AGRIVITA VOLUME 34 No. 3 OCTOBER - 2012 ISSN : 0126-0537

TWO ACTIVE STAGES OF AMBROSIA BEETLE, *Platypus quercivorus* MURAYAMA ESTIMATED FROM FRASS PRODUCTION

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Received: March 12, 2012/ Accepted: July 9, 2012

ABSTRACT

Beetle activity deep inside wood was studied in field (Mt. Yoshida, Kyoto) and laboratory conditions by monitoring the type and amount of frass ejected from beetle galleries of *Platypus quercivorus*. Twenty-one galleries were selected from five infested *Quercus serrata* trees for field studies. In the laboratory, frass production was monitored for three galleries using a computer-aided electrical balance (A&D Co. Ltd.). Long-term patterns of frass production from field observation revealed that there are three distinguishable stages; a fibrous frass stage, an intermediate stage, and a powdery frass stage. The duration of the fibrous frass production was clearly different between galleries, ranging from five to twenty one days. The intermediate stage was also different from gallery to gallery, ranging from two to twenty days. Accordingly, the starting time of the third stage, powdery frass production, ranged from the 19^{th} to the 27^{th} day. Under laboratory conditions, the long-term patterns of frass production revealed that in galleries of logs no. 1 and 2 the fibrous frass period was stopped on the 23^{rd} and 22^{nd} day, respectively. Field monitoring revealed there was a negative relationship between the length of fibrous frass stage and the length of the intermediate stage.

Keywords: fibrous frass stage, intermediate stage, *Platypus quercivorus,* powdery frass stage

INTRODUCTION

Signs and symptoms such as holes and frass produced by bark and wood boring beetles are important to diagnose pests and disease attacks. The presence of the woodborer beetle *Megaplatypus (=Platypus) mutatus* (Chapuis) in Brazilwood, (*Caesalpinia echinata*) is detected by the occurrence of holes and two different types of frass coming out of the trunks (Girardi *et al*., 2006). Coarse boring dust produced by *M. mutatus* can be observed on newly attacked trees, which usually exude sap through the entrance holes (Alfaro *et al*., 2007). Other examples are the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Safranyik and Carroll 2006) and ambosia beetles such as *Platypus flavicornis* Fabricius (Atkinson, 2004; Clarke and Menard, 2006), *Xyleborus glabratus* Eichhoff and *X. crassiusculus* (Motschulsky) (Hanula *et al*., 2008). All produce sawdust or frass resulting from their activity and large amounts pile up at the base of standing trees.

Like other ambrosia beetles, *Platypus quercivorus* Murayama also produces gallery excrement/frass when attacking fagaceous trees and logs such as *Quercus crispula* and *Q. serrata* (Ueda and Kobayashi, 2001; Tarno *et al*., 2011). Adult males start the formation of galleries in June and females begin to deposit eggs at the terminal parts of tunnels two to three weeks after the commencement of gallery formation (Sone *et al*., 1998). More eggs are laid in healthy logs than infected logs by *Raffaelea quercivora* of *Q. crispula*, or in logs of any other tree species (Tarno *et al*., 2011).

Frass production by the beetles is related to the feeding activity on suitable host trees (Atkinson, 2004; Girardi *et al*., 2006; Kim *et al*., 2009). Beetle activity is an important factor to predict infestation stages and beetle development.

Accredited SK No.: 81/DIKTI/Kep/2011

http://dx.doi.org/10.17503/Agrivita-2012-34-3-p207-214

However, the activities of ambrosia beetles are difficult to observe because they live mainly deep inside wood. Simple indicators are needed to study the activity of ambrosia beetles in oak trees. Svihra and Kelly (2004) used the number of emerged beetles and frass amount to evaluate the effect of insecticide (permethrin) on both western oak bark beetle *Pseudopityphthorus pubipennis* Swaine and the oak ambrosia beetles *Monothrum scutellare* and *M. dentiger*. Svihra and Kelly (2004) reported that the differences in frass volume ejected on the bark surface are clearly visible: the sections sprayed with insecticide are almost free of frass, indicating that beetle activity was reduced or had been stopped completely by the insecticide.

