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Suitability of Potential Host Plants in Japan for Immature Development of the Coconut Hispine Beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae)

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Abstract

The coconut hispine beetle, *Brontispa longissima* is a foliage feeder of the coconut palm, *Cocos nucifera* and other palm plants. Because *B. longissima,* supposedly native to Indonesia and Papua New Guinea, is now distributed in Ishigaki, Okinawa and Ogasawara, mainland Japan faces the potential risk of invasion by this pest. Suitability of palms other than *C. nucifera* for development and reproduction of *B. longissima* has been neglected, and the possibility of the other palms as alternative food plants for mass-rearing of this insect has not been evaluated. To evaluate the potential risk to palms in Japan and the possibility of the palms as alternative food plants, we examined host plant suitability for immature development of *B. longissima*, using the following exotic and native plants in Japan, *Chrysalidocarpus lutescens*, *Collinia elegans, Livistona chinensis*, *Phoenix canariensis*, *Rhapis excelsa, Rhapis humilis*, *Washingtonia filifera*, and *Typha latifolia*. When given each of those plant leaves, the percentage survival from first instar to adult emergence was 70% on *C. lutescens*, 55% on *L. chinensis,* 45% on *P. canariensis*, 80% on *W. filifera*, and 45% on *T. latifolia*. The adults given one of these suitable plants during larval and adult stages oviposited. Although larvae fed on *C. elegans*, *R. excelsa* or *R. humilis,* they never developed to pupae. When given a choice among the suitable plant species, *B. longissima* adults preferred *W. filifera* over the other plants. Considering suitability for immature development and reproduction of *B. longissima*, the ornamental palms such as *C. lutescens, L. chinensis*, *P. canariensis*, and *W. filifera* can be potential hosts in mainland Japan.

Discipline: Insect pest
Additional key words: alternative plant, coconut leaf beetle, *Cocos nucifera*, invasive pest, mass-rearing

Introduction

The coconut hispine beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) is a serious pest of the coconut palm *Cocos nucifera* L. or other palms[2,5,9,13,16]. *Brontispa longissima*, supposedly native to Indonesia or Papua New Guinea, has recently invaded Thailand, Vietnam, Hainan Island, and other Southeast Asian countries, causing serious damages on *C. nucifera*[9,13]. The adults lay eggs on leaflets of young unopened fronds, and all the developmental stages stay in or between leaflets of young unopened or half opened fronds. As the leaflets separate when the frond expands, the larvae and adults move to other young fronds. The feeding scars caused by larvae and adults enlarge to form irregular brown blotches as the frond opens[5]. Although at least 20 palm species have been listed as hosts of *B. longissima*[5,16], field and laboratory studies on host suitability of palms other than *C. nucifera* for *B. longissima* have been neglected. It remains unknown how often palms other than *C. nucifera* are attacked by *B. longissima* in natural conditions and how suitable those palms are for immature development and adult reproduction in *B. longissima*, except for only a few studies[11,23].

In Japan, *B. longissima* was so far found in the islands off mainland Japan. It was first found on *C. nucifera* on the main island of Okinawa in 1978[2]. Then, it was found attacking only *C. nucifera* on Ishigaki Island in 1982, Miyako, Iriomote and Yonaguni Islands in 1984, and Ogasawara Island in 1989[1,14,22]. However, the authors recently confirmed that this insect had been established in

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the Yaeyama Islands, attacking the Yaeyama palm *Satakentia liukiuensis* (Hatusima) H. E. Moore, endemic to Ishigaki and Iriomote Islands[17].

Although occurrence of *B. longissima* in mainland Japan has never been reported so far, the mainland faces a potential risk of invasion by this pest. *Brontispa longissima* is often brought to a new area together with palm seedlings infested with this pest because all the stages of this pest live inside unopened fronds of the seedlings and the infection often goes unnoticed[5,9,13,17].

