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FIELD BIOLOGY, SETAL MAP AND ADULT GENITALIA OF THE CITRUS RIND BORER *Prays endolemma* Diakonoff (Lepidoptera: Yponomeutidae) ON PUMMELO *Citrus maxima* (Burm) Merr. (Rutaceae) IN REGION XI, PHILIPPINES[1]

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ABSTRACT

Citrus rind borer (CRB) is a major threat to pummelo, particularly in Region XI. Very little is known about the biology, identifying features for proper species identification, including detailed description of the insect. Hence, the biology of CRB was investigated under laboratory and field condition. The egg hatches in 3-4 days. The larval period ranges from 13 to 15 days, the end indicated by the coming out of the last-instar larva from the rind to pupate. The pupal period lasts for 4 days. In summary, the total developmental period from egg laying to adult emergence was 20 to 23 days.

Eggs are flat, almost circular, cloudy white, measuring .048 mm in diam and laid singly on the surface of the pummelo fruits as early as fruit setting. Last instar larva (5.5 ± 0.22 mm) crawls out of the rind. It is dark to light green in color with the red stripes very prominent intersegmentally from first thoracic to the 9th abdominal segment. Pupa measures 4.5 ± 0.513 mm long, enclosed in a lacelike, large-meshed cocoon. Adult measures 4.05 ± 0.605 mm long and generally grayish brown in color with dark brown markings.

Dissected female genitalia shows distinct ductus bursae with short tubular upper third and sclerotized wide lower part formed by two broad sclerites (cestum), each bearing a dentate ridge the species was named after, *Prays endolemma* Diakonoff.

Key words: citrus rind borer, pummelo, cestum, female genitalia, ductus bursae, lacelike cocoon, dentate ridge

INTRODUCTION

As early as 1913, E. O. Essig recorded a larva of a moth infesting the rind of the fruit, forming galls and causing serious damage on Philippine oranges (as cited by Fletcher 1920). Thereafter, it was reported as a serious pest of citrus cultivars such as cajel *Citrus sinensis*, lucban *C. decumana* (*C. maxima*), lime *C. aurantifolia*, citron *C. medica*, lemon *C. limonia*, and sour orange *C. hystrix* (San Juan 1924, Gavarra et al. 1990, Godoy and Fuentes 1992). At present, citrus rind borer (CRB) remains a major threat to pummelo, particularly in Region XI, the largest pummelo-producing area in the country (Pangan et al. 2007). The earliest studies on CRB (San Juan 1924, Garcia 1939) identified the pest as *Prays citri* Milliere. However, Gavarra et al. (1990) reported the CRB as *Prays endolemma* Diakonoff, *as identified by* R. G. Robinson of the British Museum based on specimens they sent. These early workers also gave brief accounts of the description and duration of different stages, nature of damage, distribution and other biological studies conducted, including control measures.

According to the National RDE Network for Fruit Crops (2002), there is a big demand for pummelo in the domestic market. It also has a big potential for export, considering that Malaysia and Thailand are actively selling pummelo to Singapore, Hong Kong and other countries. More importantly, the competition created by the imported China pummelo threatens the small- and big-scale pummelo growers in Mindanao and intensifies the need to produce high quality fruits. Unfortunately, the prevailing problem posed by CRB limits its economic potential and should be addressed.

Although there were previous studies conducted on the pest, very little is known about its biology in the field and most of the information was generated 20 years ago. San Juan (1924) and Garcia (1939) described the different stages of the moth which they identified as *Prays citri* Milliere. In 1990, Gavarra et al. identified the moth attacking pummelo as *P. endolemma* Diakonoff and worked on its biology in the laboratory. Therefore, more detailed information about the biology of the insect on pummelo appears necessary. The reproductive organs of the moth have to be examined to confirm the identity of the species, an important prerequisite to planning of effective management strategy against the pest.

This study was conducted with the following objectives: (1) to establish the identity of the CRB attacking pummelo in Region XI and (2) to determine the field biology of CRB in pummelo and describe the different developmental stages of the insect.

METHODOLOGY

The study was conducted at South Davao Development Company (SODACO) Farm, Sitio Dayan, Brgy. Talandang, Calinan, Davao City from May to December 2010. The farm is generally planted to fruit trees such as pummelo, mango, 'Cavendish' and 'Lakatan' banana, mangosteen, durian and lanzones. Laboratory studies were conducted at the Crop Protection Laboratory of the University of Southeastern Philippines-Tagum Campus, Apokon, Tagum City.

Thirteen years old fruit-bearing pummelo trees, var. Magallanes, were used for the study. The trees intended for field observations were randomly selected and tagged, as well as trees for laboratory studies. In the month of August, following the rains of June and July, flowering occurred on the selected ten trees. From these, four trees were used for field biology study from August to September, 2010 and the remaining six served as source of sample insects for description and dissection in the laboratory.

Field Biology of CRB on Pummelo

Flower buds and developing fruits from tagged pummelo trees were inspected daily for the presence of eggs using hand lens. When eggs were observed, the flower buds or young fruits were tagged and the terminal where the inflorescence arose (Figure 1) was enclosed in 40 cm x 40 cm fabricated cylindrical cage made of fine nylon mesh. A total of 20 cages were set up. The caged flowers and fruits were inspected daily for egg hatching, entry points and exit holes, mature larvae, pupation and adult emergence. The duration of each developmental stage was recorded. Five fruits per sample tree with larval exit holes were randomly picked and brought to the laboratory. Each abnormal protruding portion of the rind (gall) was dissected to determine the number and sizes of head capsules inside as indicators of molting and number of larval instars for each individual insect.

Figure 1. Pummelo terminal, with tagged fruits/flower buds, enclosed in 40 cm x 40 cm nylon mesh cylindrical cage for use in studying the field biology of the citrus rind borer, *Prays endolemma* Diakonoff.

Figure 2. Field-collected pummelo fruits damaged by the citrus rind borer *Prays endolemma* Diakonoff in 6 cm. x 6 cm. x 25 cm. nylon mesh cages for exit of mature larvae and emergence of adults.

Unenclosed fruits during the same fruiting period were also collected and kept in a cage in the laboratory (Figure 2) until the developing larvae in galls pupated and adults emerged inside the cage. Other unenclosed fruits with newly laid eggs were gathered, brought to the laboratory and examined. The eggs were measured using an ocular micrometer, while the larvae in late instar, pupae and adults were measured using caliper micrometer. Each specimen representing a developmental stage was described by shape, size, and color and photographed using Canon DIGITAL IXUS 95 IS (10.0 mega pixels). Line drawings of the important morphological details of the specimens were made. Pinned specimens of CRB adults were preserved. All samples were viewed under a dissecting microscope (with WF10X, 1X to 3X objective lens, Ken-a-Vision, USA). Images were processed and analyzed using ImageJ Program. The mean duration of each stage was compared to the counterpart data reported by Gavarra et al. (1990) and Godoy et al. (1992) using one sample t-test in SPSS 13.0 for Windows.

Last-instar larvae were collected the moment they exited from the rind and placed in vials with 70% alcohol, then placed in boiling water for at least 5 minutes, and transferred back to 70% alcohol. Using stereo microscope, the larvae were examined for important characters. Setal map of larva was constructed and the crochet arrangement was described.

The following symbols were used in labeling and describing the setal map of thoracic and abdominal segments adopted from Hinton (1946) system of naming setae for identification (as cited by Stehr 1998). These setae were divided into six groups according to their location as follows:

XD group – two setae found only on the anterior margin of the T1 shield.
Dorsal (*D*) group – two setae usually present near the dorsal midline on all segments.
Subdorsal (*SD*) Group – two setae present on all segments except on A9.
Lateral (*L*) Group – three setae usually present on all segments.
Subventral (*SV*) Group – either unisetose or bisetose setae found above the coxa and often on large pinacula.
Ventral (*V*) Group – a single seta usually present on all segments, closer to the ventral meson than the SV group.

In addition, abbreviations are used to describe the placement of the chaetotaxy, T1 – T3 represent thoracic segments and A1 – A10 represent abdominal segments. The setal map and its description was submitted to Dr. Jessamyn R. Adorada, Curator, UPLB Museum of Natural History for review and confirmation. The type of cocoon of the pupa was also described.

To get more samples, additional fruits where adults were observed ovipositing were tagged and the dates recorded. Fruits were no longer caged but monitored daily for gall development (as a sign of larval activity inside). After about 13 days from hatching, 10 infested fruit samples, characterized by tumor-like rind, were picked. To keep the samples fresh, the peduncles were soaked in tap water in a jar, and then placed individually in a 6 cm. x 6 cm. x 25 cm. nylon mesh cage. The fruits were kept until all the larvae have crawled out of the galls, and pupated individually in a loosely woven cocoon attached on the cage wall or on the fruit rind. The pupae were marked individually and kept undisturbed in the same cages until adult emergence. Newly-emerged adults were collected and transferred to separate cages according to emergence date. Mating was observed and each pair was carefully transferred to a separate cage for oviposition of the female moth.

