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A TECHNIQUE FOR MASS REARING THE COCONUT LEAF BEETLE, *Brontispa longissima* (Gestro) (COLEOPTERA: CHRYSOMELIDAE), ON *Cocos nucifera* L.

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ABSTRACT

A technique is described for rearing the coconut leaf beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomellidae) on young coconut leaflets under laboratory conditions. The method is simple, economical, and convenient to use. Large numbers of individuals of uniform ages can be generated for use in the production of biological control agents and for other experiments. A total of 120,539 eggs, larvae, pupae and adults may be produced by about 10,000 adult females and males within three months.

Key words: *Brontispa longissima*, *Cocos nucifera*, mass rearing technique

INTRODUCTION

The coconut leaf beetle (CLB), *Brontispa longissima* (Gestro) (Coleoptera: Chrysomellidae), is an invasive pest that was first reported on coconut in the Philippines in 2005 (Ooi et al., 2005; Cuyacot et al., 2014). Feeding by both larvae and adults causes scarring on leaves. Prolonged infestations damage all emerging fronds that may result in drying-up of leaves. Heavy infestations can sometimes cause coconut trees to die (Waterhouse, 1993; CABI, 1999; Recuenco-Adorada et al., 2006; Viet 2006; Rethinam & Singh, 2007; Navasero et al., 2008a,b). The injury also serves as entry points for pathogens that cause diseases, which further contribute toward more severe damage. Countries like Vietnam, Samoa, Indonesia, Thailand, Maldives, and China that were invaded earlier by this pest had experienced serious losses in revenues from widespread infestations and eventual death of many of their coconut trees (FAO, 2004). In Vietnam, for example, the pest caused losses of USD 40 million per year and up to 5% of the infested trees died (Liebregts et al., 2006).

Different control tactics have been tried including chemical control and classical biological control. The use of insecticides provided only short-term suppression and this approach is expensive and causes health risks to farmers and their families, and consumers. Moreover, the application of insecticides may disrupt the successful establishment and effectiveness of natural enemies, whether introduced or indigenous. Nevertheless, chemical control is still the

most widely used management option for CLB in the Philippines. However, the majority of coconut growers cannot afford chemical pesticides. The Philippine Coconut Authority (PCA) in August 2007, conducted massive insecticide treatment of infested trees in all affected areas, providing free insecticides and lending drilling equipment for insecticide trunk injection. PCA reported to have treated half a million infested trees with 80% recovery. Application of insecticides provided only temporary relief because pest populations build-up when residual activity of the applied pesticide stops. Nevertheless, campaigns against CLB have been continuously carried out by researchers in government institutions using biological control agents such as the predatory earwig *Chelisoches morio*, pupal parasitoid *Tetrastichus* sp., and the fungal entomopathogen, *Metarhizium anisopliae* (Recuenco-Adorada & Navasero, 2007; Navasero & Navasero, 2008, 2010, 2015; Orense et al., 2009; PCA, 2012).

Basic studies on the response of CLB to leaf volatiles of the coconut palm revealed that a blend of β-myrcene and (-)-limonene elicits aggregation and oviposition in females (Fang et al., 2011). Sugeno et al. (2011) evaluated some monocots for rearing CLB and found that *Trachycarpus wagnerians* (Arecaceae), *Cyperus esculentus,* and *C. serotinus* (both Cyperaceae) are food plants in addition to *C. nucifera* (Arecaceae) and *Typha latifolia* (Typhaceae) for rearing the insect. Earlier, Yamashita et al. (2008) reported on the use of mature leaves of coconut and narrow leaf cattail, *Typha angustifolia,* for laboratory rearing CLB, with a follow-up on the suitability of potential host plants in Japan for immature development (Yamashita & Takasu, 2012). Lü et al. (2012) developed an artificial diet based on the analysis of nutrient content of the leaves. The diet consisted of 4% sucrose, 2% dried coconut powder, 2% soybean powder, 10% coconut old leaf powder, 2% yeast, 0.3% vitamin E, 0.2% vitamin C, 0.2% methyl p-hydroxybenzoate, 4% agar, 0.03% streptomycin and 75% water, with a survival rate of 36% from hatching to pupation. Liebregts et al. (2006) published a mass rearing technique for CLB and its larval parasitoid, *Asecodes hispinarum* Bouček, using young leaves of coconut.

