Studies on ecology and control measures against the invasive wood-boring beetle

Aromia bungii **(Coleoptera: Cerambycidae)**

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ABSTRACT

Chapter 1 General Introduction

Invasive non-native species have become a global problem with the increased international trade and transportation of goods and humans. Species unintentionally invade new regions in addition to their original distribution and significantly impact region-specific ecosystems, agriculture, forestry, fisheries, and human-living environments in invaded regions. Longhorn beetles are the most common invasive wood-boring species. For example, the Asian longhorn beetle, which is native to Asia, has spread to North America and Europe, wreaking havoc on maple, poplar, willow and a variety of broad-leaf trees.

The red-necked longhorn beetle *Aromia bungii* (Faldermann 1835) (Coleoptera: Cerambycidae) has recently invaded Japan, Germany, and Italy, causing severe damage to Rosaceae trees. The beetle is indigenous to China and the surrounding countries, and the trees of *Prunus* spp. are the most common hosts of this beetle. The beetle has been identified as a significant pest in fruit orchards, including peach, plum, ume, and apricot, in China. However, the biology of *A. bungii* remains unknown. In Japan, the primary hosts of *A. bungii* are flowering cherry trees (*Cerasus* spp.), peach, plum, and ume. Host flowering cherry trees are planted everywhere in urban areas in Japan, which is a significant difference from its native regions. Consequently, considerable impacts of *A. bungii* infestation have been observed in on urban greenings, particularly flowering cherry trees. In these areas, the development of effective methods for detecting *A. bungii* and effective control measures are urgently needed to eliminate the target beetle.

This research was conducted in Osaka Prefecture, where *A. bungii* was first discovered in 2015 and flowering cherry trees were widely planted in urban areas. This research aims to uncover reliable information for protecting flowering cherry trees from the invasive wood-boring beetle *A. bungii* by identifying its basic ecology in the invaded area and developing effective control measures. In particular, the detailed life cycle of *A. bungii*, which is presumed to have a 2-year life cycle in Japan, as well as the seasonal larval feeding and tunneling activities in host trees was clarified. Thereafter, based on the ecology of the beetle, a systemic insecticide treatment in the form of trunk injection against *A. bungii* was developed and its effect on *A. bungii* larvae in infested trees was evaluated.

This thesis is composed of eight chapters. Chapters 1 and 8 are the general introduction and discussion, respectively. In Chapter 2, the characteristics of infested flowering cherry trees were examined based on the presence or absence of tree damage in pre- and post-surveys along with tree characteristics (e.g., bark roughness, size, species, and vigor). The adult emergence periods from trees and the flight periods of adults in the field in Osaka Prefecture were discussed in Chapter 3. *A. bungii* was counted based on the number of adults emerging from and sighted on flowering cherry trees, respectively. In Chapter 4, feeding locations of *A. bungii* larvae in wood tissue were studied through direct observations of cross-sections of infested logs and X-ray CT observations of artificially reared larvae in the host branches. Chapter 5 describes the formation process of the pupal chamber with calcareous entrance lids by *A. bungii* identified by X-ray CT observations of larvae reared artificially in host branches and energy-dispersive X-ray fluorescence analysis of the inside of a larval body. In Chapter 6, the activity periods of *A. bungii* larvae in the field, particularly the larval frass ejection and pupal chamber formation periods, were documented through daily follow-up surveys on the number of frass-holes with frass ejection and newly formed adult emergence-holes on infested flowering cherry trees in the field. Moreover, life cycles of *A. bungii* in Osaka Prefecture were estimated based on the results of Chapters 3–6. In Chapter 7, the effects of insecticide injection in tree trunks infested by *A. bungii* in the field were evaluated. The optimal application timing of the injection treatment was then discussed in light of the estimated life cycle of *A. bungii*.

Chapter 2: Characteristics of Flowering Cherry Trees Infested by *Aromia bungii*

Tree characteristics associated with invasive wood-boring beetle infestation provide helpful information for selecting and monitoring trees and sites for intrusion detection surveys and prioritizing the application of preventive control measures.

Public parks in Osaka Prefecture near the areas estimated to be the beginning of the infestation by *A. bungii* were the study sites in this chapter. The presence or absence of tree damage was identified using the frass ejected from the monitoring trees in the 2017 pre- and the 2018 post-surveys in each study site. Four tree characteristics (e.g., bark roughness, tree size, species, and tree vigor) were also examined between the pre- and post-surveys. With such methods, it was possible to understand better the characteristics of flowering cherry trees infested with *A. bungii*. The generalized linear mixed model were applied based on the study site categories: uninvaded study sites (survey for the first trees to be damaged) and invaded study sites (survey for the next trees to be damaged) in the 2017 pre-surveys (i.e., a first tree model and a next tree model), to evaluate tree characteristics (explanatory variables) affecting tree damage by *A. bungii* (response variable). Three characteristics were chosen as explanatory variables in the most predictive models (the first-trees model and the next-trees model), indicating that trees with rough surface bark, large size, and weakened conditions were more susceptible to the infestation of *A. bungii*.