Egg Laying on Pummelo

Ten pairs of male and female adults in copulation were carefully collected and placed in separate 6 cm. x 6 cm. x 25 cm. plastic cages containing an oviposition substrate. The adults were provided daily with 10% honey solution as food. Oviposition substrates were as follows: flower bud and young fruits of different sizes (10-12 mm, 15-20 mm, 25-30 mm and 35-45 mm diam). Treatments were laid out in a completely randomized design.

Egg deposition was observed daily for two to five days after mating. The number and location of eggs were recorded. Specific oviposition sites were categorized as: on the receptacle, lower, middle or upper part of fruit. Data were analyzed using ANOVA and treatment means compared using Tukey's Honest Significant Difference.

A similar experiment was set up in the field. Prior to bud break, each flower bud was enclosed with 60 cm. long x 27 cm. diam cylindrical nylon mesh cage to ensure that the flowers have not been oviposited on. One pair of CRB adults in copulation was carefully collected and introduced to each cage. Cages were labeled according to the fruit stage when female CRB was introduced. A total of three caged samples for each oviposition substrate; namely, - flower bud, and fruits measuring 10-12 mm, 15-20 mm, 25-30 mm and 35-45 mm diam were used. The female was allowed to lay eggs until death. The same data were collected as in laboratory set up. No eggs were observed in all the set up.

Adult Longevity

To test whether adults will survive longer with food provision, a total of 20 newly emerged adults per treatment were placed in a cage and provided with either sterilized water alone, 10% honey solution, or 20% honey solution absorbed in a cotton wad. Each treatment was replicated thrice. Longevity of the adults (number of days) and number of individuals that died in each treatment were recorded. Data were subjected to ANOVA and treatment means were compared using Tukey's Honest Significant Difference (HSD).

Dissection and Examination of Genitalia

A total of 365 adults were dissected to identify the species and describe the male and female genitalia following the procedure of Barrion (2004). Newly emerged adult CRB was killed in 70% alcohol and immediately, the abdomen was severed from metathorax using fine forceps. The detached abdomen was soaked in aqueous KOH solution. After 12-24 hrs, the softened abdomen was removed from KOH solution, and transferred to a watch glass or palette with 30-50% ethyl alcohol. Descaling and cleansing were done by pushing out with needle and brush the body contents from the genital area to the more anterior portion of the abdomen. After initial cleaning, the tip of the abdomen was transferred to a small Petri dish with fresh alcohol and the genitalia dissected out using fine forceps or needles, and then stained using Chlorazol Black E.

Photographs of the genitalia were taken and line drawings were made to illustrate and characterize each sex morphologically. Important parts were processed and analyzed using ImageJ Program. Samples of genitalia, both male and female, were submitted to Prof. Aimee Lyn B. Dupo, Curator, UPLB Museum of Natural History for confirmation of species identification.

RESULTS AND DISCUSSION

Field Biology of CRB on Pummelo

The duration from egg laying to adult emergence ranged from 20 to 23 days (Table 1) with mean of 21.18 ±0.51. Incubation period was 3 to 4 days. Of the 167 eggs laid, only 91% hatched to first-instar larvae. Each neonate larva bore into the rind and remained inside until the mature larva exited from the gall after 13 to 15 days, with a mean of 13.33 ±0.53. The larva passed through 5 instars based on the number of head capsules retrieved from the 20 damaged fruits. The mature larva enclosed in a lacelike, large-meshed cocoon, fastened itself to a solid surface before turning into a pupa 4 to 5 days later. Eighty-eight percent of the eggs laid successfully developed into adults.

When compared with the data reported by Gavarra et al. (1990) and Godoy & Fuentes (1992) using one sample t-test, the differences were highly significant (Table 2). The previous two studies were conducted in the laboratory while the present study was under field condition. Nonetheless, number of days at each stage in the present study falls within the range of values reported by the previous workers. This is because the fruit is a protected habitat, whether it is in the field or in the laboratory. Inside the rind, the larvae probably do not experience extreme environmental changes that could influence their development.

Table 1. Durations of the developmental stages of *Prays endolemma* Diakonoff on pummelo under field conditions (SODACO Farm, Davao City, 2010)

STAGES	MEAN (days)	STD. DEV.	NUMBER OF INDIVIDUALS EXAMINED	DURATION OF STAGES (days)	NUMBER OF INDIVIDUALS EXAMINED
Egg Stage	3.47	± 0.51	167	3	86
				4	81
Larval Stage	13.33	± 0.53	152	13	115
				14	34
				15	3
Pupal Stage	4.28	± 0.46	148	4	108
				5	40
Egg incubation to adult emergence	21.15	± 1.04	147	20	58
				21	42
				22	33
				23	14

Table 2. One sample t-test analysis comparing the current findings and those of Gavarra et al. (1990) and Godoy and Fuentes (1992) on the duration of different stages of the citrus rind borer *Prays endolemma* Diakonoff in Region XI

STAGES	MEAN OF PRESENT STUDY	STD. DEV.	GAVARRA et al. (1990)		GODOY & FUENTES (1992)	
			Mean	P value	Mean	P value
Egg	3.47	± 0.51	4.5	.000*	4.06	.000*
Larva	13.33	± 0.53	13.55	.845^{ns}	11	.000*
Pupa	4.28	±0.46	4.8	.000*	5.2	.000*
Egg incubation to Adult emergence	21.15	± 1.04	22.13	.000*	20.34	.000*

* highly significant difference; ns = not significant difference

Deduced Duration of CRB Larval Instars

Based on the number of head capsules and larvae of different instars retrieved weekly from uncaged fruits in one fruiting season (Table 3), it appears that three (3) batches of egg laying occurred and could be supported by the developing pummelo fruits. Further, the age of the rind influences the oviposition behavior of the moth because among the samples only one fruit (35-40 mm diam) collected on 28 DAFS had second instar larvae. Hence, moths laid eggs only on fruits around three weeks-old or with diameter of 25-35 mm or smaller. Figure 3 presents the deduced duration of CRB larval period based on the data reflected in Table 3. From egg hatching, it took 1 to less than 7 days before the first molting occurred, then approximately on the seventh day at most, another molting occurred. Thereafter, third and fourth moltings occurred before the 14th day and subsequently, the mature larva exited from the gall to pupate. An illustration (Figure 4) was constructed showing the developmental process of one complete life cycle of CRB deduced from the data on the durations of different stages of CRB in the field and the projected durations of larval instars.

Table 3. Number and stage of development (instar) of citrus rind borer *Prays endolemma* Diakonoff larvae, based on the number of head capsules counted inside the dissected pummelo fruit samples of different sizes collected from five trees in SODACO Farm, Brgy. Talandang, Calinan, Davao City from June 21 to July 19, 2010

NO. OF SAMPLE FRUITS	NUMBER OF LARVAE AT INDICATED LARVAL INSTARS (2ND TO 5TH) PER COLLECTION DATE																								
	7 DAFS					14 DAFS					21 DAFS					28 DAFS					35 DAFS				
	2nd	3rd	4th	5th	T	2nd	3rd	4th	5th	T	2nd	3rd	4th	5th	T	2nd	3rd	4th	5th	T	2nd	3rd	4th	5th	T
t 1-1	3						5					2	4				7							6	1
t 1-2	2							3				1	5						3	3				8	
t 1-3	1					9						2	8				9							5	
t 2-1		1				8						1	4				8							7	
t 2-2	2					6							6						8	4				7	2
t 2-3	4							2				2	8				10							9	
t 3-1	1						6					1	7						5	1				10	
t 3-2	2					8						2	9			5								5	6
t 3-3	3							4				3	7						7	1				5	
t 4-1	1						5					1	7				8							8	
t 4-2	4					5						1	9						3	2				9	2
t 4-3	1						6						10						5	3				10	
t 5-1	2					6						1	7				7							5	
t 5-2	1					7						2	8						7	3				5	3
t 5-3	3						7						5						8	2				7	4
Total	30	1				49	29	9				19	104			5	49		46	19				106	18
Fruit Size	10-15 mm					15-25 mm					25-35 mm					35-45 mm					50-55 mm				

