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Effect of Pine Wilt Disease Control on the Distribution of Ground Beetles (Coleoptera: Carabidae)

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Abstract

We chose the Mt. Dalum area (located in Gijang-gun, Busan, Korea) for our survey, particularly The pine wilt disease zone and the non-permanent control area. This study investigates the effect of pine wilt disease on the distribution of beetle species in the process of ecosystem change due to insect control; pine forests treated for pine wilt disease were divided into insect control and non-control sites, respectively. The results of this study are as follows. Twen tyseven species belongs to 12 families were identified from 969 ground beetles collected from this sites. Species richness was the highest in Coleoptera (6 species, 469 individuals). In the control site, 21 species belongs to 10 families were identified from 228 individuals, while 24 species of 11 families from 533 individuals in the non-control area. The highest number of species were noted in June and July from the non- control and control sites, respectively. The highest number of insects in control and non-control sites was observed in July, while the lowest in September. *Sipalinus gigas gigas*, *Spondylis buprestoides*, *Plesiophthalmus davidis*, *Calosoma maximowiczi*, *Damaster jankowskii jankowskii,* and *Damaster smaragdinus* were captured in both study sites. *Episomustur ntus* and *Glischrochilus ipsoides* were only captured in the control site, while *Macrodorcas rectus rectus* and *Pheropsophus javanus* were only captured in the non-control site. Six beetles and five species (such as *Calosoma maximowiczi*) were found in the control site and six species (including *Damaster smaragdinus*) in the non-control site. The species distributions by altitude were 163, 518, and 258 individuals, at 100, 200 and 300 m sites, respectively. The diversity, evenness, and dominance indices in the control area were 0.764, 0.812, and 0.367, respectively. The diversity, evenness, and dominance indices in the non-control area were 0.927, 0.837, and 0.352, respectively. The similarity index between the control and non-control area was 80%.

Key Words: pine wilt disease, ground beetles, insect control, diversity index, similarity index

Introduction

Pine is a tree that shares its life and death with the history of the Korean people. It has been used as a construction material in historical palaces and in temple architecture; as the production site of pine mushrooms originating in the forest area, they contribute to the income of the local residents. It is a valuable species that provides economic, social, and ecological benefits.

Pine forests account for about 24.7% of the forest area in Korea as of 2017 (Korea Forest Service 2017). There are many pests that can damage the pine trees. Among them, the pests causing extreme damage are *Dendrolimus spectabilis*, *Thecodiplosis japonensis*, and *Matsucoccus thunbergianae*.

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In addition, the pine wood nematode caused the greatest damage to pine tree in the form of pine wilt disease which has been spreading nationwide since the outbreak in Busan Dongrae at 1988.

Pine trees native to North and Central America (Steiner and Butter 1933; Knowles et al. 1983; Dwinell 1993) are known to be relatively resistant to pine wilt disease. The pine wood nematode (PWN) causes wilt of the pine tree, and it is named as pine wilt disease (PWD) in Korea. It was reported that PWN originated in North America and spread to Japan in the early 20th century (Mamiya 1988). There are more than 15,000 species recorded so far on the earth, and there are no places in the soil, sea, salt water, and polar regions where their presence is unknown (Moom 1995). Pine wood nematode, *Bursaphelenchus xylophilus*, does not have the ability to move itself to another pine tree. Thus, after moving onto insect's body, it is transferred to a sound tree through a wound that occurs when the insect eats into the bark of a healthy tree branch and the skin is torn and scattered (Mamiya and Enda 1972).

The occurrence and prevention of the pine wilt disease is thought to be caused by improving the resistance to forest diseases and insects due to intragastric exhaust. These results can increase the biodiversity of forests through the provision of diverse dietary resources and habitats for insects in forest ecosystems (Smith and Hawley 1986; Igarashi and Kiyono 2008). In mountainous areas, it is a report of biodiversity. It is necessary for the conservation of biological species such as insects and wild animals, distribution of biological resources and population change (Jung et al. 2011). There are relatively few studies on beetles serving as an environmental indicator species of forest ecosystem.