The presence of adult or larval stages of ambrosia beetles such as *M. mutatus* and *P. quercivorus* was evaluated by the presence of the different types of frass in the holes or on the base of the trunks (Girardi *et al*., 2006 and Tarno *et al*. 2011). There were two types of frass produced by *P. quercivorus* beetle: fibrous and powdery frass. Whenever powdery frass was produced, larvae were found in the galleries, while fibrous frass was present only in galleries with just adults. The mouthparts of adults are completely sclerotized, which likely accounts for the fibrous frass production (Tarno *et al*., 2011). Because detailed observations of the life cycle are difficult, as sampling is necessarily destructive (Kent and Simpson, 1992), we proposed to monitor the frass production both in the field and under laboratory conditions to study the long-term changes in *P. quercivorus* activity carried out deep inside the wood.

MATERIALS AND METHODS

Field Experiment

Field research was conducted at a low-elevation (125 m) on Mt. Yoshida ca. 300 m south of Graduate School of Agriculture, Kyoto University, Kyoto city. Five infested *Q. serrata* trees were selected as experimental trees. In the middle of June, 2010, *P. quercivorus* adults began to attack healthy trees by boring galleries to produce fibrous frass. Monitoring of the daily frass production started on June 23rd 2010.

In the initial digging of entrance holes by adult males, they produced brown fibrous frass made from bark of the tree. After copulation, females continued to elongate the tunnel, producing white fibrous frass made from sap wood. When the production of white fibrous frass started, a plastic tube was attached into the entrance hole. The plastic tube, 10.7 cm long and 1.7 cm in diameter, was closed with a screw cap attached with a short metal tube, 1 cm long and 4 mm in diameter. The metal tube was inserted into the entrance tunnel, which had a diameter of ca. 2 mm. For each experimental tree, 4-5 tunnels successfully colonized by *P. quercivorus* were selected; in total, 21 tunnels were included in the field study. Every day frass was collected from each tube and desiccated in the drier at 41°C for two hours, before weighting.

Laboratory Experiment

To confirm the long-term pattern of frass production observed in the field, logs were placed in a room with light and temperature control. The experiments were conducted under 14 hours' daylight and 10 hours darkness, at 26°C.

Three logs, 50 cm long and 15-25 cm in diameter, two of *Quercus crispula* and one of *Q. serrata*, were obtained from healthy trees in late June 2010. The logs were immersed in tap water for 5 days before use to increase their water content and maintain humidity necessary for growth of symbiotic fungi (Kobayashi *et al*., 2004), and five holes (10 cm between each hole, 3 mm diameter, 5 mm depth) were punctured with a sharp needle. Then male beetles were introduced into the logs using a plastic tube, 6.5 cm in length and 0.9 cm in diameter with a tapered tip that was inserted into the needle holes. To maintain the humidity, the logs were kept in plastic boxes with wet bog moss. One to two days later, when frass production by the males was observed, female beetles were additionally introduced to the holes, one for each hole. Before the monitoring of frass production started, both ends of the logs were cut so the total lengths of the logs were 30 cm.

A computer-aided electrical balance (A and D Co. ltd.) was used for continuous measurement of the weight of frass produced by *Platypus quercivorus* (Figure 1). The frass weight data were stored in a computer and analyzed using Microsoft Office Excel 2007 to show the results as a cumulative curve.

