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SHORT COMMUNICATION

Oviposition site selection of *Doubledaya bucculenta* (Coleoptera: Erotylidae: Languriinae) in relation to wall thickness of bamboo internode

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Abstract

Thickness of woody tissue of host plants may affect behaviors of wood-using insects that excavate the woody tissue during oviposition. To determine whether females of the bamboo-using lizard beetle, *Doubledaya bucculenta* (Coleoptera: Erotylidae: Languriinae) choose thin-walled areas of *Pleioblastus simonii* bamboo internodes to penetrate the wall for oviposition, we investigated the diameter and wall thickness at each of the relative heights (0 to 10 from basal node) within internodes and the distribution pattern of oviposition marks of *D. bucculenta* within each internode. Internodes of *P. simonii* were tapered and the wall was thinnest around the relative heights 7 and 8. Most oviposition marks were made where the wall was thinnest. Therefore, it is suggested that *D. bucculenta* females prefer thin-walled areas of *P. simonii* internodes for oviposition.

Key words: cultivation mutualism, fungiculture, Languriini, lizard beetle, *Pleioblastus simonii*.

In wood-using insects that excavate woody tissue to access their oviposition sites, thickness of woody tissue may affect behaviors of female insects, as excavating hard and thick tissue requires time and energy of ovipositing females. For example, females of the Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) bore the bark of host pine trees and lay eggs under the bark (Nakamura *et al.* 1995). The females prefer thin-barked wood to thick-barked wood for oviposition (Nakamura *et al.* 1995). Females of the kuroshio shot hole borer, *Euwallacea kuroshio* Gomez and Hulcr (Coleoptera: Curculionidae: Scolytinae) bore the bark of host willow trees to make galleries in xylem for reproduction and attack thin bark more frequently than thick bark (Boland & Woodward 2021). Females of the woodwasp, *Sirex nitobei* Matsumura (Hymenoptera: Siricidae) use their ovipositors to pierce the bark of host pine trees and lay eggs into the sapwood (Fukuda & Hijii 1998). Small females (with relatively short ovipositors) lay eggs more frequently in thin- than thick-barked wood, whereas large females do not discriminate between thin and thick bark (Fukuda & Hijii 1998). Therefore, females of such wood-using insects may choose thin woody parts to reach the oviposition substrates efficiently, resulting in the improvement of oviposition success.

The lizard beetle, *Doubledaya bucculenta* Lewis (Coleoptera: Erotylidae: Languriinae) oviposits on the internodes of recently dead bamboo culms of the genera *Pleioblastus* and *Semiarundinaria* (Hayashi 1974; Toki & Togashi 2011; Toki *et al.* 2021) and farms the Saccharomycetes yeast *Wickerhamomyces anomalus* on the inner surface (i.e. pith tissue) of the internode cavities (Toki *et al.* 2012, 2013). The female adults carry the yeast using a specialized pocket-like organ for fungal transportation (termed the mycangium or mycetangium) located in the abdomen (Toki *et al.* 2012). In spring, the female adult excavates a small hole in the internode wall using her mandibles, inserts her ovipositor into the cavity and deposits both

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an egg and the symbiotic yeast (Toki *et al.* 2013). Finally, she plugs the hole with the excised bamboo fibers, forming a completed oviposition mark (COM) (Toki & Togashi 2013; Miyazaki & Toki 2020). The hatched larva spreads the symbiotic yeast all over the cavity to make a fungal garden and feeds on the yeast grown by consuming free sugars, mainly glucose and fructose, in the pith (Toki *et al.* 2012, 2013; Toki & Aoki 2021). Within the internode, only a single individual develops, ecloses and overwinters (Toki & Togashi 2013). The adult female incorporates the yeast of her natal garden into the mycangium. The adults exit the internodes in the spring (Toki *et al.* 2012; Toki & Togashi 2013).

In most cases, an internode receives a single oviposition mark (OM), which could be either a COM or an abandoned oviposition mark (AOM), the latter indicating when a female ceased excavation, in nature (Toki & Togashi 2013). The female adults preferably lay eggs onto internodes with no OMs compared to those with COMs, indicating that the COM deters the females from oviposition (Miyazaki & Toki 2020).

What part of an internode wall does a female adult of *D. bucculenta* bore for oviposition? The internode cavity is accessible from any parts of the surrounding woody wall. Females spend a long time (over 1 h) excavating the wall prior to oviposition (Miyazaki & Toki 2020). If the wall thickness is not uniform, it can be hypothesized that female insects choose the thin-walled part to penetrate the wall for oviposition to improve oviposition efficiency. To examine this hypothesis, we investigated the shape of internode of the host bamboo and the distribution pattern of OMs of *D. bucculenta*.

