Ambrosia beetle guild attacking a deciduous oak tree *Quercus serrata* in the Central Japan and species risk assessment in relation to potential invasiveness and aggressiveness based on niche analysis

（中部日本においてコナラに寄生するアンブロシアキクイムシのギルド構造およびニッチ解析による侵入生物と攻撃性のリスクアセスメント）

サングァンスップ　スニサ

Ambrosia beetle guild attacking a deciduous oak tree *Quercus serrata* in the Central Japan and species risk assessment in relation to potential invasiveness and aggressiveness based on niche analysis

by

Sunisa SANGUANSUB

Dissertation

Submitted to the University of Tokyo
for the degree of
Doctor of Philosophy

Graduate School of Agricultural and Life Sciences

2012

Advisory Committee:

Naoto Kamata
Katsumi Togashi
Tadashi Miyashita
Hisashi Kajimura
Masahi Murakami

ABSTRACT

For decades, secondary ambrosia beetle species have been increasingly observed to attack apparently healthy trees. Some of them cause mortality to living trees. In Japan, *Platypus quercivorus* that is widely distributed in Asia has caused mass mortality of trees belonging to the family Fagaceae by vectoring *Raffaelea quercivora*. Similar disease has been in epidemic in Korea by attacks of *Platypus koryoensis*. On the other hand, unexpected introduction of ambrosia beetles to non-indigenous area has been increasing because of globalization. Some has become major components of ambrosia beetle fauna in the non-indigenous area. The worst example among invasive ambrosia beetles is *Xyleborus glabratus* that causes laurel wilt in US by vectoring *Raffaelea lauricola*. The laurel wilt has caused enormous economic damage to avocado farmers in US. However, ecological backgrounds of these phenomena are still unclear. Therefore, ecological information on ambrosia beetles is needed from a view point of biodiversity conservation as well as economic importance. The purpose of this study was to determine ambrosia beetle guild, factors influencing the guild, and niche of each species attacking on an oak tree *Quercus serrata*. Risks of invasion and of vectoring tree-killing disease were evaluated for each species.

The study was conducted in three locations of the University of Tokyo Forests in the Central Japan. Timing of cutting trees (= TC) was controlled by preparing bolts monthly from March to September in Chichibu. They were covered by metal mesh to protect from insect attacks and left on the forest floor in the three locations: Aichi (LOC-A), Chiba (LOC-B), and Chichibu (LOC-C) (= LOC). The LOC-A had Japanese oak wilt (JOW). However, the LOC-B and the LOC-C had no historical records of JOW. Timing of exposure (= TE) was controlled by removing the metal mesh so that wood oldness (= WO) at the timing of exposure after cutting tree was also experimentally controlled. Bait bolts were exposed to insect attacks for one month in a Normal experiment, while they were left until the end of September in a Conditioning experiment. Twenty eight regimes, each consisted of four bolts, were set in each of a Normal and a Conditioning experiment so that the total number of bolts on each location was 224. Ambrosia beetles were collected by dissecting the bolts after the exposure and identified into species. The number of entry holes was used as an indicator for abundance of each species.

In the Normal experiment, twenty morphospecies of Scolytinae and six species of Platipodinae were collected. In the Conditioning experiment, six Scolytid and two Platypodid species disappeared,

whereas one Scolytid species recruited so that fifteen species of Scolytinae and four species of Platypodinae were collected. In total, twenty one morphospecies of Scolytinae and six species of Platipodinae were collected.

Species richness and abundance peaked on bolts prepared in April–May, on bolts exposed in July, and on 2–3-month-old bolts. Eliminating greatest influences of LOC on abundance, results of hierarchical partitioning showed that TC had a strong influence on both species richness and abundance. LOC-A (Aichi), in which Japanese oak wilt disease (JOW) incidence occurred, showed the greatest species richness and the smallest value of Pielou's evenness. Abundance of the most abundant ("the major") species was more than twice that of the second major species, which was a likely cause of the smallest evenness in LOC-A. Trees killed by JOW may have increased the abundance of the major species. On the contrary, in LOC-C (Chichibu), Pielou's evenness and alpha and gamma diversity and the Shannon index were greatest among the three locations although species richness was smallest. High similarity between guilds in LOC-A and LOC-B (Chiba) was probably caused by similarity in vegetation. The LOC had the greatest effect on determining guild structure. Effect of TE was greater than TC. The effect of WO was negligible. A hierarchical structure among the three factors was a likely cause of their relative importance determining guild structure.

Niche center and niche breadth were estimated for the three niche contexts. Colonizing ability of ambrosia beetles for bolts that had been exploited by other species. Abundance was compared between the Normal and the Conditioning experiments before each species stated to attack in Normal experiment. If the number in a regime No. 1 was smaller than 3 and no galleries were found in a regime No.2 and 8, the species was included in the analysis. A paired t-test was employed to test if the difference was significant. Species that have been reported as an alien species tended to have broader niche. All Scolytid species collected in my study have a habit of haplodiploidy and sibling mating so that Scolytid have a higher risk of invasion than Platypodid. Platypodid species tended to attack fresh bolts compared to Scolytid so that Platypodid have higher risk to vector tree-killing disease than Scolytid. Among Platypodinae, *Crossotarsus niponicus* and *Platypus calamus* significantly increased in the Conditioning experiment so that they are good competitors and high risk species of invasion. However, they did not show strong preference to fresh bolts compared to other Platypodid species. *Xylosandrus germanus* and *Xylosandrus crassiusculus*, which

attack living trees in non-indigenous area, also did not show a strong preference to fresh bolts so that there are no guarantees that *C. niponicus* and *P. calamus* are safe enough in non-indigenous area. *Xylosandrus germanus* and of *X. crassiusculus* supported that they became invasive alien species. *Euwallacea validus* significantly decreased in the Conditioning experiment compared to the Normal experiment so that this species seemed a poor competitor, whereas this species tended to attack fresh bolts. *Xyleborus ganshoensis* was considered as the highest potential to establish population in non-indigenous area because of its broad niche. *Xyleborus ganshoensis* was also likely to attack living trees since many individuals attacked fresh bolts but unlikely an invasive alien species. *Platypus quercivorus*, a vector of the Japanese oak wilt, showed the highest mean abundance on 0-month-old bolts. On the other hand, *Xyleborus seiryorensis* had the smallest value of WO niche center, indicating that this species could be aggressive enough to attack living trees. The method developed in this study would be applicable to similar risk assessment of ambrosia beetles on other host species and in other countries.

ACKNOWLEDGMENTS

This thesis arose in part out of years of research that has been done since I came to the University of Tokyo. By that time, a great number of people have supported my research in assorted ways and the making my thesis deserved special memorial. It is a pleasure to convey my gratitude to them all.

First and foremost, my utmost gratitude to my supervisor, Professor Dr. Naoto Kamata, for the intellectual supports all my study in Japan, without his help it is impossible for me to have been gained my knowledge. I am indebted to his patience with my careless, foolish, and for the logical and intellect that he impressed upon me.

I would also like to thank Dr. Hideaki Goto from Kyushu Research Center, Forestry and Forest Products Research Institute, providing guidance and support ambrosia beetles identification throughout my research. Special thanks to Dr. Roger Beaver for information on taxonomy. Special thanks also to all the members of the supervisory committee: Professor Dr. Kazumi Togashi, Professor Dr. Tadashi Miyashita, Professor Dr. Hisashi Kajimura, and Professor Dr. Masahi Murakami who gave valuable comments and suggestions for my thesis.

This thesis would not have been possible without the help and their kind support in field experiments of other staffs of the University forest, the University of Tokyo: Ken Watanabe, Yoshitaka Arakida, Haruo Sawada, Makoto Inoue, Motoshige Goto, and Michio Saiki in the Research Forests of the Ecohydrology Research Institute in Aichi. Kazunobu Iguchi, Masanori Suzuki, and Isamu Karakama in the University of Tokyo Chiba Forest. Shinsaku Shibano, Kazuya Omura, Takesshi Chishima, Ryusei Haraguchi, and Kazu Hirano in the University of Tokyo Chihibu Forest.

Thanks also must be given to Assistant Professors Dr. Toshihide Hirao for helpful advice on statistical analysis of ambrosia community structure, and to Assistant Professors Dr. Tadashi Maehara and Dr. Dai Kutsumoto for their help and allowance to use facility at the University of Tokyo Tanashi Forest. I thank to Dr. Hayato Masuya, Forestry and Forest Products Research Institute, for his advice for fungal isolation from ambrosia beetles. I also thank to Dr. Naoko Miura for checking English. The teaching staffs, office staffs, and friends in the Ecosystem Studies laboratory who helped me during my stay in Tokyo.

My graduate study was supported by The Ministry of Education, Culture, Sports, Science, and Technology under the Japanese Government and a Grant-in-Aid for Scientific Research [No. 20405027]

and [No. 23255011] from the Japanese Society for the Promotion of Science (JSPS) to Professors Dr. Naoto Kamata.

I am pleased to thank my parents for their unending love and everything in my life. Special thanks to Thai friends who gave moral supporting, encouragement, and sharing good time during my study in Japan.

TABLE OF CONTENTS

ABSTRACT i
ACKNOWLEDGMENTS iv
TABLE OF CONTENTS vi
LIST OF TABLES vii
LIST OF FIGURES ix
CHAPTER 1: INTRODUCTION 1
Figure 6
CHAPTER 2: GUILD STRUCTURE 7
2.1. Introduction 7
2.2. Materials and Methods 9
2.2.1. Study areas 9
2.2.2 Experimental design 9
2.2.3. Data analysis 10
2.3. Results 12
2.4. Discussion 16
Tables 22
Figures 29
CHAPTER 3: NICHE ANALYSIS AND RISK ASSESSMENT 33
3.1. Introduction 33
3. 2.Materials and Methods 35
3.2.1. Field experiments 35
3.2.2. Data analysis 36
3.3 Results 38
3.4. Discussion 41
Tables 49
Figures 54
CHAPTER 4: GENERAL DISCUSSION AND CONCLUSION 58
BIBLIOGRAPHY 62

LIST OF TABLES

Table 2-1 Experimental design of an artificial control for timing of cutting and timing of exposure of *Quercus serrata* bolts by covering and removing with wire net. 22

Table 2-2 The number of ambrosia beetle galleries and that of ambrosia beetle species found in Aichi (LOC-A), Chiba (LOC-B), and Chichibu (LOC-C), located in the Central Japan. 23

Table 2-3 Frequency of regimes and mean abundance in relation to the number of species in each regime (Aichi: LOC-A, Chiba: LOC-B, Chichibu: LOC-C). 24

Table 2-4 List of ambrosia beetle species baited by *Quercus serrata* bolts in Aichi (LOC-A), Chiba (LOC-B), and Chichibu (LOC-C), located in the Central Japan. Abundance and frequency of regimes, in which each species was found, are shown. 25

Table 2-5 Independent (*I*) and joint (*J*) contributions (the percentage of the total explained variance) of each predictor variable estimated from hierarchical partitioning for (a) the abundance and (b) the species richness. Significance of the independent contributions was tested by *Z*-scores obtained using 1,000 repeated randomizations. Asterisks indicate significant independent contributions ($P < 0.05$). 26

Table 2-6 Alpha, beta, and gamma diversity (species richness and Shannon index) of ambrosia beetle guilds on *Quercus serrata* bait bolts set in Aichi (LOC-A), Chiba (LOC-B), and Chichibu (LOC-C), located in the Central Japan. 27

Table 2-7 The results of multiple regression on distance matrices (MRM) analysis to test effects of factors determining community patterns of ambrosia beetles attacking *Quercus serrata* bait bolts in Aichi, Chiba, and Chichibu, located in the Central Japan. 28

Table 3-1 Abundance of ambrosia beetle species collected from *Quercus serrata* bait bolts in the three locations of the Central Japan. The number of entry holes was used as an indicator of the abundance of each species. Results for a Normal experiment (N) and for a Conditioning experiment (C) are shown. A paired *t*-test was employed to test the difference in the abundance between the two experiments in each location. 49

Table 3-2 Colonizing ability of ambrosia beetles for bolts that had been exploited by other species. Abundance was compared between the Normal and the Conditioning experiments before each species stated to attack in Normal experiment. If the number in a regime No. 1 was smaller than 3 and no galleries were found in a regime No.2 and 8, the species was included in the analysis. A paired t-test was employed to test if the difference was significant. 50

Table 3-3 Comparison between Normal and Conditioning experiments to test if species is good or bad in competition. (a): species that increased in a Conditioning experiment. (b): Species that decreased in Conditioning experiment. 51

Table 3-4 Summary table for categorized abundance, categorized niche breadth, and biology of ambrosia beetle species collected from *Quercus serrata* bait bolts in the three locations of Central Japan. Biology is shown for characteristics of arrhenotoky and of sibling mating, records of alien species, and aggressiveness attacking living trees and killing trees or branches, Schedl (1964), Wood (1982), Hoebeke (1991),Wood and Bright (1992), Vandenberg et al. (2000), Mudge et al. (2001), Haack (2006), Kajimura (2006), Lightle et al (2007), Biederman et al. (2009), Faccoli et al., 2009, Kirkendall and Faccoli (2010), Reed and Muzika (2010). 52

LIST OF FIGURES

Figure 1-1 Schematic illustration showing stages of biological invasion (Lockwood et al. 2007) 6

Figure 2-1 Scatter graphs of relationships between three factors (TC, TE, and WO) of abundance and species richness of ambrosia beetle. A dash line is a least square linear regression and black line is a smooth regression. (a)-(c) show relationship between $\log_{10}(X+0.5)$ of total abundance on each independent factors: (a) TC = timing of cutting tree (3-9: March - September), (b) TE = timing of exposure (3-9: March - September), (c) WO = wood oldness (0-6). (d)- (f) show relationship between species richness on each independent factors: (d) TC = timing of cutting tree (3-9: March - September), (e) TE = timing of exposure (3-9: March - September), (f) WO = wood oldness (0-6). 29

Figure 2-2 Pielou's evenness: J' and rank-abundance curve for ambrosia beetle species collected from *Quercus serrata* bait bolts that had been set in three locations, Central Japan: (a) Aichi (LOC-A), (b) Chiba (LOC-B), and (c) Chichibu (LOC-C).

$J' = -\sum_{i=1}^{S} p_i \ln p_i / \ln(S)$, where p_i is the proportion of each species in the sample, and S is the number of species.

1: *Ambrosiodmus lewisi* (Blandford), 2: *Ambrosiodmus rubricollis* (Eichhoff), 3:*Debus emarginatus* (Eichhoff),4: *Euwallacea validus* (Eichhoff), 5:*Leptoxyleborus depressus* (Eggers), 6: *Microperus kadoyamaensis* (Murayama), 7: *Planiculus bicolor* (Blandford), 8: *Truncaudum agnatum* (Eggers), 9: *Xyleborinus saxesenii* Ratzeburg, 10: *Xyleborus defensus* (Blandford), 11: *Xyleborus ganshoensis* Murayama, 12: *Xyleborus laetus* Niisima, 13: *Xyleborus pfeili* (Ratzeburg), 14: *Xyleborus seiryorensis* Murayama, 15: *Xyleborus seriatus* Blandford, 16: *Xyleborus volvulus* (Fabricius), 17: *Xylosandrus crassiusculus* (Motschulsky), 18: *Xylosandrus germanus* (Blandford), 19: *Crossotarsus niponicus* Blandford, 20: *Crossotarsus simplex* Murayama, 21: *Platypus calamus* Blandford, 22: *Platypus modestus* Blandford, 23: *Platypus quercivorus* (Murayama), 24:*Treptoplatypus solidus* (Walker). 30

Figure 2-3 Boxplot of ranked dissimilarity in ambrosia beetle guild collected by location. Pairwise comparisons were grouped as "Between" locations or within location (individual groups shown in the plot). There are significant differences among the three locations(R = 0.555, P = 0. 001). Boxes represent the median and interquartile range (IQR). Dissimilarity among three locations was greater than those within location. LOC-A: Aichi, LOC-B: Chiba, and LOC-C: Chichibu. 31

Figure 2-4 Non-metric multidimensional scaling (NMDS) ordination of ambrosia beetle guilds attacking *Quercus serrata* bait bolts in each experimental regime (01-28) in Aichi (LOC-A), Chiba (LOC-B), and Chichibu (LOC-C), located in the Central Japan. The NMDS is based on a distance matrix computed with Chao's dissimilarity index. The result with "noshare = 0.4" is shown. 32

Figure 3-1 A diagram of competitive ability of each of ambrosia beetle to exploit bolt. 53

Figure 3-2 Niche center and niche breadth of each of ambrosia beetles collected from *Quercus serrata* bait bolts in the three location of the Central Japan. The data are shown in a descendent order of niche breadth. Total abundance and mean abundance at each value of the niche context are also shown. (a) timing of cutting trees (TC = 3, 4, …, 9 indicate March, April,…, September, respectively), (b) timing of starting exposure (TE = 3, 4, …, 9 indicate March, April,…, September, respectively), (c) wood oldness (WO = 0, 1,…, 6) 54

Figure 3-3 Non-metric multidimensional scaling (NMDS) ordination of ambrosia beetle guilds attacking *Quercus serrata* bait bolts in three locations Central Japan, based on ambrosia beetle species (species codes are detailed in Table 3-1). The NMDS is based on a distance matrix computed with Chao's dissimilarity index. The result with "noshare = 0.1" is shown 57

CHAPTER 1: INTRODUCTION

Ambrosia beetles are an ecological guild, which is known as a group of fungus-farming beetles (Paracer and Ahmadjian 2000; Batra 1963; Kirkendall 1993; Mueller and Gerardo 2002) . They cultivate fungi on surface of tunnels that they construct in xylem and feed on them. Fungus-farming insects were known in three insect groups: termites (higher termite group) found in tropics of the Old World, a leaf cutter ant (*Atta cephalotes*) in tropics the New World and ambrosia beetles spread widely from tropics to cool temperate zone but prosperous mostly in tropics and sub-tropics.

More than 3,400 species of ambrosia beetles were described (Ganter 2006). Ambrosia beetles are found in two subfamilies of the family Curculionidae. Almost all species of the subfamily Platypodinae and some species in the subfamily Scolytinae are ambrosia beetles (Wood 1973; Beaver 1989). In the subfamily Scolytinae, the most major insect guild is a bark beetle, which lives in and feeds on phloem (inner bark) and has a similar ecological niche to an ambrosia beetle by playing a role in early stages of wood decay. One of great differences between ambrosia beetles and bark beetles is food: Ambrosia beetles feed on fungi whereas bark beetles feed on plant tissues. However, some bark beetles have symbiotic relationship with fungi. The relationship between bark beetles and fungi is classified into three categories: The first group is bark beetles that have no symbiotic relationships with fungi. The second group is phloeophagous bark beetles that have a symbiotic relationship with aggressive phytopathogenic fungi to come over host plant defense when they attack healthy living trees (Paine et al. 1997). The third group is mycophloeophagous bark beetles that develops better when they feed on plant materials conditioned by symbiotic fungi or completely depends on the conditioned plant materials (Klepzig and Six 2004). Ambrosia beetles are in one end of the spectrum of the insect-fungus symbiosis, in which both beetles and fungi depend completely each other (Beaver 1989) . It is estimated that fungal farming of ambrosia beetles has evolved at least seven times independently in the two subfamilies (Mueller and Gerardo 2002).

Although both leaf cutter ants and higher termites cultivate only fungi (Katoh et al. 2002; Poulsen and Boomsma 2005), ambrosia fungi are mixture of mycelial fungi, yeasts, and bacteria (Batra 1966; Haanstad and Norris 1985). However, the roles of microbes other than primary ambrosia fungi are, to date, not well known (Poulsen and Currie 2006). Ambrosia beetles have an organ called “mycangia” that is a set of morphological structure including pits to carry ambrosia fungi from brood tree to a new host plant

(Batra 1963; Beaver 1989). Mycangia are found in many parts of body such as mandibular, pronotum/mesonotum, propleuron, coxal cavity, or elytra (Beaver 1989; KnÍŽek and Beaver 2004). The position of mycangia depends on species but does not appear to be related to taxonomic position (Berryman 1989).

Bark beetles consume plant materials (phloem), which make them specific to host plant species, size, age, bark thickness, and part of host stem (Rudinsky 1962). On the contrary, ambrosia beetles are less specific with host plant because they feed on ambrosia fungi (Nobuchi 1974; Beaver 1989; Nobuchi 1994). It seems likely that ambrosia beetle can use host trees, in which symbiotic ambrosia fungi can colonize. For example, *Platypus quercivorus* (Murayama) have been reported to attack more than 50 species of woody plants (Kamata et al. 2008) although it can reproduce only on species belonging to the family Fagaceae excluding the genus *Fagus* (Ito 2008).

Plants have developed defense mechanisms against pathogens and herbivores (e.g. Walters 2011). Phloem is an important tissue for vascular plants to carry photosynthetic from foliage to roots. Woody plants produce defensive materials, such as resins, saps, and latex when they receive attack on branches and trunk. The attackers must overcome the plant defense so that all species cannot colonize on healthy living trees. Bark and ambrosia beetles can be classified into three groups according to their aggressiveness. Primary bark and ambrosia beetles can colonize full vigor and healthy host plants, and eventually kill host trees by mass attacks (Rudinsky 1962; Kühnholz et al. 2001). Secondary attackers colonize trees with unusual physiological condition such as freshly cutting logs, weakened by injury, suppressed, or dying trees (Rudinsky 1962), but sometimes attack healthy living trees and kills them in epidemics. Most ambrosia beetles are referred to as a secondary attacker. Saprophytic bark/ambrosia beetles can colonize dead trees, which includes most species of bark beetles.

In recent years unusual secondary ambrosia beetle species have been increasingly observed to attack living trees (Kühnholz et al. 2001). The epidemic populations of ambrosia beetles have been implicated as causes of mortality of woody plants. In Japan, *P. quercivorus* and *Raffaelea quercivora* complex kills healthy-looking trees of many species belonging to the family Fagaceae (the Japanese oak wilt: JOW) (Kamata et al. 2002). *Platypus quercivorus* is distributed in Asia Pacific Region, such as Taiwan (Murayama 1925), Indonesia (Schedl 1972), Thailand (Hulcr et al. 2008), India, Papua New

Guinea (Wood 1972), and Vietnam (Naoto Kamata, personal communication). Outside Japan, *P. quercivorus* is a secondary attacker infesting fallen trees or broken branches without causing problems to healthy-looking trees. Similar disease has suddenly emerged in Korea, in which *Quercus mongolica* Fischer ex Ledebour was killed by *Raffaelea quercus-mongolicae* K.H. Kim, Y.J. Choi, et H.D. Shin carried by *Platypus koryoensis* (Murayama) (Korean oak wilt: KOW) (Kim et al. 2009). These ambrosia beetles attack and kill apparently healthy-looking trees so that tree composition in forest stands will greatly be altered by the epidemics of those beetles. Furthermore, it is very likely that epidemics of these devastating disease change ambrosia beetles guilds directly by increase of vector insects and indirectly by producing resources for species that attack unhealthy and/or killed trees.

Wood-boring behavior of ambrosia beetles likely promotes opportunity to invade non-indigenous areas. Actually, many species have been intercepted at Japanese ports (e.g. Browne 1986) and US ports (Haack 2001; Haack 2006). Many ambrosia beetle species have established in US (Haack 2006) and in Europe (Kenis et al. 2009). Some alien ambrosia beetles have become major components of ambrosia beetle assemblages in invaded ranges (Reed and Muzika 2010). Furthermore, some alien ambrosia beetles have a tremendous impact on economics especially in US and EU (Gandhi et al. 2010; Kenis et al. 2009). *Xylosandrus crassiusculus* (Motschulsky) attacks and kills a wide variety of hosts in US (Horn and Horn 2006). However, the greatest economic impact of ambrosia beetles is the laurel wilt of avocado crops in US (Koch and Smith 2008). This is caused by *Raffaelea lauricola* T.C. Harr., Aghayeva, & Fraedrich. carried by *Xyleborus glabratus* (Eichhoff) (Hanula et al. 2008) though *X. glabratus* is a rare species in native of India, Japan and Taiwan (Harrington et al. 2011). To date the three species belonging to the genus *Raffaelea* are known to cause plant diseases (namely the JOW, the KOW, and the laurel wilt). The great difference of the laurel wilt compared to the JOW and the KOW is its virulence. As for the JOW, multiple inoculation by *R. quercivora* caused by mass attacks by *P. quercivorus* is needed to kill a healthy living oak tree (Kinuura and Kobayashi 2006) as well as the KOW (Kim et al. 2009). On the contrary, *R. lauricola* can kill avocado trees by a single infection by the fungus (Fraedrich et al. 2008 ; Ploetz et al. 2012). It probably depends on a lack of coevolutionary process between the plant (avocado trees) and the fungus (*R. lauricola*) (Kamata et al. 2002) by overreaction of trees killing tree itself (Hulcr and Dunn

2011). It is very likely that the lethal encounters between exotic symbionts and naive trees (Hulcr and Dunn 2011) will increase by climate change and globalization in future.

I determined ambrosia beetle guild attacking on an oak tree *Quercus serrata* Murray by controlling timing of cutting trees (TC), seasonality (timing of exposure = TE), wood oldness (WO), and in three locations in the Central Japan. My major objectives are as follows:

1) To analyze patterns of diversity based on the guild structure in relation to location, wood oldness, and seasonality.