This paper describes a rearing method using coconut leaves, commercially available plastic containers, and other requirements, all readily available under Philippine conditions, for successful mass rearing of CLB in the laboratory.

MATERIALS AND METHODS

The improvised rearing container

Commercially available plastic containers (21 cm long, 15 cm wide, and 10 cm deep) were used after cutting and removing a 14 cm x 9 cm area on the lid covers. This opening was covered with the fine mesh.

Each container was lined with paper towels cut to fit the bottom. Plain paper may be used also. The piece of tissue paper or plain paper absorbed excess

moisture. It also serves to contain CLB excreta which can be easily discarded. Rearing containers were emptied, cleaned, and disinfected with either 70% ethyl or isopropyl alcohol before re-use.

The tops of the rearing containers were lined or covered with fine mesh muslin cloth (28cm x 24cm) before placing its cover, to prevent escape of larvae and adults through the lids.

Stock culture

Infested young and unopened coconut leaves, called spears, were collected from a farm in Sariaya, Quezon. These were placed in Mylar™ tubes to confine CLB prior to use in the laboratory. The initial stock was composed of 7,854 larvae, 560 pupae, and 5,178 adults.

Mass-rearing procedure

The entire mass-rearing procedure and set-up, from collection of eggs to feeding of larvae, through to pupation and emergence of adults are illustrated/ featured in Figure 1.

Collection of eggs. About 300 unsexed adults were placed in each rearing container with three sets of stapled (i.e., fastened with staple wires) leaflets. These served as food for adult beetles as well as oviposition substrates. After 24 hours, leaflets were replaced with fresh ones. Care was taken to ensure that CLBs were completely removed from the leaflets by brushing off the beetles with a camel-hair brush or slightly tapping the leaflets to dislodge them. The leaflets containing eggs were combined and placed in another rearing container for holding prior to incubation.

Eggs were clipped from the leaflets and then placed in rearing containers lined with 0.5 cm thick cotton saturated with tap water and lined on top with filter paper. Plain paper may be used also. The filter or plain paper absorbed excessive moisture and prevented direct contact of eggs with the saturated cotton while under incubation. Eggs were placed at the rate of 1,000, 1,500, or 2,000 per pan for incubation for about two days, after which they were placed in another rearing container with five sets of stapled coconut leaflets.

Larval feeding, pupation, and adult emergence. When almost all the eggs had hatched (after two days), leaflets with unhatched eggs were discarded. These leaflets were replaced with new ones every two days. A camel-hair brush was used to transfer larvae to the new substrates. The culture was divided into two or more rearing containers to prevent overcrowding and larvae were fed every two days. Larvae pupated after two weeks. Pupae collected on the same day were combined and placed in new rearing containers for adult emergence.

Food for the feeding stages

Larval food consisted of young and un-infested and unopened spears of coconut. Although fresh spears were preferred, the basal parts of the spears were soaked for a week in tap water in a container to keep them from wilting and ensure continuous supply of food.

Leaflets from the spears were cut 15 cm long and 4 to 5 pieces were fastened at one end by stapling to approximate natural condition of the spear which is furled at the base. Three sets were placed in each rearing pan. These served as food for the developing larvae and adults of CLB.