Coleoptera is a taxon belonging to the phylum Arthropoda and class insecta, which accounts for about 40% of all insect species and approximately 400,000 species have been discovered worldwide. In Korea, approximately 4,000 insect species have been reported, which is the largest taxon of approximately one-quarter of the insects inhabited in the country. It represents the highest species diversity and population among ecosystem fauna (Kang et al. 2013). Beetles and insects are often carried on foot, and their movement can be severely affected if cuttings and fillings occur in their habitats due to various deforestation activities. Hence, it is recognized as an important environmental indicator insect at home and abroad because of rapid population changes in isolated or confined spaces (Thiele 1977; Ishitani and Yano 1994). It is highly likely to be used as a measure of species diversity depending on the environment of the habitat (Kremen et al. 1993; Samways 1994; McIntyre 2000; Yi and Modenke 2005; Yi and Modenke 2008).

Kwon et al. (2011) recently studied on the interaction of climate change and vegetation, using beetles mainly found in forests, and there was no study on the distribution of beetles in the areas where pine-repellent disease is prevalent, which accounts for most of the forest damage areas in Korea.

This study was carried out to investigate the effect of pine tree reeves disease on the distribution of beetle species in the process of ecosystem change caused by pine wilt disease. This study also compared the changes in population and diversity of beetle species as a basic data for the control of pine wilt disease.

Materials and Methods

Survey selection and overview

We chose the Mt. Dalum area (located in Gijang-gun, Busan, Korea) for our survey, particularly the pine wilt disease zone and the non-permanent control area. The total number of different species and the total number of insects present in each species were ascertained. The effect of PWD control on the distribution of beetle species was investigated in traps set in a sphere of 20 m × 20 m sized squares designated at a location of 100, 200 and 300 m altitude in the control and non-control areas (Fig.1).

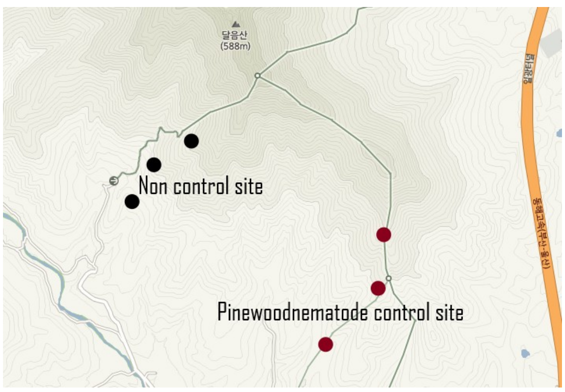

Fig. 1. Location map of the survey sites.

Pinus densiflora dominates the area surrounding Mt. Dalumsan in Kijang-gun. The upper vegetation includes *Pinus densiflora, Carpinus laxiflora, Alnus japonica, Quercus serrata,* and *Quercus aliena*. *Ternstroemia gymnanthera, Styrax japonicus, Styrax obassia Siebold &Zucc,* and *Lindera erythrocarpa Makino* inhabit in the middle layers. The lower layers contain *Zanthoxylum schinifolium, Corylus heterophylla, Lindera obtusiloba, Stephanandra incisa var. incisa, and Eurya japonica* (Table 1).

Research methods and classification

The trap was installed on April 20, 2018, and the pitfall survey was first started on May 4, and finished the survey on September 21, when the beetle was inactive. In the case of rainfall forecast, traps were collected on the previous day and re-installed on a clear day (Table 2).

The insect survey was conducted in the PWD control and non-control area, using the pitfall trap method (Lövei and Sunderland 1996; Niemelä et al. 2000; Jung et al. 2011) using characteristics of the environmental index species. Plastic food containers (depth: 12 cm; entrance diameter: 8 cm; floor diameter: 5 cm) with mixed fruits and jams were used as pitfall traps to collect beetles. The investigation was conducted using both the pitfall trap and the direct grab method.

The investigations were started from April 2018, when the beetles begin to move, and continued 10 times a month until September, when the beetles were almost inactive (Table 2).

A sphere of 20 m × 20 m was designated at the locations of 100 m, 200 m, and 300 m altitude each in the control area, where five traps were installed in each square hole.

The beetles collected by the pitfall trap at the survey site were naturally radioactive to the forest after they were classified. The direct investigation involved identification of the beetle species found in the field, and the samples of the species that were difficult to control were taken at the site and then classified by a conductor in the laboratory. We used the "checklists of insects from Korea" (Korean Journal of Entomology; Korean Journal of Applied Entomology 1994) and the insect ecology (Kim 1998) to categorize beetles.

