# Suitability of Monocots for Rearing Alien Coconut Pest *Brontispa longissima* (Coleoptera: Chrysomelidae)

W. SUGENO,[1] K. KAWAZU,[1] S. TAKANO,[2] S. NAKAMURA,[3] AND A. MOCHIZUKI[1,4]

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**ABSTRACT** The suitability for larval development of the alien coconut pest *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) on the following 15 monocots was investigated. Arecaceae: *Cocos nucifera*, *Trachycarpus wagnerianus*; Typhaceae: *Sparganium erectum*, *Typha latifolia*; Poaceae: *Avena sativa*, *Echinochloa esculenta*, *Hordeum vulgare*, *Oryza sativa*, *Sorghum bicolor*, *Zea mays*; and Cyperaceae: *Carex morrowii*, *Cyperus alternifolius*, *Cyperus esculentus*, *Cyperus serotinus*, and *Rhynchospora colorata*. The larval survival rate to the adult stage was significantly higher when reared on *C. esculentus* and *C. serotinus* (95%); however, it was not significantly different compared with *C. nucifera* (75%). Sixty, 45, 45, and 10% were observed when reared on *T. wagnerianus*, *S. erectum*, *T. latifolia*, and *C. alternifolius*, respectively. The larval developmental period reared on *C. serotinus* was as short as on *C. esculentus* and on *C. nucifera* up to adult emergence. The fecundity of adults was examined using *C. nucifera*, *T. wagnerianus*, *T. latifolia*, *C. esculentus*, and *C. serotinus* as food plants. In these five plants, the females reared on *T. wagnerianus* started to lay eggs earliest. However, the preoviposition periods on *C. esculentus* and *C. serotinus* were not significantly different from those on *C. nucifera* and *T. latifolia*. Numbers of eggs laid per female did not differ significantly among these five plants. Our present results suggest that *T. wagnerianus*, *C. esculentus*, and *C. serotinus* can be used as new food plants in addition to *C. nucifera* and *T. latifolia* for rearing *B. longissima*.

**KEY WORDS** *Brontispa longissima*, invasive alien species, alternative diet, mass rearing, host selection

The beetle *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) is one of the most destructive pests causing leaf damage to the coconut palm, *Cocos nucifera* L., and also attacks other palm species (Waterhouse and Norris 1987, Wu et al. 2006, Rethinam and Singh 2007). Both larvae and adults feed on young unopened fronds of the coconut palm and the damaged leaves wither. Infestation of the beetle decreases fruit production and successive heavy attacks will lead to death of the palm tree, which for instance within only one country, Vietnam, causes an estimated annual loss of US$$30–40 million (FAO 2004; Nakamura et al. 2006, 2008). This beetle is reportedly native to Indonesia and Papua New Guinea (Waterhouse and Norris 1987), but it is now widely distributed throughout the Pacific Islands, Australia, and Asia (Takasu et al. 2010). The beetle also has spread to parts of Japan: it was discovered on the main island of Okinawa in 1978 (Azuma and Kinjo 1978); Ishigaki Island in 1982; and on Miyako, Iriomote, and Yonaguni islands in 1984 (Azuma 1986). It is also known to be invading the Ogasawara (Ohbayashi 2002) and Kohama islands (Takasu et al. 2010). Currently, on the main island of Okinawa, *B. longissima* can be observed attacking *C. nucifera* planted along roadsides and in nurseries (W.S., unpublished data). Takasu et al. (2010) reported that *B. longissima* was using an endemic palm, *Satakentia liukiuensis* (Hatusima) H.E. Moore, which is listed as "Near Threatened" on the Red List of the Government of Japan's Ministry of the Environment (http://www.biodic.go.jp/english/rdb/rdb_f.html), as a main host on Ishigaki, Iriomote, and Kohama islands. Damage to palm trees in Japan by this beetle should clearly not be ignored.