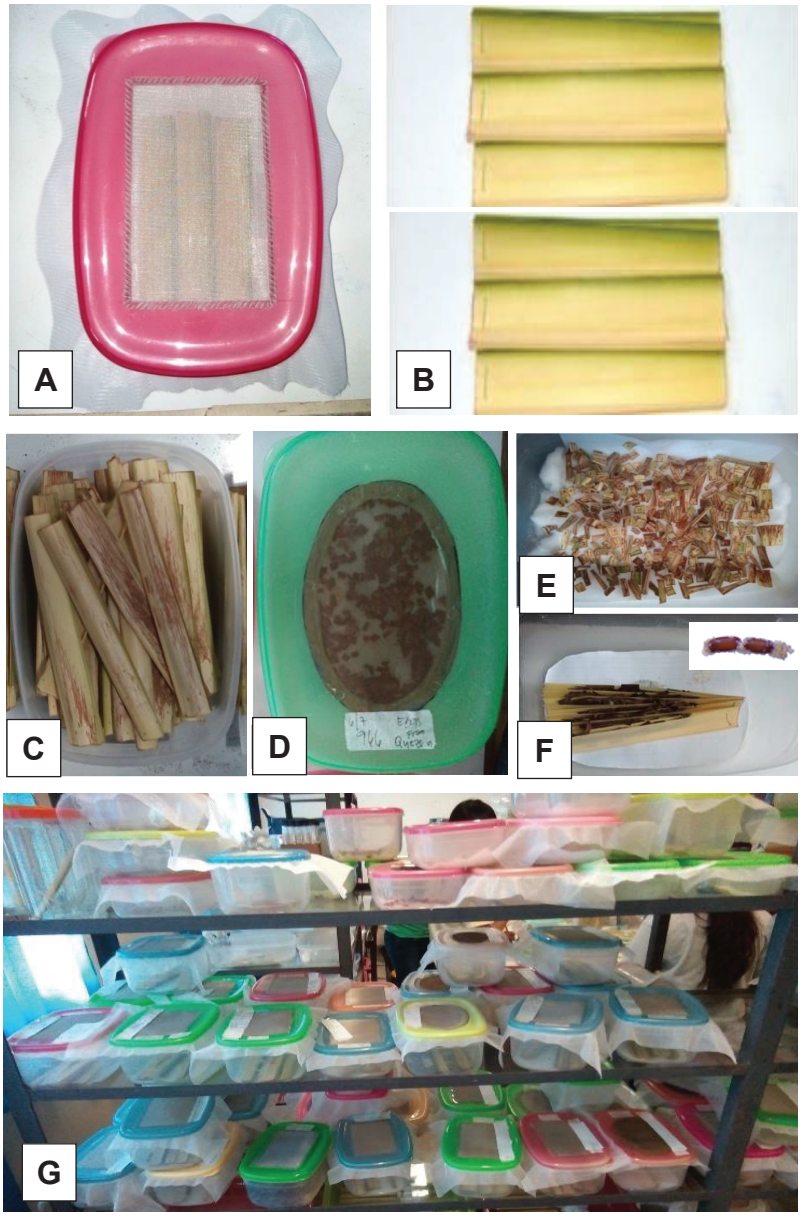

Figure 1. Set-up for mass-rearing *Brontispa longissima* (Gestro) showing the different materials used: A. plastic container. B. young coconut leaflets stapled at the end. C. newly harvested eggs. D. incubating newly laid eggs in containers. E. close-up of eggs inside containers. F. eggs placed in between opened leaflets when about to hatch (inset: close-up of eggs). G. shelf for stacking rearing containers.

RESULTS AND DISCUSSION

Laboratory rearing was optimized and the specimens representing different life stages were kept separately in rearing pans (Figure 2). The approximate durations of various life stages under an ambient temperature of 26°-29°C and relative humidity of 70-75% were as follows: egg, 3-4 days (d); larva: 1st instar, 4d; 2nd, 4d; 3rd, 5d; 4th, 6d; and 5th, 12-14d; pupa, 4-5d; and adult, 218d, in mass culture.

Eggs (Figure 1 F) kept in pans lined with moistened cotton provided humid conditions and prevented them from drying up. In the field, eggs are deposited between young, unopened leaflets where humidity is close to 100% (Liebregts et al., 2006). Apparently, the moist cotton lining of the pan approximated the condition in the field resulting in high hatchability of eggs, up to 100%.