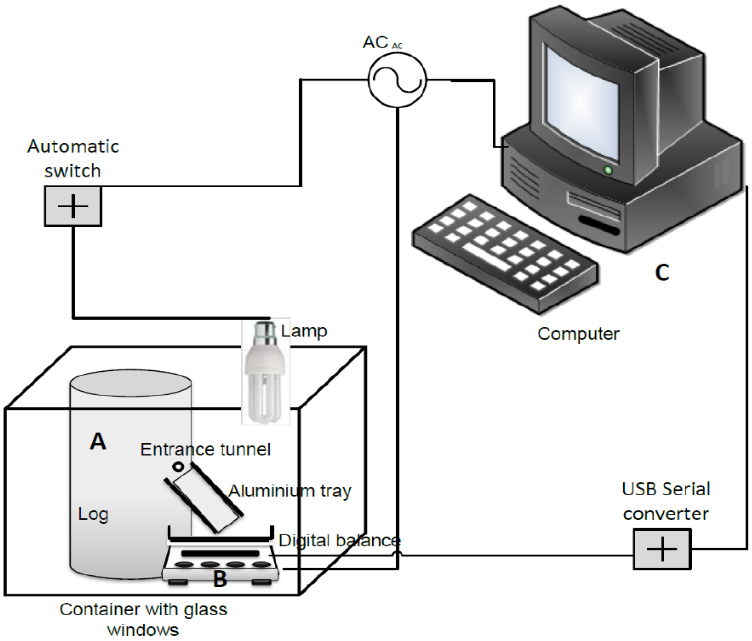

Figure 1. An automated measurement system for frass production
A: a log infested with *P. quercivorus*, **B**: electirical balance, **C**: computer

RESULTS AND DISCUSSION

RESULTS

Based on the types of frass ejected from galleries in the field experiment, results show there were three stages of the frass production activities; a fibrous frass stage when adults were digging galleries, an intermediate stage without any frass production, and a powdery frass stage with larval digging activity (Figure 2).

The mean length of fibrous frass stage was 11.14 days (± 4.83), ranging from five to twenty one days. The mean length of inter-mediate stage with no-frass production, between cessation time of fibrous frass and starting time of powdery frass production was 11.95 days (± 4.87) ranging from two to twenty days. Accordingly, starting time of powdery frass production ranged from day 19 to day 27.

As shown in Figure 3, the length of fibrous frass stage was negatively correlated with the length of intermediate stage. The speed of frass production calculated from the field data on a dry weight basis of 21 galleries was 14.5 mg/day (± 6.5) and 62.8 mg/day (± 35.6) for fibrous frass and for powdery frass respectively.

In the laboratory experiment, the long-term patterns in frass production were different among the three galleries (logs) as shown in Figure 4. The fibrous frass stage in log no.1 continued for 23 days producing 384 mg fibrous frass. The following intermediate period was shorter than one day, and then powdery frass production started on the same day. Similar to log no. 1, the fibrous frass stage in log no. 2 continued for 22 days producing 155 mg fibrous frass, followed by a short intermediate stage. The long-term patterns in frass production behaved quite differently in log no. 3 (Figure 4). The fibrous frass production stopped on the 11th day after producing 59 mg of fibrous frass. The intermediate period continued for 21 days from day 11 to day 32 and the powdery frass production started on the following day.