Seedlings of *C. nucifera* and *S. liukiuensis*, which are known as hosts of *B. longissima* on Okinawa and Ishigaki Islands, are often shipped from nurseries in the Ryukyu Islands to the mainland. Although *C. nucifera* and *S. liukiuensis* are not planted as outdoor plants but indoor ornamental plants in the mainland, some exotic and native palms known to be hosts of *B. longissima* are planted as outdoor ornamental plants, including *Livistona chinensis* (N. J. Jacq.) R. Br. ex Martius *var. subglobosa* (Hask.) Martius*, Phoenix canariensis* Hort. ex Chabaud and *Washingtonia filifera* (Linden ex Andre) H. Wendl.[12]. Once introduced to the mainland, *B. longissima* may become established in mainland Japan, using those palms.

To assess the potential risk of *B. longissima* to palms in mainland Japan, it is necessary to examine availability and suitability of those palms for development and reproduction of this insect. Host suitability for *B. longissima* should also be studied to find alternative food for mass-rearing of *B. longissima*. *Brontispa longissima* has been reared in the laboratory, using young, unopened leaves of *C. nucifera*[20]. However, conventional laboratory rearing of this insect has not been effectively productive, because there are some problems with the use of the young, unopened leaves, including limited availability and fungal contamination[21]. Since effective mass-rearing of this insect is vital for biological control programs using natural enemies as well as ecological and physiological studies of this insect, alternative food plants are needed for more productive mass-rearing of *B. longissima*.

The objectives of the present study are to assess the potential risk of *B. longissima* to palms in mainland Japan and to evaluate potential use of the plants as alternative food plants for mass-rearing of this insect. Therefore, we examined suitability of seven palm species grown in Japan for immature development of *B. longissima*. We also examined suitability of the cattail *Typha latifolia* L. (Poales: Typhaceae) for immature development and adult reproduction of this pest because a recent study showed that this pest can be reared with the narrow leaf cattail, *Typha angustifolia* (L.) in Thailand[20]. For plants suitable for its immature development, we also examined larval preference among the plants and adult reproduction.

Materials and methods

1. Insects

Brontispa longissima adults and larvae were originally collected from *S. liukiuensis* on Ishigaki Island, Japan. Although the regular hosts of *B. longissima* are *C. nucifera* and *S. liukiuensis* leaves, we used common cattail *Typha latifolia* leaves as the food plant for rearing this pest in the present study conducted at Kyushu University, Fukuoka, Japan, because fresh leaves of these two palms were not available. However, *T. latifolia* is a relative species of *T. angustifolia*, which is known as an alternative host plant of *B. longissima*[20]; it is a very common wetland weed and therefore leaves are available in large amounts from the field in Japan[8,12]. Since *B. longissima* larvae and adults always stay in still-folded leaflets or between leaflets, we used “leaf bundles” where the beetles can hide and feed on for rearing and experiments. To make the leaf bundles, fresh *T. latifolia* leaves were cut in 4 cm length, and three pieces of leaves were bundled and stapled at the center of the leaves. *Brontispa longissima* larvae and adults stayed between leaves of the leaf bundles and fed on the leaves. The eggs were placed on filter paper saturated with water in a Petri dish (5.5 cm diameter, 1.5 cm high) until hatching. The hatched larvae were provided with *T. latifolia* leaves until the following experiments. The rearing and the following experiments were conducted at 25°C and 16L:8D.

2. Plants used for experiments

The plants listed in Table 1 were used for the following experiments. *Livistona chinensis* (N. J. Jacq.) R. Br. ex Martius *var. subglobosa* (Hask.) Martius*, Phoenix canariensis* Hort. ex Chabaud and *Washingtonia filifera* (Linden