2) To evaluate ecological niche of each ambrosia beetle species for risk assessment in relation to invasiveness and aggressiveness.

1. Pattern of diversity based on guild structure of ambrosia beetles community

Many attempts have been conducted to describe community/guild structure and determine factors that prescribe the structure (Kikkawa and Anderson 1986; Lawton and MacGarvin 1985). Because of influences the most of the question when I conduct research in community ecology is how organisms change when they received effort by variable conditions of environments. Community structure has been emphasizes on environmental conditions that regulate community type and population dynamics (Schowalter 2011).To test which factors influencing diversity pattern of ambrosia beetle I determine the importance of four variable factors as predictors for ambrosia beetle community structure: food available conditions (WO is closely related to wood conditioning), the succession of ambrosia beetles based on seasonal occurrence of beetles species (Timing of exposure (TE)), and Timing of cutting (TC) is related to the both timing of cutting, timing of exposure and location related to spatial scales of geographic distance (beta diversity).

2. Niche analysis of ambrosia beetles and risk assessments

From chapter two, niche partition based on four controlled factors has effect to generate diversity in ambrosia beetles. The number of publications of alien ambrosia beetles increased and play as pests in new environment. I estimated ecological niche of ambrosia beetles species that infest oak trees, to evaluate the ability itself to establish in new area. My hypotheses are species with broader niche are advantageous and species preferred wood soon after cutting have a potential for causing a vector insect.

Regarding to a process of biological invasion, there are several discrete stages that an alien species passes through. Fig. 3-1-1 is a schematic illustration picturing the stages (Lockwood et al. 2007). According to Lockwood et al (2007), I separated the process into four stages: transport, establishment, spread, and impact.

According to the Conservation on Biological Diversity (CBD) (http://www.cbd.int/), I used the two terms as follows: ‘Alien species’ indicates species that has established its population in non-indigenous area. ‘Invasive alien species’ indicates an alien species whose introduction and/or spread threaten biological diversity.

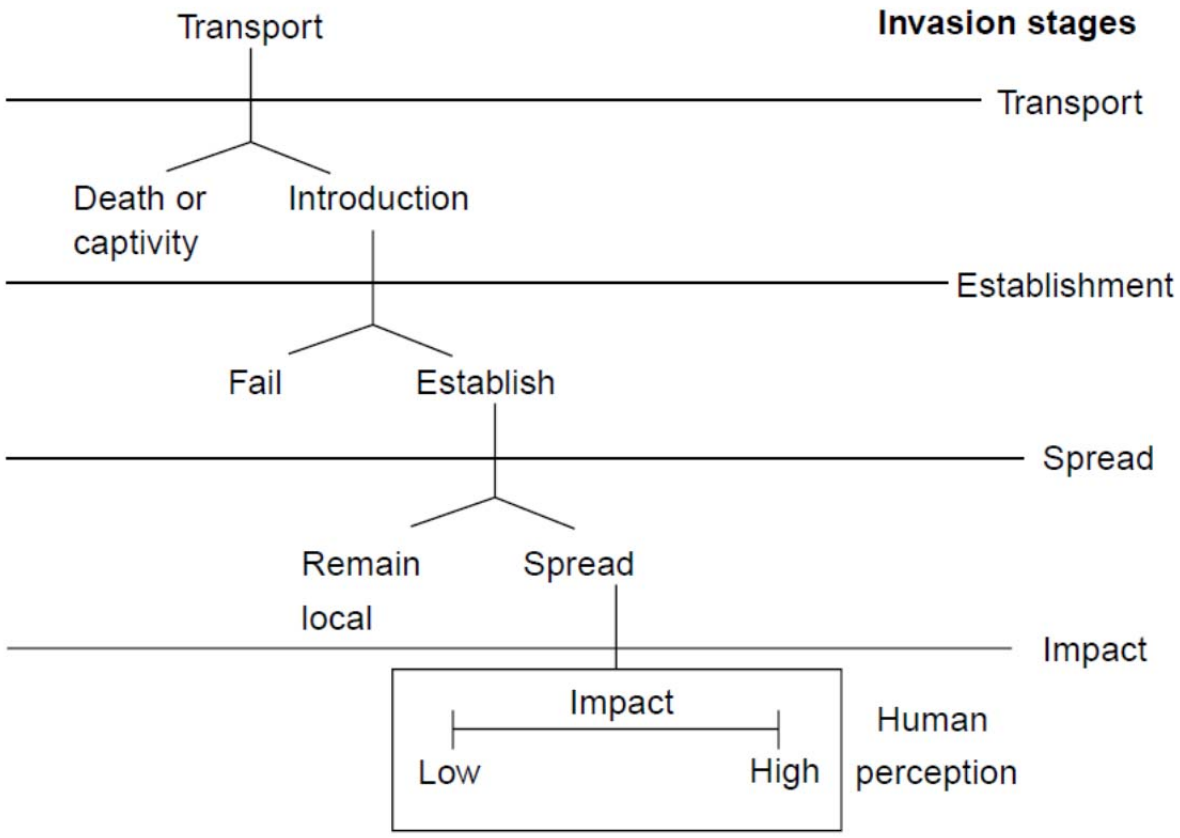


Figure 1-1 Schematic illustration showing stages of biological invasion (Lockwood et al. 2007).

CHAPTER 2: GUILD STRUCTURE

2.1. Introduction

Host plant is one ecological niche of ambrosia beetles (Kabe 1960; Beaver and Shih 2003). However, in general ambrosia beetles have broad host ranges compared to other wood-boring insect groups that attack similar status of host trees because ambrosia beetles feed on fungi not on plant materials (Nobuchi 1974; Beaver 1989; Nobuchi 1994). Some species can attack several tree families. Others attack trees in a particular plant family: For example, the woody plants belonging to the family Fagaceae are considered as essential hosts of *P. quercivorus* because the beetle can reproduce only on Fagaceae trees although its attacks are found in other family of plants (Ito 2008).

Another ecological niche axis important for ambrosia beetles is seasonality. Adults of each species fly at a certain season depending on its life history parameters relating to timing of emergence, such as the number of generation per year, development rate, and diapause. For example, in US, attacks of *X. crassiusculus* are found from May to July (Oliver and Mannion 2001). *Xylosandrus germanus* (Blandford) is a multivoltine insect that is most abundant in ethanol traps in mid-May and early August (Oliver and Mannion 2001; Ranger et al. 2010). *Platypus quercivorus* is basically univoltine but partially bivoltine in Japan (Sone et al. 1998). Emergence of the main overwintering generation is observed from May to September with a peak in early July, and emergence of the second generation has been observed from late August to early December (Kamata et al. 2002).

There is a definite succession of wood-feeding insects in logs as the chemical and physical character of the wood changes during the process of disintegration and decay (Graham 1925; Yoshikawa 1987). Knight and Heikkenen (1980) classified wood-feeding insects into two categories: primary and secondary. Primary insects are able to attack a healthy, living tree and complete their normal development, whereas secondary insects are incapable of attacking and completing normal development in healthy trees. Some ambrosia beetles can attack healthy trees (Kühnholz et al. 2001; Kajimura 2006). However, most ambrosia beetles attack weakened trees, fallen trees, or broken branches and play roles in early stages of wood decay (Carpenter et al. 1988; Edmonds and Eglitis 1989). Tree vigor is important when host trees are still alive because only primary attackers infest healthy trees. When trees receive stress and become unhealthy, then the secondary attackers can colonize. After the tree mortality, the chemical and physical

characters of the wood changes during the process of disintegration and decay is important (Graham 1925; Yoshikawa 1987). Therefore the occurrences of a favorable condition of hosts and their timing seem essential to determine ambrosia beetle guild. Yoshikawa et al. (1986) and Yoshikawa (1987) studied wood- boring insects on a Japanese red pine, *Pinus densiflora* (Siebold et Zuccar), in relation to oldness and seasonality. Many Coleopteran insects including Cerambycidae, Curculioninae, Scolytinae, were collected in their study although no ambrosia beetles were collected. However, relative importance of each factor (oldness and seasonality) in determining the insect guilds were not evaluated (Yoshikawa et al. 1986; Yoshikawa 1987). Annual temperature, humidity, and host tree vigor were investigated in relation to bark beetle and ambrosia beetle community (Hulcr et al. 2008; Reed and Muzika 2010). However, no studies have been conducted to evaluate relative importance of each of factors in determining community structure of ambrosia beetles.

Recently, epidemic populations of ambrosia beetles have been implicated as causes of massive mortality of woody plants: Laurel wilt disease that is caused by *R. lauricola* carried by *X. glabratus* has been spreading in avocado plantation in US (Koch and Smith 2008). *Xylosandrus crassiusculus* also attacks and kills a wide variety of hosts in US (Horn and Horn 2006). In Japan, *P. quercivorus* and *R. quercivora* complex kills many tree species belonging to the family Fagaceae (the Japanese oak wilt: JOW) (Kamata et al. 2002). Similar disease has suddenly emerged in Korea, in which *Q. mongolica* was killed by *R. quercus-mongolicae* carried by *P. koryoensis* (Kim et al. 2009). These ambrosia beetles attack apparently healthy-looking trees. It is very likely that epidemics of these devastating disease change ambrosia beetles guilds directly by increase of vector insects and indirectly by producing resources for species that attack unhealthy and/or killed trees.

An objective of this study is to determine ambrosia beetle guilds attacking *Q. serrata* in relation to seasonality and wood oldness. My experiment was carried out in one location with JOW and in two locations without JOW, all located in the Central Japan. I investigated ambrosia beetle guilds attacking *Q. serrata* bait logs in which timing of cutting trees and timing of exposure were artificially controlled. Influences of location, timing of cutting, timing of exposure, and wood oldness on the guild structure were analyzed. Relative importance of each factor was also discussed.

2.2. Materials and Methods

2.2.1. Study areas

The study was conducted in three University Forests, the University of Tokyo, located in the Central Japan: Aichi (LOC-A, 35˚22´N, 136˚59´E, 95 m asl), Chiba (LOC-B, 35˚09´N, 140˚08´E, 280 m asl), and Chichibu (LOC-C, 35˚54´N, 138˚59´E, 820 m asl). Average of the monthly mean temperature from March to September 2009 was 20.0ºC at Mino-Kamo (c. 10km apart from LOC-A) (Japan Meteorological Agency 2009), 17.5ºC at Kiyosumi (c. 2 km apart from LOC-B), and 14.6°C at Ochigawa (exactly the same location as LOC-C) (The University Forest 2011). Among the three locations, only LOC-A had JOW. The other two locations had no incidence or historical records of JOW.

2.2.2 Experimental design

Every month from March to September 2009, healthy *Q. serrata* trees (c. 25 years old, 15-25 cm in DBH) were cut in an oak stand 20 km apart from LOC-C and prepared to 60-cm-long bolts for baits. Those were individually covered with wire net to protect from insect attacks after being coated the cut ends of bolts with paraffin wax to prevent desiccation, and were left on forest floor in each of the three locations. Each bolt was exposed to insect attacks by removing the wire net for one month. Table 2-1 shows the experiment design indicating timing of cutting tree (TC) and timing of exposure (TE), and wood oldness (WO) of each experimental regime. The WO is given by difference in the number of months between timing of exposure and timing of cutting trees. Twenty eight regimes were set in each of the three locations. Each regime in each location consisted of four bolts. After the one-month exposure, ambrosia beetle guild in each bolt was determined by dissection. I marked all entry holes on the surface of inner bark and that of the most outer part of sapwood after debarking to collect ambrosia beetles. The number of the entry holes, which is equal to the number of galleries, was counted by matching inner bark and sapwood. Insects in the bark were checked at first, and insects in the sapwood were checked by breaking the bolts into small pieces. Insects collected from each gallery were kept separately in a bottle with 70% ethanol. Thereafter, adults were removed from the bottle and prepared to pin specimens for further identification. Ambrosia beetle species was identified for each gallery. If adults were badly damaged for identification or not collected

from a gallery, the gallery was dealt with as “unidentified gallery”. Voucher specimens were deposited in the main office of the University of Tokyo Chichibu Forest (UTCF).

2.2.3. Data analysis

All the following analyses were done using the statistical software R version 2.11.1 (R Development Core Team 2010).

To determine relationship of ambrosia beetle abundance and that of their species richness with each of independent factors (TC, TE, WO), scatter graphs with a regression line and a smooth curve (span for smooth = 0.5) were obtained by a library ‘car (Version: 2.0-10) (Fox and Weisberg 2011, 2011a)’ for the statistical software R. Regarding to abundance, the values (X) were transformed by $\log_{10}(X+0.5)$ to fit normal distribution (Yamamura 1999).

Hierarchical partitioning (HP) was employed to determine effects of independent factors (TC, TE, WO, and location (LOC)) on ambrosia beetle abundance and their species richness because high level of multicollinearity among the three independent factors derived from experimental design (WO = TE -TC) should be avoided (Chevan and Sutherland 1991; Mac Nally 1996, 2000). Because both abundance and species richness were likely to have a nonlinear relationship with the three independent factors (TC, TE and WO), squares of these values were added to the HP procedure as independent variables (TC2: square transformed TC; TE2: square transformed TE; WO2: square transformed WO). The HP protocol fitted a series of generalized linear models with a Poisson error distribution and log link function. As the goodness-of-fit measure, R^2 was used. The distribution of the explanatory values (independent influence: I) was calculated by randomizing the data matrix 1000 times to determine the significance for each variable (Mac Nally 2002). The results of this significance test are expressed as Z-scores. If the observed I value fell above the upper 95% confidence limit of the normal distribution of Z-scores (1.65), I considered the independent factor was significant. This HP procedure was carried out for each of species richness and abundance in each experimental regime.

Distributions of abundance of ambrosia beetles in a regime was~~were~~ compared among three locations by using Chi-square test. Fisher's exact test was used to compare proportion of “endemic species” between Platypodinae and Scolytinae, which were found only in one location.

To evaluate community diversity, Shannon index (H') were obtained by the following equation:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i ,$$

where P_i is the proportion of each species in the sample (Shannon and Weaver 1964).

The number of galleries of each ambrosia beetle species and the number of species in each regime were calculated from the original data. First, rank-abundance curves and Pielou's evenness (J') (Pielou 1966) were obtained to compare species evenness among three locations. The Pielou's evenness (J') was provided by the following equation:

$$J' = -\sum_{i=1}^{S} p_i \ln p_i / \ln (S) = H'/ \ln (S),$$

where S is the number of species (Pielou 1966).

The differences of all diversity indices among three locations were tested by Kruskal-Wallis multiple comparisons by using a subroutine program for the statistical software R by Aoki (2009) .

Whittaker (1972) described three terms for measuring biodiversity over spatial scales: alpha, beta, and gamma diversity. Gamma diversity is a measure of the overall diversity for the different community within a region, which is partitioned into within-community (alpha diversity) and between-community components (beta diversity). Regarding to species richness, gamma diversity is multiplicatively partitioned into alpha diversity and beta diversity (Crist et al. 2003; Jost 2007):

Gamma = alpha · beta.

In my experiment, the gamma species richness was a total number of species found in overall regimes. The alpha species richness was a weighted mean of the number of species in each regime, in which the weights were determined by the proportion of the total number of individuals found in each regime. To obtain the beta species richness for each location, the gamma value was divided by the alpha value (Jost 2007). On the contrary, regarding to Shannon index, gamma diversity is additively partitioned into alpha diversity and additive beta diversity (Jost 2007):

Gamma = alpha + beta.

In my experiment, the gamma was Shannon index (H') that was calculated from whole data in each location. The alpha Shannon was the weighted mean of H', in which the weights were determined by

the proportion of the total number of individuals found in each regime. To obtain the beta Shannon for each location, the alpha was subtracted from the gamma (Jost 2007).

Sensitivity analysis for Pielou's *J′* in LOC-A was conducted by removing the major species in the location. Because the value in LOC-A was much greater than those in LOC-B and LOC-C, changes in the values were checked when major species were excluded from calculation for Pielou's *J′*.

To test dissimilarity of guild structure among and within locations, analysis of similarities (ANOSIM) (Clarke 1993) was performed using Chao's abundance-based Jaccard index (Chao et al. 2005). Dissimilarity within location was also compared among the three locations. Non-metric multidimensional scaling (NMDS) (Minchin 1987) was employed to determine similarity patterns in ambrosia beetle guild among regimes. Regimes with no identified galleries were omitted from the NMDS analysis. Ordination may be difficult if a large proportion of sites have no shared species so that step-across dissimilarities were employed to improve the problem by stepwise 0.1 decrements of the parameter "noshare" (1 to 0.1) in a library 'vegan (version 1.17-3) (Oksanen et al. 2010a, b)' of the statistical software R. To determine effects of factors on dissimilarity between ambrosia beetle guilds, multiple regressions on distance matrices (MRM) (Lichstein 2007) was performed using Chao's abundance-based Jaccard index. Significant effects of TC, TE, WO, and LOC were tested. Standardized partial coefficient of each of the significant independent factors was also calculated to determine their relative importance.

A package 'vegan' version 1.17-3 (Oksanen et al. 2010a, b) was used for Pielou's *J′*, Shanon-Wiener *H′*, ANOSIM, NMDS, Chao's abundance-based Jaccard index, and MRM. A package 'car' Version: 2.0-10 (Fox and Weisberg 2011, 2011a) was used for scatterplot matrices. A package 'hier.part' Version: 1.0-3 (Walsh and Mac Nally 2008a, b) was used for HP.

2.3. Results

In total, 2,333 galleries of ambrosia beetles were found from three locations. Among these, 1,765 were identified. Five hundred sixty eight were not able to be identified into species because of empty holes including missing insects, or of mechanical damage of specimens (Table 2-2). Twenty four species were identified as the subfamily Scolytinae (18 species; 1,371 galleries) and Platypodinae (6 species; 394 galleries). The species richness was 18 in LOC-A, 16 in LOC-B, and 14 in LOC-C.

Tables 2-3 shows frequency of regimes and mean abundance in relation to the number of obtained ambrosia beetle species in each regime. The mean abundance tended to be great with the number of species. Median of the number of species was greatest in LOC-A (median = 4), followed by LOC-B (median = 3), and smallest in LOC-C (median = 2). In LOC-C, no ambrosia beetles were caught in two regimes. Only one species was found in nine regimes with low abundance (mean =2.0). A proportion of regimes where only one species was found differed significantly among three locations (Chi-square test, Chi-squared = 31.0, $df = 2$, $P < 0.001$). The proportion in LOC-C (9/26 = 0.35) was greater than that in the other two locations (LOC-A: 1/26 = 0.04, LOC-B: 5/27 = 0.19). Significant difference was found between LOC-A and LOC-C (Chi-square test, Chi-squared = 6.07, $df = 1$, $P = 0.014$) but not between LOC-B and LOC-C (Chi-square test, Chi-squared = 1.04, $df = 1$, $P = 0.309$).

Table 2-4 shows a list of ambrosia beetle species with their abundance and the numbers of regimes, in which each species was found. Seven species were found only in one location (7/24 = 0.29). Ten were found in two locations (10/ 24 = 0.42). Seven were found in all the three locations (7/24 = 0.29). Regarding to Scolytinae, two species were found in one location (2/18 = 0.11). Nine were found in two locations (9/18 = 0.50). Seven were found in the three locations (7/18 = 0.39). One Platypodine species was found in two locations (1/6 = 0.17). Five Platypodine species (5/6 = 0.83) were found only in one location. The percentage of the "endemic species", which were found only in one location, was significantly greater in the subfamily Platypodinae than in Scolytinae (Scolytinae: 2/18 = 0.10, Platypodinae: 5/6 = 0.83, Fisher's Exact Test, $P = 0.010$). In LOC-A, in which the JOW was epidemic, the most abundant species was *M. kadoyamaensis*, followed by *P. quercivorus*, a vector of JOW, *Planiculus bicolor* (Blandford), and *Xyleborus defensus* Blandford. Their numbers were greater than those of the most abundant species in LOC-B: *Xyleborus ganshoensis* Murayama and LOC-C: *Platypus modestus* Blandford. The most major species in LOC-A was more than twice as an abundant as *P. quercivorus*, the second major species. The major species in the LOC-A were found in many regimes, with one exception of *P. quercivorus*. LOC-A and LOC-B had 62.0% of shared species, namely thirteen among twenty one species were found in both the two locations (13/21 = 0.620). Between LOC-A and LOC-C, the percentage was 45.5% (10/22 = 0.455). However, between LOC-B and LOC-C, the percentage was 40.0% (8/20 = 0.400). Two species out of eighteen (11.1%) were exclusively found in LOC-A: *P. quercivorus* (second major species in LOC-A),

and *Truncaudum agnatum* (Eggers) (the most minor species). Two species out of sixteen (12.5%) were found only in LOC-B: *Leptoxyleborus depressus* (Eggers) (fourth major species in LOC-B), and *Crossotarsus simplex* Murayama (seventh major species). Three species out of fourteen (21.4%) were found only in LOC-C: *P. modestus* (the most major species in LOC-C), *Crossotarsus niponicus* Blandford (tenth major species), and *Treptoplatypus solidus* (Walker) (the most minor species).

Figure 2-1 shows relationships between each of independent factors (TC, TE, and WO) and ambrosia beetle abundance and their species richness. All graphs had a peak indicating non-linear relationships. Abundance peaked in April for TC, in July for TE and two-months old for WO. Species richness peaked in May for TC, in July for TE and three-months old for WO.

Table 2-5 shows a result of HP analysis showing the relative contributions of independent and joint contribution of each factor in abundance and species richness. The majority of the explained variation was related to independent effects of the variables in both abundance (97.3%) and species richness (98.3%). Regarding to abundance (Table 2-5a), independent effects were significant only for LOC (55.3%), TE2 (11.2%), and TC2 (10.4%).The LOC had the highest total effects (55.3%) followed by TC2 (17.6%) and TC (14.3%). The TE2 had the second highest independent effects, while it had small total effects depending on negative joint effects. Variables relating to timing of exposure (TE, TE2) and wood oldness (WO, WO2) had small effects with one exception of the independent effect of the TE2. On the contrary, regarding to species richness (Table 2-5b), independent effects of all factors were significant except for WO2. The greatest total (independent + joint) value was shown in TC2 (34.2%) followed by TC (25.8%). The TC2 also had the highest independent contribution (23.9%) followed by LOC (15.9%) and TE2 (15.5%). The WO and the WO2 had small total effects depending on small independent effects and negative joint effects.

Figure 2-2 shows a rank-abundance curve and Pielou's evenness *J′*. A rank-abundance curve was sharpest in LOC-A with the smallest Pielou's evenness *J′* (0.59) (Fig. 2-1a). Pielou's evenness *J′* in LOC-A increased to 0.62 by excluding the most major species (*M. kadoyamaensis*) and to 0.69 by excluding four major species (*M. kadoyamaensis, P. quercivorus*, *P. bicolor*, *X. defensus*). On the contrary, those of LOC-B and LOC-C showed shallow gradient indicating more even species composition (Figs 2-1b and 2-

1c). The result was supported by the greatest Pielou's evenness J' in LOC-C (0.78) followed by LOC-B (0.70).

Table 2-6 shows alpha, beta, and gamma species richness and those for Shannon index with their arithmetic mean. Regarding to species richness, the arithmetic mean was greatest in LOC-A, and smallest in LOC-C (Kruskal-Wallis multiple comparisons, LOC-A vs. LOC-B: Chi-square = 3.12, $P = 0.21$; LOC-B vs. LOC-C: Chi-square = 0.43, $P = 0.80$; LOC-C vs LOC-A: Chi-square = 5.88, $P = 0.05$). In LOC-A, alpha and gamma species richness were greatest among the three locations. Alpha species richness was smallest in LOC-B. Beta species richness and proportion of beta species richness in gamma were smallest in the LOC-C among the three locations. Regarding to Shannon index H', the arithmetic mean for each regime was greatest in LOC-A, and smallest in LOC-C although no significant differences were found (Kruskal-Wallis multiple comparisons, LOC-A vs. LOC-B: Chi-square = 1.74, $P = 0.42$; LOC-B vs. LOC-C: Chi-square = 0.22, $P = 0.90$; LOC-C vs LOC-A: Chi-square = 3.18, $P = 0.20$). Beta Shannon index was greatest in LOC-B and smallest in LOC-A. Alpha Shannon index (the weighted mean) and gamma Shannon index were greatest in LOC-C. When I exclude species from the calculation, of which abundance was one in each location, beta Shannon was greatest in LOC-B (0.96), followed by LOC-C (0.89), and smallest in LOC-A (0.69).

Figure 2-3 shows a result of ANOSIM. Significant dissimilarity in guild structure was found among locations ($R = 0.555$, $P = 0.001$). Dissimilarity among three locations was greater than those within location. Among-regime dissimilarity within each location was smallest in LOC-A and greatest in LOC-C.

Figure 2-4 shows a result of NMDS analysis. Because a stress value in NMDS decreased greatly from 0.215 to 0.140 when "noshare" value changed from 0.4 to 0.5, I adopted the NMDS result with "noshare = 0.4". The result indicated that guilds were grouped by location. However, LOC-B group and LOC-A group crossover each other indicating that similarity between LOC-A and LOC-B was higher than that with LOC-C. LOC-C showed a unique ambrosia beetle guilds because regimes in LOC-C made a cluster separate from the other two locations. In LOC-A, many regimes were packed compactly, which indicates that similarity among regimes in LOC-A was high, supporting a result of ANOSIM (Fig. 2-3).