Statistical analysis

The species and population of beetles collected in PWD control and non-control areas were classified according to month and altitude. Composition and diversity of species were analyzed in each field. The analysis was conducted using the Diversity Index (D) of Simpson (1949), the Coefficient Index (D) of Pielou (1975), and the Diversity Index of Shannon-Wiener. The equations representing these indexes are as follows:

Shannon-Wiener's diversity index
$$H` = -\Sigma Pilog\ (Pi);$$

Simpson's diversity index
$$D` = 1 - (\Sigma ni\ (ni-1))/(N\ (N-1);$$

The coefficient index
$$D = \Sigma Pi2;$$

Pielou's homogeneity index
$$E = H`/log\ (S),$$

Table 2. Collection date and method for this study

Times	Collection date	Collection method
1	May 4	Pitfall trap and direct collection
2	May 18	
3	June 1	
4	June 15	
5	July 5	
6	July 21	
7	August 3	
8	August 17	
9	September 7	
10	September 21	

Table 1. General characteristics of survey site

Site	Alt. (m)	Slope (°)	Aspect	DBH (cm)	H (m)	Density (No./ha)
PNW control	100-300	32	N	27.5	12.5	250
Non control	100-300	37	NW	25.4	11.5	570

where Pi=ni/N, ni: the population of the ith species, N: the total population, and S: the sum of the species.

In addition, a similarity index was analyzed between the two irradiated regions, using the results from a non-control and the control areas in Geomyeong-gun, where the formula applied is SI=2C/(A+B) and Si=2C/(A+B) (A: the Pine Wilt Disease Zone appeared as a common control).

Results and Discussion

Habitat distribution of beetles

In the PWD control and non-control areas, from May to September 2018, the beetles collected using trap and direct methods (Table 3) were classified into 12 families, 27 species and 939 individuals. The most abundant species were Caradidae (6 species, 469 individuals); Curculionidae (2 species, 130 individuals), Harpalidae (5 species, 115 individuals), Tenebrionidae (3 species, 56 individuals), Silphidae (1 species, 39 individuals), Cetoniidae (1 species, 37 individuals), Brachinidae (2 species, 34 individuals), Elateridae (1 species, 28 individuals), Lucanidae (1 species, 11 individuals), Cerambycidae (2 species, 11 individuals), Ruelidae (2 species 8 individuals), and Nitidulidae (1 species 1 individuals) (Table 4).

In the PWD control area, 386 individuals be longs to 21 species, 10 families were observed. The results were 5 species (208 individuals) of Caradidae, 2 species (87 individuals) of Curculionidae, 3 species (24 individuals) of Tenebrionidae, 4 species (19 individuals) of Harpalidae, 1 species (11 individuals) of Silphidae, 1 species (11 individuals) of Elateridae, 1 species (10 individuals) of Cetoniidae, 2 species (8 individuals) of Cerambycidae, 1 species (7 individuals) of Brachinidae, and 1 species (1 individuals) of Nitidulidae. The beetle distribution in PWD control area was 208 individuals out of 386 total population (54%), followed by Curculionidae (23%) and Tenebrionidae (6%), and more than half of the surveyed population were Carabidae in the PWD control area. The followed by Curculionidae and Tenebrionidae. Two families (Lucanidae and Ruelidae) and six species were not observed during the survey in the control area. These results indicate that the control of *Bursaphelenchus xylophilus* affected the pine forests.

Table 3. Species composition of Carabid beetles in the study site

Site	Family	No. of species	No. of individuals
PWD control	Curculionidae	2	87
	Tenebrionidae	3	24
	Cetoniidae	1	10
	Cerabidae	5	208
	Harpalodae	4	19
	Elateridae	1	11
	Cerambycidae	2	8
	Nitidulidae	1	1
	Silphidae	1	11
	Brachinidae	1	7
Non-control	Rutelidae	2	8
	Curculionidae	1	43
	Tenebrionidae	3	32
	Cetoniidae	1	27
	Cerabidae	6	261
	Harpalodae	5	96
	Elateridae	1	17
	Cerambycidae	1	3
	Silphidae	1	28
	Brachinidae	2	27
	Lucanidae	1	11

Table 4. Species and individuals of insects belonging to key families from the survey sites