Chapter 3 Seasonal Prevalence of *Aromia bungii* Adults in Osaka Prefecture, Japan

Understanding the seasonal prevalence of invasive pests in newly invaded areas is critical for developing an appropriate and localized control plan for successful eradication. It has been reported in Japan that the seasonal prevalence of *A. bungii* adults in the field varies according to the invaded regions.

The adult flight and emergence periods of *A. bungii* from infested flowering cherry trees in

Osaka Prefecture were discussed in this chapter. The number of *A. bungii* adults sighted on flowering cherry trees at three study sites (one site from 2019–2021 and two sites from 2020–2021) was surveyed one or two times per week from late May or early June to late August. According to these surveys, the adult flight period of *A. bungii* can last 2 months (approximately early June to early August). Furthermore, the highest number of adults observed in the field was in late June. Moreover, adults were more abundant in the early stages of their seasonal prevalence (around peak number dates) and had almost disappeared by August. It was suggested by these findings that late June is one of the best times to control *A. bungii* adults in Osaka Prefecture.

Adults emerging from infested trees were surveyed daily in 2021 at one study site. According to the survey results, the emergence period of *A. bungii* adults could last approximately one month from the initial emergence date of the season (early-June to early-July), supporting the above idea of adult abundance concentration in the early phase of seasonal prevalence. A linear mixed model was used to assess the characteristics of emerging adults of *A. bungii*. The explanatory variables used in this analysis were sex, body weight, and tree types. The influence of these variables on the elapsed days from the initial emergence date of adults, which was the response variable, was assessed in this analysis. Two explanatory variables, sex (male or female) and tree type (standing tree or tree stump), were chosen as explanatory variables in the most predictive model. This result implied that males emerged earlier than females and adults emerged earlier from stumps than standing trees.

Chapter 4: Feeding Locations of *Aromia bungii* Larvae inside Host Trees

Longhorn beetles cause tree damage in larval stages by feeding or tunneling. Identifying larval feeding locations in wood tissue is critical for selecting insecticide control measures as it helps determine which inner woody parts of the trees must be reached by insecticides.

To identify the feeding locations of *A. bungii* larvae in wood tissues, the tissues of sliced wood disks from flowering cherry trees infested by immature and mature larvae of *A. bungii* were directly observed. Furthermore, nondestructive observations were made using X-ray CT on the branches of *Cerasus* × *yedoensis* ‘Somei-yoshino’ and *P. cerasifera* var. *atropurpurea*, in which the early larvae were artificially reared. Both observations revealed that *A. bungii* larvae are found near the cambium during their early and immature developmental stages and feed from the inner bark to the surface layer of the xylem while digging vertically, whereas the mature larvae tunnel deeply into the xylem.

Chapter 5: Formation Process to Complete Pupal Chamber in *Aromia bungii* Larvae

Longhorn beetle larvae form pupal chambers at maturity, where they spend vulnerable pupal stages. The structures and locations of the pupal chambers differ between species. These differences in chamber forms are considered adaptations to external biotic (e.g., natural enemies) or abiotic (e.g., humidity) factors in the environment of each species. The larvae of *A. bungii* constructed a cell-type pupal chamber with a calcareous entrance lid at the end of a xylem tunnel. However, little is known about the process by which the larvae of *A. bungii* form calcareous lids and pupal chambers.

A. bungii larvae were artificially reared in branches of *P. cerasifera* var. *atropurpurea* for 100 days under 28°C long-day conditions. The developmental stages of each larva were evaluated using X-ray CT observations of the larval location in the wood tissue. Thereafter, the larvae from the test branches were collected and their weight was determined. According to the findings, approximately 40% of the larvae completed their pupal chambers, and large larvae tended to tunnel into the xylem to form pupal chambers with entrance lids. Except for small larvae with low weight, it was revealed by the dissection of the collected larvae that immature larvae that did not complete the pupal chamber had two out of the six Malpighian tubules (MTs) enlarged and white. However, no enlargement of the two MTs was observed in the larvae inside the pupal chambers. An energy-dispersive X-ray fluorescence analytical microscope was used to examine the elemental distribution on the midgut with

MTs of an immature larva and the entrance lid of a pupal chamber. It was revealed that the two white-enlarged MTs stored calcium in the proximal segments. At the same time, the midgut had little calcium, and the MTs and entrance lid had a significant amount of this element. *A. bungii* larvae could accumulate calcium in MTs through wood-feeding activities, and mature larvae would use the calcium stored in the MTs to form the calcareous lid at the pupal chamber entrance.