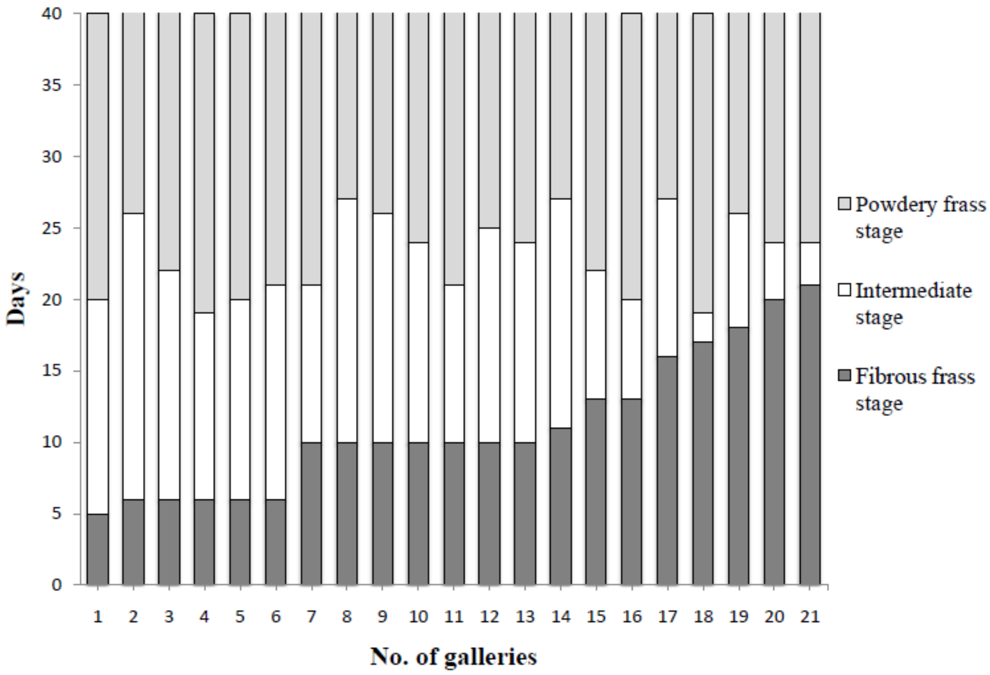

Figure 2. Lengths of three stages of frass production, a fibrous frass stage when adults were digging galleries, an intermediate stage without any frass production, and a powdery frass stage with larval digging activity, observed for 21 galleries

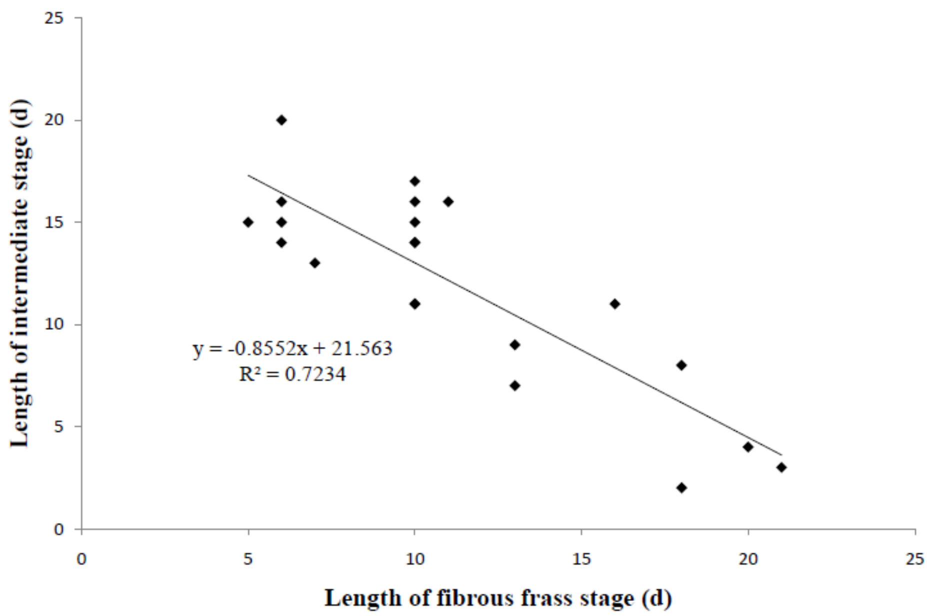

Figure 3. Relationship between the length of the fibrous frass stage (adult digging activity) and the length of the intermediate stage (no frass production)

DISCUSSION

Field and laboratory experiments revealed that there are three stages of activity in frass production by *P. quercivorus*, namely fibrous frass stage, intermediate stage and powdery frass stage. The fibrous frass stage is the period of adult digging activity that can produce fibrous frass. The intermediate stage is the time between the cessation of fibrous frass production and the starting of powdery frass production (no-frass production period). The powdery frass stage is the period of larval digging activity that can produce powdery frass.

Boring a tunnel to make a gallery system is initiated with fibrous frass production. Tarno *et al*. (2011) reported that fibrous frass indicated both male and female activity in the boring tunnel for reproduction. First a male makes a simple tunnel. After copulation the female substitutes for the male in boring the tunnel and lay eggs near the terminus of the tunnel and then moves back to tunnel new branches and lay more eggs (Kobayashi and Ueda, 2002; Tarno *et al*., 2011).

