

# Feeding Preferences and Reproduction of the False Powder Post Beetle, *Sinoxylon anale* Lesne, on Two Clones of the Pará Rubber Tree

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## ABSTRACT

The feeding preference and reproduction of *Sinoxylon anale* on two rubberwood clones (PB235, RRIM600 and the main branch of clone RRIM600) were investigated. No-choice and multiple-choice feeding tests were designed to determine the feeding preference of the beetle, whereas laboratory reared beetles were used for reproductivity measurements. Adult *S. anale* preferred rubberwood clone PB235 over both types of wood from clone RRIM600. In a multiple choice test, 60% of the beetles chose rubberwood clone PB235, and of the remainder, 24% and 16% chose wood from the trunk and branch of clone RRIM 600, respectively. The adult beetles also showed a highly significant ( $P < 0.001$ ) preference for feeding on wood from clone PB235 ( $0.295 \pm 0.028$  g) over trunk wood from clone RRIM 600 ( $0.253 \pm 0.029$  g) but there was no significant difference in the preference shown for wood from the RRIM600 branch ( $0.275 \pm 0.021$ g). *S. anale* also produced the greatest number of progeny in clone PB235 ( $39.07 \pm 17.30$  individuals), significantly greater ( $P < 0.01$ ) than the number produced in the trunk wood ( $25.73 \pm 8.41$  individuals) and the branch wood ( $22.47 \pm 2.73$  individuals) from the RRIM600 clones. The results indicated that *S. anale* seemed to prefer (as well as being more reproductive in) wood with a higher starch content.

**Keywords:** *Hevea brasiliensis*, Bostrichidae, Coleoptera, progeny numbers, rubberwood

## INTRODUCTION

Rubberwood (*Hevea brasiliensis* Müll. Arg.) has become an important substitute for other types of natural wood in the timber trade and wood-based industries (Hong, 1996). Rubberwood has a substantial overall advantage in its woodworking and mechanical characteristics (Kangkamanee *et al.*, 2010). However, its susceptibility to fungus and insect attacks are the principal drawbacks of the wood (Wong *et al.*, 2005). Dried and seasoned

rubberwood is susceptible to powder post beetle infestation (Ho and Hashim, 1997; Sittichaya and Beaver, 2009; Sittichaya *et al.*, 2009; Kangkamanee *et al.*, 2010). This is due to high levels of starch in the tissues (Creffield, 1991; Peters *et al.*, 2002) and also a low wood extractives content (Harmatha and Nawrot, 2002). The wood-boring powder post beetle, *Sinoxylon anale* Lesne (Coleoptera: Bostrichidae), was recently reported as being one of the most destructive pests of dried and seasoned rubberwood sawn timber in Thailand and Malaysia

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(Ho and Hashim, 1997; Sittichaya and Beaver, 2009; Sittichaya *et al.*, 2009; Kangkamanee *et al.*, 2010) and wood products made from this timber are now shipped to worldwide destinations (Fisher, 1950; Skalski, 1971; Joly *et al.*, 1994). This species is also an important insect pest of dying and dead trees, freshly cut logs and firewood in India, Israel and Thailand (Stebbing, 1914; Argaman, 1987; Hutacharn and Choldumrongkul, 1989). *S. anale* is strongly polyphagous, and both adults and larvae feed on the woody tissue of the host plants and derive nutrition by feeding on the starches and sugars in the plants' tissue (Tomimura, 1993). These starches and soluble sugars are the most important energy sources of powder post beetles (Tomimura, 1993).

*S. anale* is a wood-boring insect; its larvae cannot compensate for poor host quality by moving to new hosts, so the choice of host by the female is critical for the beetle (Chew, 1977; Thompson, 1988). Therefore, if any difference in the suitability of host species exists, females should choose the species which results in the highest offspring fitness (Lederhouse *et al.*, 1992; Rank *et al.*, 1998). To ensure that the selected wood material is suitable for progeny development, powder post beetles use contact chemo-orientation, in which the parental females bite into the wood, to determine if it is suitable breeding material (Gay, 1953)

*S. anale* is considered to be an important insect pest of rubberwood sawn timber, although its biology and the potential damage it can cause have rarely been investigated. Therefore, the present study looked at the preference and reproductivity of this beetle on rubberwood clones.