approximately 14 days from entry of 1st instar to exit of mature larva

approximately 14 days from entry of 1st instar to exit of mature larva

approximately 14 days from entry of 1st instar to exit of mature larva

DAFS = days after fruit set
T = tunnels
t = tree

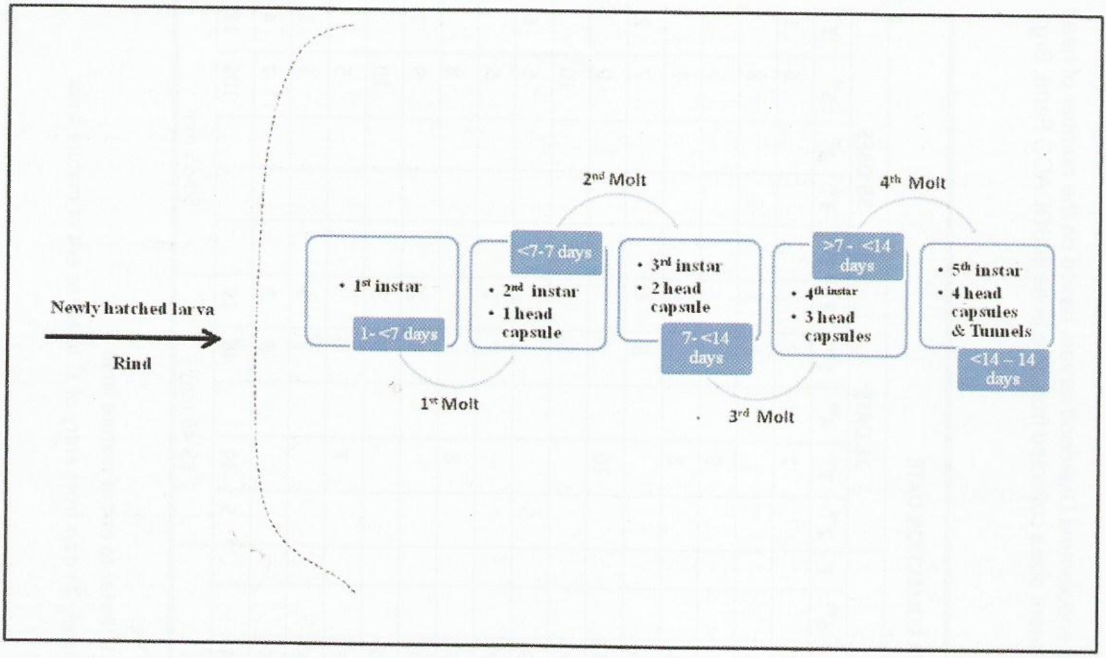

Figure 3. Deduced durations of larval instars of citrus rind borer *Prays endolemma* Diakonoff on pummelo fruits, extrapolated from the number and size of head capsules counted from the dissected damaged fruits collected from mid June to mid July of 2010.

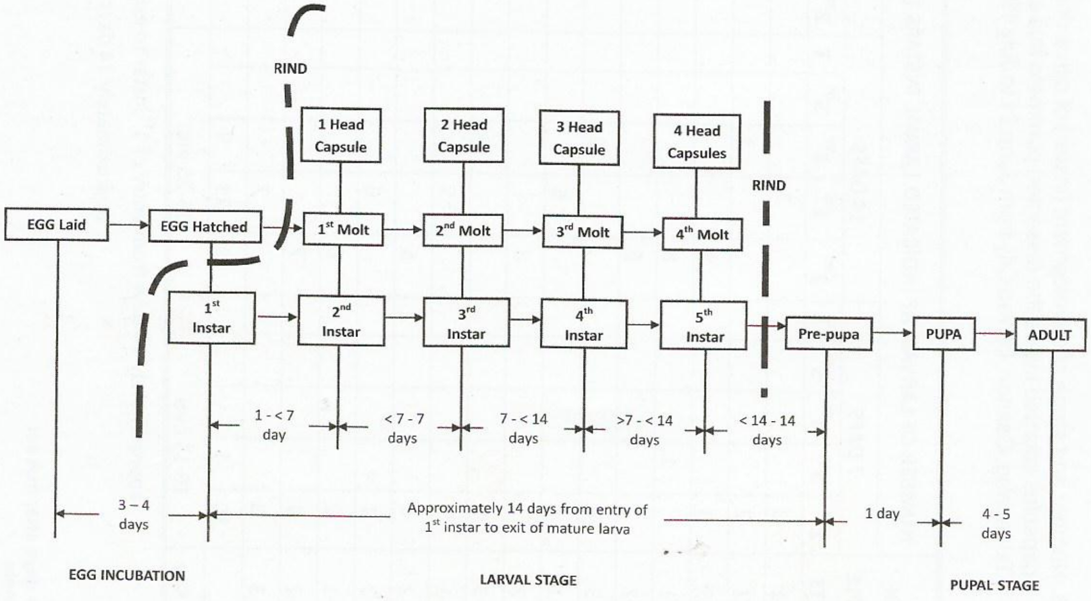

Figure 4. Illustration of the developmental process of one complete life cycle of the citrus rind borer *Prays endolemma* Diakonoff on pummelo fruits

Description and Habits of Different Developmental Stages

Egg. Dome-shaped, almost circular, cloudy white when newly laid to light brown when about to hatch, and measures 0.48 mm ±0.03 in diam. It is laid singly on the surface of the pummelo fruit as early as fruit setting (Figure 5) or on leaves adjacent to the flower bud. Despite careful examination, no eggs were observed on the flower bud itself. Egg shell becomes flaccid and transparent after the lava escapes after hatching.

The egg description by Garcia (1934) is similar to the present description while San Juan (1924) described it as sub-elliptical, slightly tapering towards one of the poles.

Larva. The newly-hatched larva penetrates into the rind and remains inside until fully mature. The young larva is light green with two brown markings on the dorsum of prothorax and four at the meso- and metathoracic segments and the head is brown (Figure 6). The third and fourth instar larvae look similar to young larvae but bigger. Before the fifth instar larva exits from the rind, thin red lines extending to the lateral margins of the tergum, are apparent from first thoracic to the 9^{th} abdominal segment. The head capsules (Figure 7) of the first (n=20), second (n=20), third (n=20) and fourth (n=20) instar larvae retrieved from the dissected damaged fruits measure an average of 0.22 ±0.02, 0.33 ± 0.02, 0.43 ± 0.01 and 0.64 ± 0.03 mm, respectively. The last-instar larva or prepupa (Figure 8), with an average length of 5.5 ± 0.51 (n=20), crawls out from the rind to pupate. Head is round to squarish, epicranial suture is depressed giving an overall slightly heart-shaped appearance. Body is roughly round in cross section, dark to light green dorsally and ventrally. Presence of brownish red lines extending to the lateral margins on either side on the abdominal tergites is distinct on CRB larva. As in the early stages, it bears four conspicuous dark brown markings, inner two are smaller, on each thoracic segment of the dorsum.

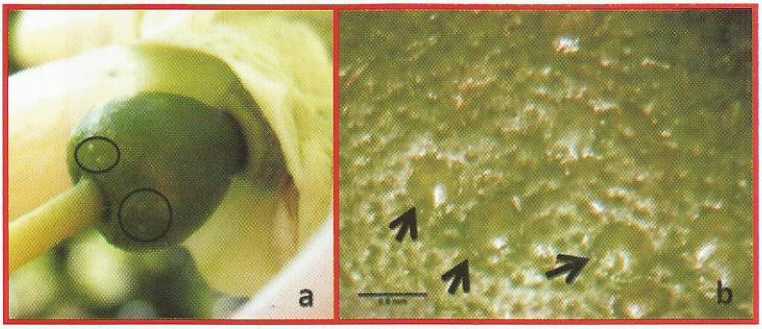

Figure 5. (a) Eggs (encircled) of the citrus rind borer *Prays endolemma* Diakonoff on the surface of 12 mm diam pummelo fruit; (b) Eggs on pummelo fruit (300X) at tips of arrows

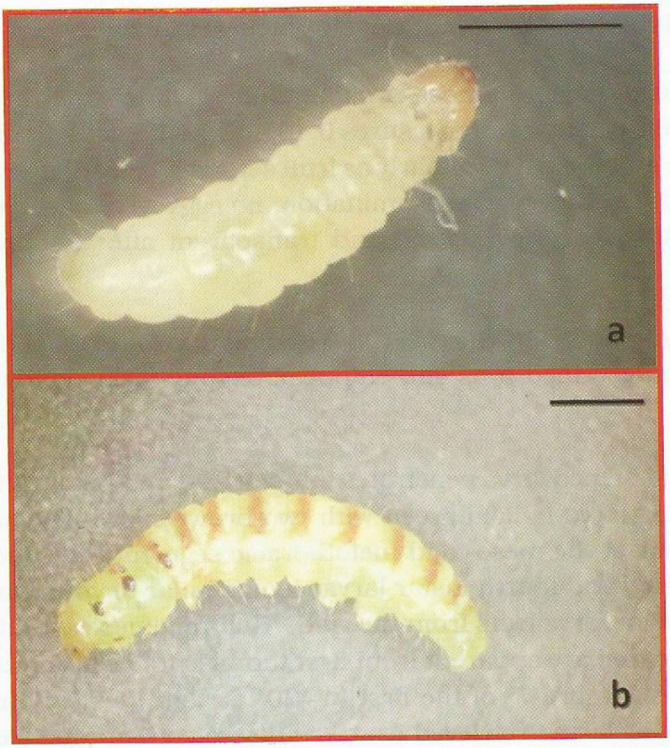

Figure 6. (a) Early instar larva of the citrus rind borer *Prays endolemma* Diakonoff, dorsal view (30x); (b) Fifth instar larva of *Prays endolemma* Diakonoff , lateral view (20 X) Scale Bar = 1 mm

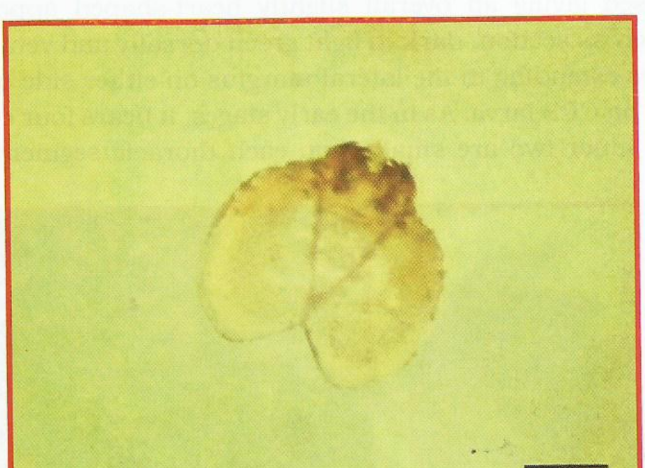

Figure 7. Dorsal view of a head capsule of an early instar larva of the citrus rind borer *Prays endolemma* Diakonoff (45X). Scale Bar = 1 mm.