A result of MRM for all factors except for WO significantly influenced guild structure ($P < 0.05$) (Table 2-7). Standardized partial regression coefficients indicated that effect of location was greatest

among the four factors, supporting the result of NMDS (Fig. 2-4). The effect of LOC was 4.84 times as great as that of TC, 3.08 times as great as TE, and 30.8 times as great as WO. The effect of TE was 1.57 times as great as that of TC.

2.4. Discussion

Funnel traps or intercept panel traps with attractants are commonly used to study ambrosia beetle communities (Saito et al. 2005; Hulcr et al. 2008; Reed and Muzika 2010). In the University Forests in LOC-C, Saito et al. (2005) investigated Platypodinae and Scolytinae assemblages by ethanol bait traps for nine years, in which subfamily Platypodinae (not identified into species) and twelve species of Scolytinae ambrosia beetles were recorded. I checked the voucher specimens that was deposited in the main office of UTCF and confirmed that species belonging to the subfamily Platypodinae were completely the same as I collected in this study (Table 2-4): *C. niponicus*, *P. modestus*, and *T. solidus*. Regarding to Scolytinae ambrosia beetles, eleven species were observed in my experiment and twelve species in Saito et al. (2005). Six species of the Scolytinae were shared between Saito et al. (2005) and my study. They are *Ambrosiodmus lewisi* (Blandford) (=*Xyleborus lewisi*), *Euwallacea validus* (Eichhoff) (=*Xyleborus validus*), *Xyleborinus saxesenii* (Ratzeburg), *Xyleborus seriatus* Blandford, *X. crassiusculus*, and *X. germanus*. Six Scolytinae species were not found in my experiment but in Saito et al. (2005). They are *Xylosandrus brevis* (Eichhoff), *Cnestus mutilatus* (Blandford)(=*Xylosandrus mutilatus*), *Xyleborus attenuatus* Blandford, *Scolytoplatypus daimio* Blandford, *Scolytoplatypus mikado* Blandford, and *Scolytoplatypus tycon* Blandford. However, the tree species belonging to the genus *Quercus* were not recorded as host trees of these ambrosia beetles (Kabe 1960). Furthermore, these species were not collected from my experiments in LOC-A and LOC-B. These species do not seem likely to attack *Q. serrata*. On the contrary, five Scolytinae species in LOC-C were not collected in Saito et al. (2005) though my study was conducted in only one season. They are *X. defensus, X. ganshoensis*, *Xyleborus pfeili* (Ratzeburg), *Xyleborus seiryorensis* Murayama, and *Xyleborus volvulus* (Fabricius). These results indicate that ethanol did not attract these species attacking *Q. serrata* and that bait logs were effective to study ambrosia beetle guild on a target tree species compared to ethanol baited trap.

All relationships of both abundance and species richness to the three independent factors (TC, TE, WO) had one peak (Fig. 2-1), which was supported by great and significant 2nd order effects of these independent factors (TC2, TE2, WO2) obtained by the HP (Table 2-5). Both abundance and species richness peaked on bolts exposed in June-July (TE) (Fig. 2-1). Species richness and abundance that were obtained by ethanol traps deployed in several locations in the UTCF also peaked in the same season (Saito et al. 2005). Annual flight patterns and diversity of ambrosia beetles attracted to bait logs in Zambia showed that number of attack by ambrosia beetles was extensive especially during the wet season (October and November) (Beaver and Löyttyniemi 1991). In Japan they have rainy seasons in June and July so that it is likely that flights of ambrosia beetles are intense in wet seasons. Abundance peaked in bolts that were cut in April (Fig. 2-1). Smaller numbers of attacks were observed on the March bolts. It is true that too much decayed woods are not suitable for reproduction of ambrosia beetles because ambrosia fungi cannot colonize on these woods. However, this is probably not a cause for the small beetle abundance on the March bolts in my experiment. Sprouts came out late in the seasons from some of the March bolts late in my experiments (August and September). It is likely that these March bolts had been in good conditions during the experiment, which is a likely cause of small beetle abundance on the March bolts for the same reasons as fresh bolts (0-1 month old) received smaller numbers of attacks (WO in Fig. 2-1). In the results of HP, effects of the timing of cutting (TC+TC2) on species richness and abundance were greatest among the three independent factors (TC+TC2, TE+TE2, WO+WO2). On the contrary, in MRM, TE had the strongest effect in determining differences of guild (Table 2-7). Among the three independent factors, WO is closely related to wood condition. Timing of exposure (TE) is related to seasonal occurrence of the beetles. Timing of cutting is related to the both. Because each species has its own seasonality (Chapter 3), and because MRM was influenced by abundance of each species, an effect of TE was strongest in MRM. On the contrary, in HP the total abundance of ambrosia beetles were tested so that effect of each species became smaller by being averaged.

The diversity in guild differed greatly among locations (Fig. 2-2; Table 2-6). In LOC-A, species richness was greatest for an arithmetic mean and all the three types of diversities (alpha, beta, and gamma) (Table 2-6). The species diversity-energy hypothesis predicts that local species diversity increases with energy availability (Wright 1983). Results of this study followed the hypothesis because species richness

was greatest in LOC-A where mean temperature during the season was highest and poorest in LOC-C where mean temperature was lowest. The warmest climate was a possible cause of the greatest species richness in LOC-A. However, a rank-abundance curve indicated that guild structure in LOC-A was most biased, which was supported by the smallest evenness *J′* (Fig. 2-2). In LOC-A, the most major species (*M. kadoyamensis*) was twice as abundant as the second major species. Such dominance of the most major species was related to the small *J′* value. However, the increment of *J′* was small when only the most abundant species was excluded from the calculation. On the contrary, when the four major species were excluded, the *J′* increased greatly (0.72), which was greater than the original *J′* value in LOC-B (0.70). The four major species was the most likely cause of the smallest *J′* in LOC-A (great skewness). The JOW is epidemic in LOC-A. *Platypus quercivorus* is a vector insect of the disease so that it is reasonable that the species was abundant in LOC-A. Hijii et al. (1991) investigated trees killed by JOW in Fukui Prefecture (c. 300 km northwest from LOC-A), west coast of Japan, and reported that the dead trees were colonized by *P. quercivorus* and *P. calamus*. In this study, although the number of *P. calamus* was small in LOC-A (seven galleries) (Table 2-4), much greater numbers of galleries and species were found in LOC-A than in LOC-C and LOC-B. Furthermore, the abundance of the four major species were greater than 100, which is greater than the number of the most abundant species in LOC-B and LOC-C (Table 2-4). Trees that were killed by JOW may have increased the abundance of these major species in LOC-A. Those trees may also have contributed to great species richness in LOC-A (Tables 2 and 5). Further studies are needed.

In LOC-A, beta Shannon was smallest (Table 2-6), which was consistent to the lowest dissimilarity in a result of ANOSIM (Fig. 2-3) and to the narrowest range in a result of NMDS (Fig. 2-4). The major species in LOC-A were found in many regimes (Table 2-4), which was a likely cause of the small beta Shannon (Table 2-6) and the small dissimilarity among regimes (Figs. 2-2 and 2-3). As the results, a whole diversity within location (gamma diversity) was also smallest in LOC-A (Table 2-6).

In LOC-C, the abundance of the most major species (*P. modestus*) was sixty nine (Table 2-4). Frequencies of major species in LOC-C were much smaller than those in LOC-A. The abundance was smaller than ten for seven species out of thirteen (Table 2-4). These are causes of the greatest evenness *J′*, alpha and gamma Simpson in LOC-C (Fig. 2-2 and Table 2-6). On the contrary, the arithmetic mean of Shannon indices was smallest (Table 2-6). This discrepancy between the greatest alpha Shannon (the

weighted mean) and the smallest arithmetic mean of the same indices depended on the facts that no insects were found in two regimes out of twenty eight and that one species ($H' = 0$) were found in nine regimes (Table 2-3), which reduced the arithmetic mean greatly although their influence on weighted mean (alpha Shannon) was small because of their small number of individuals (mean = 2.00, min = 1, max = 3) (Table 2-3). Although their causes are not clear, the coolest climate in LOC-C may have been related to the events.

Beta Shannon in LOC-B was greatest (Table 2-6), which was not consistent to a result of ANOSIM. I have no idea about the reason of the difference.

The result of ANOSIM (Fig. 2-3) also indicated that dissimilarity among the three locations was greater than those within location, which was supported by the results of NMDS (Fig. 2-4), the largest standardized partial coefficient of location obtained by MRM (Table 2-7). The results indicate that location was the strongest factor to determine the ambrosia beetle guild. The results of NMDS indicated that the guilds in LOC-C and LOC-B differed greatly and that in LOC-A was intermediate between them (Fig. 2-4). Furthermore, similarity between LOC-A and LOC-B was greater than that between LOC-A and LOC-C because overlap between LOC-A and LOC-B was greater (Fig. 2-4), which was supported by results in Table 2-4. The number of species shared by two locations was greatest between LOC-A and LOC-B (62.0%), followed by between LOC-C and LOC-A (45.5%), and lowest between LOC-B and LOC-C (40.0%). In addition to these, the most major species in LOC-C was “endemic species” found exclusively in the LOC-C. Geographic distance cannot explain these similarities in guilds among locations because LOC-C is located between LOC-A and LOC-B. Among the three locations, the vegetation in LOC-C differs greatly from those in LOC-B and LOC-A. Regarding to the genus *Quercus*, only two deciduous species, *Q. serrata* and *Quercus crispula* Blume, grow in LOC-C (Igarashi and Iwata 2004). However, in addition to the above two species, two deciduous species, *Quercus valiabilis* Blume, *Quercus acutissima* Carruthers, and several evergreen oak species grow both in LOC-A and LOC-B (The University Forests in Chiba 1985; Ecohydrology Research Institute 2011). Kabe (1960) listed host plant species of Scolytinae and Platipodinae in Japan. For eight ambrosia beetle species that contain three major species from each of the three locations (*M. kadoyamaensis* was the first major species in LOC-A and the third in LOC-B so that the number was not nine but eight.), sixty three host plant species grow in the three locations (The University Forests in Chiba 1985; Igarashi and Iwata 2004; Ecohydrology Research Institute 2011).

Among the sixty three species, the number of shared species between two locations was forty three between LOC-A and LOC-B, twenty four between LOC-B and LOC-C, twenty three between LOC-C and LOC-A. Temperature was lowest in LOC-C among the three locations followed by LOC-B. Because vegetation is strongly influenced by temperature, vegetation related to climate seemed to be a likely cause of these similarity and dissimilarity in ambrosia beetle guilds among locations.

On the contrary, in lowland rainforests in Papua New Guinea, beta diversity among three locations, in which furthest distance between the two locations was c. 1,000 km, was smaller than alpha diversity (diversity within each location) (Hulcr et al. 2007). Actually, the percentage of the species that were present in the three locations was greater in Hulcr et al. (2007) than my experiment. I speculate that small difference among the three locations in the Hulcr et al. (2007) depended on their similar elevation (between 100-200 m above the sea level), with minimal seasonal variation, and similar amounts of annual rainfall (3,000-4,000 mm), which probably caused similar vegetation among the sites. Despite of shorter distances among locations, differences in vegetation and in climate among the three locations were greater in my study because of great difference in elevation (between 95-820 m above the sea level), which was a likely cause of the great dissimilarity in ambrosia beetle guild among the locations.

The subfamily Platypodinae had higher proportion of "endemic species" than Scolytinae (Table 2-4), indicating that an effect of location was stronger in Platypodinae than Scolytinae. Further information is needed to test if this is universe phenomenon.

This is the quantitative studies to investigate relative importance of TC, TE, and WO in determining ambrosia beetle guilds attacking one tree species. Standardized partial coefficient obtained by MRM indicated that relative importance among TC, TE, and WO. Effect of TE was greatest among the factors: 1.79 times as strong as that of TC, and 5.64 times as strong as that of WO (Table 2-7). This is very likely because each species of ambrosia beetle flies at a certain period depending on its life cycle. For example, attacks of *P. quercivorus*, the second most abundant species in LOC-A, were highly concentrated in July (e.g.Ezaki 1957). On the contrary, oldness had the smallest effect (Table 2-7). Timing of cutting wood and wood oldness (period after cutting) are both factors to determine wood condition. I found that some sprout comes out late in the season from bolts cut in March, which indicates that these bolts were kept in a good condition with living cells. Bolts that were prepared before the plants waked up from

overwintering could probably be maintained in a good condition for long time compared to those cut in growing season. This can be one of mechanisms that timing of cutting influenced ambrosia beetle guild via effect of wood conditions. Preference to wood condition determined by timing of cutting and wood oldness might not be strict for most of ambrosia beetle species. However, I hypothesize that hierarchical structure among these factors was a likely cause of the results: No attacks occur if no adults are flying even though the conditions of hosts remain suitable. Furthermore, no attacks occur if the insect species is not distributed. Therefore, local fauna of ambrosia beetles are the highest hierarchy, seasonality is the second and wood condition determined by timing of cutting and wood oldness is the lowest in determining their attacks. Higher hierarchal factors were likely to mask effects of the lower ones. I conclude that such a hierarchical structure among factors is likely to influence relative importance in determining guild structure of ambrosia beetles attacking *Q. serrata* trees.

Table 2-1 Experimental design of an artificial control for timing of cutting and timing of exposure of *Quercus serrata* bolts by covering and removing with wire net.

Regime no.	Timing of cutting trees (TC)	Timing of exposure (TE)	Wood oldness (WO)	Regime no.	Timing of cutting trees (TC)	Timing of exposure (TE)	Wood oldness (WO)
1	March	March	0	15	May	June	1
2	March	April	1	16	May	July	2
3	March	May	2	17	May	August	3
4	March	June	3	18	May	September	4
5	March	July	4	19	June	June	0
6	March	August	5	20	June	July	1
7	March	September	6	21	June	August	2
8	April	April	0	22	June	September	3
9	April	May	1	23	July	July	0
10	April	June	2	24	July	August	1
11	April	July	3	25	July	September	2
12	April	August	4	26	August	August	0
13	April	September	5	27	August	September	1
14	May	May	0	28	September	September	0

Wood oldness (WO) is difference in the number of months between timing of exposure and timing of cutting trees.

Table 2-2 The number of ambrosia beetle galleries and that of ambrosia beetle species found in Aichi (LOC-A), Chiba (LOC-B), and Chichibu (LOC-C), located in the Central Japan.

	No. of galleries			No. of species		
	Identified	Unidentified	Total	Scolytinae	Platypodinae	Total
LOC-A	1,251	319	1,570	16	2	18
LOC-B	276	131	407	14	2	16
LOC-C	238	118	356	11	3	14
Total	1,765	568	2,333	18	6	24

Table 2-3 Frequency of regimes and mean abundance in relation to the number of species in each regime (Aichi: LOC-A, Chiba: LOC-B, Chichibu: LOC-C).

No. of species in a regime	LOC-A		LOC-B		LOC-C	
	Frequency of regimes	Mean abundance	Frequency of regimes	Mean abundance	Frequency of regimes	Mean abundance
0	2	0.0	1	0.0	2	0.0
1	1	1.0	5	2.0	9	2.0
2	2	44.5	5	2.8	*3	4.7
3	7	30.3	*7	10.1	5	7.8
4	*5	23.2	4	10.0	3	11.0
5	2	33.0	2	29.5	2	8.0
6	2	80.5	3	14.7	1	11.0
7	3	39.3			2	26.5
8	1	96.0				
9	2	94.5	1	38.0		
10	1	203.0			1	54.0

*: median

Table 2-4 List of ambrosia beetle species baited by *Quercus serrata* bolts in Aichi (LOC-A), Chiba (LOC-B), and Chichibu (LOC-C), located in the Central Japan. Abundance and frequency of regimes, in which each species was found, are shown.

No.	Species	LOC-A		LOC-B		LOC-C	
		Abundance	Frequency of regimes	Abundance	Frequency of regimes	Abundance	Frequency of regimes
1	*Ambrosiodmus lewisi* (Blandford)	15	8	1	1	13	11
2	*Ambrosiodmus rubricollis* (Eichhoff)	8	3	6	6	0	0
3	*Debus emarginatus* (Eichhoff)	1	1	3	3	0	0
4	*Euwallacea validus* (Eichhoff)	3	3	0	0	38	15
5	*Leptoxyleborus depressus* (Eggers)	0	0	22	11	0	0
6	*Microperus kadoyamaensis* (Murayama)	517	20	47	13	0	0
7	*Planiculus bicolor* (Blandford)	227	18	4	4	0	0
8	*Truncaudum agnatum* (Eggers)	1	1	0	0	0	0
9	*Xyleborinus saxesenii* Ratzeburg	1	1	3	3	5	3
10	*Xyleborus defensus* Blandford	111	20	1	1	5	3
11	*Xyleborus ganshoensis* Murayama	9	4	87	21	3	3
12	*Xyleborus laetus* Niisima	1	1	1	1	0	0
13	*Xyleborus pfeili* (Ratzeburg)	1	1	0	0	3	2
14	*Xyleborus seiryorensis* Murayama	36	5	3	3	11	6
15	*Xyleborus seriatus* Blandford	2	2	0	0	34	4
16	*Xyleborus volvulus* (Fabricius)	0	0	1	1	1	1
17	*Xylosandrus crassiusculus* (Motschulsky)	58	13	10	6	6	3
18	*Xylosandrus germanus* (Blandford)	13	9	16	7	43	11
19	*Crossotarsus niponicus* Blandford	0	0	0	0	4	2
20	*Crossotarsus simplex* Murayama	0	0	5	2	0	0
21	*Platypus calamus* Blandford	7	3	66	6	0	0
22	*Platypus modestus* Blandford	0	0	0	0	69	15
23	*Platypus quercivorus* (Murayama)	240	9	0	0	0	0
24	*Treptoplatypus solidus* (Walker)	0	0	0	0	3	3

Table 2-5 Independent (*I*) and joint (*J*) contributions (the percentage of the total explained variance) of each predictor variable estimated from hierarchical partitioning for (a) the abundance and (b) the species richness. Significance of the independent contributions was tested by *Z*-scores obtained using 1,000 repeated randomizations. Asterisks indicate significant independent contributions ($P < 0.05$).

Depended factor	Factor	% *I*	% *J*	% Total
(a) abundance	TC	7.2	7.1	14.3
	TC2	10.4 *	7.2	17.6
	TE	6.0	-4.7	1.3
	TE2	11.2 *	-8.3	2.9
	WO	5.0	0.8	5.8
	WO2	2.2	0.6	2.8
	LOC	55.3 *	0.0	55.3
	Sum	97.3	2.7	100.0
(b) species richness	TC	14.2 *	11.6	25.8
	TC2	23.9 *	10.3	34.2
	TE	11.0 *	-3.7	7.3
	TE2	15.5 *	-4.2	11.7
	WO	8.9 *	-4.0	4.9
	WO2	9.0	-8.7	0.3
	LOC	15.9 *	0.0	15.9
	Sum	98.3	1.7	100.0

TC = Timing of cutting oak tree from March to September, TE = Timing of exposure of bait bolts from March to September, WO = wood oldness, which is given by difference in the number of months between timing of exposure and timing of cutting trees, LOC = location.

Table 2-6 Alpha, beta, and gamma diversity (species richness and Shannon index) of ambrosia beetle guilds on *Quercus serrata* bait bolts set in Aichi (LOC-A), Chiba (LOC-B), and Chichibu (LOC-C), located in the Central Japan.

	Species richness				Shannon index			
	Mean (SD)	α	$m\beta$ (prop)	Γ	Mean (SD)	α	$a\beta$ (prop)	γ
LOC-A	4.35 (2.53)	6.06	3.00 (0.33)	18	0.93 (0.49)	1.05	0.66 (0.39)	1.70
LOC-B	3.18 (2.00)	4.75	3.37 (0.41)	16	0.79 (0.47)	0.97	0.91 (0.48)	1.94
LOC-C	2.93 (2.42)	5.68	2.46 (0.30)	14	0.72 (0.62)	1.17	0.88 (0.43)	2.05

Species richness: the number of species.

Shannon index: $H' = -\sum_{i=1}^{S} p_i \ln p_i$ where p_i is the proportion of each species in the sample, and S is the number of species (Shannon and Weaver 1964).

α: weighted mean by abundance in each regime.

γ: calculated from whole data in each location.

mβ: multiplicative beta calculated for species richness by γ/α (Jost 2007).

aβ: additive beta calculated for Shannon index by $\gamma-\alpha$ (Jost 2007).

Table 2-7 The results of multiple regression on distance matrices (MRM) analysis to test effects of factors determining community patterns of ambrosia beetles attacking *Quercus serrata* bait bolts in Aichi, Chiba, and Chichibu, located in the Central Japan.

	Coefficient		Standardized partial coefficient
	asin (sp.dissim)	*P*	
Intercept	0.66	1.0000	0.00
TC	0.04	0.0003	0.11
TE	0.05	0.0001	0.17
WO	0.01	0.5403	0.02
LOC	0.52	0.0001	0.52
R^2	0.31	<0.0001	
F	349	<0.0001	

asin (sp.dissim) = arcsine of species dissimilarity, TC = Timing of cutting oak tree from March to September, TE = Timing of exposure of bait bolts from March to September, WO = wood oldness, which is given by difference in the number of months between timing of exposure and timing of cutting trees, LOC = location.

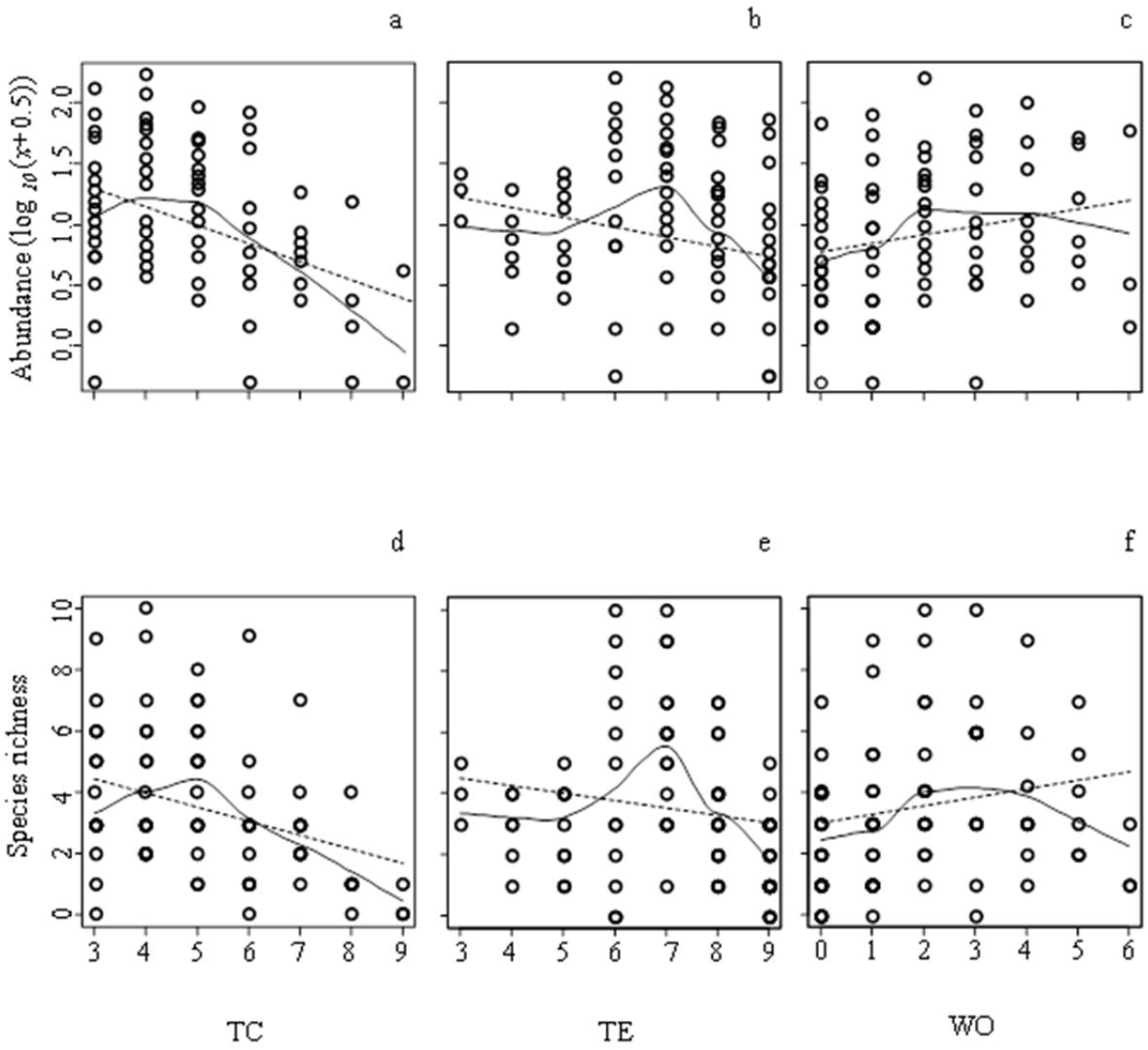


Figure 2-1 Scatter graphs of relationships between three factors (TC, TE, and WO) of abundance and species richness of ambrosia beetle. A dash line is a least square linear regression and black line is a smooth regression. (a) - (c) show relationship between $\log_{10}(X+0.5)$ of total abundance on each independent factors: (a) TC = timing of cutting tree (3-9: March - September), (b) TE = timing of exposure (3-9: March - September), (c) WO = wood oldness (0-6). (d)- (f) show relationship between species richness on each independent factors: (d) TC = timing of cutting tree (3-9: March - September), (e) TE = timing of exposure (3-9: March - September), (f) WO = wood oldness (0-6).

