

## 論文 (Original Article)

# Long-term attractiveness of autoclaved oak logs bored by male *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) to male and female beetles

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### Abstract

*Platypus quercivorus* (Murayama) beetles attack oak trees in large numbers and kill them in Japan. Thus, understanding the phenomenon of aggregation is important in understanding the infestation process, but the short aggregation period of oak logs bored by the beetles has made it difficult to understand the process through field experiments. Therefore, we tested whether it was possible to preserve the attractiveness of autoclaved logs. A comparison between autoclaved logs and no-treated fresh logs in numbers of beetles attracted per entry hole showed no differences between these two until 10 days after the initial release of males to make them bore in the logs, but the number attracted to the autoclaved logs was significantly higher than the number attracted to the fresh logs after that. A second release of males on the fresh logs after the log attractiveness had declined increased the attractiveness of the logs, suggesting that it was the presence of males that produced the attractiveness. The majority of the entry holes were occupied by living males in the autoclaved log at the end of the study period and the ratio of holes with living males to holes without living males differed significantly between fresh and autoclaved logs, suggesting that males in the autoclaved logs experienced increased longevity. These results suggest that the extended attractiveness of oak logs resulted from increased male longevity. The ability to extend the period of attractiveness by autoclaving logs will be useful in future studies of aggregation of this beetle.

**Key words :** aggregation, ambrosia beetle, mass attack, oak wilt, *Quercus*, platypodid beetle

### Introduction

*Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) is a male-initiating, monogamous ambrosia beetle (Kobayashi et al., 2001). Mass mortality of oaks attacked by large infestations of these beetles has occurred continuously in Japan since the first reports of damage in Kyushu in 1934, Shikoku in 1950, and Honshu in 1952 (Ito & Yamada, 1998). Ecological study of the beetle, focusing on mass attacks, is important to clarify how the damage occurs and to develop pest management strategies.

In some platypodid species that attack their hosts in large numbers, the males produce an aggregation pheromone in their entry holes, and this pheromone attracts conspecific males and females (Madrid et al., 1972; Milligan, 1982; Milligan et al., 1988; Milligan & Ytsma, 1988). Ueda and Kobayashi (2001a) suggested that male *P. quercivorus* also released this pheromone. Bark beetles of the family Scolytidae cease calling or

release an antiaggregation pheromone after one or a few mates have arrived at entry holes (e.g., Furniss et al., 1972; Sasakawa & Sasakawa, 1981). In the Platypodidae, however, there have been no studies of changes in the signals emitted by the beetles after mating.

Ueda and Kobayashi (2001a) suggested that aggregation by *P. quercivorus* disappeared after mating because fewer beetles were attracted to logs colonized by males that had mated after boring than to logs colonized by unmated males. However, they could not verify this hypothesis because there was little difference in numbers of the beetles attracted between logs with mated and unmated males, even if a significantly higher number of beetles were attracted to logs with unmated males than to logs with mated males. This was because the attractiveness of logs colonized by unmated males began to decrease within 5 days after boring.

It takes at least a few days to demonstrate whether differences between treatments exist in trap catches in the field because the traps must be rotated several times

原稿受付：平成 15 年 4 月 23 日 Received Apr. 23, 2003 原稿受理：平成 16 年 1 月 5 日 Accepted Jan. 5, 2004

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to reduce the possibility of a bias associated with their location. To verify whether aggregation after mating ceases in field experiments using logs, a means of preserving the attractiveness of logs for more than 10 days must be developed; this number is derived from the estimation that it takes at least 5 days to detect no differences between logs with unmated males which will mate and which will not mate, and at least 5 days afterward to detect the differences between logs with mated and unmated beetles.

The short longevity of unmated males after boring into logs (Ytsma, 1989; Kobayashi et al., 2001) may be responsible for the quick decline in log attractiveness. Kobayashi et al. (2002) demonstrated the high survival rate of males introduced into autoclaved logs, and this result suggests increased longevity of unmated males in these logs, and thus a greater period of attractiveness. In the present study, we attempt to verify this hypothesis.