Table 1. Plants used for this study

Family	Species	Exotic (E) or Native (N) in Japan
Arecaceae	*Chrysalidocarpus lutescens*	E
Arecaceae	*Collinia elegans*	E
Arecaceae	*Livistona chinensis*	N
Arecaceae	*Phoenix canariensis*	E
Arecaceae	*Rhapis excelsa*	E
Arecaceae	*Rhapis humilis*	E
Arecaceae	*Washingtonia filifera*	E
Typhaceae	*Typha latifolia*	N

ex Andre) H. Wendl. have been planted in Southeastern Japan as roadside trees or in parks[12]. *Chrysalidocarpus lutescens* (Bory) H. Wendl., *Collinia elegans* Liebm., *Rhapis excelsa* (Thumb. Ex Murray) Henry ex Rehder, and *Rhapis humilis* Blume are mainly indoor ornamental plants[12]. *Livistona chinensis* and *T. latifolia* are also native to Japan and naturally grown on Kyushu Island and the Ryukyu Islands[8,12]. For all the plants tested, only green, mature leaves were collected from Hakozaki campus, Kyushu University, Fukuoka, Japan (33°37'N 130°25'E) and used for the experiments below because the young leaves from unopened fronds were not available.

3. Host suitability

Immature development of *B. longissima* reared with each of the plants listed in Table 1 was examined. The leaf bundles consisting of three pieces of fresh plant leaf (4 cm length) were made as described above for *T. latifolia*. Soon after hatching, 10 first instar larvae were provided the leaf bundle as a food in a Petri dish (5.5 cm diameter, 1.5 cm high) until they pupated or died, because *B. longissima* larvae always stayed in still-folded leaflets or between leaflets. The leaf bundle was renewed every two days. The development time and survival up to pupation and adult emergence were examined. This experiment was repeated five times for *T. latifolia* (a total of 50 larvae tested) and twice for other plants (a total of 20 larvae tested) at 25°C and 16L:8D.

After emergence, we continued to rear the adults with the same plants as during larval stages, and examined if adults reared with those plants can reproduce. The adults were reared with the leaf bundle of the same plants as given in larval stages as a food in a Petri dish (5.5 cm diameter, 1.5 cm high) until 40 days after emergence at 25°C and 16L:8D. The leaf bundles were renewed, and the number of eggs laid on the leaf bundles was counted every two days. Although *B. longissima* adults live for more than two months at 25°C, we examined their fecundity in only 40 days after emergence because we could not obtain enough plants during this experiment. The number of adults reared was as follows. Two males and six females on *C. lutescens,* two males and one female on *L. chinensis,* two males and three females on *P. canariensis,* three males and four females on *W. filifera*, three males and two females, two males and five females, and one male and three females on *T. latifolia* for each dish.

4. Host preference by *B. longissima* larvae

When provided with *C. lutescens*, *L. chinensis*, *P. canariensis*, *W. filifera*, and *T. latifolia*, *B. longissima* larvae developed to adults. Therefore, preference by *B. longissima* larvae among those plants was examined, using first, second, third, and fourth instars reared with *T. latifolia* leaves before this experiment. For each instar, 20 larvae were introduced into a Petri dish (9 cm diameter, 1.5 cm high) in which a total of five leaf bundles of a plant species were placed in a row with 0.5 cm intervals. The order of the bundles placed in a row was at random. One day later, the number of larvae present in each leaf bundle was counted. This experiment was repeated three times at 25°C and 16L:8D.

Results

When given each of the palms or *T. latifolia,* first instar larvae of *B. longissima* fed on all the plants. However, the percentage survival from first instar to pupation or adult emergence was significantly different among plants (Chi-square = 63.9, df = 7, $p < 0.01$) (Table 2). Although first instar fed on *C. elegans, R. excelsa* and *R. humilis,* they never developed to second instar on *C. elegans* and *R. humilis* or to third instar on *R. excelsa*. More than 75% of first instar reared with *C. lutescens*, *L. chinensis*, *P. canariensis*, *W. filifera*, and *T. latifolia* molted to second instar, but 20-44% of larvae on *L. chinensis*, *P. canariensis* and *T. latifolia* died between second instar and fourth instar. As a result, 70-80% of *B. longissima* larvae that fed on *C. lutescens* and *W. filifera* developed to adults, while 45-55% that fed on *L. chinensis, P. canariensis* and *T. latifolia* did so. The percentage survival from first instar larvae to pupation or adult emergence was significantly lower on *C. elegans, R. excelsa* and *R. humilis* than on the other plants (multiple comparisons for proportions based on arcsine transformation, $p < 0.05$).