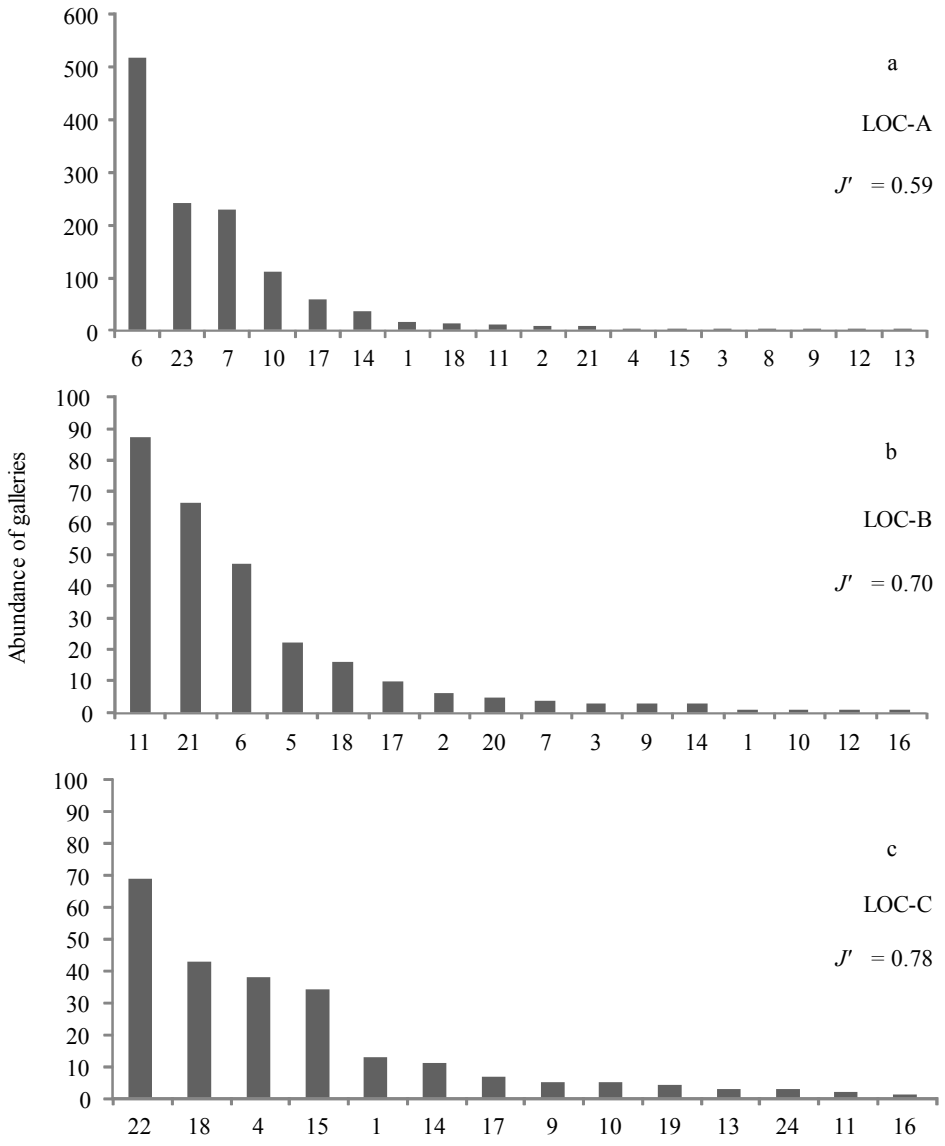


Figure 2-2 Pielou's evenness: *J′* and rank-abundance curve for ambrosia beetle species collected from *Quercus serrata* bait bolts that had been set in three locations, Central Japan: (a) Aichi (LOC-A), (b) Chiba (LOC-B), and (c) Chichibu (LOC-C).

$J' = -\sum_{i=1}^{S} p_i \ln p_i / \ln(S)$, where p_i is the proportion of each species in the sample, and S is the number of species.

1: *Ambrosiodmus lewisi* (Blandford), 2: *Ambrosiodmus rubricollis* (Eichhoff), 3: *Debus emarginatus* (Eichhoff), 4: *Euwallacea validus* (Eichhoff), 5: *Leptoxyleborus depressus* (Eggers), 6: *Microperus kadoyamaensis* (Murayama), 7: *Planiculus bicolor* (Blandford), 8: *Truncaudum agnatum* (Eggers), 9: *Xyleborinus saxesenii* Ratzeburg, 10: *Xyleborus defensus* (Blandford), 11: *Xyleborus ganshoensis* Murayama, 12: *Xyleborus laetus* Niisima, 13: *Xyleborus pfeili* (Ratzeburg), 14: *Xyleborus seiryorensis* Murayama, 15: *Xyleborus seriatus* Blandford, 16: *Xyleborus volvulus* (Fabricius), 17: *Xylosandrus crassiusculus* (Motschulsky), 18: *Xylosandrus germanus* (Blandford), 19: *Crossotarsus niponicus* Blandford, 20: *Crossotarsus simplex* Murayama, 21: *Platypus calamus* Blandford, 22: *Platypus modestus* Blandford, 23: *Platypus quercivorus* (Murayama), 24: *Treptoplatypus solidus* (Walker).

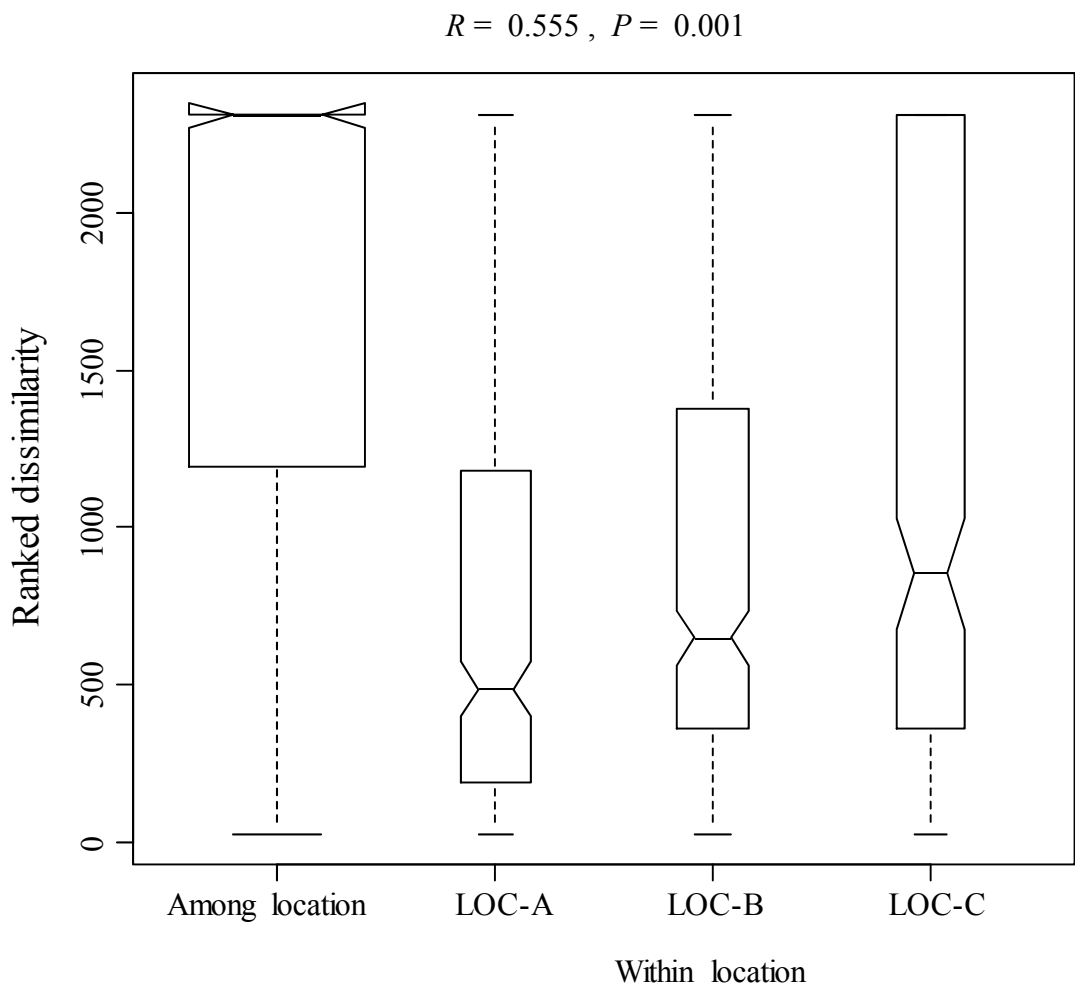


Figure 2-3 Boxplot of ranked dissimilarity in ambrosia beetle guild collected by location. Pairwise comparisons were grouped as "Between" locations or within location (individual groups shown in the plot). There are significant differences among the three locations (R = 0.555, P = 0. 001). Boxes represent the median and interquartile range (IQR). Dissimilarity among three locations was greater than those within location. LOC-A: Aichi, LOC-B: Chiba, and LOC-C: Chichibu.

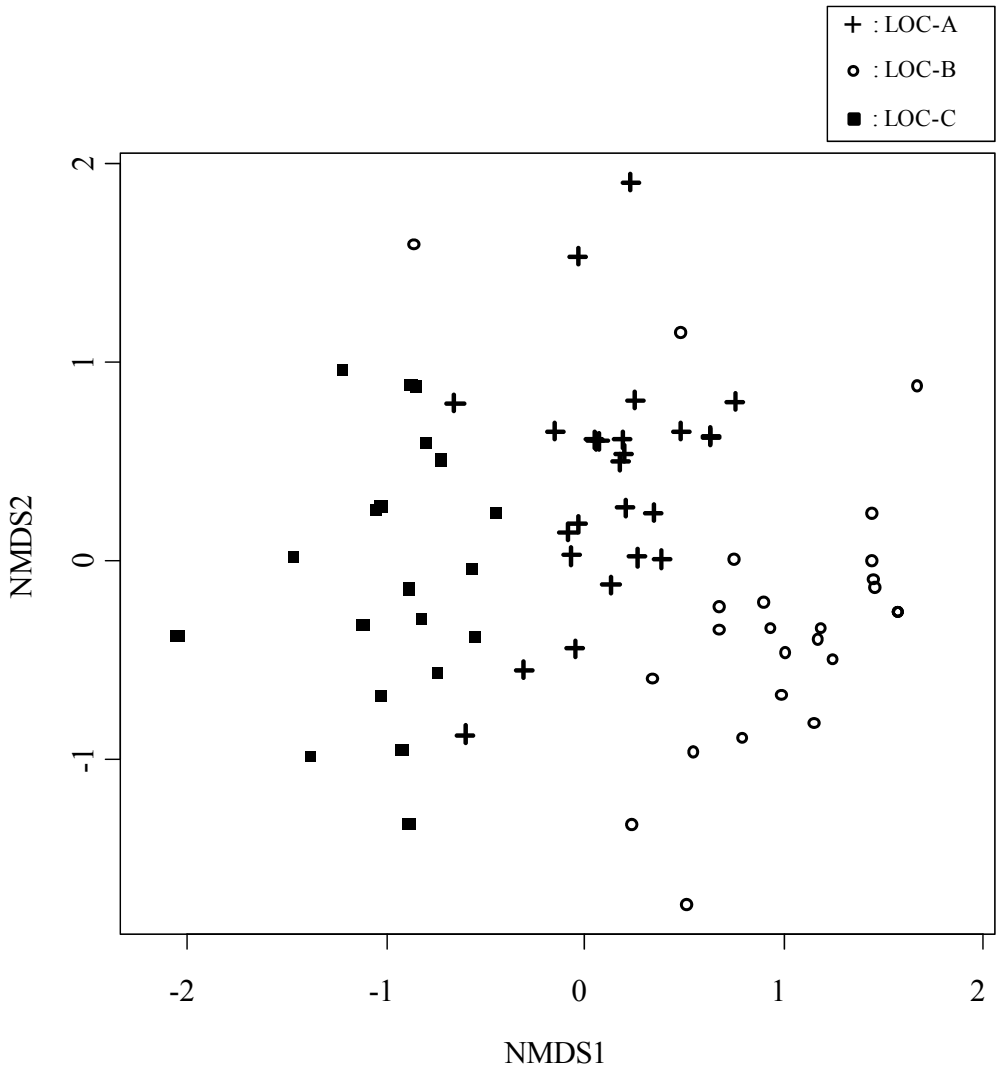


Figure 2-4 Non-metric multidimensional scaling (NMDS) ordination of ambrosia beetle guilds attacking *Quercus serrata* bait bolts in each experimental regime (01-28) in Aichi (LOC-A), Chiba (LOC-B), and Chichibu (LOC-C), located in the Central Japan. The NMDS is based on a distance matrix computed with Chao's dissimilarity index. The result with "noshare = 0.4" is shown.

CHAPTER 3: NICHE ANALYSIS AND RISK ASSESSMENT

3.1. Introduction

Ambrosia beetles can be divided into two groups by physiological conditions of their host plants. One group is called a primary attacker that is more aggressive and sometimes kills healthy living trees. The other group is a secondary attacker attacking unhealthy or dead trees. Typically, most of the ambrosia beetles are known as saproxylic insects, non-pest species that utilize dead trees, logs, fallen trees, broken branches, and play a key role in nutrient cycling and ecosystem functioning in the forest (Lassauce et al. 2011), so that most of the ambrosia beetles are secondary, non-pest species (Wood 1972). In recent years unusual secondary ambrosia beetle species have been increasingly observed to attack living trees (Kühnholz et al. 2001). For example, *P. quercivorus* attacks healthy-looking trees in Japan and kills them in combination with *R. querivora* (Japanese oak wilt disease: JOW), which is a secondary attacker infesting fallen trees or broken branches in the other countries, such as Taiwan (Murayama 1925), Indonesia (Schedl 1972) ,Thailand (Hulcr et al. 2008), India (Beeson 1937), Papua New Guinea (Wood 1972), and Vietnam (Naoto Kamata, personal communication). *Platypus koryoensis* in Korea also causes similar mortality to several species belonging to the genus *Quercus* and *Carpinus laxiflora* (Sieb. et Zucc.) Blume. by an accompanied fungus, *R. quercus-mongolicae* (Kim et al. 2009).

On the other hand, during the last two decades the number of alien insect species has been increasing greatly with globalization (Liebhold et al. 1995). Cases of interceptions of ambrosia beetles at ports have been reported in the world (Browne 1986; Haack and Cavey 2000). Many ambrosia beetle species have been established their population in US (Haack 2006): *X. crassiusculus*, *X. germanus*, *X. saxesenii*. Reed and Muzika (2010) captured *X. saxesenii*, *X. crassiusculus*, *X. germanus*, *Xyleborus atratus* Eichhoff, and *Xyleborus californicus* Wood in ethanol baited interception traps in Missouri, US, where these species were believed to be exotic, and *X. saxesenii* was predominant in the ambrosia beetle assemblage. Each of ambrosia beetles has specific niche characteristics that determine its ability to survive in the environment. Traits that are common for alien species include: high potential of population growth (fast growth, ability of both asexual and sexual reproduction, high fecundity, many generations), high dispersal ability, phenotypic plasticity, broad habitat niche, broad food niche, broad niche for seasonality, high tolerance to environment, and cryptic behavior (Davis 2009). Regarding to ambrosia beetles, they

spend most of their life inside wood, which makes them escape from human quarantine and from natural enemies (Kühnholz et al. 2001) and also provides them more constant environment than outer world (Aukema et al. 2010). Alien ambrosia beetle and fungus complexes have sometimes become horrendous threat to forest ecosystems (Hulcr and Dunn 2011). Some of the alien ambrosia beetles have a tremendous impact on economics especially in the US and the EU (Kenis et al. 2009; Gandhi et al. 2010). Probably the greatest economic impact of alien ambrosia beetles is the laurel wilt of avocado crops in the US. This is caused by *R. lauricola* carried by *X. glabratus* (Hanula et al. 2008), though *X. glabratus* is a rare species in its native range: India, Japan and Taiwan (Harrington et al. 2011). *Megaplatypus mutatus* (Chapuis) is an ambrosia beetle native to South America, which has recently become a serious pest in commercial poplar and hazelnut fruit tree plantations in Italy as a vector of *Raffaelea santoroi* Guerrero (Funes et al. 2011). In the Eastern US, two Asian ambrosia beetle species (*X. germanus* and *X. crassiusculus*) attack sapling in nurseries (Oliver and Mannion 2001; Ranger et al. 2010). *Euplatypus parallelus* (Fabricius) with *Fusarium oxysporum* Schlecht, which is native to tropical America (Bright and Skidmore 2002), attacks *Pterocarpus indicus* Willd in Thailand (Bumrungsri et al. 2008). All these cases are caused by alien ambrosia beetle species that are non-pest species in indigenous areas. One exception is that *M. mutatus*. *Megaplatypus mutatus* attacks only living standing trees and causes a serious problem in commercial plantations of poplars (*Populus deltoides* Bartr. ex Marsh.) in Argentina (Funes et al. 2011). The galleries degrade the lumber and weaken the tree stems, which often then break during windstorms (Alfaro et al. 2007). These cases indicate that the fungi were highly pathogenic to host plants due to their lack of coevolutionary process with the host plant and/or that the insects shifted from colonizing dying trees to attacking living trees (Hulcr and Dunn 2011).

Ambrosia beetles do not feed on plant materials but feed on fungi, so that host plant ranges of ambrosia beetles are wider than close insect groups such as bark beetles that feed on plant material (Kolar and Lodge 2001). This broad range of host plants was considered to be a major reason why they were successful as invaders (Kirkendall et al. 2008; Stokkink 2008). Actually two-thirds of alien Scolytinae and Platypodinae that have been found in Europe from Asia or North America are ambrosia beetles although the number of species are smaller in ambrosia beetles than in bark beetles in the source fauna (Kirkendall 1993). Most ambrosia beetle species are subsocial insects with a few exceptions that were recently

reported to have eusociality (Smith et al. 2009), which enable the founder females to lay eggs for a long period and high reproductive success (Peer and Taborsky 2007; Biedermann et al. 2011). Some ambrosia beetle species are known to use sex pheromone for mating or aggregation pheromone for attracting both sexes for mass attacks and mating (Kühnholz et al. 2001), which contribute to successful colonization. True arrhenotoky has been demonstrated in two tribes (Dryocetini and Xyleborini) of the subfamily Scolytinae (Kirkendall 1993): Some species in the tribe Dryocetini and all species belonging to the tribe Xyleborini are haplodiploidy, of which virgin females can lay all-male broods (Kirkendall 1993). Inbreeding polygyny, whereby a few males fertilize all their sisters before emerging from the brood trees, is found throughout the tribe Xyleborini and some species of the tribes Dryocetini, Hyorrhynchini and Xyloterini (Stokkink 2008; Biedermann 2010; Alfaro et al. 2007; Kirkendall and Faccoli 2010). These features enable these species to establish new populations from a single female and contribute to colonization success (Kirkendall and Odegaard 2007).

In this chapter, I estimated ecological niche of ambrosia beetles that infested oak trees, *Q. serrata* in three locations in the Central Japan. I prepared freshly cut bolts of *Q. serrata* and controlled wood freshness and timing of exposure to evaluate ecological niche for wood freshness and seasonality. My major hypotheses were; (1) species with broader niche were advantageous in colonization success, (2) species that attacked bolts soon after the bolts were cut had a potential for causing a wilt disease if some of their symbiotic fungi were pathogenic enough to kill trees. I discussed my results in terms of a risk of alien species and invasive alien species as well as that of vector insects of tree wilt disease in relation to their ecological niche.

3. 2.Materials and Methods

3.2.1. Field experiments

The data used in this study were partially obtained by the same experiment as the Chapter 2 of this thesis. I tried to determine three dimensions of ecological niche for ambrosia beetles attacking *Q. serrata*. Three factors, timing of cutting tree (TC), timing of starting exposure (TE), and wood oldness (WO), were considered.

In addition to the experiment in the Chapter 2, which I call “Normal experiment” in this chapter, another twenty one regimes (“Conditioning experiment”) were prepared at the same regimes that started to be exposed before September. Each regime of the Conditioning experiment was consisted of four bolts that were exposed to insect attack until the end of September so that bolts of the Conditioning experiment were exposed for two months to seven months depending on the TE. The Conditioning experiment did not include regimes that started to be exposed from September because they were identical as the same regimes of the Normal experiment. To collect ambrosia beetles, I marked all entry holes on the surfaces of inner bark and of sapwood after debarking. The number of the entry holes, which was equal to the number of galleries, was counted by matching inner bark with sapwood. Insects were checked first in the bark, then in the sapwood by breaking the bolts into small pieces. Ambrosia beetles were collected from each entry hole and kept separately in an individual container filled with 70% ethanol. Thereafter, each individual was made into pin specimens for identification.

3.2.2. Data analysis

As suggested in the Chapter 2, the ambrosia beetle guild was strongly influenced by location. The objective of this chapter was to analyze ecological niche of each ambrosia beetle species in relation to the possibility of alien species and/or carrying tree-killing disease. Therefore, I excluded effects of location from the following analysis. All the analyses were done using the statistical software R 2.13.1 (R Development Core Team 2011).

I differentiated the twenty-eight regime of the Normal experiment using three dimensional niche contexts (TC, TE, and WO). Niche center and niche breadth of each ambrosia beetle species were obtained for each of the three dimensional niche contexts. I did not use normalized values but real numbers of month. Therefore, the number, 3, 4, 5, 6, 7, 8 and 9 in the TC and the TE indicate in March, April, May, June, July, August, and September, respectively. The WO was given by the difference in the number of months between TE and TC, which ranged from 0 to 6 month old. Niche center was given as relative preference value of species that was obtained by weighted mean of resource use as follows; first relative abundance of each regime was calculated. Then average relative abundance was calculated for each value of a niche axis (regime characteristic value: 3 - 9 for TE and TC, 0 - 6 for WO). Finally relative preference

value was obtained by summing up products of the regime characteristic value and the average relative abundance.

According to De Cáceres et al. (2011), the notation for Rao's quadratic entropy (Rao 1982) was adopted for niche breadth.

$$B_D = \frac{1}{2} \sum_{j=1}^{28} \sum_{k=1}^{28} f_j f_k \, d_{jk}^2,$$

where f is a relative abundance of each regime, and d_{jk} is a distance between regimes j and k. The distance was given by difference of the niche characteristic values between the two regimes. The scale of niche broadness was also a month. Confidence interval of the niche breadth was incorporated in the bootstrap framework described by Mueller and Altenberg (1985). Both the species resource use and resource availability were treated as random variables in the calculation. This procedure was conducted in the package "resniche (v. 1.3.0)" (De Cáceres 2011) for the statistical software R.

Based on my hypothesis, effects of location and treatments (Normal experiment vs. Conditioning experiment) were tested by a linear mixed model with a random effect of regimes, for each species by paring the abundance in the two experiments of the same numbered regime. Difference in abundance between the two experiments (Normal experiment vs. Conditioning experiment) was tested by paired t test for each species by paring the abundance in the two experiments of the same regime numbering. Before the paired t-test, all abundance values were transformed by $\log_{10}(X+0.5)$ to improve normality of the distribution (Yamamura 1999). Mann-Whiteny U test was employed to test the difference in niche breadth for TC, TE, and WO between Scolytinae and Platypodinae and between species that were known as alien species and those that were not by using subroutine program by Aoki (Aoki 2009) for the statistical software R.

Regimes that started to be exposed in September (regimes No. 7, 13, 18, 22, 25, 27, 28) of the Normal experiment were excluded from the further analysis because I did not set these regimes in the Conditioning experiment. Competitive ability of each of ambrosia beetle species was evaluated from two different viewpoints as follows:

(1) Can the species attack the bolts that have already been exploited by other species?

(2) Is the species a strong competitor or a weak one?

The first question was checked by the following procedure (Fig 3-1):

(1)-1. The species that attacked from beginning of the season were basically excluded from this procedure. However, if the abundance in a regime No.1 was small (<3) and if no galleries were found in regimes No. 2 and 8, the species was included in this procedure.

(1)-2.The number of galleries was compared between the Normal and the Conditioning experiments for regimes before the species started to attack in the Normal experiment. For species that was collected in small abundance (< 3) in the regime No. 1 but not in regimes No. 2 and 8, the comparison was done for regimes before the species started to be collected thereafter.

(1)-2-A. If the species was collected in regimes of the Conditioning experiment before the species started to attack in the Normal experiment, the species was judged as to colonize trees that had been exploited by other species.

(1)-2-B. If the species was not collected both in the Normal and Conditioning experiment, the species was thought to avoid trees that had been exploited by other species.

The second question was checked by comparing abundance between the Normal and the Conditioning experiments for the regimes, in which each species was collected in the Normal experiment.

A paired *t*-test was employed for the comparison between the Normal and Conditioning experiments of each species.