### Materials and Methods

We conducted our study on a south-facing slope in Ohura Natural Park (lat. 35° 32'N, long. 135° 23'E, 470 m asl), in Maizuru City of Honshu's Kyoto Prefecture in 2000. The dominant canopy species were *Quercus crispula* Blume, *Q. serrata* Thunb. ex Murray, *Acer rufinerve* Sieb. et Zucc., *Castanea crenata* Sieb. et Zucc., and *Q. acuta* Thunb. ex Murray. Mass mortality of oak trees began in 1997 in the study area, and tree mortality was still increasing in 1999 (Kobayashi & Hagita, 2000). *Q. crispula* exhibited the highest mortality among the tree species in the study area. Mortality of *Q. serrata* was also relatively high, but only a few trees of *C. crenata* and *Q. acuta* died (Kobayashi & Hagita, 2000).

High mortality of *Q. crispula* and *Q. serrata*, which are deciduous oaks, has been reported from most infested forests in Honshu (Shiomi & Osaki, 1997; Kobayashi & Hagita, 2000, 2001; Inoue et al., 2000, 2003; Kobayashi & Ueda, 2001). Since both *Q. crispula* and *Q. serrata* have been used in many studies of *P. quercivorus* (e.g., Ueda & Kobayashi, 2001a), it is necessary to develop a suitable method for both tree species. Thus, logs of both *Q. crispula* and *Q. serrata* were used in the present study.

On 8 June 2000, we established a dodecagonal plot with 5-m sides at the study site. Wooden chairs with a seat height of 50 cm were used to form the vertices of the dodecagon. For each chair, we built a cage by inverting a steel stool (40 cm in diameter and 58 cm tall) and fully covering it with 0.5-mm nylon mesh netting (Ueda & Kobayashi, 2001a). These cages were placed on the wooden chairs as follows: a fresh log from a *Q. crispula* tree (Qc 1) was placed on the northernmost (first) chair,

and an autoclaved log from the same tree was placed on the next (second) chair clockwise from the first chair. The third and fourth chairs held fresh and autoclaved logs from a *Q. serrata* tree (Qs 1), respectively; similarly, the fifth and sixth chairs held fresh and autoclaved logs from another *Q. crispula* (Qc 2), the seventh and eighth chairs held fresh and autoclaved logs from another *Q. serrata* (Qs 2), and so on.

On 20 June, we cut down three living trees of *Q. crispula* and three of *Q. serrata*. From each tree, we prepared two logs, each 17 to 21 cm in diameter and 50 cm long. One of these logs was immediately placed in a cage after the cut ends were coated with paraffin (melting point: 54 to 56 °C) to reduce drying. Hereafter, these logs are referred to as the "fresh" logs. The second log from each tree was brought to the laboratory and autoclaved for 90 min at 121°C and 1.2 atm. The next day, the cut ends of the "autoclaved" logs were coated with paraffin to reduce drying, and the logs were returned to the study site and kept in the cages.

*P. quercivorus* beetles that flew to a standing *Q. serrata* tree were collected by hand or with insect nets on the mornings of 29 and 30 June in the study area, and the sex of each beetle was determined. One hundred males were released into each cage on 30 June. Hereafter, this is referred to as the "first release". An 8-cm-wide adhesive paper ('Kamikirihohoi', Earth Bio-chemical Co. Ltd., Tokyo) was bound round on the nylon net 30 cm above the bottom of each cage. The beetles captured on these papers were counted on 3, 5, 10, 14, and 17 July. After each counting, the positions of the cages with fresh and autoclaved logs were reversed for each tree so as to reduce the possibility of a bias associated with the locations of the cages, and the adhesive papers were replaced. On 21 July, we counted both the number of beetles on the papers and the number of entry holes in the logs that had been bored into by the released males.