Family	Species	Individual	Family	Species	Individual
Rutelidae	2	8	Elateridae	1	28
Curculionidae	2	130	Cerambycidae	2	11
Tenebrionidae	3	56	Nitidulidae	1	1
Cetoniidae	1	37	Silphidae	1	39
Cerabidae	6	469	Brachinidae	2	34
Harpalodae	5	115	Lucanidae	1	11

Also, 553 individuals be longs to 24 species, 11 family were found in the control area without PWD. The results were 6 species (261 individuals) of Caradidae, 5 species (96 individuals) of Harpalidae, 1 species (43 individuals) of Curculionidae, 1 species (28 individuals) of Silphidae, 2 species (27 individuals) of Brachinidae, 3 species (32 individuals) of Tenebrionidae, 1 species (27 individuals) of Cetoniidae, 1 species (17 individuals) of Elateridae, 1 species (11 individuals) of Lucanidae, 2 species (8 individuals) of Ruelidae, and 1 species (3 individuals) of Cerambycidae. In the control group, Nitidulidae in *Glicochilchilusipsoides*, Curculionidae in *Episomusturisus*, Cerambycidae in *Moechotypadiphysis*, and 3 family and 3 species were not identified. These results show that *Glischrochilusipsoides* dominate the pine forests and *Moechotypadiphysi, Episomusturritus* is considered to have little activity in the healthy pine forest. These results are similar to that reported from the sound forest ecosystem (Lee 2010) because *Moechotypadiphysis* in Cerambiodae and *Episomustruritus* in Curculationidae are common in the area around the dead trees (Table 3).

Sipalinus gigas gigas and *Damaster smaragdinus* accounted for about 13% of the total collected species, followed by *Calosoma maximowiczi* and *Carabussternbergi*. Therefore, the pine wilt disease controand a control, the population of Carabidae amounted to 469 individuals, of which approximately 50% were distributed, which is similar to the studies reporting a high ratio of Carabidae in the healthy forest areas (Lee et al. 2005; Lee 2010; Kim 2011).

Therefore, the total number in PWD control area was 1.4 times higher than that in control area, which is considered to affect the distribution of beetle caused by PWD control. Changes in forest ecosystems due to PWD control have been influenced by species diversity and abundance due to the influences of populations of small animal insects in forest ecosystems and temporary loss of arboreal pine trees treated for pine wilt disease that are still remaining in the forest had a combined effect on the overall ecosystem of the forest, which is presumably revealed in the change of population in the Carabinae habitat distribution (Greenberg and McGrane 1996; Ha and Lee 2017).

So far, no distribution of beetles in the Pine Wilt disease Embankment area has been investigated, but the results of the survey show that the density of the beetles was higher in areas that were less than insignificant in the forestry area (Lee 2010), the distribution of beetles in the forest ecosystem (Kim 2005), and the results of the survey on Bangtae-san similar to the distribution of the beetle of the control area in Busan Mountain in the forest area located on the coast and south of the temperate zone (Park 2013), there was a slight difference in the number of collectives and populations from the control area.

The dominant species appearing in forest areas were reported by *Synchus cycloders* and *Synchus nitidus* (Yeon et al. 2005; Jung et al. 2011). *Damaster smaragdinus*, *Calosoma maximowiczi*, and *Carabussternbergi* were dominant in Caradidae, followed by *Planetespuncticeps* in Harpalidae. These differences were due to PWD control. Therefore, the beetles, an insect whose habitat is sensitive to changes in the forest environment ecosystem, is a classification group close to the habitat distribution (Thiele 1977; Park et al. 2017), which necessitates some differences in the location of the survey site, the structure of the forest vegetation, and the survey methods (Jung et al. 2017).

In present study, beetle populations were inhabited by pine trees in the control area because pine trees were temporarily lost in the PWD area (Lee and Lee 1995). This result shows that the temporary loss of pine trees due to PWD affects the beetle community of environmental indicator species, together with the change of forest ecosystem.

Distribution of species by survey period

The family, species, and individuals observed in a monthly survey conducted from May to September 2018 in

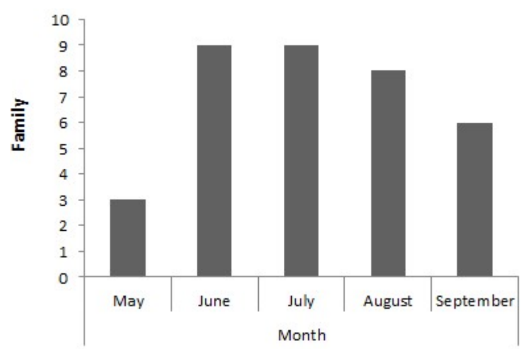

Fig. 2. Monthly number of families of collected beetles in the control site of pine wilt disease.

the PWD control area are shown in Figs. 2-4.