Non-metric multidimensional scaling (NMDS) was used to illustrate niche similarity patterns among ambrosia beetle species. Regimes with no identified galleries were omitted from the analysis. Ordination may have been difficult if a large proportion of species were not shared among multiple regimes, so step-across dissimilarities were employed to improve the problem by stepwise 0.1 decrements of the parameter "noshare" (1 – 0.1) in a library "vegan (v. 1.17–3) " (Oksanen et al. 2011) for the statistical software R.

3.3 Results

Table 3-1 shows abundance of ambrosia beetle for a Normal experiment (N) and for a Conditioning experiment (C) for regimes that started to be exposed from March to August. The abundance was strongly influenced by treatment (Normal vs. Conditioning experiment) and location (LOC) for ten species: *A. rubricollis*, *L. depressus*, *M. kadoyamaensis*, *P. bicolor*, *X. defensus*, *X. ganshoensis*, *X.*

seiryorensis, C. niponicus, P. calamus, and *P. quercivorus.* Three of these (*L. depressus*, *C. niponicus*, and *P. quercivorus*) were found only in one location. In LOC-A, twenty species were found in the Normal and eleven species in the Conditioning experiment. Seven species that were found in the Normal experiment disappeared in Conditioning experiment; these were *Ambrosiodmus rubricollis, Debus emarginatus*, *Truncaudum agnatum*, *X. saxesenii*, *Xyleborus laetus*, *Xyleborus pfeili*, *Xyleborus seriatus*. Six species increased their abundance in the Conditioning experiment; *M. kadoyamaensis* increased 1.60 times (abundance in the Conditioning experiment/ abundance in the Normal experiment = 727/453), *P. bicolor* increased 2.21 times (*ditto* = 323/146), and *X. defensus* increased 1.36 times (*ditto* = 120/88). On the contrary, excluding the seven species that disappeared in the Conditioning experiment, four species decreased in abundance: *A. lewisi*, *X. ganshoensis*, *X. seiryorensis*, and *P. quercivorus*. Especially the decrease in the number was greatest in *P. quercivorus* (from 240 to 72). Significant difference in the abundance was found between the Normal and the Conditioning experiments for *A. rubricollis, M. kadoyamaensis, P. bicolor*, and *P. quercivorus* (paired *t*-test, $P < 0.05$). In LOC-B, nine species were found in the Conditioning experiment. Eight species that were collected in the Normal experiments disappeared in the Conditioning experiment. They were *A. lewisi, D. emarginatus*, *X. saxesenii, X. defensus, X. laetus*, *X. seiryorensis, X. volvulus,* and *C. simplex*. Abundance of five species increased in the Conditioning experiment; *M. kadoyamaensis* increased 1.18 times (= 53/45), *Xyleborus festivus* Eichhoff, and *X. pfeili* increased from 0 to 2, *X. ganshoensis* increased 1.79 times (= 125/70), and *P. calamus* increased 2.62 times (= 173/66). On the contrary, excluding the eight species that disappeared in the Conditioning experiment, four species decreased in abundance: *A. rubricollis*, *L. depressus*, *X. crassiusculus*, and *X. germanus*. The difference was marginally significant for *D. emarginatus*, *L. depressus*, *X. saxesenii, X. seiryorensis, X. germanus*, and *P. calamus* (paired *t*-test, $P < 0.1$). In LOC-C, ten species were found in Conditioning experiment. Four species that were collected in the Normal experiment disappeared in the Conditioning experiment. They were *X. ganshoensis, X. pfeili*, *X. volvulus*, and *T. solidus*. Six species increased their abundance in the Conditioning experiment; *X. saxesenii* increased 2.50 times (= 5/2), *X. defensus* increased 1.60 times (= 8/5), *X. crassiusculus* increased 4.14 times (=29/7), *X. germanus* increased 1.09 times (= 46/42), *C. niponicus* increased 7.50 times (= 30/4), and *P. modestus* increased 1.09 times (= 62/57). Excepting the four species that disappeared in the

Conditioning experiment, *E. validus*, *X. seiryorensis* and *X. seriatus* decreased in abundance in the Conditioning experiment. Significant difference in abundance was found between the Normal and the Conditioning experiments for *E. validus* (paired *t*-test, $P < 0.05$). The difference was marginally significant for *C. niponicus* and *T. solidus* (paired *t*-test, $P < 0.1$).

Table 3-2 shows an ability of ambrosia beetles to colonize bolts that had been exploited by other species. Eleven species colonized bolts that had been exploited by other species. Six species among the eleven showed significant difference (paired *t* test, $P<0.05$) and evaluated as good at colonizing bolts that had been exploited by other species. These were *M. kadoyamaensis, P. bicolor, X. seriatus, X. crassiusculus, P. calamus,* and *P. modestus*. On the contrary, *L. depressus* and *P. quercivorus* avoid trees that have been occupied by other species because these species did not attack bolts that started to be exposed before they started to attack (Table 3-2).

Table 3-3 shows competitive ability of each species indicated by comparison in abundance between the Normal and Conditioning experiments during the season, in which each species were captured in the Normal experiment. Table 3-3(a) shows species that increased in abundance in the Conditioning experiment. They were *M. kadoyamaensis, P. bicolor, X. crassiusculus, C. niponicus, P. calamus, and P. modestus*, *X. defensus*, and *X. ganshoensis*. Among the eight species, only *X. crassiusculus* showed a significant difference in the abundance between the Normal and the Conditioning experiments. Table 3-3(b) shows sixteen species that decreased in abundance in the Conditioning experiment. Four species, *A. rubricollis*, *D. emarginatus*, *E. validus,* and *P. quercivorus,* had a significant difference ($P < 0.05$). *Leptoxyleborus depressus* had marginally significant difference ($P < 0.1$).

Fig. 3-2 shows niche center and breadth for TC (Fig. 3-2a), TE (Fig. 3-2b), and WO (Fig. 3-2c). In each, the species were shown in a descendent order by niche breadth. Platypodinae species tended to have narrower niche with one exception of *P. modestus* for all three niche axes. Between Scolytinae and Platypodinae, no significant difference was found in niche breadth of TC (Mann-Whitney *U* test, $U = 73.0$, $P = 0.45$). However, significant difference was found in TE (Mann-Whitney *U* test, $U = 97.0$, $P = 0.03$) and WO (Mann-Whitney *U* test, $U = 95.5$, $P = 0.03$). Most Platypodinae species attacked late in the seasons with one exception of *C. simplex* of which niche center was 4.8 (early May) (Fig. 3-2b). *Xyleborus seiryorenis* had the smallest value of niche center for WO, which indicated that the species had a tendency

toward attacking most fresh status of wood. This was followed by *P. quercivorus*. *Xyleborus volvulus* had the third smallest value of niche center for WO, though the abundance was small (N = 2). *Platypus calamus* followed. On the contrary, some species that had greater values of niche center with broad niche for WO also attacked fresh bolts: *X. ganshoensis*, *E. validus*, *A. lewisi*, *X. defensus*, *D. emerginatus,* and *A. rubricollis*. Their lower limit value of niche broadness was smaller than that of *P. calamus*. Mean abundance in the 0-month-old bolts was greatest in *P. quercivorus*, which was followed by *X. ganshoensis*, *X. seiryorensis*, *M. kadoyamaensis*, *X. defensus*, *E. validus*, and *X. germanus* (mean per bolt > 0.1).

Figure 3-4 shows a result of NMDS, indicating ordination of each ambrosia beetle species based on experiment regimes. Species with similar niche were plotted closely in the graph. Species with broad niche and/or great abundance tended to be plotted near the origin. Abundance seemed to influence x-axis (NMDS1). Nine species that were plotted near the origin were found to be in many regimes with great abundance: They are *A. lewisi* (Sp.1), *E. validus* (Sp.4), *L. depressus* (Sp.5), *M. kadoyamaensis* (Sp.6), *P. bicolor* (Sp.7), *X. defensus* (Sp.10), *X. ganshoensis* (Sp.11), *X. crassiusculus* (Sp.17), and *X. germanus* (Sp.18). On the contrary, those with narrow niche or small abundance tended to be plotted far from the origin. *Platypus quercivorus* and *P. calamus* were plotted peripherally despite of their great abundance (*P. quercivorus*; N = 240, *P. calamus*; N = 73). The TE was likely to influence an angle of each plot in an anti-clockwise manner, starting with *X. seiryorensis* that was collected earliest in the season and had the smallest niche center of TE.

3.4. Discussion

Table 3-4 shows a summary of abundance categorized by the order of magnitude (S<1, M<2, L<3), ability to attack trees that was exploited by other species, competitive ability, niche breadth classified by an arithmetic mean (AM) and geometric mean (GM) of three niche breadth (broad niche (B): AM > 1 and GM > 1, narrow niche (N): AM < 1 and GM < 1, and the rests were intermediate niche (I)), arrhenotoky, sibling mating system, records as an alien species in non-native areas, features of attacking living trees, and features to killing living trees. Regarding to the species with small abundance, note that narrow niche was likely an artifact depending on their small abundance. All Scolytid species that were caught in my experiment have a habit of haplodiploidy and sibling mating system. On the contrary, all Platypodid species do not. Furthermore, Scolytid species had broader niche than Platypodid species (Table 3-2).

Therefore, Scolytid species are likely to have greater potential as alien species. Actually nine species out of the nineteen Scolytid species have been recorded as alien species in the world: *A. lewisi* (Hoebeke 1991), *A. rubricollis* (Lightle et al. 2007), *E. validus* (Coyle et al. 2005), *X. crassiusculus* (Kirkendall and Odegaard 2007), *X. saxesenii* (Reed and Muzika 2010), *X. germanus* (Mandelshtam 2001; Wood and Bright 1992), *X. seriatus* (Haack 2006), *X. pfeili* (Vanhanen 2008), and *X. volvulus*. Among those, *X. volvulus* is originated from North America and invasive to Japan (Wood 1982; Wood and Bright 1992). The rest is native in Japan and known as alien species in North America and/or Europe.

With two exceptions of *X. seriatus* and *X. pfeili*, species that are known as alien species outside Japan tended to have broad niche of TC. They were plotted near the origin of NMDS plot (Fig. 3-2). However, abundance of *X. pfeili* was so small that I cannot conclude that its niche is narrow. Regarding to TE, no consistent tendency was found for species that were known as alien species. However, species with broad seasonality are probably advantageous in invading and establishing their population in a new world. Four species, *X. ganshoensis*, *E. validus*, *X. germanus,* and *X. defensus*, were found throughout the season from March to September. *Xyleborus seiryorensis* was found for five months from March to August.

The species that attacked 0-month old bolts (Table 3-2) are thought to be aggressive species that potentially attack living trees. Actually, *A. rubricollis*, *E. validus*, *X. germanus*, *X. crassiusculs, T. solidus*, and *P. quercivorus* were known to attack living trees (Kajimura 2006; Chey 1996). Among these species, *P. quercivorus*, *X. crassiusculus*, and *X. germanus* were reported as vector insects of the sap wood stain fungus that caused wilt disease (Sreedharan et al. 1991; Kamata et al. 2002).

It is expected that there are relationships between ability to attack trees that was exploited by other species and preference to wood oldness. Mean value of niche center for WO was 2.18 for all twenty-four species, 2.38 for three 'able' species, and 2.79 for 'good' species for attacking bolts that had been exploited by other species. On the contrary, the value of *P. quercivorus* was 0.96 (Fig. 3-2c), which avoided bolts that had been exploited by other species (Table 3-2). However, *L. depressus* also did not attack bolts that had been exploited by other species though its niche center value for WO was 2.68 (Fig. 3-2c). Abundance of *L. depressus* in regimes that started to be exposed by August was 20 for the Normal experiment and 5 for the Conditioning. The number was so small that it seems impossible to conclude that the species do not attack bolts that were exploited by other species.

Species belonging to the subfamily Platypodinae tended to have narrow niche compared to those belonging to the subfamily Scolytinae. Platypodid species do not have features of arrhenotoky or sibling mating. Therefore Platypodid species has been thought to have a lower risk of invasiveness than Scolytid species. Among Platypodid species *P. modestus* showed broad niche in TC and WO (Fig. 3-2a & c), so that *P. modestus* seem to have the highest risk of invasion in the subfamily Platypodinae. My results of niche for WO (Fig. 3-2c) suggested that Scolytid ambrosia beetles showed stronger preference to fresh bolts than Platypodid ambrosia beetles did, though Speight and Wylie (2001) described that most of species belonging to Platypodinae preferred fresh wood.

Xylosandrus crassiusculus was only a species that evaluated as good at direct competition (Table 3-3a). On the other hand five species were evaluated as bad at direct competition (Table 3-3b). Seventeen species decreased their abundance in the Conditioning experiment whereas only seven species increased (Table 3-1). Seven out of nine minor species that were categorized as "S" in abundance in Table 3-4 decreased in the Conditioning experiment (Table 3-1, 3-2), suggesting that these were not good at competition, and which was a likely cause why these species were minor. *Platypus quercivorus* was major in LOC-A, in which the JOW is epidemic. However, *P. quercivorus* was "very bad" at competition because this species significantly decreased in the Conditioning experiment (Table 3-3b). This species could not colonize trees that had been exploited by other species. *Platypus quercivorus* is distributed in Asia-Pacific Region including Taiwan (Murayama 1925), Indonesia (Schedl 1972), Thailand (Hulcr et al. 2008), India (Beeson 1937), Papua New Guinea (Wood 1972), and Vietnam (Naoto Kamata, personal communication). However, outside of Japan, there had been no JOW incidence and the population density is generally low. It is likely that *P. quercivorus* can increase their number by creating its own resource by means of a symbiotic pathogenic fungus *R. quercivora* as some bark beetles do.

There are many Scolytid ambrosia beetles that are known as alien species (Haack 2006). However, there are not so many alien Platypodid species. One exception is *M. mutatus*, which was introduced from Argentine to Italy (Tremblay et al. 2000) and has caused serious problems in both of the locations (Alfaro et al. 2007). *Crossotarsus niponicus* and *P. calamus* are likely to establish their population if they will be introduced to non-indigenous-area because of significant increase in their abundance in the Conditioning experiment compared to the Normal (Table 3-1). However, infestations of those two species were both

found for a short period of late in the season (Fig. 3-2b) so that timing of introduction seems critical to succeed in colonization.

Ambrosiodmus lewisi is an Asian species with a broad range of hosts including more than thirty broadleaf trees and shrubs (Murayama 1930, 1933; Ezaki 1957) with preference for Dipterocarpaceae and Fagaceae (Browne 1961). Despite of its low abundance in my study, *A. lewisi* showed broad niche: 1.52 for TC, 2.32 for TE, and 2.06 for WO (Fig. 3-2). Because of the broad niche, the species seems likely to be a high risk species of invasion. Actually, *A. lewisi* is a non-native species in North America but was first found in 1990 from dead branches of an old black oak (*Quercus velutina* group) in Philadelphia (Hoebeke 1991). Small abundance and weak competitive ability of *A. lewisi* (Table 3-3) in my study may have depended on the fact that *A. lewisi* mostly breeds in branches (Hoebeke 1991) and prefers smaller portions. *Ambrosiodmus lewisi* was not included in a list of ambrosia beetles attacking a living tree made by Kajimura (2006).

Ambrosiodmus rubricollis is native to East Asia and was introduced to Australia, North America (Rabaglia et al. 2006), and Italy (Faccoli et al. 2009). Despite of its small abundance in my study (N = 14), *A. rubricollis* showed TC (4.64), TE (6.78), and WO (3.27) (Fig. 3-2) and colonize trees that had been exploited by other species (Table 3-2). There was a report that this species was collected throughout the season in South Carolina, US (Coyle et al. 2005). This species was collected from 0 to 4-month-old bolts, indicating the possibility of attacking living plant tissues. In Italy, *A. rubricollis* was actually reported to attack living trees of horse chestnut (*Aesculus hippocastaneum* (L.)) and peach (*Prunus persica* (L.) (Faccoli et al. 2009). In my study, competitive ability of *A. rubricollis* was evaluated as "very bad" (Table 3-3). It was reported that *A. rubricollis* tended to attack smaller stems (2 - 15 cm in diameter) (Browne 1961). This may be a cause of the small abundance of this species in my experiment, since the size of bolts was mostly larger than 15 cm in diameter.

Euwallacea validus is native to Asia and established its population in New York in 1976. It was also reported from Pennsylvania in 1980 (Wood 1977, 1982), from Louisiana in 1984 (Chapin and Oliver 1986), and from South Carolina in 2003 (Coyle et al. 2005). In my experiment, *E. vcalidus* showed niche breadth of 0.92 for TC, 2.19 for TE, 1.87 for WO (Fig. 3-2) and "very bad" competitive ability (Table 3-3). In western Japan, *E. validus* was captured by alpha-pinene and ethanol baited traps from April to summer

with a peak in May and June (Shibata et al. 1992), which coincides with a result of my study. The insect attacked 0-month-old bolt in my study (Fig. 3-2c). Actually, *E. validus* attacked living trees in the US although there were no records of attacking living trees in native area according to Kajimura (2006).

Xyleborus pfeili occurs in Asia, and was introduced to New Zealand and to the US (Bright and Skidmore 2002; Wood and Bright 1992). Although *X. pfeili* had been believed to be native in Europe (e.g. Eichhoff 1878), Kirkendall and Faccoli (2010) proposed that this species was non-native to Europe as well as to Northern Africa and Turkey but native to southern China, Japan, and Korea, and was introduced into Europe before 1837. In my experiment, the abundance was so small (N = 4) (Table 3-1) that I could not argue with niche breadth of this species. Two individuals were collected from 3 and 4-month-old bolts (Fig. 3-2c) so that no evidence to attack fresh bolts or living trees was obtained.

Xyleborus seriatus is native to the Northern China (Shanxi, Sichuan), Japan, North and South Korea, Taiwan, and the Russian Far East (Kuril Islands and Primorsk Territory) (Chu 1964; Choo and Woo 1985; Hua 2000; Mandelshtam 2001, 2006; Wood and Bright 1992) and newly recorded in Massachusetts, the US (Hoebeke and Rabaglia 2008). This species could colonize trees that had already been exploited by other species and was evaluated as a "good" colonizer. Its host range is extensive including both coniferous and broadleaved trees (Hoebeke and Rabaglia 2008), which is likely to be advantageous as an alien species. In my experiment, niche breadth was narrow (0.58 for TC, 0.05 for TE, and 0.58 for WO) (Fig. 3-2). In Massachusetts, the species was trapped from mid-April through mid-July with a strong peak in May (Hoebeke and Rabaglia 2008), which coincides with my results. Its broad host range enabled *X. seriatus* to colonize after arrival in the new area.

Xylosandrus germanus is known as an alien species in North America (Oliver and Mannion 2001) and Europe (Kirkendall and Faccoli 2010). In my experiment, *X. germanus* had broad niche for TC (1.29), TE (1.33), and WO (1.58) (Fig. 3-2) which are likely to be advantageous when they arrive in a new world. *Xylosandrus germanus* decreased significantly in the Conditioning experiment compared to the Normal experiment in LOC-B (Table 3-1). I speculate that some conditions differed in the LOC-B from the other two locations but have no specific idea. Furthermore, *X. germanus* is a generalist with broad host range: attacking both coniferous and broadleaved species (Weber and McPherson 1983). Under temperate climate, this species is bivoltine or trivoltine depending on temperature (Gandhi et al. 2010; Oliver and Mannion

2001), so that adults of this species appear from spring to fall, which was supported by my results (Fig. 3-2c). This species was also reported to attack apparently healthy trees: In early 1960s, *X. germanus* caused mass mortality of tea trees by attacking living trees in tea plantations in Japan (e.g. Kaneko et al. 1965). The species is an important pest of stored spruce in Switzerland (Graf and Manser 1996, 2000), and of walnut (*Juglans nigra* (L.)) and chestnut (*Castanea mollissima* Blume) plantations in the US (Weber 1979; Katovich 2004; Oliver and Mannion 2001). In this study *X. germanus* infested 0-month-old bolts. However, estimated niche breadth of WO ranged from 1.28 to 2.83, indicating that the species preferred slightly older bolts to fresh bolts. Some bark beetles are known to become aggressive enough to attack healthy trees during epidemics, although they infest only weakened or fallen trees during endemic periods (Lieutier et al. 2009). It is supposed that *X. germanus* was in endemic in my experiment so that the species may have not showed strong preference to fresh bolts. Another possibility is that my results may have depended on a diameter of bait bolts greater than the preferred size because there was a report that *X. germanus* preferred branches with a diameter of 5-7 cm (Kappes and Topp 2004).

Xyleborus saxesenii is a Eurasian species and is believed to be the first alien ambrosia beetle species in the US. It was first detected at the US port in 1915 (Haack 2001; Rabaglia et al. 2006). *Xyleborus saxesenii* showed broad niche despite of small abundance of ($N = 6$): estimated niche breadths were 1.58 for TC, 1.55 for TE, and 2.62 for WO. This broad niche of *X. saxesenii* contributed to great success as an invasive alien species in the US. In fact, in Missouri, *X. saxesenii* represented 41% of ambrosia beetles attracted by black oak trees killed every month from March to August (Roling and Kearby 1975) and 33% of collection by ethanol baited traps (Reed and Muzika 2010). An ability of *X. saxesenii* for colonization and competition was probably not evaluated correctly because it was minor species in my experiment (Table 3-1-3). In spite of its great abundance, *X. saxesenii* was not reported to attack living trees. *Xyleborus saxesenii* was probably not aggressive enough to attack living trees, although Cebeci and Ayberk (2010) speculated that *X. saxesenii* was a potentially serious pest of fruit orchards, hardwoods and conifers in Turkey because of their great abundance there. In my study only one individual of *X. saxesenii* among six was found from 0-month-old bolt (Fig. 3-2c).

Xyleborus ganshoensis showed the broadest niche for TC and TE, and the third broadest niche for WO (Fig. 3-2). Niche center for WO was 1.67 with estimated niche breadth ranging from 0.02 to 3.17. The

abundance of *X. ganshoensis* on the 0-month-old bolts was 157. These results indicate that *X. ganshoensis* attacks fresh to intermediately fresh bolts, which also suggests that this species is likely to attack living trees despite of no records of attacking living trees in the list made by Kajimura (2006). *Xyleborus ganshoensis* can be a vector of tree killing disease if the species establish a relationship with a fungus pathogenic to living trees. *Xyleborus ganshoensis* was found throughout the season (Fig. 3-2b). Furthermore, this species can reproduce by arrhenotoky and is highly inbred. In LOC-B, abundance increased greatly from 81 in the Normal experiment to 132 in the Conditioning experiment (Table 3-1), Considering its broad seasonality and habits of inbreeding and arrhenotoky, *X. ganshoensis* is likely a high risk species as an invader to non-indigenous areas and as a possible vector insect of tree killing disease. However, *X. ganshoensis* seems to be a good competitor because its increased its abundance in Conditioning experiment (Table 3-3a). Therefore a risk of *X. ganshoensis* as alien species causing great influence on other ambrosia species is possibly high if it carries tree-killing disease.

Niche breadth of *L. depressus* was 1.07 for TC, 1.03 for TE, 1.94 for WO. The species was found in 0- to 5-month-old bolts indicating that the species is possible to attack living trees. However, estimated niche center for WO was 2.68 (niche breadth 1.71 – 3.65) (Fig. 3-2c), which suggest that the species preferred intermediately fresh wood. *L. depressus* was intercepted at Japanese ports from timbers imported from Solomon to Nagoya and reported as its synonym, *Xyleborus sejugatus* Schedl (Browne 1981). The species was also collected from *Elaeocarpus ferrugineus* (Family, Elaeocarpaceae) from Perak, Pennisula Malaysia (Browne 1961) but has not previously been reported from Japanese forests so that this species is likely an alien species in Japan (Roger Beaver, personal communication).

Six species, *X. pfeili*, *X. volvulus*, *L. depressus*, *C. niponicus*, *T. agnatum*, and *T. solidus* did not attack 0-month-old bolts (Table 3-3). Abundance of each species was smaller than ten with one exception of *L. depressus*. Among those, *T. solidus* was recorded to have attacked living trees in tropics (Speight and Wylie 2001).

Platypus quercivorus, a vector insect of the JOW in Japan, showed the greatest mean abundance per 0-month-old bolt (1.06) (Fig. 3-2c). A result of NMDS showed that *P. calamus* had the most similar niche to *P. quercivorus* (Fig. 3-3). Some studies showed that both the two species were collected from the identical trees killed by the JOW (Hijii et al. 1991; Inoue et al. 1998). *Platypus quercivorus,* tends to attack

earlier than *P. calamus* (Ueda and Kobayashi 2001). *Platypus quercivorus* attacks living trees, whereas *P. calamus* does not (Inoue et al. 1998). These were supported by my results of WO (Fig. 3-2c) and TE (Fig. 3-2b); *P. calamus* started to attack the bolts one month later than *P. quercivorus*, showing a peak at 1-month-old bolt. *Platypus quercivorus* may play an important role as a primary attacker to produce a great amount of resource for the species that infest later than the primary attacker by attacking living trees and killing these. However, the results of Conditioning experiment (Table 3-1) indicated that *P. calamus* was a good competitor, while *P. quercivorus* was a quick colonizer but a weak competitor (Table 3-1) and avoided resource that had already been exploited by other species. It seems likely that *P. quercivorus* has evolved for attacking trees soon after starting to be weakened before other species. The abundance in the Conditioning experiment increased in *P. calamus* but decreased in *P. quercivorus* compared to the Normal experiment. If they interact with each other, it is likely that *P. calamus* would drive out *P. quercivorus*. In the LOC-A, where JOW occurred, abundance of *P. calamus* was so small in a Normal experiment (seven individuals) (Table 3-1) that I could not test the competitive exclusiveness hypothesis. There was a report that *P. quercivorus* tended to attack lower part of the tree trunk, whereas *P. calamus* tended to attack higher (Hijii et al. 1991). Therefore, competition between the two species does not seem so severe that competitive exclusion does not occur.