It is possible that bored logs attract beetles solely on the basis of the odor of the logs. However, if the attractiveness of bored logs which had been verified not to attract the beetles increased after an additional release of the males, it is more likely that the released males are responsible for the increased attractiveness. To quantify this change, we added 50 males collected on the mornings of 20 and 21 July to each cage on 21 July. Hereafter, this is referred to as the "second release". On 24 July, we again counted the number of beetles captured on the adhesive papers and the number of entry holes in the logs. We also dissected a pair of logs from Qc 3 and observed whether the released males were present in the entry holes so as to compare the longevity of the released males that had bored in the autoclaved log and the fresh log.

Because the numbers of males and females attracted to logs correlates well with the number of entry holes bored into the logs (Ueda & Kobayashi, 2001a), we used the number of beetles captured per entry hole as the variable for statistical analysis. We used the Wilcoxon signed-ranks test to compare the differences between the treatments in terms of the numbers of captured males and females per entry hole on each collection date ( $N = 6$  for each date). This test was also used to determine whether the fresh and autoclaved logs differed in terms of the numbers of entry holes bored by the males of respective releases ( $N = 6$  for each release), and whether the first and second releases differed in terms of the number of entry holes per released male ( $N = 6$  for each treatment). We also compared the number of entry holes occupied by a living male with the total number of empty entry holes plus entry holes occupied by a dead male for the dissected logs from Qc 3 in two treatments by using Fisher's exact probability test. StatView (ver. 5.0) software (SAS Institute, 1998) was used for these analyses.

### Results

The number of entry holes did not differ significantly between the fresh and autoclaved logs in either the first or the second release (Table 1;  $P > 0.05$ , Wilcoxon signed-ranks test), but changes in the numbers of male and female beetles captured per day differed between treatments (Figs. 1 and 2). The numbers of male and female beetles captured per day per entry hole both decreased until about 10 days after the first release on both the fresh and the autoclaved logs (Figs. 1 and 2). During the first 5 days, the numbers of beetles captured per entry hole did not differ significantly between the fresh and autoclaved logs ( $P > 0.05$ , Wilcoxon signed-ranks test). The difference remained non-significant on the 10th day for females ( $z = -1.36$ ,  $P = 0.17$ , Wilcoxon signed-ranks test), but the number on autoclaved logs became significantly greater than that

on fresh logs on the 10th day for males ( $z = -1.99$ ,  $P = 0.046$ , Wilcoxon signed-ranks test).

Numbers of males and females remained low on fresh logs until the second release (on the 21st day), at which point they increased again (Figs. 1 and 2). In contrast, the numbers of males and females on autoclaved logs increased and remained relatively high (Figs. 1 and 2) throughout the study period. The numbers of both males and females captured per entry hole differed significantly between fresh and autoclaved logs on the 14th, 17th, and 21st days ( $z = -2.20$ ,  $P = 0.028$  each, Wilcoxon signed-ranks test).

The numbers of male and female beetles captured increased sharply after the second release on both fresh and autoclaved logs, with the exception of the fresh log of Qc 1, where the numbers decreased (Figs. 1 and 2). The numbers of both males and females captured per entry hole remained significantly different between fresh and autoclaved logs on the 24th day ( $z = -2.20$ ,  $P = 0.028$  each, Wilcoxon signed-ranks test).

On Qc 3, there were many empty entry holes and entry holes occupied by a dead male in the fresh log, but the majority of the entry holes were occupied by living males in the autoclaved log (Table 2). The ratio of holes with living males to holes without living males differed significantly between fresh and autoclaved logs ( $P < 0.0001$ , Fisher's exact probability test; Table 2).

The number of new entry holes per released male after the second release was significantly smaller than in the first release for both fresh and autoclaved logs (Table 1; fresh logs:  $z = -2.02$ , and  $P = 0.043$ , Wilcoxon signed-ranks test. Autoclaved logs:  $z = -2.20$ ,  $P = 0.028$ ). The number of entry holes, the total number of male and female beetles captured, and the changes over time in the number of males and females captured per entry hole on *Q. crispula* relatively coincided with those on *Q. serrata* (Table 1, Figs. 1 and 2), although no statistical methods were applicable because of the scarcity of specimens.