## MATERIALS AND METHODS

### Insects used for experiment

Adult *S. anale* individuals were obtained from a research colony at the Prince of Songkla University, Hat Yai, Thailand consisting of insects

of mixed ancestry maintained on rubberwood sawn timber from unidentified clones under ambient conditions of mean annual temperature  $27.30 \pm 0.67$  °C and  $81 \pm 3\%$  relative humidity (RH). The insect colony was established from adult individuals originally collected from infested rubberwood sawn timber from different provinces in southern Thailand. To ensure that the adult beetles used in the reproductivity tests had not previously oviposited, only newly emerged beetles were used. Adult insects were collected and maintained in transparent plastic containers ( $27 \times 18 \times 10$  cm) with a screened cover under ambient conditions for 24 hr. The beetles were provided with small pieces of dried rubberwood as food and substrate. The adult beetles used in all experiments were selected from surviving individuals with a high activity level and approximately similar body size ( $0.50 \pm 0.02$  mm).

### Rubberwood materials

The rubberwood (*Hevea brasiliensis* Müll Arg.) used in the experiments was derived from the two most commonly planted rubber clones in Thailand, RRIM600 and PB235. The trees used in the experiments were from 25-year-old trees of clones RRIM600 and PB235, and a 25-year-old branch from a 40-year-old tree of clone RRIM600 (RRIM600-branch). All the trees were obtained from the Thepha Research Station, Faculty of Natural Resources, Prince of Songkla University, Songkhla, Thailand. Rubber trees, from the same planting site were used to eliminate any effects of the site on the chemical and physical properties of the wood. In total, 15 rubber trees, five in each replication, were used in each experiment. First, logs were cut from the trunks of the 25-year-old trees (planted in 1981) at 15 cm above the ground and 80 cm in length from both clones, together with main branches (aged  $25 \pm 2$  yr) of clone RRIM600. The positions of these main branches were approximately 6–7 m

above the ground. The age of the main branches was estimated by counting their annual rings in the xylem under a stereo microscope (Model Olympus SZ51, Olympus Imaging & Audio Ltd., Essex, UK) (Ohashi *et al.*, 2001). All the trees were cut in August 2009 and immediately sawn and kiln dried (80 °C) to 12-15% moisture content and assessed with a wood moisture and temperature meter (model CEM-DT-129, Shenzhen Everbest Machinery Industry Co., Ltd, China). The rubberwood samples used for wood chemical analysis were kept as dried wood (12-15% moisture content) at -20 °C until analysis. Only clear, straight-grained, homogenous samples without defects (such as knots, cross grains, checks and splits) were used in the experiments.

#### **Starch, lignin and wood extractives content**

A total of 15 rubberwood samples (divided into three equal subsamples) were analyzed to establish their starch, lignin and wood-extractives content. The mean values from the three subsamples were taken to represent the content of each tree or sample. The starch content was estimated using the method outlined by Humphreys and Kelly (1961). The acid-insoluble lignin content in the rubberwood was measured in accordance with the TAPPI standard T 222 om-98 (TAPPI, 2000) and the solvent extractive content of the wood (ethanol-benzene mixture) was analyzed in accordance with TAPPI standard T 204 cm-97 (TAPPI, 1997).