Future globalization would enhance chances of introduction of ambrosia beetles. To evaluate the risks for ambrosia beetle species, a similar experiment can be applied in other locations and/or to other tree species.

Tables

Table 3-1 Abundance of ambrosia beetle species collected from *Quercus serrata* bait bolts in the three locations of Central Japan. The number of entry holes was used as an indicator of the abundance of each species. Results for a Normal experiment (N) and for a Conditioning experiment (C) are shown. Total numbers for regimes that started to be exposed in March-August are shown. A paired *t*-test was employed to test the difference in the abundance between the two experiments in each location.

Code	Species	Three locations			LOC-A			LOC-B			LOC-C		
		N	C	*P*-value	N	C	*P*-value	N	C	*P*-value	N	C	*P*-value
1	*Ambrosiodmus lewisi* (Blandford)	24	18	0.356	14	9	0.460	1	0	0.329	9	9	0.782
2	*Ambrosiodmus rubricollis* (Eichhoff)	14	4	0.027	8	0	0.099	6	4	0.387	0	0	
3	*Debus emarginatus* (Eichhoff)	4	0	1	1	0	0.329	3	0	0.083	0	0	
4	*Euwallacea validus* (Eichhoff)	40	27	0.115	3	4	0.576	0	0		37	23	0.037
5	*Leptoxyleborus depressus* (Eggers)	20	5	0.006	0	0		20	5	0.079	0	0	
6	*Microperus kadoyamaensis* (Murayama)	498	780	<0.001	453	727	0.045	45	53	0.311	0	0	
7	*Planiculus bicolor* (Blandford)	147	326	<0.001	146	323	0.056	1	3	0.191	0	0	
8	*Truncaudum agnatum* (Eggers)	1	0	1	1	0	0.329	0	0		0	0	
9	*Xyleborinus saxesenii* Ratzeburg	6	5	0.763	1	0	0.329	3	0	0.083	2	5	0.190
10	*Xyleborus defensus* Blandford	94	128	0.023	88	120	0.206	1	0	0.329	5	8	0.511
*	*Xyleborus festivus* Eichhoff							0	2				
11	*Xyleborus ganshoensis* Murayama	81	132	<0.001	9	7	0.627	70	125	0.826	2	0	0.170
12	*Xyleborus laetus* Niisima	2	0	1	1	0	0.329	1	0	0.329	0	0	
13	*Xyleborus pfeili* (Ratzeburg)	4	2	0.423	1	0	0.329	0	2	0.162	3	0	0.170
14	*Xyleborus seiryorensis* Murayama	50	32	0.049	36	22	0.757	3	0	0.083	11	10	0.649
15	*Xyleborus seriatus* Blandford	36	31	0.542	2	0	0.162	0	0		34	31	0.337
16	*Xyleborus volvulus* (Fabricius)	2	0	1	0	0		1	0	0.329	1	0	0.329
17	*Xylosandrus crassiusculus* (Motschulsky)	71	89	0.156	54	56	0.742	10	4	0.292	7	29	0.140
18	*Xylosandrus germanus* (Blandford)	70	65	0.667	13	18	0.995	15	1	0.066	42	46	0.805
19	*Crossotarsus niponicus* Blandford	4	30	<0.001	0	0		0	0		4	30	0.092
20	*Crossotarsus simplex* Murayama	5	0	1	0	0		5	0	0.186	0	0	
21	*Platypus calamus* Blandford	73	186	<0.001	7	13	0.328	66	173	0.061	0	0	
22	*Platypus modestus* Blandford	57	62	0.647	0	0		0	0		57	62	0.442
23	*Platypus quercivorus* (Murayama)	240	72	<0.001	240	72	0.021	0	0		0	0	
24	*Treptoplatypus solidus* (Walker)	3	0	1	0	0		0	0		3	0	0.083

Table 3-2 Colonizing ability of ambrosia beetles for bolts that had been exploited by other species. Abundance was compared between the Normal and the Conditioning experiments before each species stated to attack in Normal experiment. If the number in a regime No. 1 was smaller than 3 and no galleries were found in a regime No.2 and 8, the species was included in the analysis. A paired *t*-test was employed to test if the difference was significant.

	LOC	Period collected in NOR	Total no. individuals captured in NOR	Period tested	No. individuals captured in NOR during test period	No. individuals captured in CON during test period	*P-value*	Evaluation
Ambrosiodmus lewisi (Blandford)	A,B,C	APR-AUG	24	MAR	0	1	-	-
Ambrosiodmus rubricollis (Eichhoff)	A,B,C	MAY-AUG	14	MAR-APR	0	2	0.423	Able
Microperus kadoyamaensis (Murayama)	A,B,C	MAY-AUG	498	MAR-APR	1	78	0.003	Good
Planiculus bicolor (Blandford)	A,B,C	MAY-AUG	147	MAR-APR	2	60	0.003	Good
Xyleborinus saxesenii Ratzeburg	A,B,C	MAY-AUG	6	MAR-APR	1	1	-	-
Xyleborus pfeili (Ratzeburg)	A,B,C	JUN-AUG	4	MAR-APR	0	2	0.184	Able
Xyleborus seriatus Blandford	A,B,C	JUN-JUL	36	MAR-MAY	0	21	0.006	Good
Xylosandrus crassiusculus (Motschulsky)	A,B,C	JUN-AUG	71	MAR-MAY	1	43	<0.001	Good
Crossotarsus niponicus Blandford	A,B,C	JUN	4	MAR-MAY	0	10	0.226	Able
Platypus calamus Blandford	A,B,C	JUN-AUG	73	MAR-MAY	0	26	0.019	Good
Platypus modestus Blandford	A,B,C	JUN-AUG	57	MAR-MAY	0	31	0.005	Good
Leptoxyleborus depressus (Eggers)	B	JUN-AUG	20	MAR-MAY	0	0	-	Avoid
Platypus quercivorus (Murayama)	A	MAY-AUG	238	MAR-APR	0	0	-	Avoid

Table 3-3 Comparison between Normal and Conditioning experiments to test if species is good or bad in competition. (a): species that increased in a Conditioning experiment. (b): Species that decreased in Conditioning experiment.

(a)

Species	LOC	Period collected in NOR	Total no. individuals captured in NOR	No. individuals captured in CON during tested period	*P-value*	Competitive ability (CA)
Microperus kadoyamaensis (Murayama)	A	MAY-AUG	563	779	0.109	
Planiculus bicolor (Blandford)	A,B,C	MAY-AUG	147	211	0.294	
Xylosandrus crassiusculus (Motschulsky)	C	MAY-AUG	7	31	0.089	Good
Crossotarsus niponicus Blandford	A,B,C	JUN	4	18	0.836	
Platypus calamus Blandford	A,B,C	JUN-AUG	73	160	0.297	
Platypus modestus Blandford	A,B,C	JUN-AUG	57	31	0.391	
Xyleborus defensus (Blandford)	A,B,C	MAR-AUG	94	105	0.339	
Xyleborus ganshoensis Murayama	A,B,C	MAR-AUG	81	132	0.362	

(b)

Species	LOC	Period collected in NOR	Total no. individuals captured in NOR	No. individuals captured in CON during tested period	*P-value*	Competitive ability (CA)
Ambrosiodmus lewisi (Blandford)	ABC	APR-AUG	20	13	0.241	
Ambrosiodmus rubricollis (Eichhoff)	ABC	MAY-AUG	14	2	0.036	Very bad
Debus emarginatus (Eichhoff)	ABC	MAR-JUL	4	0	0.042	Very bad
Euwallacea validus (Eichhoff)	ABC	MAR-AUG	40	27	0.037	Very bad
Leptoxyleborus depressus (Eggers)	B	JUN-AUG	20	5	0.078	Bad
Truncaudum agnatum (Eggers)	A	JUL	1	0	0.339	
Xyleborinus saxesenii Ratzeburg	ABC	MAR-AUG	6	5	0.774	
Xyleborus laetus Niisima	ABC	APR-JUN	2	0	0.169	
Xyleborus pfeili (Ratzeburg)	ABC	JUL-AUG	4	0	0.089	
Xyleborus seiryorensis Murayama	ABC	MAR-AUG	50	32	0.561	
Xyleborus seriatus Blandford	ABC	JUN-JUL	36	10	0.257	
Xyleborus volvulus (Fabricius)	ABC	JUL-AUG	2	0	0.167	
Xylosandrus germanus (Blandford)	ABC	MAR-AUG	70	65	0.769	
Crossotarsus simplex Murayama	ABC	APR-MAY	5	0	0.208	
Platypus quercivorus (Murayama)	ABC	MAY-AUG	238	71	0.028	Very bad
Treptoplatypus solidus (Walker)	ABC	JUL-AUG	3	0	0.208	

Table 3-4 Summary table for categorized abundance, categorized niche breadth, and biology of ambrosia beetle species collected from *Quercus serrata* bait bolts in the three locations of Central Japan. Biology is shown for characteristics of arrhenotoky and of sibling mating, records of alien species, and aggressiveness attacking living trees and killing trees or branches. Schedl (1964), Wood (1982), Hoebeke (1991), Wood and Bright (1992), Vandenberg et al. (2000), Mudge et al. (2001), Haack (2006), Kajimura (2006), Lightle et al (2007), Biederman et al. (2009), Faccoli et al. (2009), Kirkendall and Faccoli (2010), Reed and Muzika (2010).

Species	Abundance	Ability to attack trees that was exploited by other species	Competitive ability	Niche breadth	arrhenotoky	siblling	Records of alien species	attacking living tree	killing trees or branches	References
Ambrosiodmus lewisi (Blandford)	M			B	yes	yes	yes			3, 8
Ambrosiodmus rubricollis (Eichhoff)	M	Able	Very bad	I	yes	yes	yes	yes		12, 13,14
Debus emarginatus (Eichhoff)	S		Very bad	I	yes	yes				
Euwallacea validus (Eichhoff)	M		Very bad	B	yes	yes	yes	yes		9
Leptoxyleborus depressus (Eggers)	M	Avoid	bad	B	yes	yes				
Microperus kadoyamaensis (Murayama)	L	Good		B	yes	yes				
Planiculus bicolor (Blandford)	L	Good		B	yes	yes	yes	yes		11, 14
Truncaudum agnatum (Eggers)	S			I	yes	yes				
Xyleborinus saxesenii Ratzeburg	S			B	yes	yes				
Xyleborus defensus Blandford	L			B	yes	yes				
Xyleborus ganshoensis Murayama	L			B	yes	yes	yes			5, 6, 8, 13
Xyleborus laetus Niisima	S			N	yes	yes				
Xyleborus pfeili (Ratzeburg)	S	Able		N	yes	yes	yes			8
Xyleborus seiryorensis Murayama	M			N	yes	yes	yes			1, 2
Xyleborus seriatus Blandford	M	Good		N	yes	yes				
Xyleborus volvulus (Fabricius)	S			N	yes	yes				
Xylosandrus crassiusculus (Motschulsky)	M	Good	Good	B	yes	yes	yes	yes	yes	13, 14
Xylosandrus germanus (Blandford)	M			B	yes	yes	yes	yes	yes	13, 14
Crossotarsus niponicus Blandford	S	Able		N						
Crossotarsus simplex Murayama	S			N						
Platypus calamus Blandford	M	Good		N						
Platypus modestus Blandford	M	Good		B						
Platypus quercivorus (Murayama)	L	Avoid	Very bad	N				yes	yes	7
Treptoplatypus solidus (Walker)	S			N				yes		9

Abundance: **S**, abundance < 10; **M**, 10 < abundance < 100; **L**, abundance > 100.

Niche broadness: Categorized by arithmetic mean (AM) and geometric means (GM) of the three values of niche width in Fig. 3-1; **B**, broad niche (AM>1 and GM>1); **N**, narrow niche (AM<1 and GM<1); **I**, intermediate niche broadness (others).

Reference: **1**, Schedl (1964); **2**, Wood (1982); **3**, Hoebeke (1991); **4**, Wood and Bright (1992); **5**, Vandenberg et al. (2000); **6**, Mudge et al. (2001); **7**, Kamata et al (2002); **8**, Haack (2006); **9**, Kajimura (2006); **10**, Lightle et al (2007); **11**, Biederman et al. (2009); **12**, Faccoli et al.(2009); **13**, Kirkendall and Faccoli (2010); **14**, Reed and Muzika (2010).

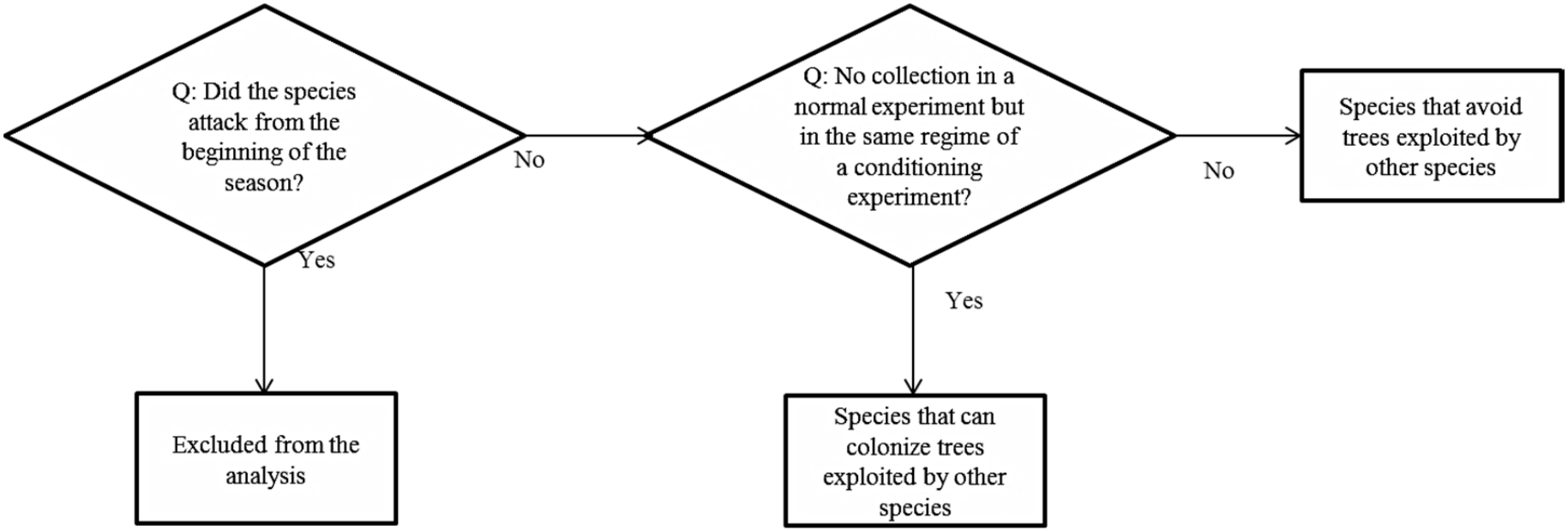
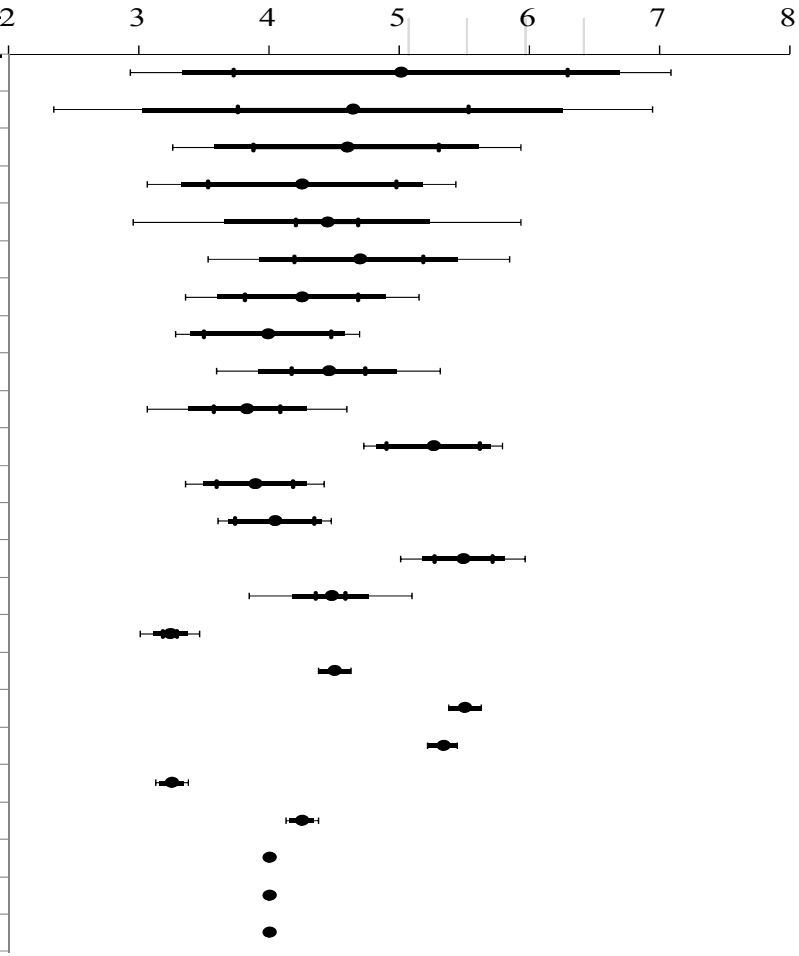


Figure 3-1 A diagram of competitive ability of each of ambrosia beetle to exploit bolt.

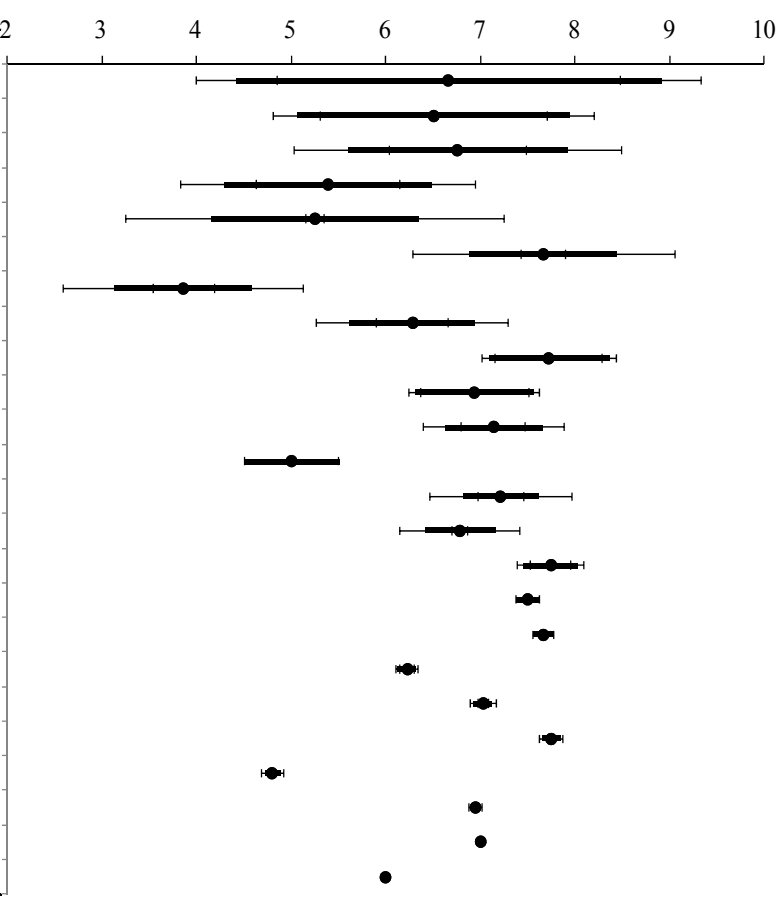
Figures
(a)

Species	Total	Mean abundance per bolt for TC						
		3	4	5	6	7	8	9
Xyleborus ganshoensis Murayama	98	0.21	0.17	0.05	0.06	0.08	0	0
Ambrosiodmus rubricollis (Eichhoff)	14	0.21	0.08	0.1	0	0.25	0.13	0
Platypus modestus Blandford	69	0	0	0.1	0.06	0	0	0
*Xylosandrus crassiusculus (*Motschulsky)	75	0	0.13	0.05	0	0	0	0
Xyleborinus saxesenii Ratzeburg	9	3.43	3.21	1.4	1.56	0.42	0	0
Ambrosiodmus lewisi (Blandford)	29	0.21	0.29	0.45	0.25	0.25	0	0
Xylosandrus germanus (Blandford)	72	0	0.21	0	0	0	0	0
Planiculus bicolor (Blandford)	231	0	0.96	0.55	0	0.17	0	0
Leptoxyleborus depressus (Eggers)	22	0	0	0.05	0.06	0	0	0
Euwallacea validus (Eichhoff)	41	0.68	0.54	0.35	0.06	0.08	0	0
Platypus quercivorus (Murayama)	240	0	0.04	0	0	0	0	0
Xyleborus defensus (Blandford)	117	0.11	0.46	0.2	0.19	0.08	0	0
Microperus kadoyamaensis (Murayama)	564	0.61	0.92	4.55	6.38	0.58	0.13	0
Platypus calamus Blandford	73	0.86	0.08	0.1	0	0	0	0
Xyleborus seriatus Blandford	36	1.25	0.13	1.15	0.56	0.33	0.13	0
Xyleborus seiryorensis Murayama	50	0	0.08	0	0	0	0	0
Xyleborus pfeili (Ratzeburg)	4	0.46	0.67	1.3	0.56	0.17	1.75	1
Xyleborus volvulus (Fabricius)	2	0.57	0.33	0.1	0	0	0	0
Treptoplatypus solidus (Walker)	3	0.11	0.04	0	0	0	0	0
Debus emarginatus (Eichhoff)	4	0	0.33	1.25	2.38	0	0.25	0
Crossotarsus niponicus Blandford	4	4.71	13.4	3.85	1.25	1.08	0	0
Truncaudum agnatum (Eggers)	1	2	2	0.85	0.38	0.08	0	0
Xyleborus laetus Niisima	2	0.64	1.25	0.75	0.19	0.5	0	0
Crossotarsus simplex Murayama	5	0.57	1.08	0.5	0.44	0.58	0.38	0



(b)

Species	Total	Mean abundance per bolt for TE						
		3	4	5	6	7	8	9
Xyleborus ganshoensis Murayama	98	0.25	1.25	0.17	0.31	0.60	1.54	0.61
Xyleborus defensus (Blandford)	117	3.75	0.63	3.00	0.88	1.10	0.54	0.82
Ambrosiodmus lewisi (Blandford)	29	0.00	0.50	0.00	0.56	0.35	0.17	0.18
Euwallacea validus (Eichhoff)	41	1.25	0.88	0.83	0.50	0.45	0.04	0.04
Debus emarginatus (Eichhoff)	4	0.25	0.00	0.08	0.06	0.05	0.00	0.00
Xyleborinus saxesenii Ratzeburg	9	1.00	0.00	0.08	0.00	0.15	0.08	0.11
Xyleborus seiryorensis Murayama	50	0.00	2.25	0.33	0.06	0.05	0.08	0.00
Xylosandrus germanus (Blandford)	72	1.00	0.13	0.17	2.19	1.15	0.21	0.07
Planiculus bicolor (Blandford)	231	0.00	0.00	0.25	1.88	3.95	1.46	3.00
Microperus kadoyamaensis (Murayama)	564	0.25	0.00	2.67	12.69	7.95	4.29	2.36
Leptoxyleborus depressus (Eggers)	22	0.00	0.00	0.00	0.50	0.25	0.29	0.07
Xyleborus laetus Niisima	2	0.00	0.13	0.00	0.06	0.00	0.00	0.00
*Xylosandrus crassiusculus (*Motschulsky)	75	0.25	0.00	0.00	0.56	1.85	1.00	0.14
Ambrosiodmus rubricollis (Eichhoff)	14	0.00	0.00	0.17	0.06	0.45	0.08	0.00
Platypus modestus Blandford	69	0.00	0.00	0.00	0.06	1.40	1.17	0.43
Xyleborus volvulus (Fabricius)	2	0.00	0.00	0.00	0.00	0.05	0.04	0.00
Treptoplatypus solidus (Walker)	3	0.00	0.00	0.00	0.00	0.05	0.08	0.00
Platypus quercivorus (Murayama)	240	0.00	0.00	0.08	11.6	2.65	0.04	0.00
Platypus calamus Blandford	73	0.00	0.00	0.00	0.38	2.95	0.33	0.00
Xyleborus pfeili (Ratzeburg)	4	6.00	0.00	0.00	0.00	0.05	0.13	0.00
Crossotarsus simplex Murayama	5	0.00	0.13	0.33	0.00	0.00	0.00	0.00
Xyleborus seriatus Blandford	36	0.00	0.00	0.00	0.13	1.70	0.00	0.00
Truncaudum agnatum (Eggers)	1	0.00	0.00	0.00	0.00	0.05	0.00	0.00
Crossotarsus niponicus Blandford	4	0.00	0.00	0.00	0.25	0.00	0.00	0.00



