	2	0.000
	B. bassiana	0.239	1	0.631	0.148	1	0.705
	Region × Season	6.706	4	0.002	5.970	4	0.003
	Region × *B. bassiana*	0.385	2	0.686	0.480	2	0.627
	Season × *B. bassiana*	0.388	2	0.684	0.709	2	0.505
	Region × Season × *B. bassiana*	1.134	4	0.372	0.714	4	0.593
Soil	Region	0.165	3	0.919	1.701	3	0.186
	Season	13.010	4	0.000	8.195	4	0.000
	B. bassiana	0.254	1	0.618	0.058	1	0.811
	Region × Season	0.458	8	0.876	1.193	8	0.334
	Region × *B. bassiana*	1.629	3	0.202	0.674	3	0.574
	Season × *B. bassiana*	1.781	4	0.157	0.268	4	0.897
	Region × Season × *B. bassiana*	0.648	8	0.732	0.562	8	0.801

Table 2. Distances between the invertebrate communities (Figs. 2, 4) on the PCA ordination space. The probabilities in t-test indicate the significant difference between distances of the communities within the homogenous conditions of a factor and those in the total. Details for the group are shown in the text.

Habitat	Group	Distance (mean ± S.D.)	n	t-test (one-tailed) p
Litter	Total	1.77 ± 0.93	630	-
	B. bassiana	1.81 ± 0.96	387	0.795
	Region	1.72 ± 0.95	198	0.256
	Season	1.27 ± 0.87	198	0.000
	B. bassiana × Region	1.78 ± 0.99	117	0.542
	B. bassiana × Season	1.35 ± 0.93	117	0.000
	Region × Season	0.85 ± 0.46	54	0.000
Soil	Total	1.79 ± 0.90	2,016	-
	B. bassiana	1.87 ± 0.93	1,248	0.993
	Region	1.73 ± 0.90	512	0.089
	Season	1.26 ± 0.68	384	0.009
	B. bassiana × Region	1.82 ± 0.93	308	0.706
	B. bassiana × Season	1.32 ± 0.74	228	0.000
	Region × Season	1.17 ± 0.68	96	0.000

Figure 3 shows the PCA ordination for invertebrate groups in soils. The first and second components had eigenvalues of 2.9 and 1.4, respectively, explaining 41.6 % and 19.5% of total variance. The ordination pattern of invertebrates was different from that in litters. Two dominant invertebrates, Acari and Collembola, were separated in the axis of PC I, where they closely located in litters (Figure 2). The close location of ant with parasitic bee was also recognized in soils as well as in litters.



\Figure 3. PCA ordination diagram of invertebrate groups in soils. Chi; Chilopoda, Col; Coleoptera, Clm : Collembola, Dip : Diptera, Hym : Hymenoptera, For : Hymenotera(Formicidae), Aca; Acari.

From the ordination of communities of the study plots in soils (Figure 4), meaningful clustering patterns did not appear according to any factors. However, the statistical analyses on the factor scores of PC I and II indicated some environmental effects on the clustering of invertebrate communities. Factor scores of both PC I and II were significantly different at only different seasons in soil (Table 1). The fungus treatment and region did not influence the ordination. In the analysis using the Euclidean distances, only season made only a significant effect on clustering of communities (Table 2). *B. bassiana* showed a significant effect in the interaction with season. However, the increase of distance in the interaction indicated that the significant effect was mainly due to the seasonal effect as well as in litter. However, the synergistic effect of region and season on community distribution was also recognized in soils as well as in litter.

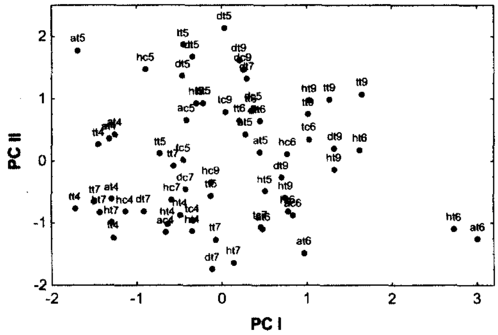


Figure 4. PCA ordination of communities of the study sites in litters. The symbols in three charactes represents characteristics of the sites as following. The first character in the legends indicates the region such as, a : Anhung, d : Dodon, h : Haanmi, t : Dunnae. The second expresses the plots treated by Beauveria bassiana as t and the control plot as c, and the last does the sampling date as follows; 4 : April 1996, 6 : June 1996, 5 : May 1997, 7 : July 1997, and 9 : September 1997.