The insects were sampled in *P. simonii* bamboo patches in Toyota City, Aichi Prefecture, Japan (35°9N, 137°13′E) on 2 October 2019 (larvae) and 6 January (newly eclosed adults), 7 February (larvae), 7 August (larvae and pupae) and 18 September 2020 (newly eclosed adults). Rearing immatures (10 larvae and 3 pupae) and sexual maturation of adults were conducted in the laboratory by following Miyazaki and Toki (2020).

The host bamboos were sampled at the same place on 6 January, 11 May and 12 May 2020. Internodes of live culms (>20) of *P. simonii* were arbitrarily collected and stored at −25°C until use to avoid desiccation and fungal contamination. As females of *D. bucculenta* deposit the eggs on recently-dead culms, internodes were placed in a dark place at room temperature for 0–63 days before use.

To determine the relationships of diameter and wall thickness to the relative height within an internode, 10 OM-free internodes (length: mean ± SD = 37.2 ± 6.5 cm, range = 26.7 to 45.8 cm, $n = 10$; diameter at the middle: mean ± SD = 17.02 ± 1.24 mm, range = 14.70 to 18.45 mm, $n = 10$) were arbitrarily selected because internodes not too large or small for the body size of the female adults were used as oviposition sites (Toki & Togashi 2011, 2013). First, each internode was cut at 1 cm distant from basal and apical nodes. Then, the remaining cylindrical part was divided into 10 pieces at equal intervals along the bamboo tissue fibers. The cut ends were numbered sequentially from 0 at the cut end nearest to the basal node to 10 at that nearest to the apical node. The number represents the relative height (RH) of the cut end which was designated as RH-number in this study. The diameter and wall thickness of each cut end (RH-0 to RH-10) were measured with calipers. The wall thickness was measured at three arbitrarily-selected parts of each cut end and averaged.

The diameter of internodes simply decreased as the RH in internodes increased (Fig. 1A). Wall thickness was greatest at RH-0, decreased sharply to RH-1 and

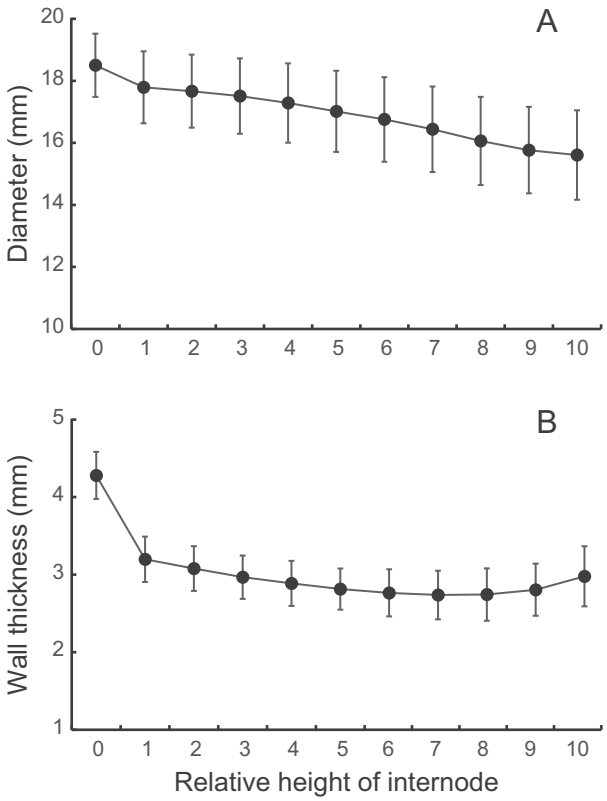


Figure 1 Mean values of the (A) diameter and (B) wall thickness at relative heights within an internode of *Pleioblastus simonii* bamboo. Data were obtained from 10 internodes. Smaller numbers on the horizontal axis represent lower positions within an internode. Error bar: SD.

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then gradually to RH-7, was lowest at RH-7 and RH-8, and increased gradually to RH-10 (Fig. 1B). Thus, an internode of *P. simonii* was not cylindrical but tapered and the wall was thinnest at around RH-7 and RH-8 from the basal node.

To determine whether females of *D. bucculenta* lay eggs on a specific position of bamboo internodes, we conducted an oviposition experiment to record the positions of OMs on internodes. One or two OM-free internodes were set against the wall (elevation angle: 35–45°) in a transparent plastic container (either 22 × 37 × 24 cm tall or 22 × 37 × 48 cm tall). The female insects (elytral length: mean ± SD = 11.17 ± 1.47 mm, range = 8.27 to 13.83 mm, $n = 29$) were singly placed on the container and allowed to oviposit for up to 72 h at approximately 25°C under natural or fluorescent light. After oviposition completion or 72 h, records were taken on the internode length (distance between basal and apical nodes), distance between basal node and OM, and vertical position of OM as explained later.