Mean times taken for larval and pupal periods of *B. longissima* that fed on *W. filifera* were shortest among the five plants suitable for immature development (one-way ANOVA, larval period: $F_{4, 69}= 54.1$, df = 4, $p < 0.01$; pupal period: $F_{4, 69} = 11.0$, df = 4, $p < 0.01$; total period: $F_{4, 69} = 48.1$, df = 4, $p < 0.01$). The mean total time from first instar larvae that fed on *W. filifera* to adult emergence was 23 days, followed by 30-32 days for insects that fed on *C. lutescens* and *P. canariensis.* The larvae that fed on *L. chinensis* and *T. latifolia* took 40-41 days to complete their development (Table 3).

When given the five suitable plants in a Petri dish, *B. longissima* larvae preferred feeding most on *W. filifera* leaves among them, regardless of instar. Second instar and fourth instar preferred *T. latifolia* more than *C. lutescens, L. chinensis* and *P. canariensis*. The distributions of larvae among the leaf bundles were significantly different from the hypothetical larval distribution predicted if they have no preference among plants (1:1:1:1:1) (Chi-square test, first instar: df = 4, $\chi^2 = 24.1$, $p < 0.01$; second instar: df = 4,

Table 2. Immature survival of *Brontispa* longissima on different plants

Plant	No. of larvae examined	% survival up to				
		2nd instar	3rd instar	4th instar	Pupa	Adult
C. lutescens	20	85.0 a	75.0 a	75.0 a	70.0 a	70.0 a
C. elegans	20	0 b	0 b	0 b	0 b	0 b
L. chinensis	20	75.0 a	65.0 a	55.0 a	55.0 a	55.0 a
P. canariensis	20	75.0 a	50.0 a	50.0 a	45.0 a	45.0 a
R. excelsa	20	10.0 b	0 b	0 b	0 b	0 b
R. humilis	20	0 b	0 b	0 b	0 b	0 b
W. filifera	20	80.0 a	80.0 a	80.0 a	80.0 a	80.0 a
T. latifolia	50	92.0 a	78.0 a	48.0 a	45.0 a	45.0 a

The percentages followed by different letters in the same columns were significantly different by Tukey's HSD test ($p < 0.05$).

Table 3. Development time of *Brontispa longissima* on different plants

Plant	Development time (days)					
	1st - 4th instar		Pupa - adult		1st instar – adult	
	N	Mean±SD	N	Mean±SD	N	Mean±SD
C. lutescens	15	22.2±2.9 b	14	9.7±2.8 c	14	31.9±3.8 b
L. chinensis	11	33.0±2.9 c	11	8.2±1.1 bc	11	41.2±3.0 c
P. canariensis	10	21.9±4.0 ab	9	8.4±1.8 bc	9	30.0±4.6 b
W. filifera	16	17.7±1.3 a	16	5.7±0.5 a	16	23.4±1.3 a
T. latifolia	24	32.0±4.8 c	23	8.0±1.6 b	23	39.9±5.7 c

The numbers followed by different letters in the same columns were significantly different by Tukey's HSD test ($p < 0.05$).

Table 4. Feeding preference by *Brontispa longissima* larvae

Instar	No. of larvae examined	% larvae feeding on					Probability*
		C. lutescens	*L. chinensis*	*P. canariensis*	*W. filifera*	*T. latifolia*	
1st	60	25.0	1.7	8.3	58.3	6.7	$p < 0.05$
2nd	60	15.0	3.3	5.0	45.0	31.7	$p < 0.05$
3rd	60	16.7	6.7	5.0	55.0	16.7	$p < 0.05$
4th	60	6.7	8.3	10.0	50.0	25.0	$p < 0.05$

*Probability that percentages of larvae on plants are not different from the percentages of larvae expected if they randomly chose plants (Chi-square test, $p < 0.05$).