Table 1. Number of entry holes bored by male *P. quercivorus* released into a cage containing a log and number of the beetles captured on the adhesive paper bound round on the cage

Tree species	Tree no.	Number of entry holes per log				Number of beetles captured			
		First release (100 males on 30 June)		Second release (50 males on 21 July)*		Male		Female	
		Fresh	Autoclaved	Fresh	Autoclaved	Fresh	Autoclaved	Fresh	Autoclaved
<i>Quercus crispula</i>	Qc 1	24	26	12	1	34	72	11	87
	Qc 2	17	43	2	4	89	193	29	115
	Qc 3	32	26	1	3	44	91	22	45
<i>Quercus serrata</i>	Qs 1	32	31	3	3	50	88	19	60
	Qs 2	27	19	3	5	62	124	21	82
	Qs 3	30	16	7	3	40	60	24	35

\* Number of additional holes bored in the log

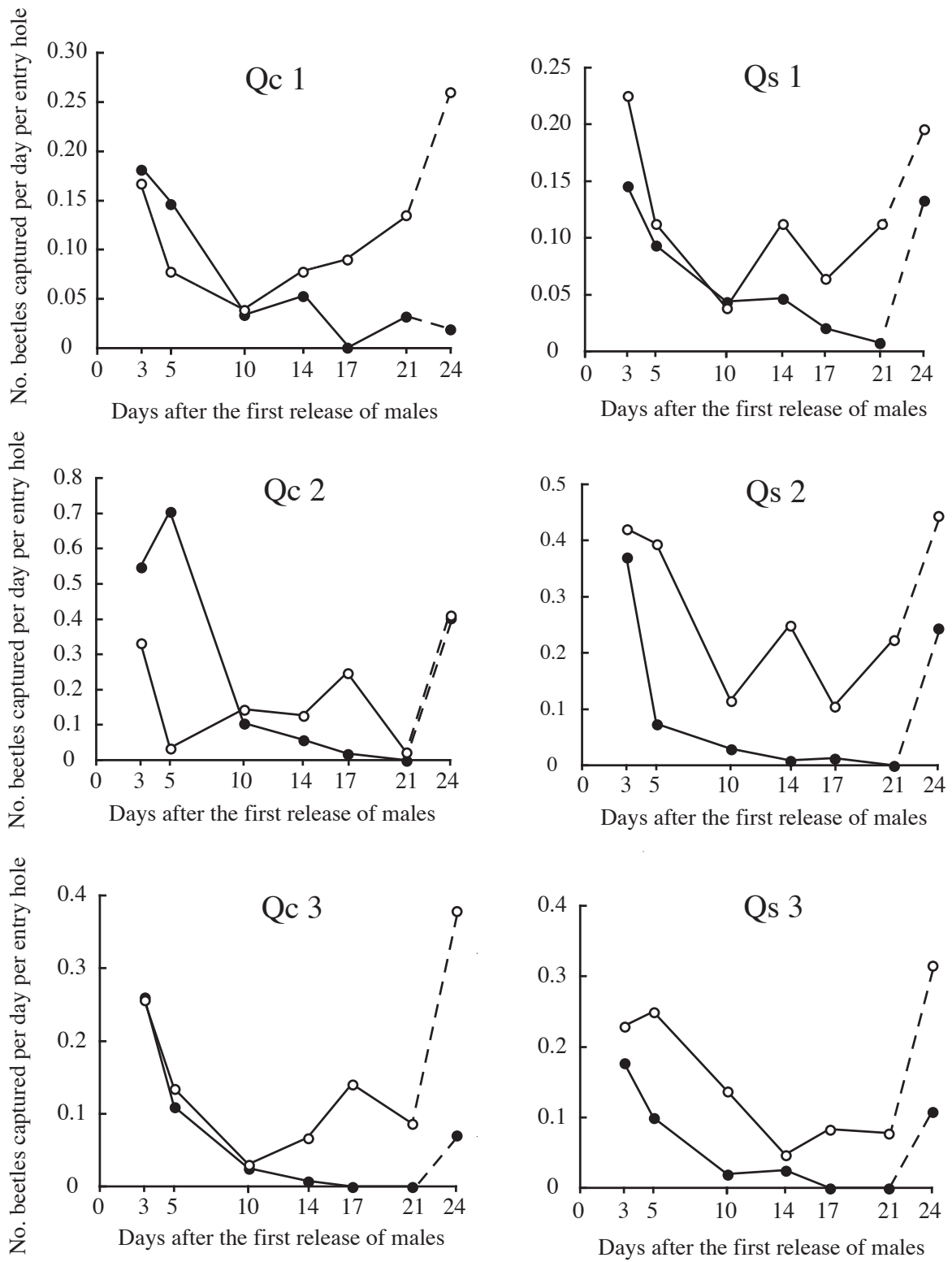


Fig. 1. Changes in the number of male *P. quercivorus* beetles captured on fresh logs (dark circles) and autoclaved logs (white circles). Dotted lines indicate the change after the second release of males on 21 July (21 days after the first release).