The fibrous frass stage consists of two important phases; the pre-copulating phase (before mating) and post-copulating phase (after mating). There are two different types of courtships in the pre-copulating phase of bark and ambrosia beetles. Among polygamous species, i.e. *Ips* and *Pityogenes*, the male arrives at the host tree first and bores an entrance hole and a mating chamber in the phloem and waits for the female (Kirkendall *et al*., 1997). On the contrary, in monogamous species, exemplified by *Scolytus, Trypodendron* and *Tomicus*, the female arrives first, bores the entrance hole and initiates tunneling of gallery where the male will join the female (Kirkendall *et al*., 1997). *Megaplatypus* and *Platypus* are exceptions for monogamous species since the males initiate boring tunnels as described for *M. mutatus* (=*Platypus mutatus*) (Alfaro *et al*., 2007) and *P. quercivorus* (Sone *et al*., 1998; Kobayashi and Ueda, 2002; Yamasaki *et al*.,2007; Yamasaki and Futai, 2008; Tarno *et al*., 2011).

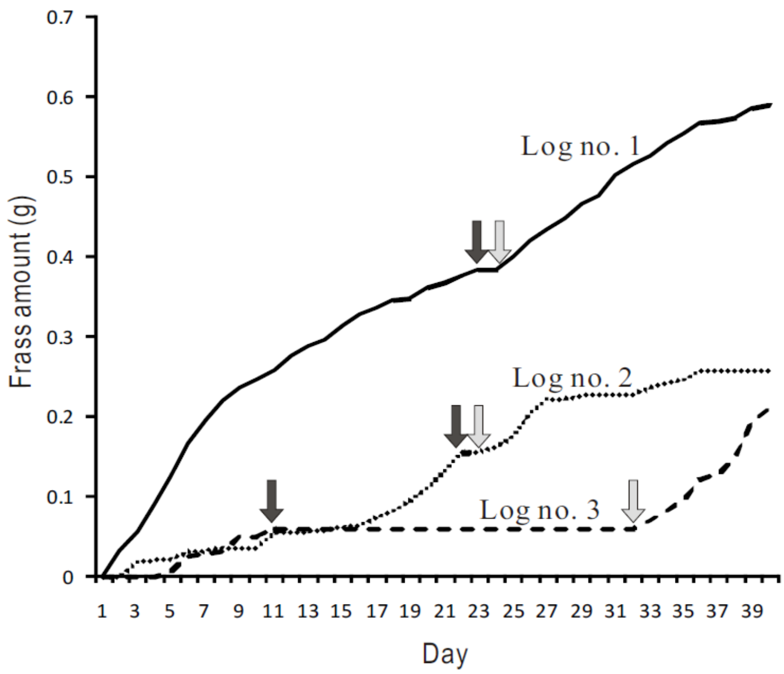

Figure 4. Long-term patterns in frass production monitored for logs in the laboratory. Black and grey arrows means the cessation time of fibrous frass production and the initiation time of powdery frass production, respectively. The distance between two arrows shows the intermediate stage with no-frass production

Fibrous frass produced by unmated males of *P. koryoensis* contains four male-specific compounds and citronellol, and a blend of these compounds acts as an aggregation pheromone (Kim *et al*., 2009). A sex pheromone of *M. mutatus* (Audino *et al*. 2005) and an aggregation pheromone of *P. quercivorus* (Tokoro *et al*., 2007) have been identified from frass produced by male beetles. Based on my observations, after the middle of June 2010, *P. quercivorus* males started to produce fibrous frass in constructing galleries. Sone *et al.* (1998) also reported that adult males of *P. quercivorus* started the formation of galleries in June. Lissemore (1997) explained that the male beetles remove frass that accumulates as the females lay their eggs in the phloem tissue of the host tree and frass clearing is necessary to maximize paternity. In the case of the pine engraver bark beetle *Ips pini*, males contribute to removal of frass and protection of eggs from predators (Reid and Roitberg, 1994).