The control of this beetle has depended chiefly on natural enemies, such as parasitoid wasps, because the beetle inhabits the crowns of tall palms that pesticides cannot reach (Nakamura et al. 2006). Biological control using the larval parasitoid *Asecodes hispinarum* Bouček (Hymenoptera: Eulophidae) has been reported to be effective in suppressing populations of this beetle in Vietnam and Thailand (Nakamura et al. 2008). For mass rearing of the parasitoid wasp, many beetle larvae need to be reared. In Thailand, young leaves of *C. nucifera* are used to rear the beetle (Winotai et al. 2007). However, obtaining sufficient fresh leaves of *C. nucifera* is highly labor-intensive. Winotai et al. (2007) and Yamashita et al. (2008, 2009) sug-

[1] Biodiversity Division, National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki 305-8604, Japan.
[2] Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 819-0395, Japan.
[3] Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki 305-8686, Japan.
[4] Corresponding author, e-mail: lepi@affrc.go.jp.

**Table 1. Plants used for this study**

| Order | Family | Species |
|---|---|---|
| Arecales | Arecaceae | *Cocos nucifera* L. |
| | | *Trachycarpus wagnerianus* Becc. |
| Poales | Typhaceae | *Sparganium erectum* L. |
| | | *Typha latifolia* L. |
| | Poaceae | *Avena sativa* L. |
| | | *Echinochloa esculenta* (A. Braun) H. Scholz |
| | | *Hordeum vulgare* L. |
| | | *Oryza sativa* L. |
| | | *Sorghum bicolor* (L.) Moench |
| | | *Zea mays* L. |
| | Cyperaceae | *Carex morrowii* Boott |
| | | *Cyperus alternifolius* L. |
| | | *Cyperus esculentus* L. |
| | | *Cyperus serotinus* Rottb. |
| | | *Rhynchospora colorata* (L.) H. Pfeiffer |

Classification of orders and families is according to the APG III System (APG III 2009).

gested that the beetle could be reared on fresh leaves of cattail (*Typha*, Poales: Typhaceae) species. Although the cattail is a common wetland weed in temperate and tropical Asia (Takahashi et al. 1990), growth is slow and large damp spaces are needed for its cultivation. More convenient plants will be needed for mass rearing of *B. longissima*. Ichiki et al. (2009) reported an artificial diet containing dried coconut leaf powder for *B. longissima*, and 40% of larvae reared on the artificial diet grew to the adult stage. However, if continuously reared on this artificial diet, the adults did not lay fertile eggs.

In this study, we investigated the suitability of 15 monocot plants as alternative diets for *B. longissima* and found three plants to be suitable for this beetle. The basic aim of this study is to find new alternative food plants to rear this beetle, but we also hope to contribute to understanding its host plant selection, which will help to develop new ways of managing *B. longissima*.

## Materials and Methods

**Insects.** The *B. longissima* colony was originally collected from an *S. liukiuensis* plant on Ishigaki Island (Okinawa Prefecture, Japan) and reared at the National Institute for Agro-Environmental Sciences (NIAES) laboratory. The beetles were reared on fresh leaves of the broadleaf cattail, *Typha latifolia* L., and the narrowleaf cattail, *Typha angustifolia* L. The larvae and adults were maintained separately on fresh leaves of the cattails in plastic containers (15.0 cm in length by 11.2 cm in width by 3.8 cm in height) with a mesh window in the lid. The eggs collected from the maintained laboratory colony were kept in petri dishes (9.0 cm in diameter by 3.0 cm in height) with a moist paper towel until hatching. Continuous rearing was conducted at 25 ± 1°C under a photoperiod of 12:12 (L:D) h.