DISCUSSION

For the principal components analysis, we ignored the minor organism groups and focused on the compositional dynamics of the major organism groups, since we were interested in change in dominant invertebrate groups rather than in that of all kinds. The selection of the relatively important invertebrate groups could reduce the high variability due to the rare invertebrate groups despite some losses of the ecological informations, and might give the robustness in the analyses. Communities usually have a few common organisms and numerous rare ones (Williams, 1964). Data matrix with all of organisms may have so numerous zero values for multivariate analysis, whilst selection of variables might change the original nature of communities. The numerous zero values in data matrix would disturb the ordinational and statistical analyses on multivariate interactions in communities (McArdle and Anderson, 2001). Accordingly, some researchers excluded the rare species or groups in the multivariate analysis (e.g., Penas and Gonzalez, 1983). As there is no logical guideline for selection of variables, we intuitively choose 40% of frequency as the selection level. For the feasible conclusions from multivariate analysis on community data matrix, more extensive studies may be necessary on the selection of variables.

The loading plots of invertebrates showed that correlational patterns among invertebrates in litter would differ from that in soils (Figures 1 and 3). At present, the meanings for such difference are not clear. This may be partly due to the difference of the physical and chemical properties between litter and soils. However, two unexpected patterns appeared from the ordination plots. Two dominant invertebrates, springtails and mites, have high correlational distribution pattern in litter but relatively low one in soils. Ants and hymenopterous parasites showed high correlations in litters and soils despite their dissimilar ecology and niche.

Seasonal effects are evident in the invertebrate communities in both litters and soils, whereas regional effects are not consistent between them (Tables 1 and 2). In temperate regions, the seasonal variations are recognized to be a main environmental factor for the dynamics of insect communities including soil mesofauna (Kwak et al., 1989; Hong et al., 1996). Hong et al. (1996) reported that peaks of abundance bimodally occurred in spring and autumn, and concluded that seasonal variations are the key force for the variation of abundance of invertebrates in soils of pine forests. In the previous study (Kwon et al., 1999), most variations in abundance and diversity of invertebrates were explained by seasonal effects. The present results also ascertain the seasonal influences on structures of invertebrate communities in litter and soils.

The descriptive and statistical analyses in the ordination patterns of communities represented that structures of invertebrate communities did not differ between treated and the untreated sites (Figures 2, 4, Tables 1). Consequently, it is concluded that the treatment of *B. bassiana* may not affect composition of components in invertebrate communities in both litter and soils. The fungus of *B. bassiana* was found in most forests in Korea (Lee, 1998), so it is highly likely that the fungus would exist in the study stands prior to the fungus treatments. Hence, the artificial infection of *B. bassiana* would give little effects on invertebrate communities (Kwon, 1999).

Despite the high pathogenicity of *B. bassiana* to some kinds of insects, the fungus *Beauveria* might be the food resources for the fungus grazing invertebrates (Choi, S.S., pers. comm.), being abundant in litter and soils (Faber, 1991). Therefore, treatment of the fungus would make the *Beauveria* feeding groups increase while the susceptible groups decrease. However, the higher taxa such as order or class used in this study may be too coarse to find such phenomena, because there can coexist the *Beaveria* feeding or susceptible invertebrates under the same order or class. In addition, it is possible that the ordination using the higher taxa groups would underestimate the variations of the community structure and thereby mask the effects of *B. bassiana*. Considering that the pathogenicity of *B. bassiana* can be species-specific according to the strain (Lee, 1998), further studies on lower taxa such as family or species should be necessary for the more feasible conclusions on the environmental effects of *B. bassiana* (Kwon et al., 1999).

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