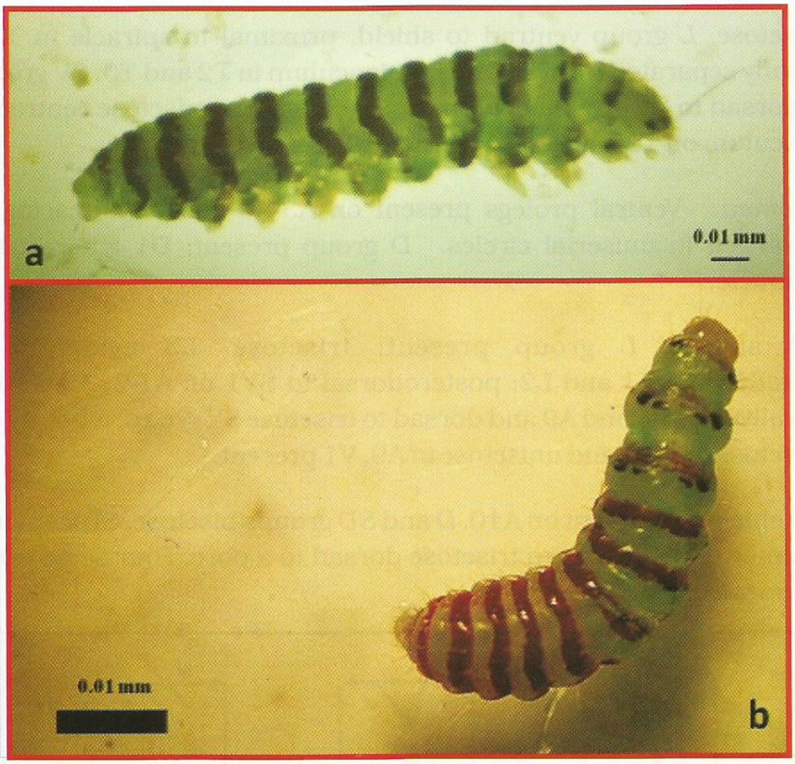

Figure 8. Last instar larva or prepupa of the citrus rind borer *Prays endolemma* Diakonoff (a) Lateral view (30X); (b) dorsal view (45X)

Peterson (1962) described the larva of most species of family Yponomeutidae to vary in color from a uniform light green to a deeply pigmented striped pattern.
Similar to the present description, San Juan (1924) also noticed the thin brownish red line extending to the lateral margins on either side of the abdominal tergites. However, he identified the species to belong to family Tineidae instead of Yponomeutidae. The mature larva is also described to bear a small subcircular dark chocolate spot on the lateral margin of the pro-, meso-, and metanota. Found in the mesonotum is a second elongate smaller marking located entally near the first on each lateral margin. Garcia (1934) also mentioned these brown markings on each thoracic segment but not the stripes on the tergites.

Figure 9 shows the schematic setal maps on the body of a mature larva of *P. endolemma* Diakonoff viewed under a stereo microscope.

Thorax. T1 shield and brown pinacula prominent; *XD* group present. *D* group distant on T1 and proximate in T2 and T3 pinaculum. D1 anterodorsad to D2. *SD* group and *D* group separated on pinaculum in T2 and T3; SD1 anterodorsad to SD2.

L group trisetose. *L* group ventrad to shield, proximal to spiracle in T1, while L3 posterodorsally separated from L1 and L2 pinaculum in T2 and T3. SV group bisetose; SV2 anterodorsad to SV1 on pinaculum in T1. *SV* group unisetose ventrad to *L* group without pinaculum on T2 and T3. V1 present; ventrad to coxa.

Abdomen. Ventral prolegs present on A3-A6 and A10 bearing uniordinal crochets arranged in uniserial circles. *D* group present; D1 anterior to D2. SD1 dorsad to spiracle,

SD2 absent. *L* group present; trisetose. L3 evidently separated posteroventrally from L1 and L2; posterodorsal to SV1 on A1-2, A7-8 while slightly distant ventrally on A3-6 and A9 and dorsad to trisetose SV group. A1-2, A7-8 *SV* group bisetose; trisetose in A3-6 and unisetose in A9. V1 present.

Anal shield prominent on A10. *D* and *SD* groups bisetose. SD2 anteroventral to SD1 approximate to D2. *L* group trisetose dorsad to a pore. Four setae present on *SV* group. V1 present.

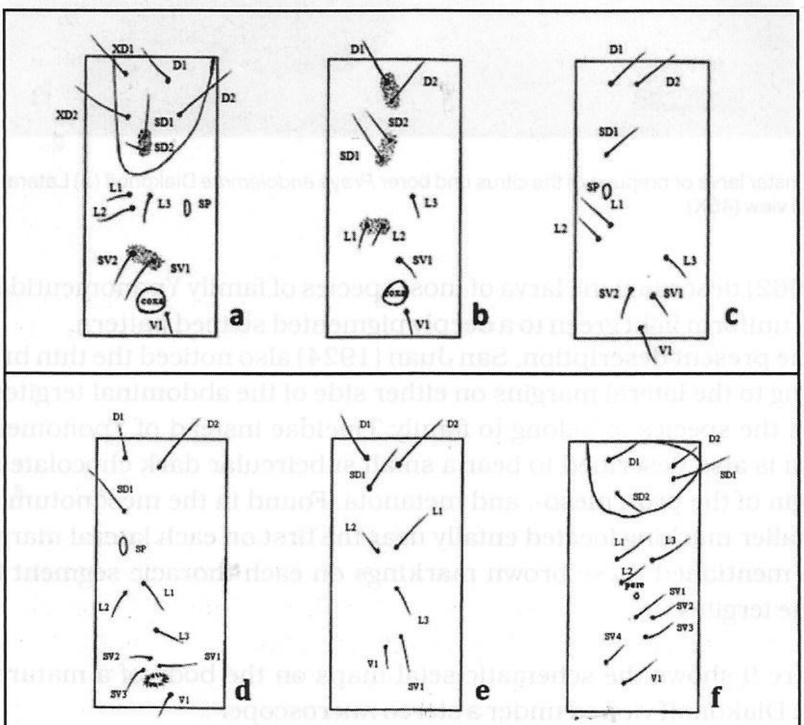

Figure 9. A schematic setal map of the last instar larva of the citrus rind borer *Prays endolemma* Diakonoff, lateral left view (a) Prothorax; (b) Meso- and Metathorax; (c) Abdominal segments 1, 2 and 7, 8; (d) Abdominal segments 3 to 6; (e) Abdominal segment 9; (f) Abdominal segment 10.

These characteristics are indeed attributed to family Yponomeutidae based on the descriptions of this family by Peterson (1962). Some of these characteristics were stated this way "On the prothorax seta beta is below alpha. The kappa (prespiracular) group is trisetose and in some species not located on a sclerotized area distinctly separated from the cervical shield. On the abdominal segments kappa and eta are well separated (a few exception). Prolegs bearing uniordinal or biordinal crochets arranged in circles that are uniserial, biserial, or triserial (rarely multiserial)".