Monthly results of the PWD control area were collected in May, including 3 families in Curculionidae, and in June and July, including 9 families in Caradidae. In addition, 8 families strains including Silphidae in August, and 6 families strains including Tenebrionidae in September were identified (Fig. 2).

There were 8 species including *Episomusturritus* in May, 17 species including *Moechotypadiphysis* in June, and 18 species including *Silpha perforata perforata* in July. There were 16 species, including *Plesiophthalmus davidis*, in August, and 10 species in September, which indicate that the collected families and species in the summer are more diverse than in the spring and autumn due to dietary (Fig. 3). In addition, the number of individuals collected in July was 123 individuals (July), 92 individuals in June, 81 individuals in August, 61 individuals in May, and 29 individuals in September (Fig. 4). These results indicate that beetle activity is most effective from early spring to early autumn. It is considered that the survey period was very appropriate for this survey, and a large number of individuals were collected during active period.

In this study, the family, species, and individuals of beetles collected monthly in the area without PWD control are shown in Fig 4. It is the same as in Figs. 5-7. The monthly results were 3 families, including Rutelidae collected in May; 10 families, including Curculionidae in June and July; and 11 families including Lucanidae in August; and 10 families were confirmed in September (Fig. 5).

In addition, the monthly number collected from the control zone was 9 species, including the *Sipalinus gigas* in May, 20 species, including the *Calosoma maximowiczi* in June, and 22 species and 23 species in July and August, respectively, and was the largest monthly tally.

In September, 13 species, including *Planetespuncticeps*, were identified (Fig. 6). These results indicate that species

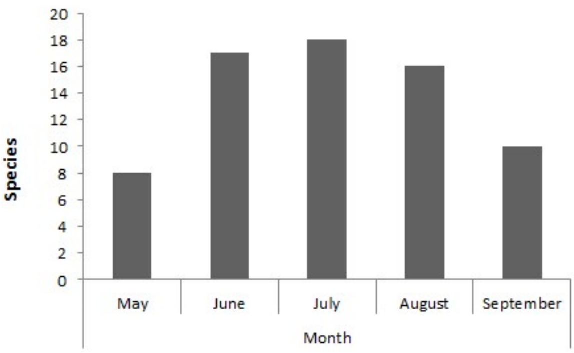

Fig. 3. Monthly number of species of collected beetles in the control site of pine wilt disease.

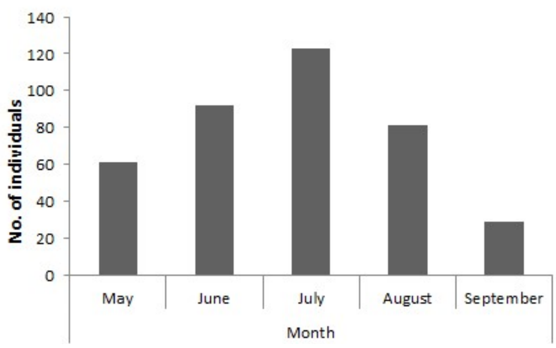

Fig. 4. Monthly number of individuals of collected beetles in the control site of pine wilt disease.

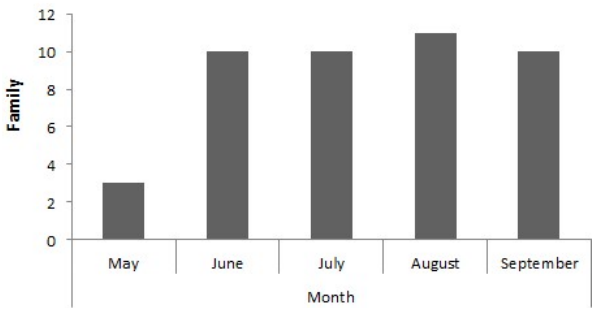

Fig. 5. Monthly number of families of collected beetles in the non-control site of pine wilt disease.