#### **Multiple choice feeding tests**

The multiple choice feeding tests were conducted in a transparent Plexiglas box (12 × 17 × 4 cm) with a screened cover and a sheet of plain white paper as the floor covering. Three rubberwood blocks (2.5 × 5 × 2 cm), one each of RRIM600, RRIM600-branch and PB235, were placed at one end of the test arena equidistant from each other and the container walls. To avoid the effect of beetle defense behavior on host selection,

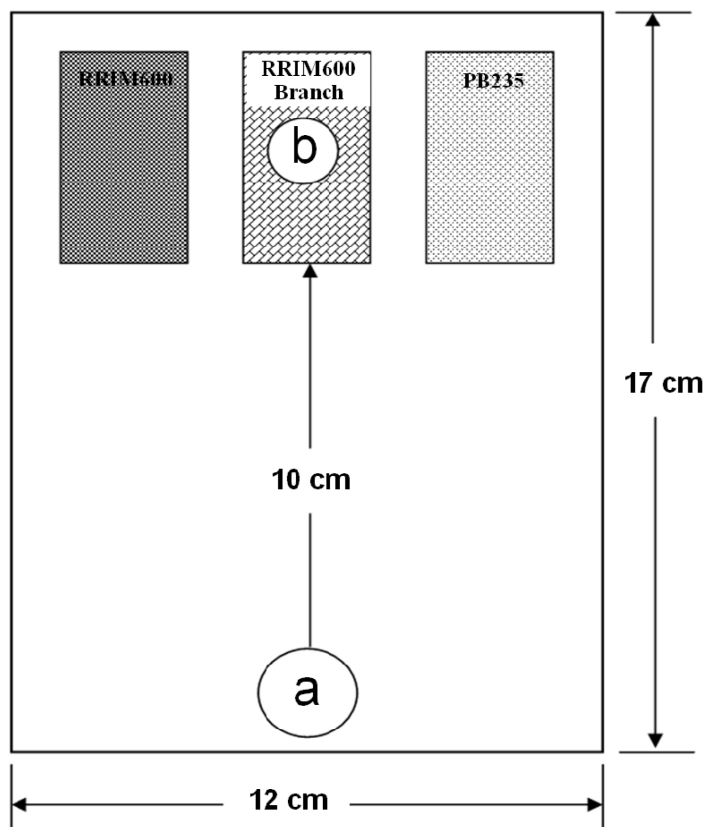
a single unsexed adult was released at the other end of the arena, 10 cm from the tested wood (Figure 1). The test was carried out for a photoperiod of 12+12 hr (light+dark) at  $28 \pm 1$  °C and  $80 \pm 3\%$  RH. After 24 hr, the adult's feeding choices were recorded. Twenty-five experiments using five samples of each rubber clone and tree were conducted. The positions of the wood blocks in the test arena were rotated selectively, by shifting the blocks one position in each clone-experiment.

#### **No-choice feeding test**

A total of 75 rubberwood blocks (2.5 × 5 × 2 cm, 12–15% moisture content) were cut, with 25 each from the RRIM600, RRIM600-branch and PB235 wood, consisting of five samples with five subsamples taken from each. A single wood block was placed at the bottom of a 10 cm diameter transparent plastic container with a screened cover. A single adult beetle was released into the test arena. The bore activity of the beetle was checked 24 hr after its release; any beetle which had not undertaken any bore activity was then replaced with a new individual. The insect bore activity was checked daily at 15:00 hours. The experiment was conducted for 30 d with a photoperiod of 12+12 hr (light+dark) at  $28 \pm 1$  °C and  $75 \pm 5\%$  RH. At the end of the experiment, all frass was collected. The frass dry mass was then determined by weighing to constant weight.

#### **Reproductivity of *S. anale***

The reproductivity of *S. anale* was established by laboratory bioassays. Defect-free rubberwood lumber (10 × 12 × 1.5 cm) with 12-15% moisture content was placed in a transparent plastic box (12 × 15 × 5 cm) with a screened cover. A pair of adults of similar size were released into the test box. The bore activity of the female individuals was checked after 48 hr and a new pair was substituted if the original female was not active. The wood substrate was then maintained with a photoperiod of 12+12 hr (light+dark) at



**Figure 1** Layout of the testing arena used in the multiple choice tests: a) starting point and b) rubberwood blocks, (the positions of the wood blocks were rotated selectively, by shifting the blocks one position in each clone-experiment).