(c)

Species	Total	Mean abundance per bolt for WO						
		0	1	2	3	4	5	6
Xyleborus defensus (Blandford)	117	0.93	1.29	0.75	0.88	1.25	2.38	2.00
Ambrosiodmus rubricollis (Eichhoff)	14	0.18	0.04	0.05	0.06	0.50	0.00	0.00
Xyleborus ganshoensis Murayama	97	0.96	0.46	0.55	1.13	0.67	0.75	0.75
Xyleborinus saxesenii Ratzeburg	9	0.18	0.00	0.10	0.13	0.08	0.38	0.00
Ambrosiodmus lewisi (Blandford)	29	0.11	0.42	0.40	0.19	0.08	0.38	0.25
Planiculus bicolor (Blandford)	231	0.14	0.63	1.80	2.06	6.17	4.13	9.00
*Xylosandrus crassiusculus (*Motschulsky)	75	0.11	0.54	0.65	0.75	1.92	1.38	0.00
Platypus calamus Blandford	73	0.07	1.50	1.45	0.38	0.00	0.00	0.00
Debus emarginatus (Eichhoff)	4	0.04	0.00	0.10	0.00	0.08	0.00	0.00
Leptoxyleborus depressus (Eggers)	22	0.04	0.17	0.30	0.19	0.50	0.25	0.00
Microperus kadoyamaensis (Murayama)	564	0.64	2.04	9.40	8.25	8.42	6.13	6.75
Euwallacea validus (Eichhoff)	41	0.36	0.54	0.45	0.31	0.17	0.25	0.00
Xylosandrus germanus (Blandford)	72	0.39	0.42	1.45	0.69	0.83	0.13	0.00
Platypus modestus Blandford	69	0.07	0.13	0.70	1.81	0.50	1.88	0.00
Xyleborus laetus Niisima	2	0.04	0.00	0.05	0.00	0.00	0.00	0.00
Xyleborus volvulus (Fabricius)	2	0.00	0.04	0.00	0.06	0.00	0.00	0.00
Platypus quercivorus (Murayama)	240	3.18	3.67	2.30	1.06	0.00	0.00	0.00
Xyleborus seiryorensis Murayama	50	0.18	0.58	0.20	0.19	0.00	0.00	0.00
Xyleborus seriatus Blandford	36	0.07	0.00	0.65	1.31	0.00	0.00	0.00
Treptoplatypus solidus (Walker)	3	0.00	0.00	0.10	0.06	0.00	0.00	0.00
Xyleborus pfeili (Ratzeburg)	4	0.86	0.00	0.00	0.19	0.08	0.00	0.00
Crossotarsus niponicus Blandford	4	0.00	0.04	0.15	0.00	0.00	0.00	0.00
Crossotarsus simplex Murayama	5	0.04	0.17	0.00	0.00	0.00	0.00	0.00
Truncaudum agnatum (Eggers)	1	0.00	0.00	0.00	0.06	0.00	0.00	0.00

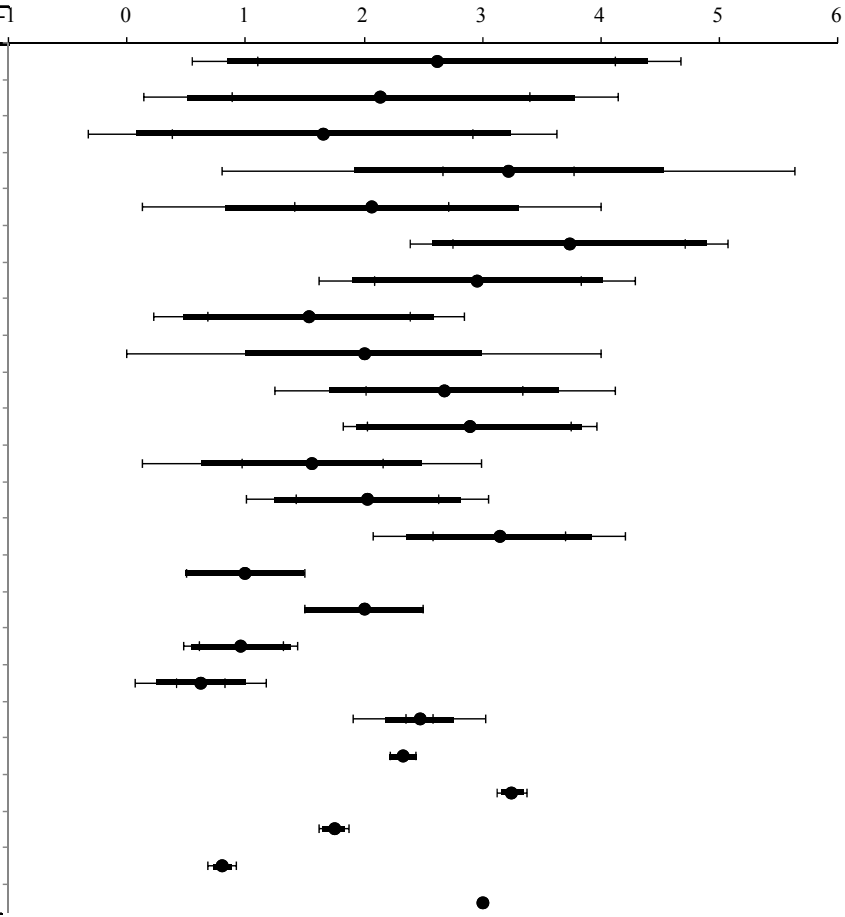


Figure 3-2 Niche center and niche breadth of each of ambrosia beetles collected from bait *Quercuss serrata* bolts in the three location of the Central Japan. The data are shown in a descendent order of niche breadth. Total abundance and mean abundance at each value of the niche context are also shown. (a) timing of cutting trees (TC = 3, 4, …, 9 indicate March, April,…, September, respectively), (b) timing of starting exposure (TE = 3, 4, …, 9 indicate March, April,…, September, respectively), (c) wood oldness (WO = 0, 1,…, 6).

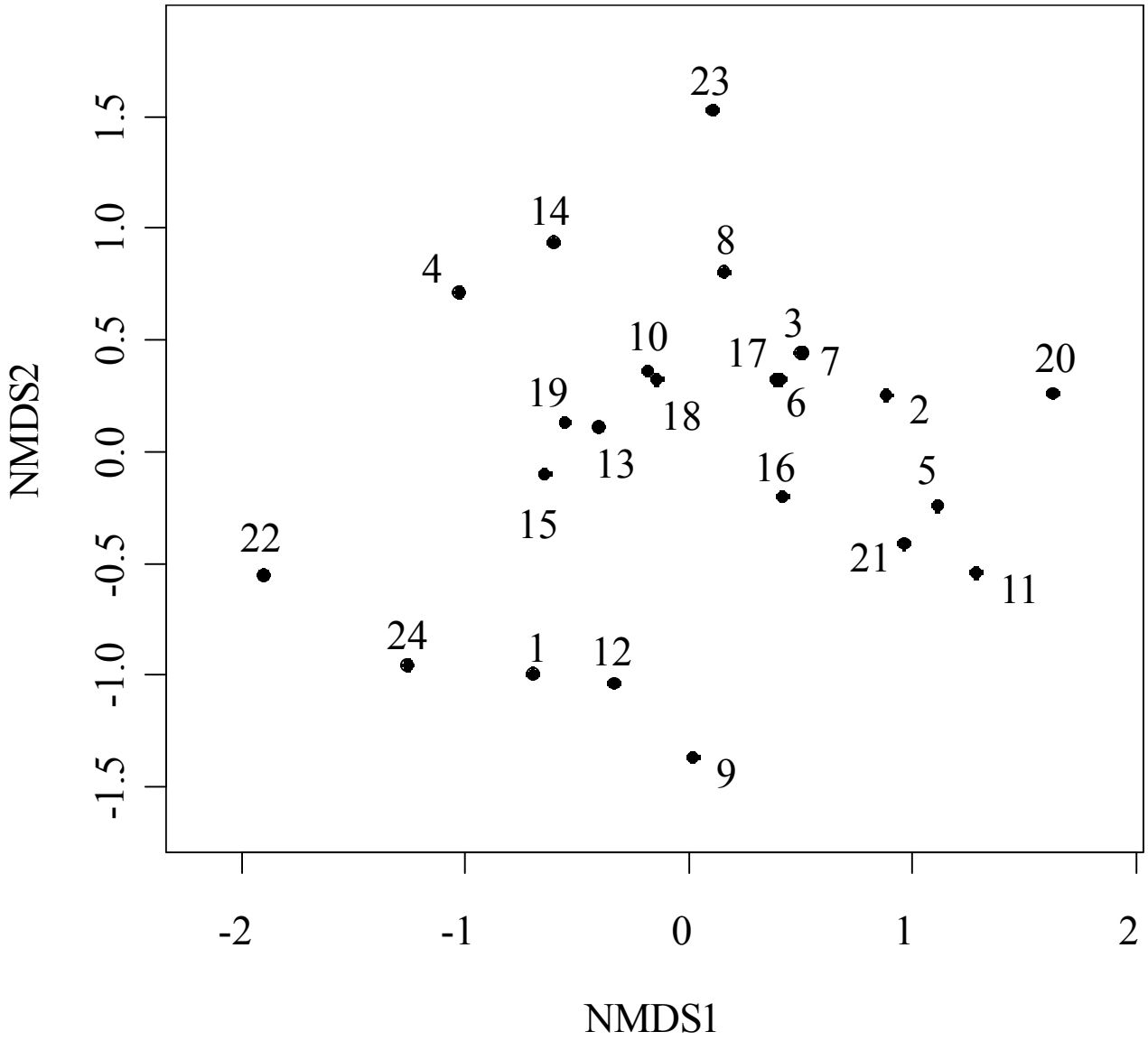


Figure 3-3 Non-metric multidimensional scaling (NMDS) ordination of ambrosia beetle guilds attacking *Quercus serrata* bait bolts in the three locations of Central Japan, based on ambrosia beetle species (species codes are detailed in Table 3-1). The NMDS is based on a distance matrix computed with Chao's dissimilarity index. The result with "noshare = 0.1" is shown.

CHAPTER 4: GENERAL DISCUSSION AND CONCLUSION

In this study, ambrosia beetles were collected using bait bolts of *Q. serrata* in three locations in the Central Japan. Timing of cutting, timing of exposure, and wood oldness were artificially controlled. In Chapter 2, factors influencing the ambrosia guild structure were determined. The LOC had the greatest effect on determining guild structure. Effect of TE was greater than TC. The effect of WO was negligible. A hierarchical structure among the three factors was a likely cause of their relative importance determining guild structure. Hulcr et al. (2007) determined ambrosia beetle community in three lowland rainforests in Papua New Guinea, in which furthest distance between the two locations was c. 1,000 km. In their study, beta diversity (diversity among locations) was smaller than alpha diversity (diversity within each location). Despite of shorter distances among locations, differences in vegetation and in climate among the three locations were greater in my study because of great difference in elevation (between 95-820 m above the sea level), which was a likely cause of the great dissimilarity in ambrosia beetle guild among the locations. In the chapter 3, the niche center and niche breadth of each ambrosia beetle species were determined for TC, TE, and WO. Species with wide niche in TC, TE, and WO were likely to be advantageous in establishment in non-indigenous area. Species with strong competitive ability are likely to have a high risk as invasive alien species. Species with small value of niche center of WO have a high risk to carry tree-killing disease.

Until 1980s, most ambrosia beetles have been believed to be pests after logging, which do not cause serious damage to living healthy trees (Coulson and Witter 1984). The situation has greatly changed since late 1980s. The JOW has been reported since 1930s in some restricted areas of Japan (Ito 2008). However, since late 1980s, epidemics have lasted for more than two decades, and the area with the JOW incidence has been spreading to new localities where the JOW incidence has never been recorded in the past (Ito 2008). A similar disease, the KOW, also suddenly emerged in the South Korea in 2003 and have spread out around the country (Kim et al. 2009). There have been arguments among scientists on the cause of recent epidemics of the JOW and the KOW. Yoshida (1994) implied a hypothesis of new associations of *P. quercivorus* with exotic *R. quercivora* that are higher virulent than the original symbionts. Kamata et al. (2002) proposed that the JOW epidemic in Japan probably resulted from global warming since the late 1980s. The unusual warm climate made possible the fateful encounter of *P. quercivorus* with *Q. cripsula*,

which was distributed most north and most susceptible to the JOW among the host trees of *P. quercivorus*, and by allowing the beetle to extend its distribution to more northerly latitudes and higher elevations. Kobayashi and Ueda (2002) also suggested that tree stress by the global warming was also a possible cause of JOW epidemics because the JOW incidence tended to start from lower elevation area and to shift to higher elevation year by year. However, Ida and Takahashi (2010) speculated that the JOW occurred in 18th century depending on the records of symptom similar to the JOW around 1750 in Nagano Prefecture, the Central Japan. Kobayashi and Ueda (2005) proposed that increase of large trees by having stopped using coppice oak stands as fuel since 1960s was crucial to the JOW epidemics because large trees are favorable resource to reproduction of *P. quersivorus*. Regarding to the KOW, underuse of fuel wood forests and stress by the global warming are thought to be a likely cause of the epidemics (Kyung-He Kim, personal communications).

The laurel wilt differs greatly from the JOW and the KOW. *Xyleborus glabratus*, a vector insect of the laurel wilt, is believed to be an alien species that was first introduced to US in 2002 (Rabaglia et al. 2006). Because of a lack of coevolutinary history between host and pathogen, virulence of the pathogen *R. lauricola* to redbay trees and to avocado trees is much greater than that of *R. quercivora* to Fagaceae trees in Japan (Fraedrich et al. 2008 ; Inch and Ploetz 2012; Kusumoto et al. 2012). Mass inoculation by *R. quercivora* is needed for the JOW to kill host trees (Ito et al. 1998). However, one-shot of inoculation by *R. lauricola* can kill redbay trees and avocado trees (Inch and Ploetz 2012).

Kühnholz et al. (2001) reviewed recent increase of ambrosia beetles attacking apparently healthy-looking trees in the world and discussed the causes. Globalization that causes new encounters of ambrosia beetles with host tree species and climate change (Kühnholz et al. 2001) are possible triggers. Phenological mismatch between ambrosia beetles and host plants caused by climate change (Kühnholz et al. 2001) is a direct cause because the beetles can attack before the host trees wake up to defense against the beetle attacks (Kühnholz et al. 2001). Cryptic behavior and chemical communication ability of ambrosia beetles (Kühnholz et al. 2001) are a background of the phenomena. Hulcr et al. (2011) reviewed recent emergence of tree killing disease carried by ambrosia beetles and proposed hypothesis as follows: "virulence of the symbioses in invaded ranges is often triggered when several factors coincide (i) invasion into territories with naive trees, (ii) the ability of the fungus to either overcome resistance of the naive host

or trigger a suicidal over-reaction, and (iii) an 'olfactory mismatch in the insect whereby a subset of live trees is perceived as dead and suitable for colonization (Hulcr and Dunn 2011)".

Shea and Chesson (2002) proposed a concept of "niche opportunity" to understand biological invasion of alien species at a community level. Community with low niche opportunity, which is supposed to result from high species diversity, is more resistant to biological invasion. Regarding to ambrosia beetles, there are many reports of establishment and spread of alien species in the world (Haack 2006). *Xyleborus glabratus* causes serious problems in non-indigenous area by vectoring *R. lauricola* that can kill redbay and avocado trees by one shot of inoculation (Fraedrich et al. 2008 ; Ploetz et al. 2012). *Xyleborinus saxesenii* has become predominant in the ambrosia beetle assemblage of its non-indigenous area without killing trees (Reed and Muzika 2010). These facts indicate that ambrosia beetle community/guild are high niche opportunity and easily allow new invasions, which probably do not depend on vacant niche but on high niche overlap.

It has been believed that relationships between ambrosia beetles and their associated ambrosia fungi have established by a long coevolutionary process so that the combinations of ambrosia beetles and ambrosia fungi are restricted compared to their compatibility (Kajimura 1995). Ambrosia beetles can grow on some ambrosia fungi, with which no symbiotic relationship in fields(Kajimura 1995). Similar specific many-to-many relationship was also reported in bark beetle-blue stain fungus complex (Yamaoka 2000). However, my understanding on bark/ambrosia beetles-their symbionts has changed recently. Dutch elm disease (DED) is caused by *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* vectored by several species of bark beetles (Sinclair and Lyon 2005). *Scolytus schevyrewi* is native in Asia but first found in US in 2003 (Negron et al. 2005). The Dutch elm disease fungus, *O. novo-ulmi*, was not accompanied by *S. schevyrewi* soon after its establishment in US but was isolated from *S. schevyrewi* that emerged from DED-diseased segments of American elm, *Ulmus americana* thereafter (Jacobi et al. 2007). Results of DNA analysis also suggested that transmission of *R. quercivora* from *Platypus taiheizanensis* (Murayama) to *P. quercivorus* in Taiwan (Naoto Kamata, personal communication). Actually "mycocleptism (Hulcr and Cognato 2010)", a strategy of acquiring symbiotic fungus by stealing fungus from inoculum of symbiotic fungi from galleries of other ambrosia beetle species, were found from several clades of the tribe Xyleborini (Hulcr and Cognato 2010) though *R. quercivora* between *P. taiheizanensis* and *P. quercivorus* is not the case of

mycocleptism. Therefore horizontal exchange of fungi between ambrosia beetle species is probably more common than it has been expected (Hulcr and Cognato 2010). Therefore, invasion of ambrosia beetles in non-indigenous area promote potentially aggressive ambrosia beetle species to create new symbiotic relationship with fungi with high virulence.

Mean annual temperature and precipitation are thought to have large effects on species composition of ambrosia beetles in the Northern Thailand (Hulcr et al. 2008). There is also a report that only approximately 2% of ambrosia beetle species in low elevations overlapped with those in high elevations (Hulcr 2010). I speculate that temperature is a direct cause of this great difference in ambrosia beetle species. Results of this study also showed that location had the greatest effect determining ambrosia beetle guild factors. Temperature and temperature related vegetation was a likely cause of the strong effects of the location. Kamata et al. (2002) hypothesized that distribution of *P. quercivorus* had expanded to more northerly latitudes and higher elevation. Therefore not only not only globalization but also climate change will possibly cause distribution shift of many ambrosia beetle species, which causes a fateful encounter of these beetles with new host species and/or new symbiotic fungi as well as alien species.

BIBLIOGRAPHY

Alfaro RI, Humble LM, Gonzalez P, Villaverde R and Allegro G (2007) The threat of the ambrosia beetle *Megaplatypus mutatus* (Chapuis) (=*Platypus mutatus* Chapuis) to world poplar resources. Forestry 80:471-479

Aoki S (2009) Kruskal-Wallis test (plus multiple comparison). Available at: http://aoki2.si.gunma-u.ac.jp/R/kruskal-wallis.html. Accessed 30 December 2011

Aukema JE, McCullough DG, Von Holle B, Liebhold AM, Britton K and Frankel SJ (2010) Historical accumulation of nonindigenous forest pests in the Continental United States. Bioscience 60:886-897

Batra LR (1963) Ecology of ambrosia fungi and their dissemination by beetles. Trans Kans Acad Sci 66:213-236

Batra LR (1966) Ambrosia fungi: extent of specificity to ambrosia beetles. Science 153:193-195

Beaver RA (1989) Insect-fungus relationships in the bark and ambrosia beetles. In: Wilding N, Collins N, Hammond P,Webber J (eds) Insect-Fungus Interactions. Academic Press, London, pp. 121-143

Beaver RA and Löyttyniemi K (1991) Annual flight patterns and diversity of bark and ambrosia beetles (Col., Scolytidae and Platypodidae) attracted to bait logs in Zambia. J Appl Entomol 112:505-511

Beaver RA and Shih H-T (2003) Checklist of Platypodidae (Coleoptera: Curculionoidea) from Taiwan. Plant Prot Bull (Taichung) 45:75-90

Beeson CFC (1937) New *Crossotarsus* (Platypodidae, Col.). The Indian Forest Records New Series Entomology 3:49-103

Berryman AA (1989) Adaptive pathways in Scolytid-fungus associations. Insect-fungus interactions, Academic Press, New York

Biedermann PHW (2010) Observations on sex ratio and behavior of males in *Xyleborinus saxesenii* Ratzeburg (Scolytinae, Coleoptera). ZooKeys 56:253-267

Biedermann PHW, Klepzig KD and Taborsky M (2009) Fungus cultivation by ambrosia beetles: behavior and laboratory breeding success in three Xyleborine species. Environ Entomol 38:1096-1105

Biedermann PHW, Klepzig KD and Taborsky M (2011) Costs of delayed dispersal and alloparental care in the fungus-cultivating ambrosia beetle *Xyleborus affinis* Eichhoff (Scolytinae: Curculionidae). Behav Ecol Sociobiol 65:1753-1761

Bright DE and Skidmore RE (2002) A catalog of Scolytidae and Platypodidae (Coleoptera), Suplement 2 (1995-1999). NRC Research Press, Ottawa

Browne FG (1961) The biology of Malayan Scolytidae and Platypodidae. Malayan Forest Records 22:1-255

Browne FG (1981) Bark beetles and ambrosia beetles (Coleoptera, Scolytidae and Platypodidae) intercepted at Japanese ports, withDescriptions of new species,VI. Kontyu 49:597-606

Browne FG (1986) Bark beetles and ambrosia beetles (Coleoptera, Scolytidae and Platypodidae) intercepted at Japanese ports, with descriptions of new species, XIV. Jap J Entomol 54:333-343

Bumrungsri S, Beaver R, Phongpaichit S and Sittichaya W (2008) The infestation by an exotic ambrosia beetle, *Euplatypus parallelus* (F.)(Coleoptera: Curculionidae: Platypodinae) of Angsana trees (*Pterocarpus indicus* Willd.) in southern Thailand. Songklanakarin J Sci Technol 30:579-582

Carpenter SE, Harmon ME, Ingham ER, Kelsey RG, Lattin JD and Schowalter TD (1988) Early patterns of heterotroph activity in conifer logs. Proceedings of the Royal Society of Edinburgh Section B-Biological Sciences 94:33-43

Cebeci HH and Ayberk H (2010) Ambrosia beetles, hosts and distribution in Turkey with a study on the species of Istanbul province. AJAR 5:1055-1059

Chao A, Chazdon RL, Colwell RK and Shen TJ (2005) A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecol Lett 8:148-159

Chapin JB and Oliver AD (1986) New records for Xylosandrus and Xyleborus species (Coleoptera: Scolytidae). Proc Entomol Soc Wash 88(4):680-683

Chevan A and Sutherland M (1991) Hierarchical partitioning. Am Stat 45:90-96

Chey VK (1996) Forest pest insects in Sabah. Sabah Forest Record 15. Sabah Forest Department, Sandakan

Choo HY and Woo KS (1985) A list of Korean bark and ambrosia beetles, and their host plants. Korean J Plant Prot 24 163-167

Chu D (1964) Geographic distribution of the class Scolytidae in Korea. Saengniulhak 3:5-14.(in Korea)

Clarke KR (1993) Nonparametric multivariate analyses of changes in community structure. Aust J Ecol 18:117-143

Coulson RN and Witter JA (1984) Forest entomology: ecology and management. Wiley,

Coyle D, Booth D and Wallace M (2005) Ambrosia beetle (Coleoptera: Scolytidae) species, flight, and attack on living eastern cottonwood trees. J Econ Entomol 98:2049-2057

Crist TO, Veech JA, Gering JC and Summerville KS (2003) Partitioning species diversity across landscapes and regions: A hierarchical analysis of alpha, beta, and gamma diversity. Am Nat 162:734-743

Davis MA (2009) Invasion biology. Oxford University Press, Oxford

De Cáceres M (2011) R package resniche (ver. 1.3). Available at: https://sites.google.com/site/miqueldecaceres/software. Accessed 4 January 2012

De Cáceres M, Sol D, Lapiedra O and Legendre P (2011) A framework for estimating niche metrics using the resemblance between qualitative resources. Oikos 120:1341-1350

Ecohydrology Research Institute, The University of Tokyo Forests, The University of Tokyo (2011) The 5th management plan on the Ecohydrology Research Institute, The University of Tokyo Forests, Graduate School of Agricultural and Life Sciences, The University of Tokyo (2011- 2020). Miscellaneous Information the Tokyo University Forests 51:305-396 (in Japanese)

Edmonds RL and Eglitis A (1989) The role of the douglas-fir beetle and wood borers in the decomposition of and nutrient release from douglas-fir logs. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 19:853-859

Eichhoff W (1878) Ratio, descriptio, emendatio eorum Tomicinorum qui sunt Dr. medic Chapuisi et autoris ipsius collectionibus et quos praeterea recognovit. 8:1-528

Ezaki T (1957) Iconographia insectorum japonicorum. Hokuryukan Publishing Co., Ltd., Tokyo

Faccoli M, Frigimelica G, Mori N, Toffolo P, Vettorazzom M and Simonato M (2009) First record of *Ambrosiodmus* (Hopkins, 1915) (Coleoptera: Curculionidae, Scolytinae) in Europe. Zootaxa 2303:57-60

Fox J and Weisberg S (2011) An R companion to applied regression. Available at: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion. Accessed 12 April 2011

Fox J and Weisberg S (2011a) Companion to applied regression. R package version 2.0-10. Available at: http://cran.r-project.org/web/packages/car. Accessed 12 April 2011

Fraedrich SW, Harrington TC, Rabaglia RJ, Ulyshen MD, Mayfield AE, Hanula JL, Eickwort JM and Miller DR (2008) A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the southeastern United States. Plant Disease 92:215-224

Funes H, Griffo R, Zerba E and Gonzalez-Audino P (2011) Mating disruption of the ambrosia beetle *Megaplatypus mutatus* in poplar and hazelnut plantations using reservoir systems for pheromones. Entomologia Experimentalis et Applicata 139:226-234

Gandhi KJK, Cognato AI, Lightle DM, Mosley BJ, Nielsen DG and Herms DA (2010) Species composition, seasonal activity, and semiochemical response of native and exotic bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in Northeastern Ohio. J Econ Entomol 103:1187-1195

Ganter PF (2006) Yeast and invertebrate associations. In: Péter G and Rosa C (eds) Biodiversity and ecophysiology of yeasts. The yeast handbook. Springer, Heidelberg, pp. 303-370

Graf E and Manser P (1996) Der Schwarze Nutzholzborkenkäfer, *Xylosandrus germanus*, in der Schweiz. Holz-Zentralblatt 122:454-456

Graf E and Manser P (2000) Beitrag zum eingeschleppten Schwarzen Nutzholzborkenkäfer *Xylosandrus germanus*. Biologie und Schadenpotential an im Wald gelagertem Rundholz im Vergleich zu *Xyloterus lineatus* und *Hylecoetus dermestoides*. Schweizerische Zeitschrift für Forstwesen 151:271-281

Graham SA (1925) The felled tree trunk as an ecological unit. Ecology 6:397-411

Haack R (2006) Exotic bark- and wood-boring Coleoptera in the United States: recent establishments and interceptions. Can J For Res 36:269-288

Haack RA (2001) Intercepted Scolytidae (Coleoptera) at U.S. ports of entry: 1985–2000. Integr Pest Manage Rev 6:253-282

Haack RA and Cavey JF (2000) Insects intercepted on solid wood packing materials at United States ports-of-entry: 1985-1998. In. International Conference on Quarantine Pests for the Forestry Sector and their Effects on Foreign Trade, Concepcion, Chile. CORMA, Concerpcion. pp. 1-16.