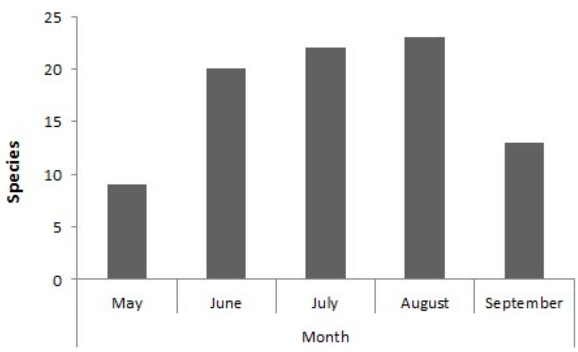

Fig. 6. Monthly number of species of collected beetles in the non-control site of pine wilt disease.

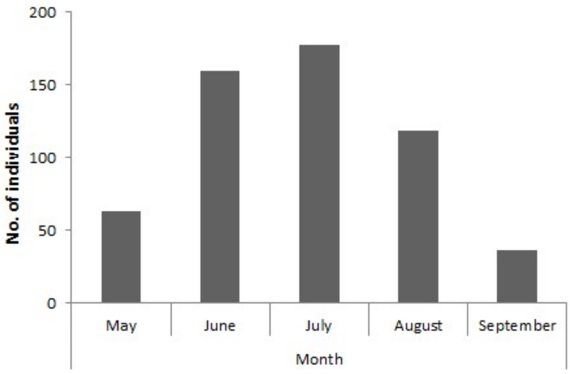

Fig. 7. Monthly number of individuals of collected beetles in the non-control site of pine wilt disease.

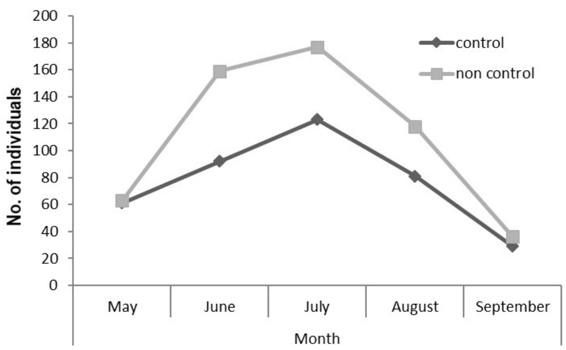

Fig. 8. Seasonal number of individual of beetles in survey site.

collected in the healthy forest ecosystems without PWD control are more likely to have healthy activities. In addition, the number of individuals was 177 individuals in July, 159 in June, 118 in August, and 63 and 36 individuals in May and September, respectively (Fig. 7).

In this study, the family, species, and indivisible areas of the beetle collected monthly showed that the collection trend was more concentrated in summer than in early spring and autumn (Fig. 8). Lee (2010) found an association between the seasons and numbers of beetles in the forested areas of pine trees: various families of beetles were identified between late spring and summer, and after September, the numbers of the beetles decreased. Kim (2004) collected the beetles from Mt. Ilwol, Yeongyang (located in North Gyeongsang Province, Korea), and found them in abundance in July and June.

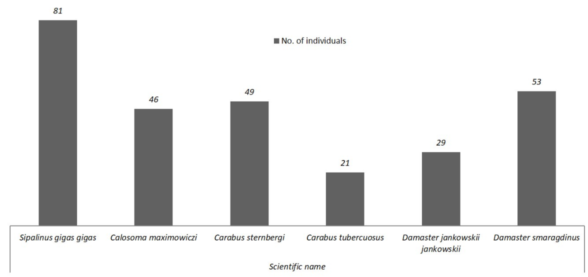

Fig. 9. Distribution of insect species in the control site of pine wilt disease.