$28 \pm 1$  °C and  $75 \pm 5$  % RH for an incubation period of two months. After the incubation period, the wood with the brood inside was then kept in a container, according to Sittichaya and Beaver (2009), and the emerging, positively phototropic beetles were trapped.

#### Statistical procedures

Data from all experiments were tested for normality using the one-sample Kolmogorov- Smirnov test and Levene's test for equality of variances. The results of the wood-chemical analysis of the rubberwood samples, the choice tests and the reproductivity tests were compared with one-way analysis of variance using Tukey's HSD (honestly significant difference) test

with the level of significance set at  $P < 0.05$ .

## RESULTS

### Starch, lignin and wood extractives content of the rubberwood

The starch content analysis indicated significant differences in the kiln-dried rubberwood both between and within the clones. The wood-starch content was significantly greater in the PB235 and RRIM600-branch than in the RRIM600 trunk samples (Table 1). No significant difference was detected in the acid-insoluble lignin content of the rubberwood samples. The ethanol-benzene extractives analysis revealed a significant difference between the RRIM600 and PB235

samples, however, the extractives content of the RRIM600-branch samples was not significantly different from either of the other samples (Table 1).

### Multiple choice tests

The adult, *S. anale*, responded positively to the rubberwood blocks provided. After setting the beetles at the start point, they moved unfalteringly in the direction of the rubberwood blocks, arriving at one of the three wood blocks randomly. Thereafter, the beetles walked around the wood blocks and in most cases bit them. After a certain time, the beetles then chose a particular wood block, by boring into the wood, or moved on to another block and repeated the process. After the 24 hr period of the experiment, all the beetles tested had chosen one of the wood blocks provided. Fifteen (60%) of the *S. anale* in the experiment selected rubberwood clone PB 235. Of the ten remaining, six beetles (24%) selected RRIM600-branch and four (16%) chose RRIM600 (Table

2). The number (mean  $\pm$  SD) of beetles which selected clone PB235 ( $3.00 \pm 1.22$  individuals) was significantly higher ( $P < 0.05$ ) than those selecting clone RRIM600 ( $0.80 \pm 0.84$  individuals) and the RRIM600-branch ( $1.20 \pm 1.10$  individuals). No significant differences were found within the clones (Table 2).

### No-choice feeding test

The amount (mean  $\pm$  SD) of frass dry mass produced by adult beetles fed on rubberwood clone PB235 ( $0.295 \pm 0.028$  g) was highly significantly ( $P < 0.001$ ) greater than that produced by clone RRIM600 ( $0.253 \pm 0.029$  g), but neither were significantly different from that produced by the beetles fed on RRIM600-branch ( $0.275 \pm 0.021$ g). The feeding preferences of *S. anale* not only varied between the different wood samples but differences were also found between individual trees within samples or clones. Adult beetles that fed on rubberwood clones RRIM600 and RRIM600-branch produced significantly more

**Table 1** Chemical analysis of rubberwood used in the experiments (%  $\pm$  SD).

Wood chemical content	Rubberwood clone		
	RRIM600	RRIM600-branch	PB235
Starch	2.26 $\pm$ 0.09 <sup>a</sup>	2.95 $\pm$ 0.21 <sup>b</sup>	3.11 $\pm$ 0.16 <sup>b</sup>
ethanol-benzene extractives	10.67 $\pm$ 0.48 <sup>a</sup>	10.86 $\pm$ 0.69 <sup>ab</sup>	11.61 $\pm$ 0.4 <sup>b</sup>
Acid-insoluble lignin	25.47 $\pm$ 1.65 <sup>a</sup>	25.93 $\pm$ 1.01 <sup>a</sup>	24.75 $\pm$ 1.55 <sup>a</sup>

Rubberwood clones with different superscript lower case letters within rows are significantly different (Tukey honestly significant difference test,  $P < 0.05$ ).