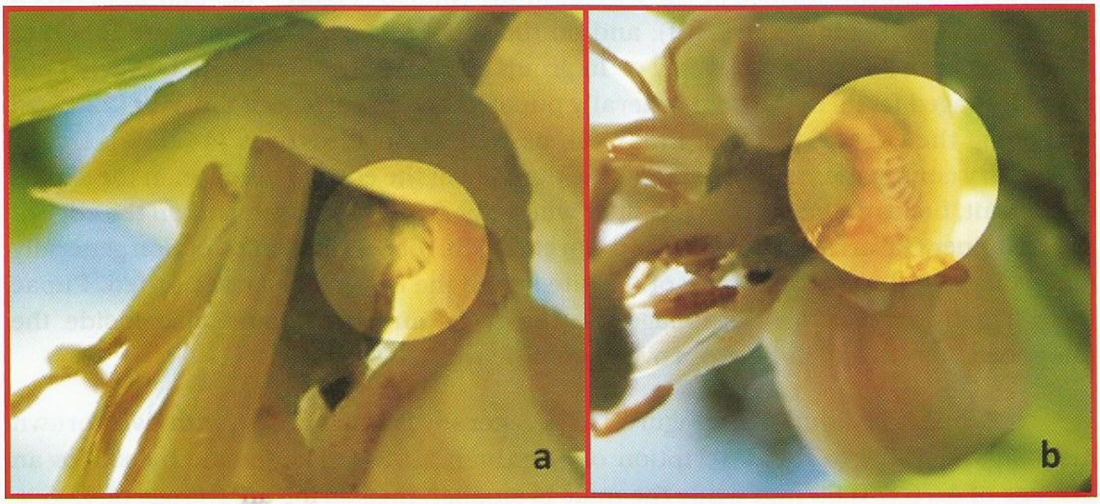

Figure 10. (a) Newly open flowers showing last-instar citrus rind borer larva (encircled) outside of the damaged developing fruit. (b) Citrus rind borer larva (encircled) on the sepal of the damaged fruit, about to pupate.

Figure 10 shows a last-instar CRB larva that came out from a developing fruit during dehiscence. When ready to pupate, mature CRB larva crawls out from the fruit and moves to nearby area to pupate. Majority of the last-instar larvae observed exited from 11:00 in the morning to 1:00 in the afternoon (n=15), in contrast to the report of Gavarra et al. (1990) that majority of larvae exited from the rind between 18:00 p.m - 1:00 a.m under laboratory conditions. As such, he concluded that light inhibits favorable emergence of the last- instar larva from infested fruits. Pupation occurred an hour after the larva exited by producing silk-like thread to fasten itself on the surface wherever it suited to pupate and finally formed the lacelike, large-meshed cocoon.

Pupa. Slightly robust, widest at thorax, tapering towards the tip of abdomen, without markings (Figure 11). It measures 4.5 ± 0.5 mm. It is light green to green in color (Figure 12) initially, gradually turning brown as it nears adult emergence, enclosed in a lacelike, large-meshed cocoon, a characteristic common to Yponomeutidae such as *Prays* spp. and similar to *Plutella xylostella* of the family Plutellidae (Landry 2007). The cocoon also changes from white to light brown. The same descriptions were given by San Juan (1924) and Garcia (1934).

In the field, they are found to pupate on the trunk of the tree where damaged fruits hang close by (Figure 13a), in-between peduncles of the inflorescence and fruits (Figure 13b), on the surface of the leaves, in the midrib (Figure 13e) and in-between petiole and leaf blade (Figure 13f), and on the sepal (Figure 13c). They are found to pupate in any of these areas singly or in group (Figure 13d). Larvae that crawled out from the caged fruits in the field generally pupated on the surface of the fruit (22.37%) and along the edge of the cage (33.55%), as well as on the leaves (44.08%). Under laboratory confinement, last-instar larvae pupated at the edge of the cage, in the sepals of the fruit, beneath the plastic container where the fruit was placed and on the edge of the container.Gavarra et al. (1990) and Garcia (1939) observed that pupation generally takes place within the proximity of the site where the last-instar larvae exited. Pupae were usually formed on the foliage, on the twigs and fruits, and also inside the unopened flowers and in-between overlapping leaves.

Adult. The adult CRB (Figure 14) is generally grayish brown with dark brown markings, parallel to the description of San Juan (1924). The female measures an average of 4.75 ± 0.69 mm while the male is smaller, measuring an average of 4.05 ± 0.605 mm (n=20).

The CRB adults emerge from the pupal case 4 to 5 days after pupation, from 9:00 in the morning until 1:00 in the afternoon (n=31) and observed both in the field and laboratory to have minimal movement after emergence (Figure 15). They stay inactive during day time and become active starting 5:00 in the afternoon until daybreak. They are found to land on the flower, fruits and nearby branches where, oftentimes, they remain unnoticed because their color is hardly distinguishable from that of the branch. Caged adults are most active when the lights are off. It is generally explained that most moths are nocturnal or inactive during the daytime but active at night, or crepuscular, primarily active during the twilight (Animal Corner 2003). The same observation was recorded by San Juan (1923) and Gavarra et al. (1990)
.

After emergence, adults were allowed to mate before they were segregated for oviposition preference test and other observation since it was difficult to determine visually the sex without damaging the wings. In the laboratory, copulation (Figure 16) was observed to occur from 6:00 o'clock in the evening until 7:00 o'clock in the

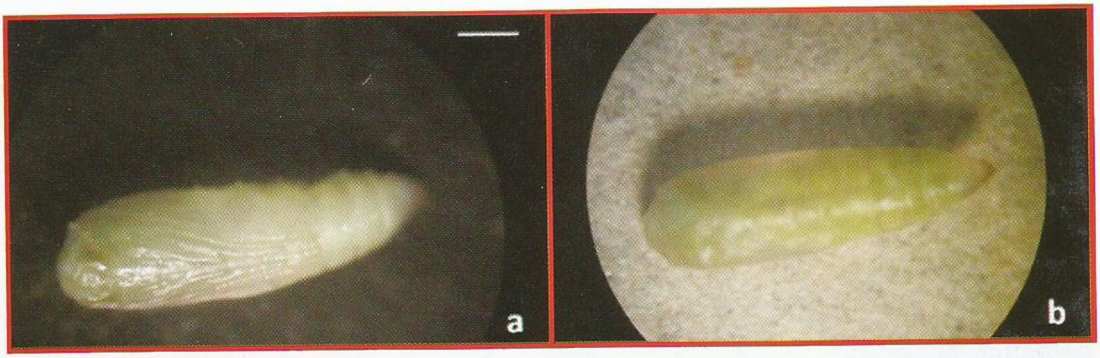

Figure 11. Pupa of the citrus rind borer *Prays endolemma* Diakonoff, (a) Ventral view (b) Dorsal view (200X). Scale Bar = 1 mm

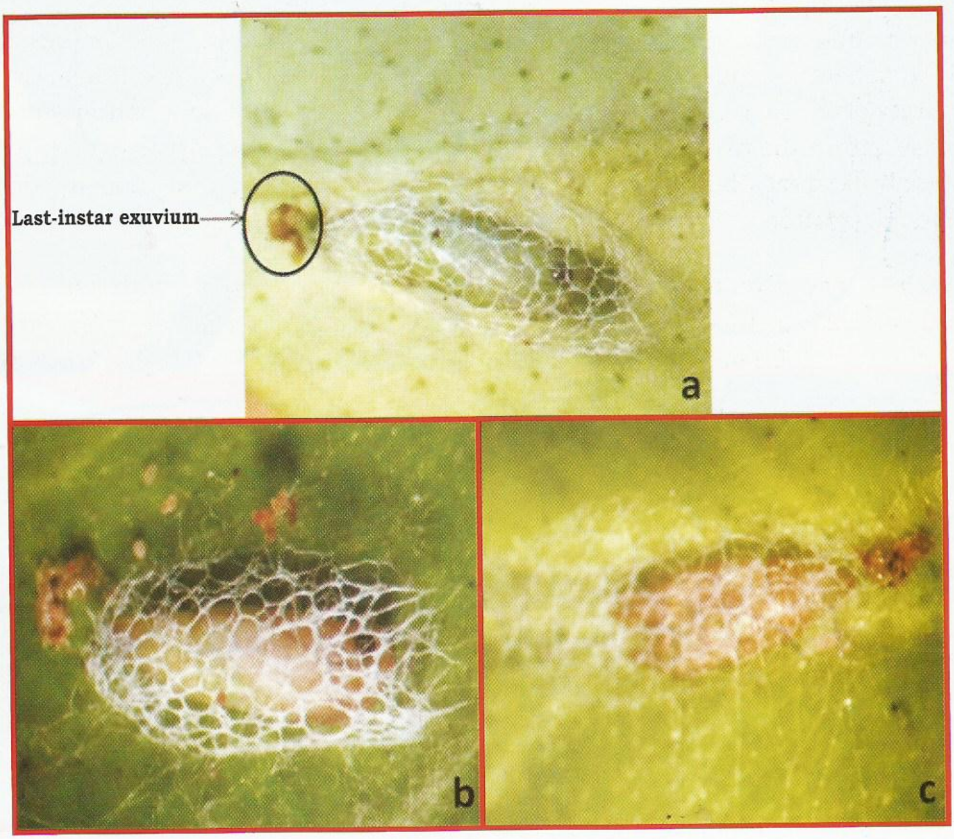

Figure 12. Pupa and pupal case of the citrus rind borer *Prays endolemma* Diakonoff. (a) Early stage, greenish pupaenclosed in lacelike, large-meshed cocoon with the last-instar exuvia found at the posterior of the pupal abdomen. (b) Late stage, brown pupa with case (c) Pupal case and cocoon after emergence of adult. (under 100X)