When the relative distance (RD) from the basal to apical nodes (i.e. internode length) was regarded as 10, the diameters at RD-2.5, RD-5 and RD-7.5, nearby apical node (as RD-10), and the location of OM were measured. In addition, the internode was divided into 10 parts at equal intervals along the bamboo tissue fibers (Fig. 2). The parts of each internode were numbered sequentially beginning with 1 for the lowermost part to represent the relative position (RP). The surface of each part was divided into four aspects (upside, underside, and right and left lateral aspects of an internode leaned against the wall of a container) (see Fig. 2). Consequently, the internode surface was divided into 40 equal-sized compartments. The presence or absence of OMs and COMs was recorded for each of the compartments. If a female ceased excavation, the OM was designated as an AOM. The internodes were then dissected using pruning clippers and a pruning saw to record the wall thickness of the internode at RD-2.5, RD-5, RD-7.5, nearby apical node (as RD-10) and the location of OMs.

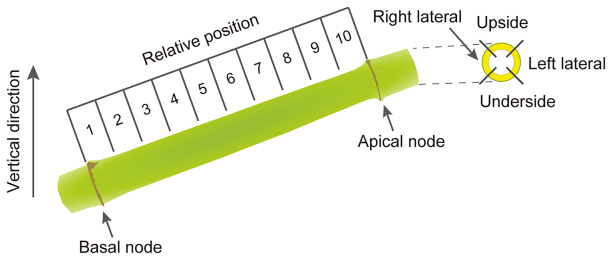


Figure 2 Schematic of an internode of *Pleioblastus simonii* bamboo used for the oviposition experiment.

For statistical analysis, Pearson's correlation coefficient was used to determine the relationship between the distance of an OM from a node and internode length. The effects of diameter and wall thickness of internodes on the location of OMs within internodes were tested using a generalized linear mixed model (GLMM). The response variable was the presence/absence of an OM (family = binomial: present = 1, absent = 0). The explanatory variables were the diameter and wall thickness of internodes; the interaction between them was not significant ($P = 0.966$) and excluded from the analysis. The random effect was the internode replicates. Calculations were performed using R 4.0.5 (R Core Team 2021). To determine the spatial distribution patterns of OMs and COMs among the 40 compartments of an internode, Morisita's I_δ index (Morisita 1959) was applied for the cumulative numbers of OMs and COMs on the 29 internodes. The I_δ values less than 1, equal to 1, and more than 1 represent uniform, random and clumped distributions, respectively (Morisita 1959).

Each of the 29 female adults of *D. bucculenta* made a single OM (in total, 20 COMs and 9 AOMs) on each internode (length: mean ± SD = 34.4 ± 6.7 cm, range = 23.8 to 49.0 cm, $n = 29$; diameter at the middle: mean ± SD = 16.83 ± 2.16 mm, range = 12.37 to 21.59 mm, $n = 29$; relative diameter to elytral length: mean ± SD = 1.52 ± 0.18, range = 1.21 to 1.87, $n = 29$). The OMs were formed on the upside of 3 internodes (3 COMs), the left lateral aspect of 1 internode (1 COM) and the underside of 25 internodes (16 COMs and 9 AOMs) (Table 1). The distance of OMs from basal node ranged from 4.2 to 39.2 cm (mean ± SD = 25.8 ± 8.4 cm, $n = 29$) and positively correlated with the internode length (mean ± SD = 34.4 ± 6.7 cm, range = 23.8 to 49.0 cm, $n = 29$) (Pearson's correlation coefficient $r = 0.785$, $P < 0.0001$). The RD of OMs from basal node compared to the internode length ranged from 1.26 to 9.23 (7.41 ± 1.67, $n = 29$) and showed no significant correlation with the internode length ($r = 0.260$, $P = 0.173$). The distance of OMs from apical node ranged from 2.0 to 29.2 cm (8.6 ± 5.2 cm, $n = 29$) and showed no significant correlation with the internode length ($r = 0.014$, $P = 0.941$).