Chapter 6: Periods of Frass Ejection and Pupal Chamber Formation of *Aromia bungii* Larvae in the Field

Understanding the developmental stages (i.e., life cycle) inside trees based on annual larval activity is helpful when planning the application of control measures against wood-boring beetle larvae. The larvae of *A. bungii* are known to eject frass frequently during feeding activities and to form adult emergence-holes just before finishing their pupal chambers. This means that seasonal timing of *A. bungii* larvae to start and stop feeding inside trees as well as the period they spend in the pupal chambers can be estimated by recording the dates of frass ejection and emergence-hole formations, respectively. Furthermore, the difference between the dates of larval emergence-hole formation and emergence from the holes as adults can be used to calculate periods spent in pupal chambers.

The number of frass ejections from frass excretion-holes and newly made adult emergence-holes on infested flowering cherry trees were surveyed daily in the field for 1 year (from March 25, 2021, to March 25, 2022). The results of the surveys were discussed in this chapter. The dates of adult emergence through each emergence-hole from May 28 to July 31, 2022 and the characteristics of emerged adults, such as sex and body weight, were also studied. Overwintering larvae in infested trees resumed feeding or tunneling activity in March, became more active until June or July, and stopped in November. From July to November, mature larvae formed adult emergence-holes (i.e., completed pupal chambers) and emerged as adults the following year, from early June to early July.

Furthermore, it was proposed that small *A. bungii* formed their pupal chambers late and emerged as adults early; the time of *A. bungii* spent in their pupal chambers was short for small ones. Note that small larvae have a short developmental period. The possibility of a 1-year cycle in the field was discussed for some of the emerging adults with lower weight in this chapter according to the weight of *A. bungii* larvae reared artificially for 100 days on host branches (Chapter 5).

The life cycle of *A. bungii* in Osaka Prefecture was depicted based on the findings of this study and those of Chapters 3–5. The 2-year life cycle of *A. bungii* is depicted in the diagram.

Chapter 7: Development of Control Measures by Trunk Injection of Systemic Insecticide against *Aromia bungii* Larvae

Trunk injection is a method of protecting the entire tree by injecting insecticides into holes drilled along the lower side of the tree trunk. The injected insecticides move upward primarily through sapwood via the water transportation associated with photosynthesis. Immature larvae of *A. bungii* feed on the surface layer of the xylem (a part of sapwood) (as shown in Chapter 4) and mature larvae tunnel into the xylem to form pupal chambers (as shown in Chapters 4 and 5); therefore, trunk injection is expected to be effective on larvae because the insecticide will be ingested when they feed on the woody tissues.

The effects of dinotefuran liquid solution injected into the trunk of infested flowering cherry trees on *A. bungii* larvae were investigated. In late April 2019, 20 test trees were injected with the insecticide. The number of larval frass excretion-holes with newly ejected frass (hereafter “active frass-holes”) in both treated and untreated trees were counted weekly from April to May 2019–2020. Additionally, adult emergence-holes in each tree were counted in 2019, 2020, and 2021. The total number of active frass-holes decreased by 21.2% in the treated trees and increased by 178.9% in the untreated trees within 4 weeks of injection. Based on the decreasing number of total active frass-holes in the treated trees, the insecticidal efficacy of the formulation was estimated to be 88.2%. Generalized linear models were applied to the number of active frass-holes and newly occurring emergence-holes on survey day in each test tree to understand the effect of the injection. This was indicated by the result that larval activity was reduced by the dinotefuran treatment for at least 4 weeks, resulting in the suppression of adult emergence the following year. However, in the following year of treatment, active frass-holes and newly occurring emergence-holes were observed in some treated trees. It was suggested by this observation that the injection treatment in late April was at least partially ineffective in controlling *A. bungii* larvae that hatched from the eggs laid during the adult reproductive (flight) period from June to August of the treatment year. It might be better to postpone insecticide injection until the adult flight period to improve the effectiveness against the overwintering larvae and possibly the newly hatched larvae of *A. bungii*.

Chapter 8 General Discussion

This study demonstrated the characteristics of flowering cherry trees infested by *A. bungii*, the life cycle of *A. bungii* in Osaka Prefecture, Japan, and the effectiveness of a dinotefuran liquid solution via trunk injection against *A. bungii* larvae. However, there remains necessary topics to promote further action for management of *A. bungii*, such as host finding process of the adults, effective use of flowering cherry trees' species diversity, environmental factors affecting on the beetle's life cycle, and other control measures for integrated pest treatment (IPM).

The author has been working on control measures for IPM from various angles, including trunk injection of insecticides with different properties than dinotefuran, trunk spraying of insecticides to kill eggs, trunk-covering of nets to prevent egg-laying, and sealing of emergence-holes with hard materials, to implement within the next few years. The author is confident that the ongoing efforts will bear fruits and eventually sprout new seeds to bloom flowering cherry trees for a long time in Japan.