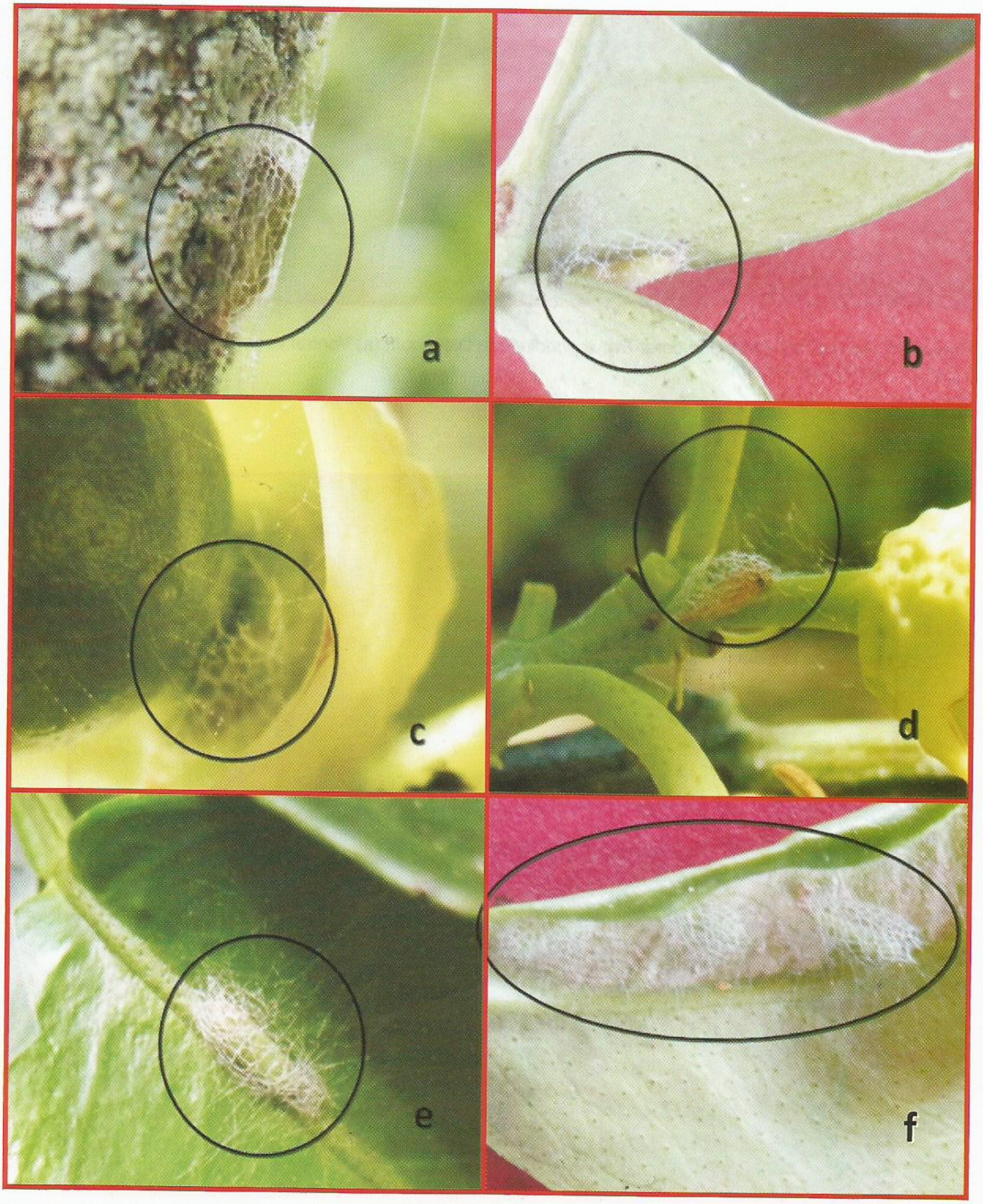

Figure 13. Different locations where the citrus rind borer *Prays endolemma* Diakonoff pupae are found: (a) on the trunk of the pummel tree (b) in-between peduncles of the fruits (c) on the fruit sepal (d) between petioles (e) on leaf midrib, and (f) on leaf surface.

Figure 14. Citrus rind borer *Prays endolemma* Diakonoff adult female (30X).

Scale Bar = 1 mm

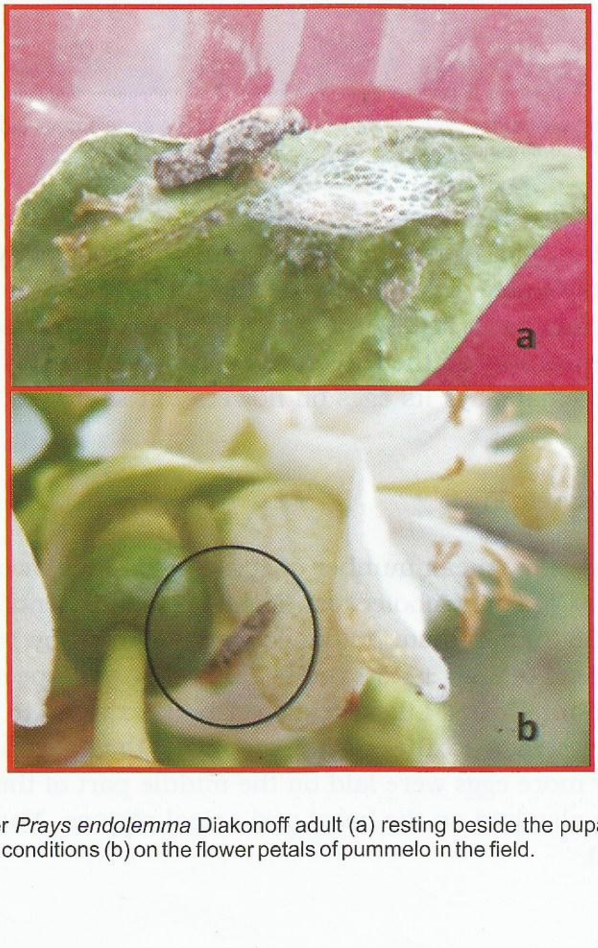

Figure 15. Citrus rind borer *Prays endolemma* Diakonoff adult (a) resting beside the pupal case after emergence under laboratory conditions (b) on the flower petals of pummelo in the field.

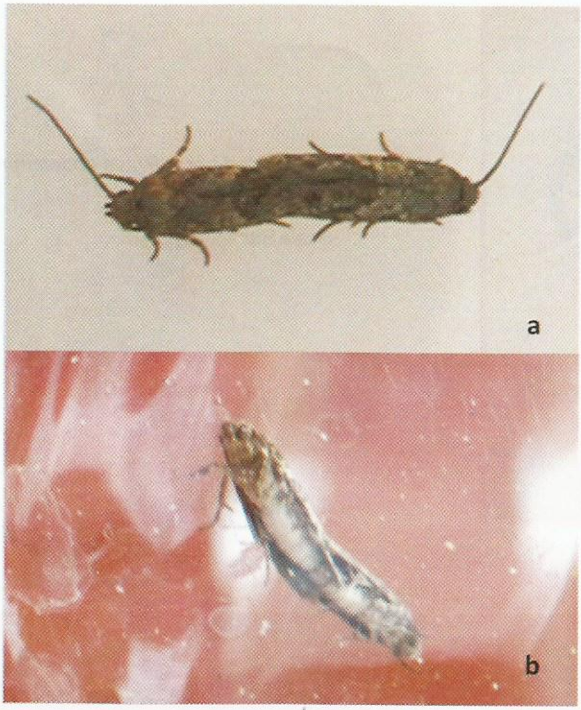

Figure 16. Dorsal (a) and ventral (b) views of citrus rind borer Prays endolemma Diakonoff adults in copulation under laboratory confinement.

morning 1-2 days after emergence, the activity lasting for an average of 1 hour and 45 minutes (n=10 pairs). This observation is parallel to that of Godoy and Fuentes (1992) wherein copulation occurred during the period 6:00 o'clock in the evening to 5:00 o'clock in the morning, each lasting for 1 to 1.5 hour. The female laid eggs two days after copulation, preferably on the fruit surface. However, Godoy and Fuentes (1992) also reported that female lays its eggs on flowers but such was not observed in the present study.