After mating (post-copulating phase), adult females of *P. quercivorus* continued to extend their tunnels and males ejected the frass from the entrance holes (Tarno *et al*., 2011). The same occurrs for *Xyleborus dispar, Dendroctonus micans* (Sauvard 2004) and *Scolytoplatypus mikado* (Kinuura and Hijii, 1991). In the case of *Megaplatypus mutatus*, the female joins the male soon after successful attack, and constructs most of the galleries as the female lays eggs (Alfaro *et al*., 2007).

Tarno *et al*. (2011) found that powdery frass was not produced immediately after fibrous frass production ceased, but began to be produced by larvae several days later. This means that there is a period without frass production, an intermediate stage, between cessation of fibrous frass production and starting time of powdery frass production. The length of the intermediate stage is variable among individual galleries as seen in this study. In the field part of the experiment it varied between two and twenty days.

There are several possibilities to explain the variation in the length of the intermediate stage.The most obvious explanation is connected to the fact that *P. quercivorus* can make various numbers of tunnel branches (Kinuura, 1995). After finishing each branch, the female lays eggs. If only one branch is done, there may be a long intermediate stage, whereas if many branches are done, larvae from the first branch may start tunneling at the same time as the female has finished her last branch. In this study the numbers of branches were not counted, but it was a negative correlation between the length of the intermediate stage and the amount of fibrous frass.

Other factors may also influence the length of the intermediate stage. During the tunneling and laying eggs, the female has to keep and protect her eggs by blocking the tunnel (post ovipositional blocking). Blocking the tunnel entrance by the adult male or female could exclude predators and possibly help regulate microclimate, and might have a much greater effect on offspring survivorship in such species than for species which breed in large aggregations (Kirkendall *et al*., 1997). Post ovi-positional blocking may be widespread in the ambrosia beetle; in some cases, this includes blocking both during and after oviposition (Kirkendall *et al*., 1997). During oviposition of *Scolytoplatypus mikado*, female beetles were likely to inoculate their symbiotic fungus spores on the gallery walls. During the gallery construct-ion and oviposition by a *S. mikado* female, the male plugged the entrance hole with his own body to prevent predators and other enemies from invading the gallery system (Kinuura and Hijii, 1991). In the case of *Xyleborus pfeili,* after gallery construction, the adult female plugged the entrance hole with her body for a long time (Mizuno and Kajimura, 2002). Females began to deposit eggs at the terminal parts of tunnels two to three weeks after the commencement of gallery formation (Sone *et al*., 1998). *Platypus quercivorus* laid more eggs on healthy logs of *Quercus crispula* than infected logs or any of the other tree species (Tarno *et al*., 2011).

The powdery frass period starts after the intermediate stage and is an indicator of larval activity (Tarno *et al*., 2011). In the case of *Scolytoplatypus mikado,* deposited eggs hatch within about a week after oviposition and the immature larvae enlarge their egg niches by themselves into larval cradles, grazing on the ambrosia fungi covering the walls (Kinuura and Hijii 1991). There are five instar larvae in *P. quercivorus*. From the 1st to the 4th stage, larval head size increased at a constant ratio, 1.35-1.38, between successive stages, and the ratio decreased to 1.22 between the 4th and the 5th stages (Sone *et al*., 1998). As with *P. quercivorus*, the larvae of *Austroplatypus* *incompertus* are xylomycetophagous and pass through five larval instars (Kent and Simpson, 1992). Last (fifth) instar platypodid larvae make their own pupal chambers in all species studied, in most species they also extend the branch tunnels which are used by larvae prior to pupating (Kirkendall *et al*., 1997). Larval galleries of *M. mutatus* are short and are oriented vertically to the parental galleries (Alfaro *et al*., 2007). Many tunnel branches made by larvae are more complicated than galleries made by adults. Sone *et al*. (1998) showed that the initial development of galleries of *P. quercivorus* required about 2–3 weeks after commencement of gallery formation, with all galleries consisting of only one main tunnel after several weeks; development of galleries was more complicated and occurs on many strata and with more branches (Sone *et al*., 1998).

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Jack Sutherland for his comments and suggestions on this paper, and Dr Takeuchi for his help in laboratory work.

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