**Plants.** The plant species used for this study are listed in Table 1. The plant species were selected from Arecales and Poales in Japan, because *C. nucifera* and *T. latifolia* belong to these orders, respectively. The coconut palm; simplestem bur-reed, *Sparganium erectum* L.; Japanese sedge grass, *Carex morrowii* Boott; umbrella papyrus, *Cyperus alternifolius* L.; and star-rush whitetop, *Rhynchospora colorata* (L.) H. Pfeiffer, were purchased from botanical shops in Japan and grown in the greenhouse at 25°C. The common oat, *Avena sativa* L.; Japanese millet, *Echinochloa esculenta* (A. Braun) H. Scholz; common barley, *Hordeum vulgare* L.; commercial rice, *Oryza sativa* L.; sorghum, *Sorghum bicolor* (L.) Moench; and corn, *Zea mays* L., were obtained as seeds, planted separately in pots, and grown in the greenhouse. Fresh leaves of a natural growing Chinese windmill palm, *Trachycarpus wagnerianus* Becc., were collected from a forest near NIAES immediately before use. Natural growing seedlings of broadleaf cattail were collected from a marsh in Ibaraki Prefecture, Japan, and grown in the greenhouse. Yellow nutsedge, *Cyperus esculentus* L., and tidalmarsh flatsedge, *Cyperus serotinus* Rottb., were provided by Dr. A. Uchino (National Agriculture and Food Research Organization) as rhizomes and grown in the greenhouse.

**Suitability for Larval Development.** First-instar larvae derived from the laboratory colony were used in the experiment within 24 h after hatching and before they had fed. Several pieces of cut leaves (≈5 cm in length) were bundled with Parafilm bands at both ends, because the larvae of *B. longissima* inhabit the closely appressed leaflets of young coconut fronds (Brown and Green 1958, Yamashita and Takasu 2010). Five individuals were reared per leaf bundle in a plastic container (6.5 cm in length by 3.4 cm in width by 1.75 cm in height), with four replicates conducted for each plant species. The leaf bundles were renewed every day until the larvae pupated or died. The survival rates and developmental periods from first instar to pupation and to adult emergence were examined. We measured the length of the elytra, as an indicator of body size, of newly emerged females and males ($n$ = 10 for each sex). The experiments were conducted at 28 ± 1°C and 65 ± 10% RH under a photoperiod of 12:12 (L:D) h.

**Suitability for Adult Reproduction.** The suitability of *C. nucifera*, *T. wagnerianus*, *T. latifolia*, *C. esculentus*, and *C. serotinus* for adult reproduction were then investigated, because the larvae reared on these five plants developed successfully into adults. *S. erectum* was not used for this experiment because sufficient amount of the leaves were not obtained. Newly emerged adults reared on the fresh leaves of the same plant as during the larval stages were used to examine the preoviposition period and the number of eggs laid by each female. One female and one male beetle were paired within 24 h of emergence and provided a leaf bundle in a plastic container. The leaf bundle was renewed every 3 d, and the number of eggs laid on the leaf bundle was counted every day for 40 d. Ten replicates were conducted for each plant species. The experiments were con-