Egg Laying on Pummelo Fruit

Table 4 shows the mean number of eggs laid on the flower buds and fruits of pummelo by female CRB in no choice test. The highest number of eggs (62.9 ± 11.64) was deposited on 25-30 mm diam pummelo fruits followed by 15-20 mm and 10-12 mm diam with a mean of 25.3 ±8.51 and 9.7 ±2.54 eggs, respectively. No eggs were deposited on 35-45 mm fruits. Likewise, flower buds exposed to CRB for oviposition had no deposited eggs. Instead, the eggs were found on the leaves adjacent to the flower buds. Significantly more eggs were laid on the middle part of the fruit, regardless of size, but eggs were also laid on the basal and apical regions. None was found on the receptacle (Table 5).

which suggests that inner gland cells are modified into boundary cells. This finding is parallel to those in other previous works cited by Knight et al. (2001) on other citrus species e.g. Eureka lemon (Ford1942) and Valencia orange (Bain 1958). Boundary cells apparently render the rind thicker and mature, making it more difficult for young larvae to bore in. It was also observed in the field that within one month from fruit set until harvest, new lumps do not develop as an indicator of CRB damage.

The same was observed in the study conducted by San Juan (1923), where eggs were commonly laid singly on young fruits and occasionally on flowers while Gavarra et al. (1990) observed that majority of the eggs were laid on ovaries of the newly opened flowers and the remaining were distributed on the style and sepal but seldom on the peduncle and floral disc.

The present data also suggest that color may play a role in the oviposition behavior of CRB. The eggs were located on the green fruits and leaves adjacent to the flower bud but not on the creamy white bud. However, portions of the flower buds fed on by neonate larvae later appeared as holes on the petals. On the other hand, eggs were observed on leaves adjacent to the flower buds. With this, it could be possible that eggs were laid on the leaves and when they hatched, the first-instar larvae moved to the nearby flower buds, then penetrated inside. Color influences insect oviposition as cited by Thompson et al. (1991). For instance, the drumming and egg-laying of *Pieris brassicae* are elicited by specific light wavelengths, implying either wavelength-specific behavior or possibly color vision (Scherer et al. 1987). Since eggs were actually observed on leaves and fruit surfaces, which are both with shades of green, this suggests that CRB female is attracted to color green and prefers to oviposit on green surfaces. Another factor could be the surface of the flower bud. It is soft and with ridges while leaf and fruit surfaces, generally glabrous and firm which most likely could affect the landing of the adult and its stability while ovipositing.

Part of the experiment was dissection of damaged flower buds and fruits. It was found that only one larva infested each damaged flower bud, unlike pummelo fruits from which as many as 11 larvae were collected from one fruit. It could be due probably to the quantity of food available for the larva on each flower bud which is much less compared to that in a fruit.

Adult Longevity

Results showed (Table 6) that 20% honey extended significantly the life span of CRB adults compared to those fed with 10% honey or water alone. Honey is composed mainly of sugars (carbohydrates), water and a great number of minor components. Sugars comprised about 95 % of honey dry weight (Bogdanov 2009). The result showed that the higher the concentration of honey, the longer it can sustain life. Two

The data suggest that CRB prefers to lay eggs on smaller and younger fruits (<35 mm, 7 to 14 days after fruit set) and on the ovary of the newly opened flower. This could possibly be due to the presence of oil glands in the rind. Knight et al. (2001) found that gland formation on orange, *Citrus sinensis* (L.) Osbeck was restricted to early stages of fruit development and the gland matures with the fruit. A mature gland consists of flattened epithelial cells increasing in thickness towards the perimeter of the gland

Table 4. Mean number of eggs laid by citrus rind borer *Prays endolemma* Diakonoff on pummelo fruits in different developmental stages and sizes

FRUIT STAGES/SIZES	MEANSTD. DEV.		AGE OF FRUIT
Flower Bud	0 D	0	
10 - 12 mm fruits	9.700 C±2.54		1 – 4 DAFS
15 - 20 mm fruits	25.300 B±8.51		5 – 8 DAFS
25 - 30 mm fruits	62.900 A±11.64		9 – 14 DAFS
35 - 45 mm fruits	0 D	0	14 – 17 DAFS

Means with a common letter in a column are not significantly different at 0.05% level using HSD.
DAFS = Days after fruit set; n = 10 Female; HSD=8.3201

Table 5. Mean number (±) and locations of eggs laid by citrus rind borer *Prays endolemma* Diakonoff female adults on young pummelo fruits of different sizes

PART OF THE FRUIT WHERE EGGS WERE LAID	Size of fruit and number of eggs laid					
	10-12 mm		-15-20mm		-25-30mm	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Receptacle	0 B	0	0 C	0	0 C	0
Lower	1.4 B	± 1.43	4.5 B	± 1.78	5 C	± 1.94
Middle	6.7 A	± 2.58	13.6 A	± 4.72	39.9 A	± 6.69
Upper	2.67 B	± 1.03	7.2 B	± 3.12	18 B	± 9.44
	LSD = 2.0164		LSD = 3.7151		LSD = 7.0657	

Means with a common letter in a column are not significantly different at 0.05% level using LSD.

other studies showed the positive effect of sugars on the longevity of CRB adults. San Juan (1929) observed that CRB adults live as long as 18 days when fed with diluted

Table 6. Longevity of citrus rind borer *Prays endolemma* Diakonoff adults fed with water alone, 10% honey and 20% honey in the laboratory (n=20)

FOOD OFFERED	SURVIVAL RANGE(in days)	MEAN	STD. DEV.
Water	3 to 9	6.7 A	± 2.57
10% Honey	1 to 12	8.85 A	± 3.93
20% Honey	4 to 17	12.35 B	± 4.12

HSD=2.748
Means with a common letter in a column are not significantly different at 0.05% level using HSD.

Adult Genitalia

Using the genitalia, Diakonoff (1960) described *Prays endolemma* n. s.p. as an endemic species in the Philippines. To confirm the identity of CRB attacking pummelo in Region XI, a total of 356 adults were dissected, 97 of which were field collected while 259 were laboratory-reared and consisting of 195 females and 161 males. Based on the genitalia of all dissected adults, the pummelo CRB belonged to only one species, *Prays endolemma* Diakonoff. The different parts of male and female genitalia of CRB are shown in Figures 17 to 20. The eight and ninth segments are sclerotized and conical.

***Male Genitalia* (n=5).** The valva measures an average of .58 mm ± .13, the sacculus 0.26 mm ± 0.09, projection exceeding cucullus has a non-clavate topbut with a short (.22 mm ± .11) and sclerotized ventral process, Saccus is long (0.4 mm ±0.07) and Anellus with slightly broader lobes (0.4 mm ± 0.10) (Figure 17).

The tegumen is trapezoidal, top emarginated (uncus absent). Socii large with an average width of 0.24 mm ± 0.05, rounded bodies, each with a spiraled porrect projection crowned with four curved thorns of diverse size. Vinculum is characterized by an inverted W-shaped part dorsally which measures an average of .54 mm ± 0.05 and a ventral part T-shaped with an average length of 0.34 mm± 0.05, the stalk being the slender saccus measuring 0.40 mm ± 0.07. Valva is bipartite and sacculus is half

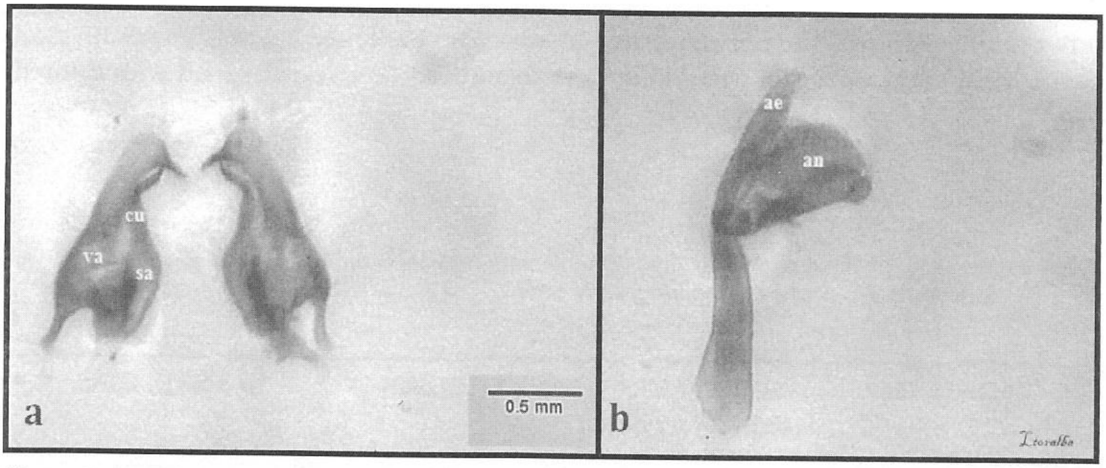

Figure 17. Male genitalia of the citrus rind borer *Prays endolemma* Diakonoff: (a) *va*=Valva with *sac*= Sacculus and *cu*= Cucullus (b) Broad *an*= Anellus supporting *ae*=Aedeagus.