Table 5. Fecundity of *Brontispa longissima* for 40 days after emergence when reared with five suitable plants

Plant	No. females examined	Pre-ovipositional period (days)	No. eggs laid for 40 days / female
C. lutescens	6	23.4	4.2
L. chinensis	1	20.0	18.9
P. canariensis	3	12.0	23.7
W. filifera	4	10.0	9.5
T. latifolia	10	16.0	13.0

$\chi^2 = 20.3$, $p < 0.01$; third instar: df = 4, $\chi^2 = 19.6$, $p < 0.01$; fourth instar: df = 4, $\chi^2 = 16.9$, $p < 0.01$) (Table 4).

When *B. longissima* were reared with one of the five suitable plants in both larval and adult stages, they reproduced on those plants. However, their pre-ovipositional period varied from 10.0 d to 23.4 d among plants (Table 5). The mean number of eggs laid by a female for 40 days after emergence was also different among plants, ranging from 4.2 for *C. lutescens* to 23.7 for *P. canariensis* (Table 5).

Discussion

Although *B. longissima* mainly attacks *C. nucifera*, it was reported to attack many other palms including *C. lutescens*, *L. chinensis* and *W. filifera* under natural conditions[5,7,9,13,16]. In the present study, we have shown that in addition to these three palm species, *P. canariensis* and *T. latifolia* are suitable for immature development and adult reproduction of *B. longissima.* Here we first compare suitability of *B. longissima* between these five plants and *C. nucifera,* a regular host used for laboratory rearing. Then, we discuss larval feeding preference, the possibility of invasion into mainland Japan, and potential use of these five plants for mass-rearing of this pest.

1. Host suitability

Immature survivals of *B. longissima* from first instar to adult emergence were significantly different among plants. Although *B. longissima* completed its development on *C. lutescens*, *L. chinensis*, *P. canariensis*, *W. filifera*, and *T. latifolia*, survival to adult emergence was 70-80% on *C. lutescens* and *W. filifera* but 45-55% on the other species. The percentage survivals on *C. lutesens* and *W. filifera* seem to be comparable to that on *C. nucifera*, 62-73% at 28°C[11] and 79% at 24°C[23].

Development time of *B. longissima* also varied among plants tested. First instar larvae reared with *W. filifera* took 23 days to develop to adult emergence, and the development time was significantly shorter than on any other plants. The development time on *W. filifera* seems to be shorter than that on young leaves of *C. nucifera*, because Yamauchi (1985) showed that when given young leaves of *C. nucifera*, first instar larvae of *B. longissima* required 33-49 days to develop to adults[22]. On the other hand, when *B. longissima* larvae were reared with *L. chinensis* and *T. latifolia*, their development time was significantly longer than other suitable plants. However, *B. longissima* larvae that were reared with a Thailand variety of *T. angustifolia*, a relative species of *T. latifolia*, developed to adults faster than those given *C. nucifera*[20,21].

2. Host preference by *B. longissima* larvae

Phytophagous insects have feeding preference for natural hosts over non-natural hosts[3,4]. In the present study, *B. longissima* larvae preferred *W. filifera* over other plants tested, regardless of instar. The fact that larvae preferred *W. filifera* although they were reared with *T. latifolia* before the experiment, indicates that the preference of *B. longissima* larvae for *W. filifera* is an innate response. Because *W. filifera* was the most suitable plant tested in terms of survival and development time, the innate preference of *B. longissima* may be correlated with host suitability for its immature development.