**Table 2** Number of beetles counted in each rubberwood sample of the multiple choice tests ( $n = 25$ ).

Sample	Rubberwood clone		
	RRIM600	RRIM600-branch	PB235
1	1	1	3
2	0	0	5
3	1	1	3
4	0	3	2
5	2	1	2
Total	4	6	15
Mean $\pm$ SD	0.80 $\pm$ 0.84 <sup>a</sup>	1.20 $\pm$ 1.10 <sup>a</sup>	3.00 $\pm$ 1.22 <sup>b</sup>

Mean values with different superscript letters are significantly different (Tukey honestly significant difference test,  $P < 0.05$ ).

frass in particular trees, whereas there were no significant differences within clone PB235 (Table 3).

### Reproductivity of *S. anale*

The number of progeny per female reared on the rubberwood in all the samples ranged from a minimum of 17 to a maximum of 78 individuals, with the mean  $\pm$  SD being  $29.09 \pm 13.68$  individuals ( $n=45$ ). The reproduction rate or numbers of progeny per female of *S. anale* on each rubberwood clone differed greatly. The maximum and minimum numbers of progeny per adult beetle in clones PB235 and RRIM600 and in RRIM600-branch were 74:19, 43:17 and 43:13, respectively. The reproductivity rate was greatest in clone PB235 with a mean ( $\pm$  SD) number of progeny of  $39.07 \pm 17.30$  individuals which was significantly ( $P < 0.01$ ) higher than both the rate in clone RRIM600 ( $25.73 \pm 8.41$ ) and RRIM600-branch ( $22.47 \pm 7.23$ ). No difference was detected in the number of progeny within the rubberwood clones (Figure 2).

## DISCUSSION

The results of the multiple-choice and no-choice tests indicated that the rubberwood

clones and the position of the wood in the tree affect the host selection and feeding preference of the powder post beetle. The results also suggested that false powder post beetles of the genus *Sinoxylon*, similarly to the related genus *Lyctus*, use host contact and bite tests to determine the optimal host suitability as reported by Rosel (1969). The wood-chemical analysis in the present study supported the findings of previous studies that beetles significantly prefer rubberwood with a high starch content (Rosel, 1969). The beetles preference to feed on the rubberwood clone with the highest starch content (clone PB235) was significantly greater than their preference to feed on the wood from the branch and trunk of clone RRIM600 which had significantly lower starch contents. Starch and sucrose are the major stored carbohydrates in woody plants (Kozłowski and Pallardy, 1997). Starch is the major energy source of wood-feeding beetles (Höller *et al.*, 2002) while sucrose is hydrolyzed and completely disappears during the manufacturing process (Tomimura, 1993; Höller *et al.*, 2002). Beetles choose a host material with a higher nutritional value to optimize progeny fitness (Lederhouse *et al.*, 1992; Rank *et al.*, 1998). In the multiple choice tests, the adult beetles moved directly and without hesitation towards the wood samples, although they did not

**Table 3** Mean frass dry mass ( $\pm$  SD) and percentage mass loss of rubberwood blocks fed by *S. anale* adults on each sample tree ( $n = 75$ ).

Tree No.	Rubberwood clone					
	RRIM600		RRIM600-branch		PB235	
	frass dry mass (g)	%	frass dry mass (g)	%	frass dry mass (g)	%
1	$0.291 \pm 0.022^c$	2.49	$0.277 \pm 0.015^{ab}$	2.02	$0.301 \pm 0.015^a$	2.11
2	$0.218 \pm 0.036^a$	2.09	$0.250 \pm 0.009^a$	2.04	$0.263 \pm 0.005^a$	1.99
3	$0.258 \pm 0.013^{abc}$	1.97	$0.275 \pm 0.017^{ab}$	2.05	$0.286 \pm 0.050^a$	2.08
4	$0.231 \pm 0.016^{ab}$	2.07	$0.307 \pm 0.076^{ab}$	2.14	$0.286 \pm 0.017^a$	2.28
5	$0.266 \pm 0.032^{bc}$	2.07	$0.265 \pm 0.017^b$	2.31	$0.340 \pm 0.056^a$	2.66
Average	$0.253 \pm 0.029^1$	2.14	$0.275 \pm 0.021^3$	2.21	$0.295 \pm 0.028^2$	2.22