When an internode was divided into 40 equal-sized compartments according to the relative position (RP-1 to RP-10) and aspect (upside, underside, and right and left lateral aspects), the OMs were found frequently at upper parts and underside of internodes leaned against the wall of a container (Table 1, Fig. 3). The OMs showed a clumped distribution among the compartments (mean density ± SD = 0.73 ± 2.21, $I_\delta = 8.97$,

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Table 1 Spatial distribution of oviposition marks (OMs) and completed oviposition marks (COMs) of *Doubledaya bucculenta* in 29 internodes of *Pleioblastus simonii*

Position of OMs (COMs) on the surface of internode	Relative position within an internode[a]										
	1	2	3	4	5	6	7	8	9	10	Total
Upside	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)	0 (0)	3 (3)
Right lateral aspect	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Underside	0 (0)	1 (0)	0 (0)	1 (1)	0 (0)	1 (0)	1 (0)	12 (9)	7 (4)	2 (2)	25 (16)
Left lateral aspect	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
Total	0 (0)	1 (0)	0 (0)	1 (1)	0 (0)	2 (1)	1 (0)	12 (9)	10 (7)	2 (2)	29 (20)

[a]Smaller numbers represent lower positions within an internode (see Fig. 2).
All values are the numbers of OMs (COMs).
Note that each internode harbored a single OM.

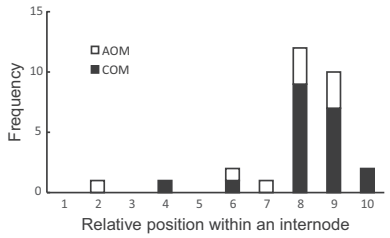


Figure 3 Frequency distribution of oviposition marks (OMs) of *Doubledaya bucculenta* on *Pleioblastus simonii* bamboo internodes. Smaller numbers on the horizontal axis represent lower positions within an internode. AOM, abandoned oviposition mark; COM, completed oviposition mark.

Table 2 Summary of generalized linear mixed model to test for the effects of diameter and wall thickness of an internode on presence of oviposition marks of *Doubledaya bucculenta*

Explanatory variable	Coefficient/ intercept	SE	*z*-value	*P*-value
(Intercept)	2.196	2.010	1.093	0.275
Diameter	0.156	0.162	0.966	0.334
Wall thickness	−2.316	1.068	−2.169	**0.030**

Significant *P*-values are in bold.

$P < 0.01$), as did the COMs (mean density ± SD = 0.50 ± 1.62, $I_\delta = 9.68$, $P < 0.01$).

The GLMM analysis, excluding two internodes due to lack of data of diameters, revealed that the probability of forming an OM decreased with increasing wall thickness of internode (coefficient = −2.316, $P = 0.030$) but the probability was not affected by the diameter (coefficient = 0.156, $P = 0.334$) (Table 2). When only wall thickness was used as the explanatory variable, the GLMM analysis also showed that the probability decreased with increasing wall thickness (coefficient = −1.664, $P = 0.015$).

Most females of *D. bucculenta* made OMs at around RP-8 and RP-9 (Fig. 3). As the RP-8 for the oviposition experiment corresponds to the area between RH-7 and RH-8 for the intact internode measurement, it is suggested that *D. bucculenta* females prefer the thin-walled area within an internode of *P. simonii* for oviposition.

An internode has a large single cavity surrounded by a woody wall. Using the mandibles, the females excavate a hole into the internode cavity through the wall to deposit an egg and the symbiotic yeast (Toki & Togashi 2011; Toki *et al.* 2012). Therefore, selecting a thin-walled site of an internode will improve the efficiency of oviposition. Like *D. bucculenta*, hard bamboo-using languriines including some congeners and *Oxylanguria acutipennis* Crotch make a single OM per internode on average, whereas *D. tonkinensis* Zia that attacks weakened and soft culms of bamboo makes two OMs per internode (Toki *et al.* 2014, 2016b, 2019, 2021). Location of OMs on an internode may be commonly affected by the wall thickness of internodes among hard bamboo users in Languriinae. Further study is needed to examine this hypothesis.

Most OMs (86.2% for OM, 80.0% for COM) were made on the underside of leaned internodes, indicating that the oviposition site selection is influenced not only by the thickness of internode wall but also by other factors. We frequently observed *D. bucculenta* females constructing the OMs on the shaded underside of internode in nature in 2019 and 2020 (pers. obs.). In the present study, the females were allowed to lay eggs under natural or fluorescent light in the laboratory. A clumped distribution of accumulated OMs suggests that the females preferred shaded parts of internodes during oviposition. Alternatively, the position of COM may be important for larval development inside the cavity. The larvae feed on the symbiotic yeast proliferating on the cavity surface (Toki *et al.* 2012). Non-symbiotic fungi sometimes enter into the cavity and at least one filamentous fungus species is harmful to the growth of the larva and the yeast (Toki *et al.* 2016a). If

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COMs were made on the upside of an internode, the larvae may suffer from the invasion of the internode cavity by such harmful fungi due to wind and rain.

In summary, thickness of woody tissue of host plants, regardless of monocots or dicots, affects behaviors of wood-using insects that excavate the woody tissue during oviposition. This phenomenon may be widespread not only in Coleoptera, including Cerambycidae, Curculionidae, and Erotylidae and Hymenoptera, but also other orders of wood-using insects.

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