In addition to innate preference, previous feeding experience of *T. latifolia* may affect subsequent feeding preference, as coleopteran larvae have been shown to develop an altered preference in favor of the plant already experienced[15]. In the present study, first instar that had never fed or only fed a little on *T. latifolia* did not have a preference for *T. latifolia*, but second and fourth instar that had fed on *T. latifolia* preferred that plant (Table 4). On the other hand, although first instar seemed to prefer *C. lutescens*, the older larvae did not have a preference for that plant (Table 4). These results may suggest induction of feeding preference for *T. latifolia* after feeding on that plant.

3. Possibility of invasion into mainland Japan

Among five suitable plants, *L. chinensis, P. canariensis* and *W. filifera* are very common ornamental palms, which are planted in Southeastern Japan as roadside trees or in parks[12]. In areas such as Thailand or Vietnam where *C. nucifera* trees are abundant and *B. longissima* recently became epidemic, the pest exclusively attacks *C. nucifera* but rarely other palms, indicating a strong preference for *C. nucifera*[6,7,10,16]. However, *B. longissima* sometimes heavily infests *S. liukiuensis,* an endemic palm on Ishigaki Island, Japan where *C. nucifera* is rarely planted[17]. This fact may also suggest the possibility that if *B. longissima* is introduced into the area where *C. nucifera* is absent or rare, the pest may attack other palms suitable for their development and survival. If so, the suitable exotic and native ornamental palms examined in the present study may be attacked by *B. longissima* once it is introduced into mainland Japan. Attack of palms in Japan by *B. longissima* under natural conditions may depend on ovipositional preference by females for the plant, suitability and availability of the plants for adult survival and reproduction as well as suitability for immature development. Also, whether *B. longissima* can overwinter in the mainland would be another important factor for it to establish because it originates from the Tropics. Further study on effects of the potential host plants in Japan on adult reproduction and behavior as well as cold tolerance of immature and adult stages in *B. longissima* is needed to determine the possibility of invasion by this pest into mainland Japan.

4. Potential use of ornamental palms or *Typha* spp. for mass-rearing

Considering immature survival and development time, mature leaves of *C. lutescens* and *W. filifera* seem more suitable than or as suitable as *C. nucifera*, which has been used for mass-rearing of *B. longissima*. Although mature leaves of *L. chinensis* and *P. canariensis* seem to be inferior to *C. nucifera* in terms of immature survival of *B. longissima*, its egg production on the former for 40 days seems to be greater than that on the latter. Therefore, mature leaves of the ornamental plants such as *C. lutescens*, *L. chinensis, P. canariensis*, and *W. filifera* could be used as alternative plants for mass-rearing of *B. longissima* in the laboratory.

Away from ornamental palms, *Typha* spp., which are common weeds in swamps or rivers in Asia[8], also may be used for mass-rearing. In the present study, *T. latifolia* sustained complete development of *B. longissima*, although the plant was not as suitable as *C. nucifera* in terms of immature survival and development. Still, we had also been successfully maintaining a laboratory colony of *B. longissima* using *T. latifolia* at Kyushu University for six months in 2007. In Thailand, *T. angustifolia* has been used as an alternative host for laboratory mass-rearing of *B. longissima*[20] (unpublished data).

Conventional mass-rearing of *B. longissima* uses young, unopened leaves from folded fronds of *C. nucifera*[20], but availability of the young leaves is limited even in areas where *C. nucifera* grows as we mentioned earlier. Besides, *B. longissima* may invade temperate areas where *C. nucifera* is absent or rare. This pest has already established and attacked the native palm *S. liukiuensis* in the Yaeyama Islands[17]. Also, infestation of *C. nucifera* by *B. longissima* was found in Chiang Mai, Thailand, and Hanoi, Vietnam where *C. nucifera* is rare (unpublished data). When and where young leaves of *C. nucifera* are not available, mature leaves of the ornamental palms such as *C. lutescens, L. chinensis, P. canariensis*, and *W. filifera* or *Typha* spp. can be used as alternative host plants for mass-rearing of *B. longissima*.

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