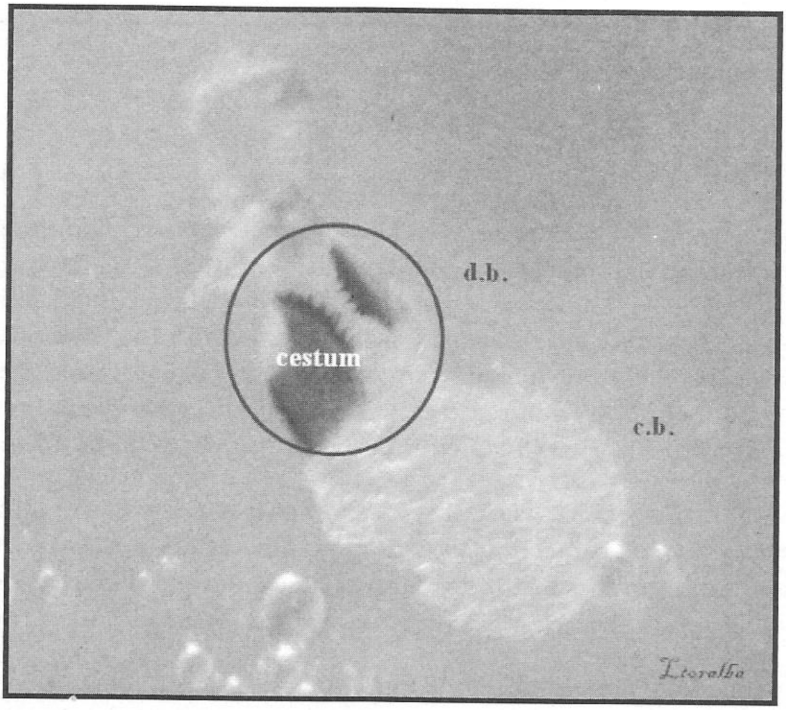

Figure 18. Ventral view of a detached part of female genitalia of citrus rind borer *Prays endolemma* Diakonoff showing the pair of *cestum* in the ductus bursae (d.b), and thecorpus bursae(c.b.) (under 45X)

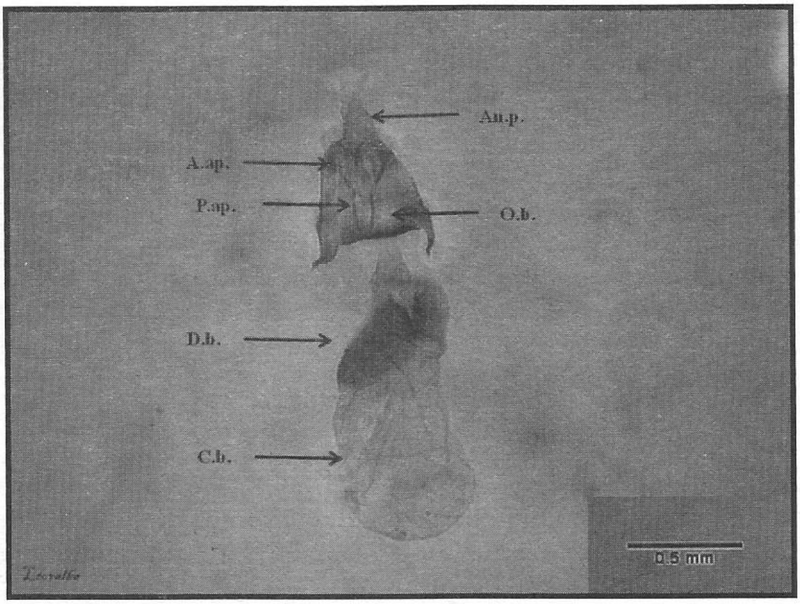

Figure 19. Ventral view of intact female genitalia of citrus rind borer *Prays endolemma* Diakonoff. *An.p*= Anal papillae, *A.ap*=Anterior apophysis, *P.ap*= Posterior apophysis, *O.b*= Ostium bursae, *D.b*= Ductus bursae, *C.b*= Corpus bursae.

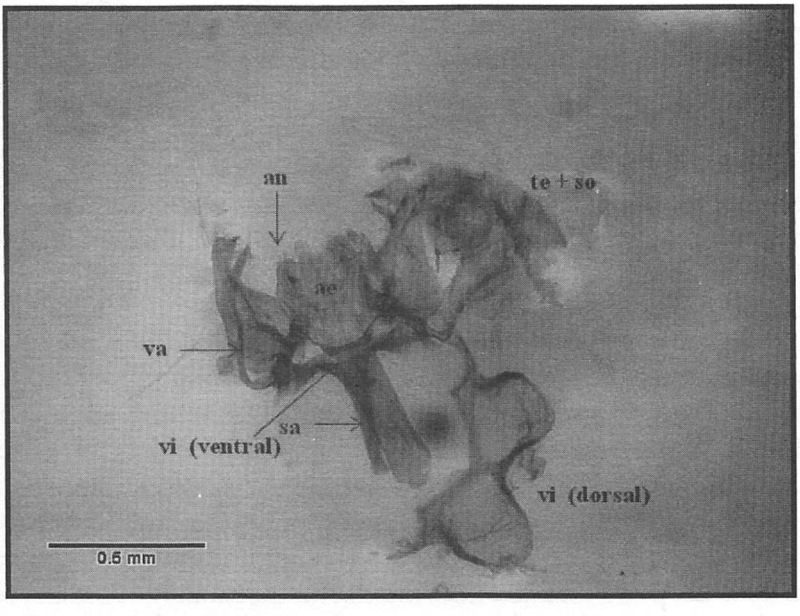

Figure 20. Dorsal view of male genitalia of the citrus rind borer *Prays endolemma* Diakonoff adult includes; *ae = aedaegus, sa = saccus, va = valva, an = anellus,* detached *vi= vinculum (dorsal & ventral view), te+so= tegumen with socii.*

shorter than valve, with an average difference of 0.58 mm ± 0.13, ending in sinuate acute process not reaching top of cucullus. While cucullus is part slender, top rounded, clavate and finely haired with an average length of 0.22 mm± 0.11, anellus is an elongate, hairy sclerite supporting aedeagus from below, top is emarginated with lateral lobes rounded and finely aciculate. Aedeagus is slender, slightly curved with an average length of 0.94 mm ± 0.23. Cornuti, apparently with one long (0.66 mm ± 0.09) and two short spines with dilated bases.

Female Genitalia **(n=5).** Ductus bursae with short tubular upper third (.18 mm ± 0.08) and sclerotized wide (.62 mm ± .19) lower part; consisted of two broad sclerites (cestum), each bearing a dentate ridge (Figure 18) and appearing like a husk (lemma).

Anapophyses are short, with an average length of 0.18 mm ± 0.08, curved and flattened, while postapophyses are slender with an average length of .42 mm ± 0.11. Ostium bursae are slender and cup-shaped. On top of this conical segment are two denticulate flattened lobes or folds. Corpus bursae are simple and weak.

SUMMARY, CONCLUSION AND RECOMMENDATION

The study on citrus rind borer (CRB), *Prays endolemma* Diakonoff, was conducted at SODACO Farm from May to November 2010, with the following objectives; (1) to confirm the identity of the CRB attacking pummelo in Region XI; (2) to document the field biology of CRB on pummelo and (3) to describe the different developmental stages. The dissected female genitalia (n=171) showed a sclerotized wide lower part of the ductus bursae forming two broad sclerites (cestum), each bearing a dentate ridge. This is characteristic of the species. Thus, the CRB on pummelo in SODACO Farm, Davao City and most likely in other areas of Region XI is *Prays endolemma* Diakonoff.

In the field, the total duration from egg laying until adult emergence of CRB is 20 to 23 days. The mean duration of each developmental stage is 3.47, 13.33, 4.28 and 21.15 days for egg, larva, pupa and adult, respectively. Larva is the destructive stage of the insect. It feeds on the tissues beneath the exocarp, particularly on the 'flavedo'. The larva undergoes five instars before it crawls out of the rind to pupate. The duration of each instar was not determined precisely due to the long interval between sampling. It is recommended that collection and destructive sampling of damaged fruits must be conducted daily to get more detailed information on the biology of the larvae inside the fruit.

The fifth instar larva is distinct from the younger ones in having a prominent pattern of red stripes on the abdominal tergites extending to the lateral margins. Most of them pupate on the sepal, nearby leaves or trunk of the host tree. Adults remain inactive during daytime and become active starting 5:00 o'clock in the afternoon. The eggs and pupae are the most exposed to the environment, including natural enemies. The eggs are laid on fruit, starting at fruit set onwards until the fruit is almost 35 mm diam. Majority of the eggs are laid on the equatorial part of the fruits, hatching after 3 to 4 days. Thus, in any CRB management strategy like chemical or biological control, this stage is the most logical target. For instance, the egg parasitoid *Trichogramma* spp. could be explored for possible introduction in pummelo fields. A pupal parasitoid was reported in earlier studies but the present study was unable to rear out this parasitoid from the pupae collected from the field.

Based on the genitalia of all dissected female moths, the pummelo CRB belongs to only one species, *Prays endolemma* Diakonoff.

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