Distribution of major species

The distribution of major species among the beetles examined in the PWD control and non-control areas was analyzed in Figs. 9 and 10. In the PWD control area, 6 species had more populations. *Sipalinus gigas gigas* were the most common with 81 individuals, and followed by *Damaster smaragdinus* (53 individuals), *Carabussternbergi* (49 individuals), *Calosoma maximowiczi* (46 individuals), *Carabus tubercuosus* (31 individuals), and *Damaster jankowskii jankowskii* (29 individuals) (Fig. 9). In addition, the results of a survey conducted by a control center found that in forest development without the PWD control, 9 species (including *Damaster smaragdinus*) were observed. The most common species was *Damaster smaragdinus*, with 70 individuals, followed by *Planetes puncticeps* (58 individuals), *Calosoma maximowiczi* (4 individuals), *Carabussternbergi* (51 individuals), *Damaster jankowski jankowski* (45 individuals), *Sipalinus gigas gigas* (43 individuals), *Carabus tubercuosus* (35 individuals), *Plesiophthalmus davidis* and *Silpha perforata perforata* (28 individuals) (Fig. 10). The major 6 species found in two irradiated regions, *Sipalinus gigas gigas*, *Calosoma maximowiczi*, *Carabus sternbergi*, *Carabus tubercuosus*, *Damaster jankowskii jankowskii*, *Planetes puncticeps*. *Sipalinus gigas gigas*, were distributed as major species in the control area. These results suggest that the population of *Sipalinus gigas gigas* increased with the disintegration of vinyl coating in the PWD control area. This is similar to the results of Lee's research on the distribution and diversity of species in the Naejang National Park (Kim 2011), and Park's study on the beetle distribution and diversity in Busan's Jangsan Mountain (Park 2013). In addition, Yeon et al. (2005) reported that the distribution of Cardiidae species was superior at Mt. Gapjang in Sangju, Korea, where the oak tree is the predominant species.

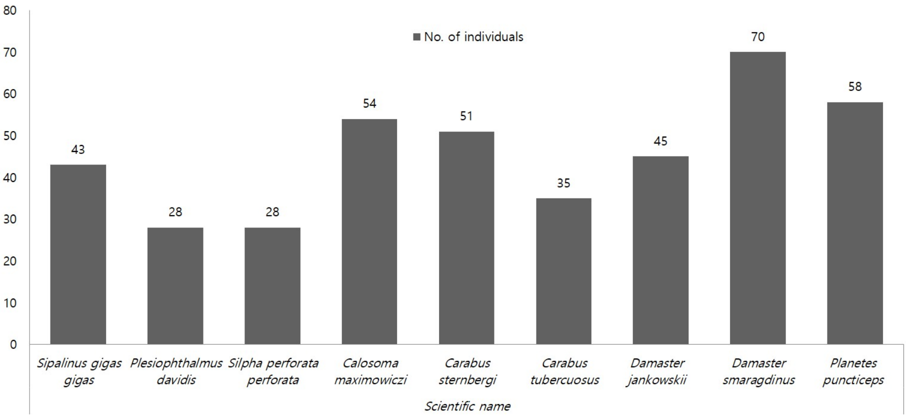

Fig. 10. Distribution of insect species in the non-control site of pine wilt disease.

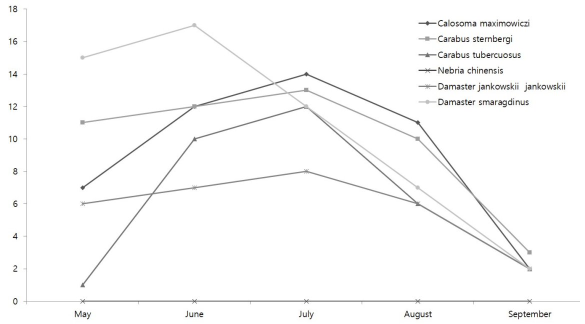

Fig. 11. Seasonal pattern of beetles (Cardiidae) species in the control site of pine wilt disease.

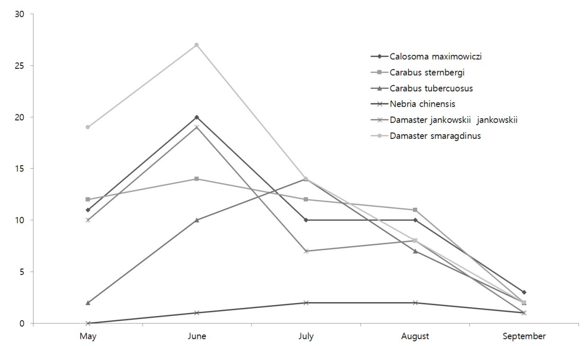

Fig. 12. Seasonal pattern of beetles (Cardiidae) species in the non-control site of pine wilt disease.