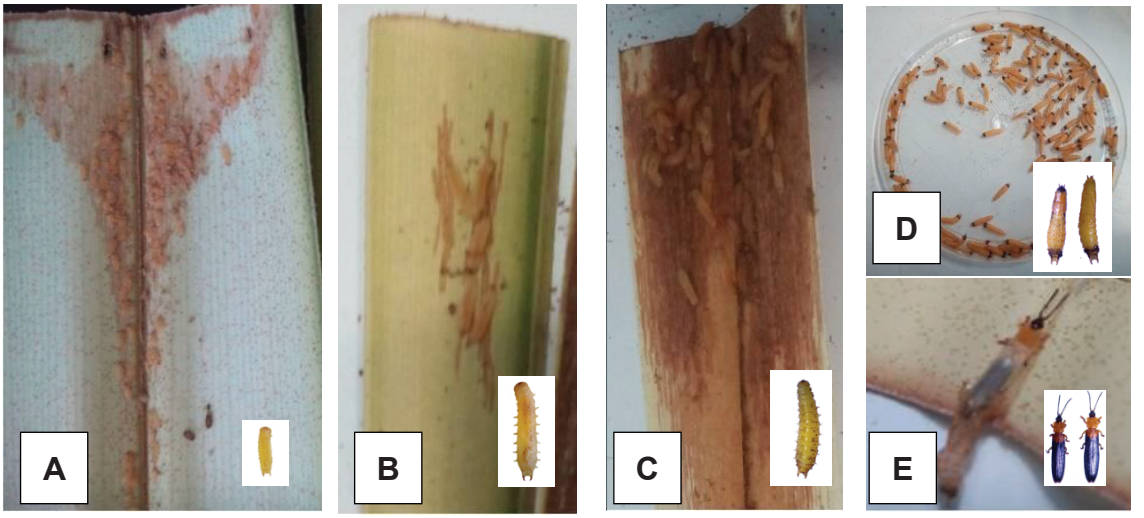

Figure 2. Different life stages of *Brontispa longissima* (Gestro) in mass culture; insets: close-up views: **A-C.** Larvae: A. first instar. B. fourth instar. C. fifth instar. D. Pupae. E. Emerging adult (inset: emerged adults: male, L; female, R).

Normally, eggs were laid in a single row of up to seven eggs, but mostly 2-3 per row, and were collected daily. When the eggs seen were mostly 1-2 only, this indicated that adult females were already entering the post-oviposition period and collection of eggs was done every two days. Keeping each day's or every other day's harvest of eggs in separate pans allowed eggs to hatch within 1-2 days of each other in each pan.

Generally, eggs laid during the early to mid-oviposition period had high percentage hatchability of up to 100 % but declined thereafter. On the average, hatchability of eggs laid by females in mass culture was 70%. When egg production by adult females in a pan drastically declined due to natural death or old age, the adults of similar condition were pooled together in a single pan for a week or so before they were discarded properly.

Newly hatched day-old larvae (Figure 2A) were the most delicate and vulnerable and mishandling them may result in high mortality, hence loss in mass- rearing. Fourth (Figure 2B) to fifth instar (Figure 2C) larvae fed voraciously. Hence, splitting the culture at this stage, rather than adding another layer of food was beneficial since ventilation was maintained. The last two instars were the longest, lasting for about three weeks and, when well-fed, produced bigger and healthy pupae (Figure 2D) and adults (Figure 2E). These, in general, would have longer reproductive period and lay more eggs.

Larvae of similar developmental stage were kept together so that extraction of the various life stages for voracity testing of *C. morio* became much easier (Navasero & Navasero, 2010).

To ensure proper growth and development of larvae, cleanliness was observed all the time. The lining of tissue paper absorbed excess moisture and served as receptacle for feces or frass, which was discarded every time food was replaced with fresh ones. Also, disinfecting the rearing pans with 70% ethyl or isopropyl alcohol before reuse reduced or eliminated the possibility of microbial contamination. Liebregts et al. (2006) cited that excessive moisture promotes the development of fungi and bacteria, which adversely affect the quality of the leaves and encourage growth of lethal pathogens.

Pupae were segregated from the previous collections to ensure uniform emergence of adults within 1-2 days so that materials for testing would be of similar age. Food was supplied when adults were expected to emerge to trap them for easy collection for testing or breeding purposes.

The post-developmental periods of adults in mass culture were as follows: pre-oviposition period, 15d; oviposition period, 172d; and post-oviposition period, 31d. Obviously, the adult is the longest stage and required feeding for a much longer time. Adults during the pre-oviposition and oviposition periods should be fed well to ensure laying of more eggs by the females. However, upon reaching the post-oviposition period, adults may be discarded properly, following basic biosafety regulations.