**Table 2. Survival rates and developmental periods of *B. longissima* reared on each plant**

| Plant | No. larvae examined | % survival up to | | Developmental periods (d) | | | |
|---|---|---|---|---|---|---|---|
| | | | | First instar–pupa[b] | | First instar–adult[b] | |
| | | Pupa[a] | Adult[a] | *n* | Mean ± SEM | *n* | Mean ± SEM |
| *C. nucifera* | 20 | 80ab | 75ab | 16 | 18.5 ± 0.4a | 15 | 23.5 ± 0.4a |
| *T. wagnerianus* | 20 | 60b | 60ab | 12 | 30.1 ± 1.3b | 12 | 35.5 ± 1.2b |
| *S. erectum* | 20 | 50bc | 45bc | 10 | 41.0 ± 2.4c | 9 | 46.3 ± 2.6c |
| *T. latifolia* | 20 | 45bc | 45bc | 9 | 24.4 ± 1.2d | 9 | 29.8 ± 1.1d |
| *A. sativa* | 20 | 0d | 0d | –[c] | | | |
| *E. esculenta* | 20 | 0d | 0d | – | | | |
| *H. vulgare* | 20 | 0d | 0d | – | | | |
| *O. sativa* | 20 | 0d | 0d | – | | | |
| *S. bicolor* | 20 | 0d | 0d | – | | | |
| *Z. mays* | 20 | 0d | 0d | – | | | |
| *C. morrowii* | 20 | 0d | 0d | – | | | |
| *C. alternifolius* | 20 | 10cd | 10cd | 2 | 52.5 ± 2.5e | 2 | 57.5 ± 2.5e |
| *C. esculentus* | 20 | 100a | 95a | 20 | 17.0 ± 0.5a | 19 | 22.4 ± 0.5a |
| *C. serotinus* | 20 | 100a | 95a | 20 | 16.6 ± 0.3a | 19 | 22.3 ± 0.3a |
| *R. colorata* | 20 | 0d | 0d | – | | | |

[a] Values followed by the same letters within the same column do not differ significantly ($P > 0.05$; Ryan's multiple range test for proportions after the chi-square test).
[b] Values followed by the same letters within the same column do not differ significantly ($P > 0.05$; Tukey–Kramer HSD test following ANOVA).
[c] No larva developed into a pupa.

ducted at 28 ± 1°C and 65 ± 10% RH under a photoperiod of 12:12 (L:D) h.

**Statistical Analysis.** Differences in the survival rates of larvae among plant species were analyzed using the chi-square test and then compared using Ryan's multiple range test for proportions. These analyses were conducted using R software, version 2.10.1 (R Development Core Team 2009). Differences in larval developmental period, preoviposition period, number of eggs laid by females, and elytra length of adults among plant species were subjected to one-way analysis of variance (ANOVA) and then compared using the Tukey–Kramer honestly significant difference (HSD) test. These analyses were conducted using JMP, version 7.0.2 (SAS Institute 2007).

## Results

**Rearing of *B. longissima* Larvae on Each Plant Species.** The developmental performance of larvae reared on 15 monocots is shown in Table 2. Larvae reared on *C. nucifera, T. wagnerianus, S. erectum, T. latifolia, C. alternifolius, C. esculentus,* and *C. serotinus* grew to adults. The survival rates up to the pupal and adult stages reared on *C. nucifera* (80 and 75%), *T. wagnerianus* (60 and 60%), *S. erectum* (50 and 45%), and *T. latifolia* (45 and 45%) did not differ significantly among these four species ($P > 0.05$; Ryan's multiple range test for proportions after the chi-square test). Among the Poaceae plants tested (*A. sativa, E. esculenta, H. vulgare, O. sativa, S. bicolor,* and *Z. mays*), no larva reached the pupal stage: all died during the first or second instar. As for the plants of the Cyperaceae family, no larva feeding on *C. morrowii* or *R. colorata* developed as far as the pupal stage. However, the larvae fed on *C. alternifolius, C. esculentus,* and *C. serotinus* grew to the adult stage at 10, 95, and 95%, respectively. The larval survival rates to the pupal and to adult stages when reared either on *C. esculentus* or on *C. serotinus* did not differ significantly from those on *C. nucifera.*

Larval developmental periods from first instar to pupation and to adult emergence were significantly different among the seven plants on which the larvae were able to develop (ANOVA: pupation, $F = 104.2$, df = 6, $P < 0.0001$; adult emergence, $F = 97.5$, df = 6, $P < 0.0001$). Larval developmental periods to pupation and to adult emergence when reared on *C. serotinus* (16.6 ± 0.3 and 22.3 ± 0.3 d) and on *C. esculentus* (17.0 ± 0.5 and 22.4 ± 0.5 d) did not significantly differ from those on *C. nucifera* (18.5 ± 0.4 and 23.5 ± 0.4 d) ($P > 0.05$; Tukey–Kramer HSD test following ANOVA). The larvae fed on *T. wagnerianus, S. erectum, T. latifolia,* and *C. alternifolius* took a significantly longer time than those on *C. nucifera* until pupation and also until adult emergence.