Periodic distribution of the Carabidae species

The results of this study are shown in Fig. 11 as a result of analyzing the time-to-time distribution of the collected species of Carabidae as environmental indicators. There were 6 species of Carabidae and 5 species of *Calosoma maximowiczi* in the PWD control area, and six species (including *Caloso mamaximowiczi*) were distributed in the control group. It was found that the distribution of the species in the PWD control area was the highest in July when *Caloso mamaximowiczi*, *Carabus sternbergi*, and *Carabus tubercuosus* were distributed, which is followed by June, August, May, and September. But *Damaster jankowskii jankowskii* had the highest distribution in May, followed by July, June, August, and September. *Damaster smaragdinus* was the most common in June, followed by May, July, August, and September, but *Nebria chinensis* was not distributed. These results suggest that *Nebria chinensis* is a sensitive species in PWD control area and it is considered to be an environmental indicator species in the same area.

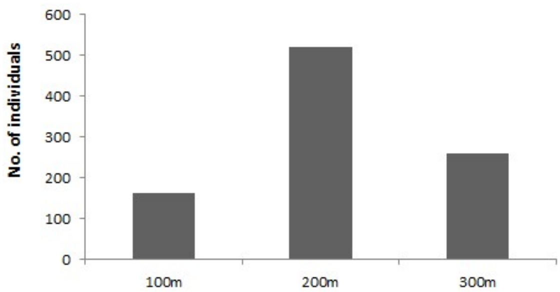

Fig. 13. Number of individuals collected from survey sites by altitude.

In the control group, Carabidae had 6 types of distribution, with no variations during the period, most common in July and June, followed by May, August, and September (Fig. 12). The change of species in the survey area is also thought to be closely related to the seasonal prevalence of beetles, and it is thought that non-distributed

Table 5. Comparison of species diversity indices of beetles in two survey sites

Site	No. of species	No. of individual	Diversity (H`)	Evenness (E)	Dominance (D)	Similarity index (%)
Insecticide control	21	386	0.764	0.812	0.367	80
Non-control	24	533	0.927	0.837	0.352	

species in the PWD control area should be continuously monitored in the future. It is also thought that the removal of vinyl sheath in the pine wilt disease control and affects the distribution of beetles.

Form distribution by elevation

In PWD control and non-control areas, the distribution of species observed in the 100 m, 200 m, and 300 m elevation zones were 163, 518 and 258 individuals, respectively (Fig. 13).

Such result shows similarity with the study result (Kim 2011) that indicates a higher population increase at 200 m or more than at 100 m by altitude. In addition, species in forests with low elevations are similar to those reported by Kim (2017), suggested that population and diversity increase at the higher altitudes than the lower altitudes.

Species diversity

Simpson's diversity index, dominance index, and Pielou's bacterial index for PWD control and non-control areas were analyzed (Table 5). The diversity index was 0.927 for the non-control, 0.764 for the PWD control, the dominance index was 0.837 for the non-control, 0.812 for the PWD control, and Pielou's index was 0.352 for the non-control and 0.367 for the PWD control. Therefore, in the survey paper, the control plane has a diversity index of 0.927, and the index value from other studies are: 0.840-0.910 (Jung et al. 2011) from Bangtaesan, Gangwon Province, 0.86-0.93 of Namdogyusan (Lee et al. 2010), 0.991-1.000 Deokyu Mountain (Jang 2008), and Yeong-yang Il-wolsan is 0.78-0.91 (Kim 2004). It turns out to be similar when compared to the back. In the PWD control area, the diversity index was lower than that of the previously studied areas and the species diversity index was considered to be low due to PWD control. The dominance index in this study area is 0.352 for the non-control and 0.367 for the PWD control area. The similarity of dominance in the two areas suggests that similar species do not form a specific cluster between two surveyed areas.

During the whole period, the evenness index was 0.837 and 0.812 between the non-control and PWD control areas. However, it is thought that the level of the evenness index is affected by the low level of PWD of deisement area and the appearance of *Episomustruritus* and *Glischrochilus ipsoides*. The dominance index was 0.367 in the non-control area and 0.352 in the PWD control area.

Kwon and Byun (1996), the dominant degree of *Gwangneung* beetle was 0.92, and the result was relatively different from that reported in the case of *S. cycloderus*, accounting for more than 80% of the total. The similarity index, on the other hand, was calculated to be 80% in order to examine the effect of PWD control on the distribution of beetle populations according to the results of the two sites (Table 5). The results of this analysis suggest that the similarity index of 80% in two sites is affected by *Bursaphelenchus xylophilus* control in the beetle cluster analysis.

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