Although artificial diets have been developed or proposed in other countries e.g. Ichiki et al. (2011), the rearing technique described above is more economical, readily available and appropriate for the intended uses, e.g. mass production of locally available natural enemies or biological control agents under Philippine conditions.

CONCLUSION

An easy, simple, and convenient to use method to mass produce *B. longissima* on young leaflets of coconut spear under laboratory conditions was devised. Large numbers of individuals of uniform ages can be produced within three months of continuous rearing for use in predator-prey experiments. Incubating eggs for several days in moist 0.5-cm thick cotton lining of a plastic

pan prevented dehydration, thus ensuring high percentage hatchability of viable eggs, up to 100%. Fastening one end of a stack of leaflets consisting of 4-5 pieces with a staple wire and placing three sets of them on a single pile in a rearing pan favored feeding, growth and development of larvae as well as oviposition by adults.

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LITERATURE CITED

[CABI] Center for Agriculture and Biosciences International. 1999. *Brontispa longissima* (coconut hispine beetle). Invasive Species Compendium. CAB International, Wallingford, UK. https://www.cabi.org/isc/datasheet/10059.

CUYACOT AR, SABADO EM & DEMAYO CG. 2014. Variability in the last abdominal sternum of *Brontispa longissima* populations using outline-based geometric morphometric analysis. International Journal of Bioscience, Biochemistry and Bioinformatics 4(4): 299-303.

FANG YL, SUN JH & ZHANG ZN. 2011. Response of *Brontispa longissima* to coconut palm (*Cocos nucifera*) leaf volatiles. Physiological Entomology 36(4): 321-326. doi: 10.1111/j.1365-3032.2011.00799.x.

[FAO] Food and Agriculture Organization of the United Nations, Regional Office For Asia and the Pacific. 2004. Report of the expert consultation on coconut beetle outbreak in APPPC member countries. 26-27 October 2004. Bangkok, Thailand. RAP Publication 2004/29. v + 33 p.

ICHIKI RT, MURATA M, TAKANO S, OO TT, NGUYEN HT, SUASA-ARD W, URAICHUEN S & NAKAMURA S. 2011. Artificial diets for rearing the coconut hispine beetle, *Brontispa longissima* (Coleoptera: Chrysomelidae), and their suitability to two specialist parasitoids, Biocontrol Science and Technology 21(12): 1423-1435. doi: 10.1080/09583157.2011.632077.

LIEBREGTS W, VIET TT & CHAPMAN K. 2006. Rearing *Brontispa longissima* and its parasitoid *Asecodes hispinarum*. FAO/TCP/VIE/2905. FAO Regional Office for Asia and the Pacific, Bangkok, Thailand. www.fao.org/world/regional/rap/highlights.asp. 25p.

LÜ BQ, JIN QA, WEN HB, PENG ZQ & DU YZ. 2009. Development of artificial diet for coconut leaf beetle, *Brontispa longissima* and the efficacy for mass rearing. Acta Phytophylacica Sinica 2012-04. http://en.cnki.com.cn/Article_en/CJFDTOTAL-ZWBF201204009.htm

NAVASERO MM & NAVASERO MV. 2008. Development, prey consumption and mass production of *Chelisoches morio* Fabr. (Chelisochidae, Dermaptera). Abstracts of papers (Oral paper presentation) presented at the 39th PMCP Anniversary and Annual Scientific Conference. Asturias Hotel, Puerto Princesa City, Palawan. May 6-9, 2008. The Philippine Entomologist 22(2): 202.

NAVASERO MM & NAVASERO MV. 2010. Biology of the black earwig *Chelisoches morio* (Fabricius) (Chelisochidae, Dermaptera). The Philippine Entomologist 24(2): 122-136.

NAVASERO MM & NAVASERO MV. 2015. *Chelisoches* production. National Crop Protection Cluster, College of Agriculture, UPLB. ISBN 978-971-8778-93-7.

NAVASERO MV, NAVASERO MM & CANDAÑO RN. 2008a. Possible natural control of the invasive coconut hispine beetle, *Brontispa Iongissima* (Gestro) (Coleoptera: Chrysomellidae: Hispinae) by its diverse indigenous natural enemies. Abstracts of papers presented at the 39th PMCP Anniversary and Annual Scientific Conference, Asturias Hotel, Puerto Princesa City, Palawan.May 6-9, 2008. The Philippine Entomologist 22(2): 222.