**Reproductive Ability of *B. longissima* Adults Reared on Each Plant Species.** Female reproductive ability when reared on *C. nucifera, T. wagnerianus, T. latifolia, C. esculentus,* and *C. serotinus* is shown in Table 3. Preoviposition periods were significantly different among each plant species (ANOVA: $F = 3.5534$, df = 4, $P = 0.0133$). The preoviposition period of adults reared on *T. wagnerianus* (12.2 ± 0.4 d) was the shortest among the five plants and not significantly different from that for *C. nucifera* (14.6 ± 0.7 d) or *T. latifolia* (13.2 ± 0.8 d) and was significantly shorter than that for *C. esculentus* (17.3 ± 1.8 d) and *C. serotinus* (17.3 ± 1.7 d), which were not significantly different from those on *C. nucifera* and *T. latifolia.*

The mean number of eggs laid per female over 40 d ranged from 37.2 to 55.1 among females reared on the five plants. There was no significant difference among them in the number of eggs laid per female (ANOVA: $F = 2.5062$, df = 4, $P = 0.0552$).

**Table 3. Fecundity and body size of *B. longissima* reared on each plant**

| Plant | No. females examined | Preoviposition period (d)[a] | No. eggs laid/female for 40 d[b] | Elytra length (mm)[a] | |
|---|---|---|---|---|---|
| | | | | Female | Male |
| *C. nucifera* | 10 | 14.6 ± 0.7ab | 42.5 ± 3.5 | 6.2 ± 0.1a | 5.4 ± 0.0a |
| *T. wagnerianus* | 10 | 12.2 ± 0.4a | 49.5 ± 3.9 | 6.2 ± 0.1a | 5.4 ± 0.1a |
| *T. latifolia* | 10 | 13.2 ± 0.8ab | 55.1 ± 4.6 | 6.1 ± 0.1a | 5.5 ± 0.1a |
| *C. esculentus* | 10 | 17.3 ± 1.8b | 37.2 ± 6.2 | 5.5 ± 0.1b | 5.0 ± 0.1b |
| *C. serotinus* | 10 | 17.3 ± 1.7b | 38.3 ± 5.4 | 5.6 ± 0.1b | 5.0 ± 0.1b |

[a] Mean ± SEM. Values followed by same letters within the same column do not differ significantly ($P > 0.05$; Tukey-Kramer HSD test following ANOVA).
[b] Mean ± SEM. There was no significant difference among the five plants (ANOVA: $F = 2.5062$, df = 4, $P = 0.0552$).

The body size of females on *C. nucifera, T. latifolia,* and *T. wagnerianus* were significantly larger than on the other plants (*C. esculentus* and *C. serotinus*) ($P < 0.05$; Tukey–Kramer HSD test following ANOVA). The elytra length of females reared on *T. wagnerianus* was 6.2 ± 0.1 mm, which was the largest among females reared on the five plants. The body size of males reared on *C. nucifera, T. latifolia,* and *T. wagnerianus* were also significantly larger than on other plants (*C. esculentus* and *C. serotinus*). The elytra length of males reared on *T. latifolia* was 5.5 ± 0.1 mm, the largest among males.