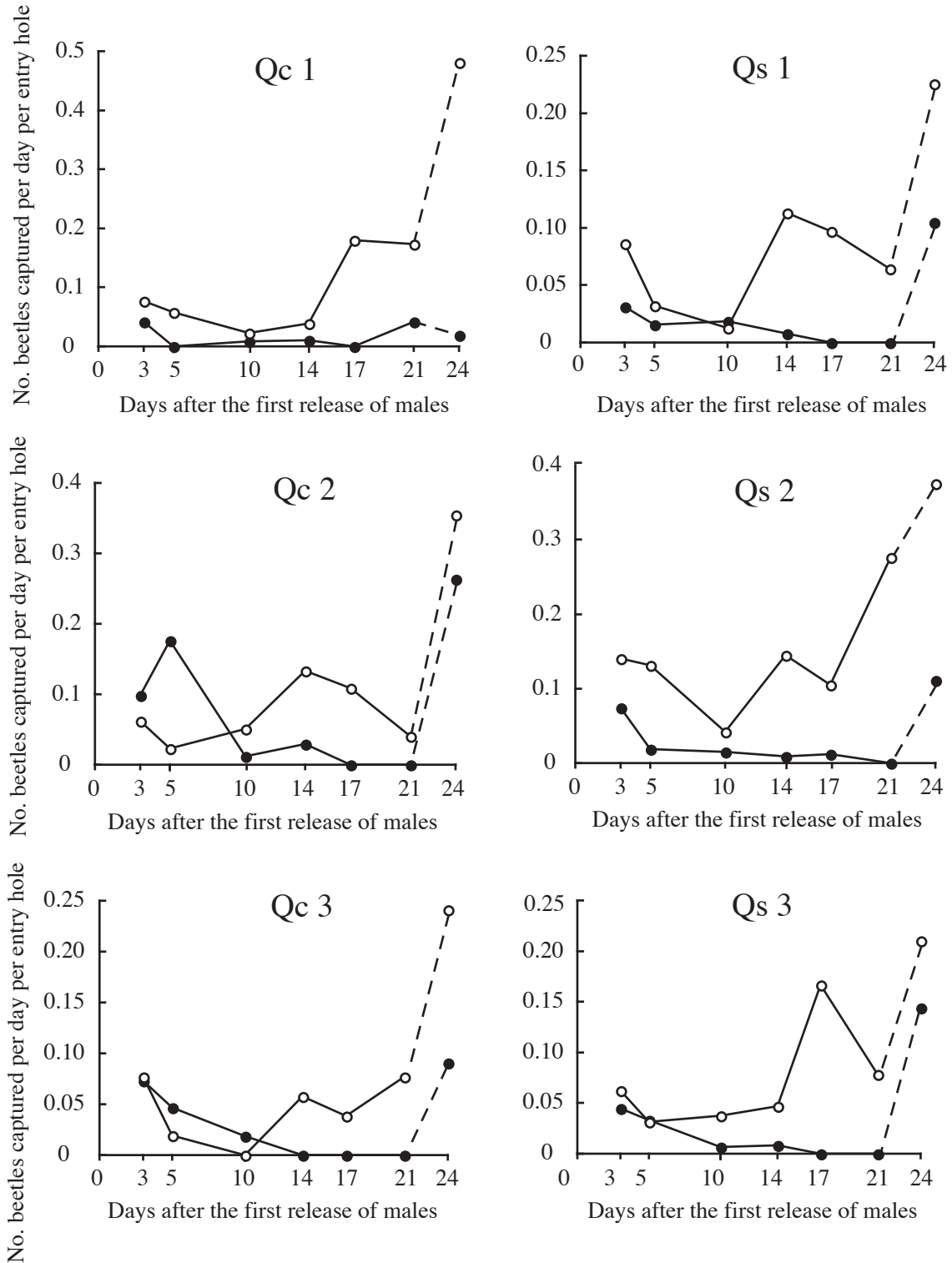


Fig. 2. Changes in the number of female *P. quercivorus* beetles captured on fresh logs (dark circles) and autoclaved logs (white circles). Dotted lines indicate the change after the second release of males on 21 July (21 days after the first release).

Table 2. Comparison of the numbers of entry holes occupied by a living male and the numbers of holes that were empty or that contained a dead male in logs prepared from Qc 3

Treatment	No. of holes occupied by a living male	Total no. of holes that were empty or contained a dead male
Fresh	10	23 (3)
Autoclaved	24	5 (1)

Numbers in parentheses indicate the number of entry holes contained a dead male  
The values of the fresh log and the autoclaved log differed significantly (Fisher's exact probability test,  $P < 0.0001$ )

### Discussion

In the present study, the attractiveness of fresh logs decreased to nearly zero just before the second release, but it clearly increased after the second release. This was likely because the males in the second release bored into the fresh logs and attracted beetles. This result suggests that it was the presence of males in the entry holes rather than the odor of the logs themselves that was responsible for the attraction. Ueda and Kobayashi (2001a, 2003) and Ueda et al. (2001, 2002b) also suggested that *P. quercivorus* has little, if any, attraction to the odor of the logs themselves.

The significantly greater number of beetles captured on autoclaved logs in the present study demonstrates an increase in the duration of attractiveness of male entry holes in autoclaved logs. However, the number of beetles captured on autoclaved logs was lowest on the 10th day after the initial release, and this gives the impression that attractiveness had varied with time rather than remaining constant.

The low number of beetles captured on the 10th day may have due to the low ambient temperatures from 6th to 10th day. Ueda and Kobayashi (2000, 2001b) observed that beetles flew in the morning, except on cool days, when few or no beetles were observed; this study demonstrated that flight of the beetle is affected by temperature. Fig. 3 shows the mean morning temperatures during the study period in Maizuru City. The observed changes in the number of beetles captured on autoclaved logs followed these temperature changes (Figs. 1, 2, and 3). These observations suggest that the attractiveness of the autoclaved logs remained high, and that low attractiveness on the 10th day occurred because of the low temperatures, which means that few beetles were flying from 6th to 10th day. Significant differences between treatments in the number of males captured on the 10th day also support our hypothesis that the attractiveness of the autoclaved logs remained high.