NAVASERO MV, SAAVEDRA NT & ZIPAGAN MB. 2008b. The coconut leaf beetle, *Brontispa longissima* (Gestro) (Chrysomellidae. Coleoptera), a serious threat to the Philippine coconut industry. Proceedings of the 39th PMCP Anniversary and Annual Scientific Conference, Asturias Hotel, Puerto Princesa City, Palawan. May 6-9, 2008. https://www.researchgate.net/publication/301327745

OOI PAC, RECUENCO-ADORADA JD, CALUMPANG SMF, NAVASERO MV, KARGANILLA GPS, MAGDARAOG FM, TAUII H, CAILO D & ZIPAGAN MB. 2005. *Brontispa longissima* (Gestro), an invasive coconut pest in the Philippines. Policy Paper Report presented by the Brontispa Action Team (BAT) to the Department of Agriculture, Philippine Coconut Authority and stakeholders. Manila, Philippines.

ORENSE JC, ALFILER ARR & IMPERIAL MLR. 2009. Prospects for biological control of *Brontispa longissimi* (Gestro) attacking coconut in the Philippines. Abstracts of papers (Poster presentation) presented at the 40th PMCP Anniversary and Annual Scientific Conference. Baguio City. May 5-8, 2009. The Philippine Entomologist 23(2): 196.

[PCA] Philippine Coconut Authority. 2012. Coconut Leaf Beetle, *Brontispa longissima* (Gestro). Technology Series No. 3 (Brochure). 6 p.

RECUENCO-ADORADA JD & NAVASERO MV. 2007. Indigenous biological control agents of *Brontispa longissima* (Gestro) in the Philippines. Abstract of posters presented at the 29th Annual Scientific Meeting National Academy of Science and Technology. Westin Philippine Plaza, Pasay City, July 9-12, 2007. Transactions of the National Academy of Science and Technology, Philippines. Supplement.

RECUENCO-ADORADA JD, NAVASERO MV & BALLENTES M. 2006. Hispine beetles of coconut and other palms in the Philippines. Abstracts of papers presented at the 37th PMCP Anniversary and Annual Scientific Conference. Grand Regal Hotel Davao City. May 2-5, 2006. The Philippine Entomologist 20(2): 180.

RETHINAM P & SINGH RP. 2007. Current status of the coconut beetle outbreaks in the Asia-Pacific region. In: Developing an Asia-Pacific strategy for forest invasive species: the coconut beetle problem - bridging agriculture and forestry. In: Appanah S, Sim HC and Sankaran KV. (eds.) Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific Publication 2007/02.Report of the Asia-Pacific Forest Invasive Species Network Workshop 22-25 February 2005, Ho Chi Minh City.

SUGENO W, KAWASU K, TAKANO S, NAKAMURA S & MOCHIZUKI A. 2011. Suitability of monocots for rearing alien coconut pest *Brontispa longissima* (Coleoptera: Chrysomelidae). Annals of the Entomological Society of America 104(4): 682-687. https://doi.org/10.1603/AN10146.

VIET TT. 2006. Classical biological control of the coconut hispine beetle, (*Brontispa longissima* (Gestro) in Vietnam. www.agnet.org/activities/sw/2006/954198798/paper-505395537.pdf

WATERHOUSE DF. 1993. The Major Arthropod Pests and Weeds of Agriculture in Southeast Asia. ACIAR Monograph No. 21. Canberra, Australia: Australian Centre for International Agricultural Research, 141 pp.

YAMASHITA A & TAKASU K. 2010. Suitability of potential host plants in Japan for immature development of the coconut hispine beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae). Japan Agricultural Research Quarterly 44(2): 143-149. https://doi.org/10.6090/jarq.44.143.

YAMASHITA A, WINOTAI A & TAKASU K. 2008. Use of mature leaves of coconut palm and narrowleaf cattail for laboratory rearing of the coconut leaf beetle *Brontispa longissima.* Bulletin of the Institute of Tropical Agriculture, Kyushu University 31(1): 61-67. https://doi.org/10.11189/bita.31.61.