## Discussion

In this study, we found that *T. wagnerianus, S. erectum,* and plant species belonging to the Cyperaceae (*C. alternifolius, C. esculentus,* and *C. serotinus*) could be used for rearing *B. longissima. Trachycarpus wagnerianus* belongs to the Arecaceae, as does *C. nucifera,* and *S. erectum* belongs to Typhaceae as well as *T. latifolia* (APG III 2009). Thus, it is rather predictable that *B. longissima* would eat these species. However, it is surprising that this beetle feeds on plant species belonging to the Cyperaceae, which are phylogenetically distant from the Arecaceae. The developmental period of larvae fed on *T. wagnerianus* was significantly longer than that on *C. nucifera,* but adults grown on this palm had similar reproductive ability and body size to those on *C. nucifera. T. wagnerianus* is an exotic palm but is presently naturalized in Japan (Satake et al. 1989). It is an evergreen, so leaves can be obtained in winter, even if planted outdoors. The observed adult fecundity and availability of *T. wagnerianus* leaves suggest it to be suitable as an alternative food plant for rearing this beetle in Japan. We assume that *S. erectum* is less suitable for rearing *B. longissima,* because the larvae fed on it took twice as long as when fed on *C. nucifera* to pupate, and *S. erectum* is neither common nor abundant in Japan (Takahashi et al. 1990).

However, 95% of larvae fed on *C. esculentus* and *C. serotinus* developed into adults, showing larval developmental periods as short as that on *C. nucifera,* one of the main host plants of this beetle. The fecundity of adults reared on *C. esculentus* and *C. serotinus* was comparable with that of adults on *C. nucifera,* although their body sizes were significantly smaller than those on *C. nucifera. C. serotinus* is a native species (Takahashi et al. 1990), and *C. esculentus* is an exotic species that is presently naturalized in Japan (Shimizu et al. 2001). These sedges are hard-to-control weeds in fields and meadows. Becausethey reproduce by rhizomes and seeds, they are easy to grow in a greenhouse and are amenable to the collection of large amounts of leaves for rearing the beetles. Our results suggest that *C. serotinus* and *C. esculentus* are feasible as an alternative food plant for *B. longissima.* One of the purposes to find alternative food plants for *B. longissima* is stable mass rearing of parasitoid wasps such as *A. hispinarum.* Further studies are needed to investigate whether there is any differences in parasitism success rate and developmental rate of *A. hispinarum* on larvae reared on Cyperaceae: *C. esculentus* and *C. serotinus* compared with parasitoids coming from beetle larvae reared on Arecaceae.

It is commonly assumed that the host selection behaviors of most phytophagous insects are restricted by secondary metabolic substances in plants (Bernays and Chapman 1994). Specifically, the presence of feeding- or oviposition-stimulating substances combined with the absence of any deterrent substances in a plant are needed to make the plant available for feeding or oviposition by insects. The feeding behaviors of several chrysomelid beetles are reported to be mediated by the secondary substances of plants. For example, *Diabrotica* and *Aulacophora* species feeding on cucurbitaceous plants have been revealed to have their feeding stimulated by triterpenoid cucurbitacins, which are generally found in cucurbitaceous plants (Metcalf and Lampman 1989, Abe and Matsuda 2005). However, the triterpenoid momordicin II in the bitter melon, *Momordica charantia* L., acts as a feeding deterrent to *Aulacophora* species (Chandravadana 1987, Abe and Matsuda 2000). The host specificity of the salicaceous-feeding leaf beetles *Chrysomela vigintipunctata costella* (Marseul) and *Plagiodera versicolora distincta* Baly have been suggested to depend on the presence of salicin, populin, and luteolin-7-glucoside contained in these host plants (Matsuda and Matsuo 1985). The feeding behavior of *Ophraella communa* LeSage was reported to be stimulated synergistically by triterpenoids and caffeic acid derivatives in the leaves of several asteraceous plants (Tamura et al. 2004). In our study, *B. longissima* were revealed to feed on cyperaceous plants. Further research is necessary to clarify why this beetle was able to eat plants in the Cyperaceae, which are phylogenetically distant from Arecaceae. However, the leaves of *C. esculentus* and *C. serotinus* probably include feeding stimulants for *B. longissima* and do not include the feeding deterrents. Identifying the feeding stimulants and deterrents to *B. longissima* would contribute to the improvement of palm cultivars that would discourage this beetle, and to the development of artificial diet and management tools.

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