Mean ( $\pm$  SD) values of frass dry mass with different superscript lower case letters in the same column indicate a significant difference between individual trees in each clone ( $P < 0.05$ , Tukey honestly significant difference test). Average (mean  $\pm$  SD) values with superscript numbers are significantly different ( $P < 0.05$ , Tukey honestly significant difference test) between rubberwood samples/clones



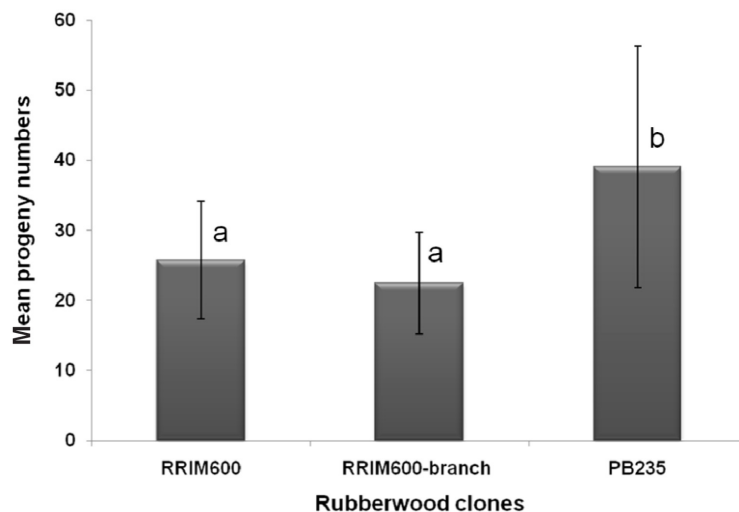
arrive at any specific wood block or clone. The beetles always considered more than one wood block or moved around all the three wood blocks provided. Most insects made a mandibular groove in the wood, sometimes in more than one of the wood blocks, before choosing one wood block in which to feed.

Insect feeding preferences are not only affected by the wood's nutritional value but are also affected by the presence of lignin (Brodeur-Campbell *et al.*, 2006) and wood extractives (Omar *et al.*, 2000) as feeding-deterrent chemicals in the host plant. In the present study, the only significant difference in wood extractive content was found between the rubberwood clones. Although the beetles preferred clone PB235 which had the highest starch content, this clone also had the highest level of wood extractives. This study did not investigate the cross effects of nutrition and defense mechanisms in general nor the situation where these two factors are optimized in the same host plant. Normally, specialist herbivores are adapted to these barriers (Awmack and Leather, 2002). Plant quality for herbivores is determined by many factors related to concentrations of

secondary metabolites (Slansky and Rodriguez, 1987). Rubberwood is generally classified as a nondurable wood type (Wong *et al.*, 2005) and has low levels of defense chemicals compared to durable wood types (Wong *et al.*, 2005). The rubberwood's low extractives content may have no importance in the feeding preference of these beetles. The reproductivity test conducted in this study supports this hypothesis, since beetles bred on rubberwood clone PB235 produced significantly more progeny than those bred on clone RRIM600. However, the mean number of progeny produced in each wood sample was not completely congruent with the starch content of the wood. Within clone RRIM600, the mean number of progeny reared in the branch was lower than the number reared in the trunk, although the branch had a higher starch content. However, a trend for a higher wood starch content to result in a higher number of progeny was evident.

## CONCLUSION

The fecundity and reproduction of the beetles are also regulated by other important



**Figure 2** Numbers of progeny per female of *S. anale* bred on rubberwood clones RRIM600, RRIM600-branch and PB235 (number per treatment = 15). Columns marked with different letters are significantly different at  $P < 0.01$ . The vertical bars indicate the standard deviation.

factors not considered in the present study (Slansky and Rodriguez, 1987). Future studies should consider (1) how the larval nutrition of adult beetles affects adult reproduction, (2) the effect of the total nitrogen or the protein content in the wood samples being different and (3) the effect of adult longevity or the oviposition period in all the samples being different.

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