Males that bored into the autoclaved logs must have

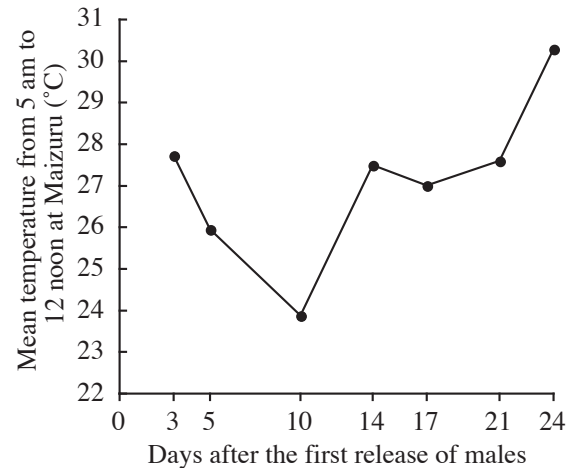


Fig. 3. Mean morning temperatures (from 5 am to 12 noon) in Maizuru City during the study period. Data from Japan Meteorological Agency (2000).

experienced increased longevity because the number of entry holes occupied by living males was significantly greater in the autoclaved log than in the fresh log. Some of these living males may have originated from the second release, because these males could have used entry holes bored and left vacant by males from the first release; this in turn may have been responsible for the significantly lower number of entry holes newly bored after the second release. However, the presence of many living males from the first release in the autoclaved log must have been responsible for the difference between the fresh and autoclaved logs in the number of living males.

From these results, it appears that the extended period of attractiveness for autoclaved logs that had been bored into by males was caused by increased male longevity in these logs. The extended period of attractiveness in these logs will be useful in future studies of the cessation of production of an aggregation pheromone and release of an anti-aggregation pheromone after mating in field experiments. This method will work for both *Q. crispula* and *Q. serrata* because there may appear to be no differences between these species in the number of entry holes bored, in the total number of male and female beetles captured, and in the changes over time in the numbers of male and female beetles captured per entry hole.

Kitajima (2000) showed high survival of *P. quercivorus* released on logs soaked in water, and Kobayashi et al. (2003) demonstrated that survival of the beetle increased with the increase of log water content. If autoclaving increases log water content, this would increase male longevity in the entry holes, thereby sustaining the attractiveness of these logs. To confirm this hypothesis, we must study changes in log water

content induced by autoclaving.

Ueda et al. (2002a) showed that unconfined *P. quercivorus* never bored into autoclaved logs in the field. However, the present study contradicts this finding; released male beetles bored into both autoclaved and fresh logs confined in a cage. This indicates that the beetles in the present experiment, which had no choice of which logs to colonize, may have bored into autoclaved logs because they had no other choice of habitat.

### Acknowledgments

We thank Ms. Ai Nozaki, Kyoto Prefectural Forestry Experimental Station, for her help in autoclaving the logs.

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## カシノナガキクイムシの雄が穿入したオートクレーブ処理ナラ丸太の雌雄に対する長期の誘引力

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### 要旨

カシノナガキクイムシは、わが国においてナラ樹を集中攻撃して枯死させる。そのため、本種の集合現象を明らかにすることは加害のプロセスを解明する上で重要であるが、本種の穿入したナラ樹丸太による誘引期間が短く、これが野外試験によるプロセス解明を困難にしていた。そこで、オートクレーブ処理した丸太を用いると誘引を維持できるかどうかを調べた。オートクレーブ処理丸太と無処理の生丸太に誘引され捕獲された穿入孔あたりの成虫数を比較したところ、丸太に穿入させるために雄を放虫してから10日目までは差がなかった。しかし、これ以降はオートクレーブ処理丸太で有意に捕獲数が多かった。誘引力が低下していた生丸太へ雄を追加穿入させると再び誘引が生じ、これは雄が誘引を生じさせていることを示唆した。また、試験終了時に生存雄がいた穿入孔数がオートクレーブ処理丸太で多く、いなかった穿入孔数との比率が生丸太と有意差があり、これはオートクレーブ処理丸太に穿入した雄が長生きであることを示唆した。これらの結果、オートクレーブ処理丸太での誘引期間の増加は、穿入した雄が長生きすることで生じたと考えられた。丸太をオートクレーブ処理することによって誘引期間を増加させる方法は、今後この虫の集合の研究において有用な手段となる。

**キーワード：**集合、養菌キクイムシ、集中攻撃、ナラ枯損、コナラ属、ナガキクイムシ

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