Haanstad JO and Norris DM (1985) Microbial symbionts of the ambrosia beetle *Xyloterinus politus*. Microb Ecol 11:267-276

Hanula JL, Mayfield AE, 3rd, Fraedrich SW and Rabaglia RJ (2008) Biology and host associations of redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae), exotic vector of laurel wilt killing redbay trees in the southeastern United States. J Econ Entomol 101:1276-1286

Harrington TC, Yun HY, Lu SS, Goto H, Aghayeva D and Fraedrich S (2011) Isolations from the redbay ambrosia beetle, *Xyleborus glabratus*, confirm that the laurel wilt pathogen, *Raffaelea lauricola*, originated in Asia. Mycologia 103:1028-1036

Hijii N, Kajimura H, Urano T, Kinuura H and Itami H (1991) The mass mortality of oak trees induced by *Platypus quercivorus* (Murayama) and *Platypus calamus* Blandford (Coleoptera: Platypodidae): the density and spatial distribution of attack by the beetles. J Jap Forestry Soc 73:471-476

Hoebeke E (1991) An Asian ambrosia beetle, *Ambrosiodmus lewisi*, new to North America (Coleoptera: Scolytidae). Proc Entomol Soc Wash 93:420-424

Hoebeke ER and Rabaglia RJ (2008) *Xyleborus seriatus* Blandford (Coleoptera: Curculionidae: Scolytinae), An Asian Ambrosia Beetle New to North America. Proc Entomol Soc Wash 110:470-476

Horn S and Horn GN (2006) New host record for the Asian ambrosia beetle, Xylosandrus crassiusculus (Motschulsky) (Coleoptera : Curculionidae). J Entomol Sci 41:90-91

Hua L-Z (2000) List of Chinese insects. Guangzhou Zhongshan (Sun Yat-sen) University Press, Guangzhou

Hulcr J (2010) Ambrosia beetle diversity. Available at: http://www.antmacroecology.org/~ambrosiasymbiosis/beetles_diversity.html. Accessed 7, February 2012

Hulcr J, Beaver RA, Puranasakul W, Dole SA and Sonthichai S (2008) A comparison of bark and ambrosia beetle communities in two forest types in northern Thailand (Coleoptera: Curculionidae: Scolytinae and Platypodinae). Environ Entomol 37:1461-1470

Hulcr J and Cognato AI (2010) Repeated Evolution of Crop Theft in Fungus-Farming Ambrosia Beetles. Evolution 64:3205-3212

Hulcr J and Dunn RR (2011) The sudden emergence of pathogenicity in insect-fungus symbioses threatens naive forest ecosystems. Proceedings of the Royal Society B: Biological Sciences 278:2866-2873

Hulcr J, Mogia M, Isua B and Novotny V (2007) Host specificity of ambrosia and bark beetles (Col., Curculionidae : Scolytinae and Platypodinae) in a New Guinea rainforest. Ecol Entomol 32:762-772

Ida H and Takahashi S (2010) Mass mortality of oak trees had already occurred at the Edo period [1603 - 1863 in Japan]. J Jap Forestry Soc 92:115-119.(in Japanese)

Igarashi Y and Iwata T (2004) The flora of University Forest in Chichibu, the University of Tokyo. Miscellaneous Information the Tokyo University Forests 44:73-119.(in Japanese)

Inch SA and Ploetz RC (2012) Impact of laurel wilt, caused by *Raffaelea lauricola*, on xylem function in avocado, *Persea americana*. Forest Pathology 42:239-245

Inoue M, Nishigaki S and Nishimura N (1998) Attack density and seasonal prevalence of two Platypodid beetles, *Platypus quercivorus* and *Platypus calamus* (Coleoptera: Platypodidae) on live, dead and logged oak trees. Applied Forest Science 7:121-126 (in Japanese with English summary)

Ito S (2008) Occurrence and severity of the Japanese oak wilt on trees in Fagaceae. Journal of Tree Health 12:57-60.(in Japanese)

Ito S, Kubono T, Sahashi N and Yamada T (1998) Associated fungi with the mass mortality of oak [*Quercus serrata*] trees. J Jap Forestry Soc 80:170-175.(in Japanese with English summary)

Jacobi WR, Koski RD, Harrington TC and Witcosky JJ (2007) Association of *Ophiostoma novo-ulmi* with *Scolytus schevyrewi* (Scolytidae) in Colorado. Plant Disease 91:245-247

Japan Meteorological Agency (2009) Weather, Climate & Earthquake Information. Available at: http://www.data.jma.go.jp/obd/stats/etrn/. Accessed 12 April 2011

Jost L (2007) Partitioning diversity into independent alpha and beta components. Ecology 88:2427-2439

Kabe M (1960) On the hosts and habitats of the Scolytid and Platypodid-beetles in Japan. Maebashi Regional Forests Office, Maebashi (in Japanese)

Kajimura H (1995) Symbiotic interactions between the ambrosia beetle, *Xylosandrus mutilatus* (Blandford)(Coleoptera : Scolytidae) and its associated fungi, and their adaptive significance. Bulletin of the Nagoya University Forests 14:89-171. (in Japanese with an English summary)

Kajimura H (2006) Overview of ecological and life-history traits in ambrosia beetles, fungus-farming insects in trees. In: Shibata E and Togashi K (eds) The fascinating lives of insects residing in tree trunks-an introduction to tree-boring insects. Tokai University, Hatano, pp. 161–186

Kamata N, Esaki K, Kato K, Igeta Y and Wada K (2002) Potential impact of global warming on deciduous oak dieback caused by ambrosia fungus *Raffaelea* sp carried by ambrosia beetle *Platypus quercivorus* (Coleoptera : Platypodidae) in Japan. Bull Entomol Res 92:119-126

Kamata N, Esaki K, Mori K, Takemoto H, Mitsunaga T and Honda H (2008) Field trap test for bioassay of synthetic (1 S ,4 R)-4-isopropyl-1-methyl-2-cyclohexen-1-ol as an aggregation pheromone of *Platypus quercivorus* (Coleoptera: Platipodidae). J For Res 13:122-126

Kaneko T, Tamaki Y and Takagi K (1965) Preliminary report on the biology of some Scolytid beetles, the tea root borer, *Xyleborus germanus* Blanford, attacking tea roots, and the tea stem borer, *Xyleborus compactus* Eichhoff, attacking tea twigs. Jpn J Appl Entomol Zool 9:23-28. (in Japanese with an English summary)

Kappes H and Topp W (2004) Emergence of Coleoptera from deadwood in a managed broadleaved forest in central Europe. Biodivers Conserv 13:1905-1924

Katoh H, Miura T, Maekawa K, Shinzato N and Matsumoto T (2002) Genetic variation of symbiotic fungi cultivated by the macrotermitine termite Odontotermes formosanus (Isoptera: Termitidae) in the Ryukyu Archipelago. Mol Ecol 11:1565-1572

Katovich S (2004) Insects attacking black walnut in the Midwestern United States. USDA, Forest Service, NC-243

Kenis M, Auger-Rozenberg M-A, Roques A, Timms L, Péré C, Cock M, Settele J, Augustin S and Lopez-Vaamonde C (2009) Ecological effects of invasive alien insects. Biol Invasions 11:21-45

Kikkawa J and Anderson DJ (1986) Community ecology: pattern and process. Blackwell Scientific Publications,

Kim KH, Choi YJ, Seo ST and Shin HD (2009) *Raffaelea quercus-mongolicae* sp nov associated with *Platypus koryoensis* on oak in Korea. Mycotaxon 110:189-197

Kinuura H and Kobayashi M (2006) Death of *Quercus crispula* by inoculation with adult *Platypus quercivorus* (Coleoptera: Platypodidae). Appl Entomol Zool 41:123-128

Kirkendall L, Dal Cortivo M and Gatti E (2008) First record of the ambrosia beetle, *Monarthrum mali* (Curculionidae, Scolytinae) in Europe. J Pest Sci 81:175-178

Kirkendall L and Faccoli M (2010) Bark beetles and pinhole borers (Curculionidae, Scolytinae, Platypodinae) alien to Europe. ZooKeys 56:227-251

Kirkendall LR (1993) Ecology and evolution of biased sex ratios in bark and ambrosia beetles. In: Wrensch DL and Ebbert MA (eds) Evolution and diversity of sex ratio in insects and mites. Chapman & Hall, New York, pp. 235-345

Kirkendall LR and Odegaard F (2007) Ongoing invasions of old-growth tropical forests: establishment of three incestuous beetle species in southern Central America (Curculionidae : Scolytinae). Zootaxa 62:53-62

Klepzig KD and Six DL (2004) Bark beetle-fungal symbiosis: context dependency in complex associations. Symbiosis 37:189-205

Knight FB, Heikkenen HJ and Graham SA (1980) Principles of forest entomology. McGraw-Hill Book Co., New York

KnÍŽek M and Beaver R (2004) Taxonomy and Systematics of Bark and Ambrosia Beetles. In: Lieutier F, Day K, Battisti A, Grégoire J-C,Evans H (eds) Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis. Springer Netherlands, pp. 41-54

Kobayashi M and Ueda A (2002) Preliminary study of mate choice in *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae). Appl Entomol Zool 37:451-457

Kobayashi M and Ueda A (2005) Wilt disease of Fagaceae trees caused bv *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) and the associated fungus: aim is to clarify the damage factor. Journal of the Japanese Forest Society 87:435-450.(in Japanese)

Koch FH and Smith WD (2008) Spatio-temporal analysis of Xyleborus glabratus (Coleoptera : Circulionidae : Scolytinae) invasion in eastern US forests. Environ Entomol 37:442-452

Kolar CS and Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199-204

Kühnholz S, Borden JH and Uzunovic A (2001) Secondary ambrosia beetles in apparently healthy trees: adaptations, potential causes and suggested research. Integr Pest Manage Rev 6:209-219

Kusumoto D, Masuya H, Ohmura K and Kamata N (2012) Virulence of *Raffaelea quercivora* isolates inoculated into *Quercus serrata* logs and *Q. crispula* saplings. J For Res 17:393-396

Lassauce A, Paillet Y, Jactel H and Bouget C (2011) Deadwood as a surrogate for forest biodiversity: Meta-analysis of correlations between deadwood volume and species richness of saproxylic organisms. Ecol Indicators 11:1027-1039

Lawton JH and MacGarvin M (1985) Interaction between bracken and its insect herbivores. Proceedings of the Royal Society of Edinburgh Section B: Biology 86:125-131

Lichstein JW (2007) Multiple regression on distance matrices: a multivariate spatial analysis tool. Plant Ecology 188:117-131

Liebhold AM, Macdonald WL, Bergdahl D and Maestro VC (1995) Invasion by exotic forest pests - a threat to forest ecosystems. For Sci 41:1-49

Lieutier F, Yart A and Salle A (2009) Stimulation of tree defenses by Ophiostomatoid fungi can explain attack success of bark beetles on conifers. Ann For Sci 66:801-801

Lightle D, Gandhi KJK, Cognato AI, Mosley B, Nielsen D and Herms DA (2007) New reports of exotic and native ambrosia and bark beetle species (Coleoptera: Curculionidae: Scolytinae) from Ohio. Great Lakes Entomol 40:194-200

Lockwood JL, Hoopes MF and Marchetti MP (2007) Invasion ecology. Blackwell Pub.,

Mac Nally R (1996) Hierarchical partitioning as an interpretative tool in multivariate inference. Aust J Ecol 21:224-228

Mac Nally R (2000) Regression and model-building in conservation biology, biogeography and ecology: The distinction between and reconciliation of 'predictive' and 'explanatory' models. Biodivers Conserv 9:655-671

Mac Nally R (2002) Multiple regression and inference in ecology and conservation biology: further comments on identifying important predictor variables. Biodivers Conserv 11:1397-1401

Mandelshtam MY (2001) New synonymy and new records in Palearctic Scolytidae (Coleoptera). Zoosyst Ross 9:203-204

Mandelshtam MY (2006) New synonymies and new combinations in Scolytidae from the Kuril Archipelago and continental territories of the Russian Far East (Coleoptera). Zoosyst Ross 15:323-325

Minchin PR (1987) An evaluation of the relative robustness of techniques for ecological ordination. Vegetatio 69:89-107

Mudge AD, LaBonte JR, Johnson KJR and LaGasa EH (2001) Exotic wood-boring Coleoptera (Micromalthidae, Scolytidae) and Hymenoptera (Xiphydriidae) new to Oregon and Washington. Proc Entomol Soc Wash 103:1011-1019

Mueller LD and Altenberg L (1985) Statistical inference on measures of niche overlap. Ecology 66:1204-1210

Mueller UG and Gerardo N (2002) Fungus-farming insects: multiple origins and diverse evolutionary histories. Proc Natl Acad Sci 99:15247-15249

Murayama J (1925) Supplementary notes on "The Platypodidae of Formosa". Journal of the College of Agriculture, Hokkaido Imperial University 15:229-235.(in Japanese)

Murayama J (1930) Re'vision des familles des Ipides et des Platypides de Core'e. Journal of the Chosen Natural History Society 11:1- 34.(in Japanese)

Murayama J (1933) Notes supplementaires a la revision des Ipides et Platypides de Core'e. II. Journal of the Chosen Natural History Society 15:14- 35.(in Japanese)

Negron JF, Witcosky JJ, Cain RJ, LaBonte JR, McElwey SJ, Lee JC and Seybold SJ (2005) The banded elm bark beetle: A new treat to elms in North America. American entomologist 51:84-94.

Nobuchi A (1974) The evolution of life styles in bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). Plant Prot Bull 28:75-81.(in Japanese)

Nobuchi A (1994) Ambrosia beetles in fresh logs. In: Shinrin K, Kobayashi F,Taketani A (eds) Forest Insects: General and Particular Theory. Yoken-Do, Tokyo pp. 204–217 (in Japanese)

Oksanen J, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Simpson G, Solymos P, Stevens M and Wagner H (2010a) Community ecology package (vegan). version 1.17-3. 1.17-3, Vienna.Available at: http://vegan.r-forge.r-project.org/

Oksanen J, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Simpson G, Solymos P, Stevens M and Wagner H (2010b) package "vegan". Available at: http://vegan.r-forge.r-project.org/web/packages/vegan/vegan.pdf. Accessed 10 December 2010

Oksanen J, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Simpson G, Solymos P, Stevens M and Wagner H (2011) Community ecology package (vegan). version 1.17-8. R Foundation for Statistical Computing, Vienna

Oliver JB and Mannion CM (2001) Ambrosia beetle (Coleoptera : Scolytidae) species attacking chestnut and captured in ethanol-baited traps in middle Tennessee. Environ Entomol 30:909-918

Paine TD, Raffa KF and Harrington TC (1997) Interactions among Scolytid bark beetles, their associated fungi, and live host conifers. Annu Rev Entomol 42:179-206

Paracer S and Ahmadjian V (2000) Symbiosis: an introduction to biological associations. Oxford University Press, New York

Peer K and Taborsky M (2007) Delayed dispersal as a potential route to cooperative breeding in ambrosia beetles. Behav Ecol Sociobiol 61:729-739

Pielou EC (1966) The measurement of diversity in different types of biological collections. J Theor Biol 13:131-144

Ploetz RC, Perez-Martinez JM, Smith JA, Hughes M, Dreaden TJ, Inch SA and Fu Y (2012) Responses of avocado to laurel wilt, caused by *Raffaelea lauricola*. Plant Pathology 61:801-808

Poulsen M and Boomsma JJ (2005) Mutualistic fungi control crop diversity in fungus-growing ants. Science 307:741-744

Poulsen M and Currie CR (2006) Complexity of insect–fungal associations: exploring the influence of microorganisms on the attine ant–fungus symbiosis. In: Bourtzis K and Miller TA (eds) Insect symbiosis, vol 2. CRC Press, Boca Raton, pp. 52-73

R Development Core Team (2010) R: a language and environment for statistical computing. www.r-project.org 2.12.0, Vienna.Available at: www.r-project.org

R Development Core Team (2011) R: a language and environment for statistical computing (v2.13.1). The R foundation for statistical computing

Rabaglia R, Dole S and Cognato A (2006) Review of American Xyleborina (Coleoptera: Curculionidae: Scolytinae) Occurring North of Mexico, with an Illustrated Key. Ann Entomol Soc Am 99:1034-1056

Ranger CM, Reding ME, Persad AB and Herms DA (2010) Ability of stress-related volatiles to attract and induce attacks by Xylosandrus germanus and other ambrosia beetles. Agric For Entomol 12:177-185

Rao CR (1982) Diversity and dissimilarity coefficients: A unified approach. Theor Popul Biol 21:24-43

Reed SE and Muzika RM (2010) The influence of forest stand and site characteristics on the composition of exotic dominated ambrosia beetle communities (Coleoptera: Curculionidae: Scolytinae). Environ Entomol 39:1482-1491

Roling MP and Kearby WH (1975) Seasonal flight and vertical distribution of Scolytidae attracted to ethanol in an oak-hickory forest in Missouri. Can Entomol 107:1315-1320

Rudinsky JA (1962) Ecology of Scolytidae. Annu Rev Entomol 7:327-348

Saito T, Ishida K, Yamane A and Sasaki K (2005) Long term monitoring record of scolytid beetle populations at the Tokyo University Forest in Chichibu, Central Japan. Miscellaneous Information the Tokyo University Forests 44:251-275.(in Japanese)

Schedl K (1964) Three new species of Scolytidae from Australia, and some introduced Coleoptera. Proc Linn Soc NSW 89:246-249

Schedl K (1972) Monographie der familie Platypodidae (Coleoptera). W. Junk, Den Haag

Schowalter TD (2011) Insect Ecology: An Ecosystem Approach. Elsevier Science, London

Shannon CE and Weaver W (1964) The mathematical theory of communication. University of Illinois Press, Urbana, IL

Shea K and Chesson P (2002) Community ecology theory as a framework for biological invasions. Trends in ecology & evolution (Personal edition) 17:170-176

Shibata E, Waguchi Y and Yoneda Y (1992) General research on wood quality degradation insect pest control for *Cryptmeria japonica* and *Chamaecyparis*.1988-1992. Rise and fall of *Xyleborus validus* attraction in *Cryptmeria japonica* and *Chamaecyparis* forests.Government subsidy. Big project research. (Sponcer: Nara Prefectural Forest Reaearch Esp. Stn.). Bulletin of the Nara Forest Experiment Station 1992:20.(in Japanese)

Sinclair WA and Lyon HH (2005) Diseases of trees and shrubs, 1. 2 edn. Comstock Pub. Associates,

Smith S, Beattie A, Kent D and Stow A (2009) Ploidy of the eusocial beetle *Austroplatypus incompertus* (Schedl) (Coleoptera, Curculionidae) and implications for the evolution of eusociality. Insectes Soc 56:285-288

Sone K, Mori T and Ide M (1998) Life history of the oak borer, *Platypus quercivorus* (Murayama) (Coleoptera : Platypodidae). Appl Entomol Zool 33:67-75

Speight MR and Wylie FR (2001) Insect pests in tropical forestry. CABI, Wallingford

Sreedharan K, Balakrishnan MM, Samuel SD and Bhat PK (1991) A note on the association of wood boring beetles and a fungus with the death of silver oak trees on coffee plantations. J Coffee Res 21:145-148

Stokkink E (2008) The environmental implications of two introduced ambrosia beetles in British Columbia. M.Sc. thesis, Royal Roads University (Canada), Victoria, British Columbia

The University Forest, Graduate School of Agricultural and Life Sciences,The University of Tokyo (2011) Annual report of meteorological observations in the University Forests, The University of Tokyo (Jan. 2009-Dec. 2009). Miscellaneous Information the Tokyo University Forests 50:73-98.(in Japanese)

The University Forests in Chiba (1985) List of plans, Tokyo University Forest in Chiba. Bull Tokyo Univ For:13-59.(in Japanese)

Tremblay E, Espinosa B, Mancini D and Caprio G (2000) Un coleottero proveniente dal Sudamerica minaccia i pioppi. L'Informatore Agrario 56:89-90

Ueda A and Kobayashi M (2001) Seasonal change of number of *Platypus quercivorus* (Murayama) and *P. calamus* Blandford (Coleoptera: Platypodidae) landing on living trees. J Jap Forestry Soc 83:77-83

Vandenberg NJ, Rabaglia RJ and Bright DE (2000) New records of two *Xyleborus* (Coleoptera: Scolytidae) in North America. Proc Entomol Soc Wash 102(1):62-68

Vanhanen H (2008) Invasive insects in Europe - the role of climate change and global trade. University of Joensuu, Helsinki

Walsh C and Mac Nally R (2008a) Hierarchical partitioning. 1.0-3: http://cran.r-project.org/web/packages/hier.part/

Walsh C and Mac Nally R (2008b) Package "hier.part". R package version 1.0-3. Available at: http://cran.r-project.org/web/packages/hier.part/hier.part.pdf. Accessed 12 December 2011

Walters D (2011) Plant defense: warding off attack by pathogens, herbivores and parasitic plants. John Wiley & Sons,

Weber B (1979) *Xylosandrus germanus* (Blandf.) (Coleoptera: Scolytidae), a new pest of black walnut: a review of its distribution, host plants, and environmental conditions of attack. vol 52. USDA Forest Service, NC-52

Weber BC and McPherson JE (1983) The distribution of *Xylosandrus germanus* in America north of Mexico (Coleoptera: Scolytidae). Great Lakes Entomol 15:171-174

Whittaker R (1972) Evolution and measurement of species diversity. Taxon 21:213-251

Wood S (1972) Monographie der Familie Platypodidae, Coleoptera. Karl E. Schedl. Junk, The Hague, 1972. vi, 322 pp., illus. 70 guilders. Science 178:1085-1086

Wood S (1973) On the taxonomic status of Platypodidae and Scolytidae(Coleoptera). Great Basin Nat Mem 33:77-90

Wood S (1977) Introduced and exported American Scolytidae (Coleoptera). Great Basin Naturalist Memorandum 37:67–74

Wood S (1982) The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. Great Basin Naturalist Memorandum 6:1-1359

Wood S and Bright D (1992) A catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: Taxonomic index. Great Basin Nat Mem 3:1-1553

Wright DH (1983) Species-energy theory - an extension of species-area theory. Oikos 41:496-506

Yamamura K (1999) Transformation using (x+0.5) to stabilize the variance of populations. Res Popul Ecol 41:229-234

Yamaoka Y (2000) Creation of resource for insect reproduction by microorganisms: symbiotic relationship between beak beetles and blue stain fungi. In: Fuktai K and Hijii N (eds) Forest microbial ecology. Asakura Shoten, Tokyo, pp. 148-162

Yoshida N (1994) Mass mortality of evergreen and deciduous oaks in Japan. Sanrin 1325:35-40.Japanese

Yoshikawa K (1987) A study of the subcortical insect community in pine trees .3. species correlation. Appl Entomol Zool 22:207-215

Yoshikawa K, Takeda H, Sone K and Shibata E (1986) A study of the subcortical insect community in pine trees .1. oviposition and emergence periods of each species. Appl Entomol Zool 21:258-268