

LABORATORY BIOLOGY OF AN IMMIGRANT ASIAN MOTH,
PARAPOYNX DIMINUTALIS (LEPIDOPTERA: PYRALIDAE),
ON *HYDRILLA VERTICILLATA* (HYDROCHARITACEAE)

GARY R. BUCKINGHAM¹ AND CHRISTINE A. BENNETT²

¹Agricultural Research Service

U.S. Department of Agriculture

Florida Biological Control Laboratory

Gainesville, FL 32614-7100

²Department of Entomology and Nematology

Institute of Food and Agricultural Sciences

University of Florida, Gainesville, FL 32611

ABSTRACT

The Asian moth *Parapoynx diminutalis* Snellen is an immigrant in Florida and Panama where it attacks hydrilla, *Hydrilla verticillata* (L. fil.) Royle, an immigrant submersed weed from Asia. Field populations of *P. diminutalis* are occasionally heavy on hydrilla but are rarely found on other plant species, including those that are laboratory hosts. Larvae build portable cases from which they feed on leaves and stems. The 7 instars can be differentiated by head capsule widths. Measurements are presented of other immature stages. In the laboratory at 26.7°C, eggs developed in 4-6 d, larvae in 21-35 d, prepupae in 1-2 d, and pupae in 6-7 d. Adults lived 3-5 d at 24.4°C.

Key Words: Biological control, fecundity, degree-days, host plants, *Bacillus thuringiensis*

RESUMEN

La polilla asiática, *Parapoynx diminutalis* Snellen es un inmigrante de la Florida y Panamá donde ataca a *Hydrilla verticillata* (L. fil.) Royle, una hierba sumergida inmigrante de Asia. Las poblaciones de campo de *P. diminutalis* son ocasionalmente altas en *H. verticillata* pero raramente son encontradas en otras especies de plantas, incluidas las que son hospedantes de laboratorio. Las larvas fabrican una funda portátil desde la cual se alimentan de las hojas y los tallos. Los siete instares pueden ser diferenciados mediante el ancho de la cápsula cefálica. Las medidas de los estadios inmaduros son presentadas. En el laboratorio, a 26.7°C, los huevos se desarrollaron en 4-6 días, las larvas en 21-35 días, las prepupas en 1-2 días y las pupas en 6-7 días. Los adultos vivieron 3-5 días a 24.4°C.

Parapoynx diminutalis Snellen is an immigrant Asian moth that attacks the submersed aquatic plant hydrilla, *Hydrilla verticillata* (L. fil.) Royle, in Florida. Hydrilla, also an Asian immigrant, is the state's most important aquatic weed and is found throughout much of the Southern USA and along the East Coast to Maryland (Langeland 1990). The native range of hydrilla includes Asia, Australia, the Rift Valley area of Africa, and Europe, where only relict populations occur. The moth was first reported in Florida in 1976, when it was discovered in hydrilla plantings at a research station in Ft. Lauderdale (Del Fosse et al. 1976). Subsequently, it was found in north Florida

(Balciunas & Habeck 1981) and in Panama, where it infested hydrilla in the Panama Canal. It has also been reported colonizing glasshouses at aquatic plant nurseries in England and Denmark (Agassiz 1978, 1981). Its native range includes much of Asia from Afghanistan to the Philippines and north to Shanghai, People's Republic of China, Africa from Ethiopia to South Africa (Speidel 1984) and Australia (Yoshiyasu 1992). Prior to its discovery in Florida, *P. diminutalis* was the subject of a study in Pakistan to determine its potential for introduction into the United States for biological control of hydrilla (Baloch & Sana-Ullah 1974).

Baloch & Sana-Ullah (1974) presented a brief description of the biology along with results of preliminary laboratory host range tests. Additional biological studies, apparently with this species, were reported by Varghese & Singh (1976) in Malaysia in support of the USA biological control program. They referred to their subject as *Nymphula* sp. in the text but as *N. diminutalis* in the table headings. *Nymphula diminutalis* is a synonym of *P. diminutalis*. Chantaraprapha & Litsinger (1986) included a table with life history data for *P. diminutalis* in their study of the host range in the Philippines.

We originally began studies with *P. diminutalis* collected in Panama and held in quarantine at the Florida Biological Control Laboratory, Division of Plant Industry (DPI), Florida Department of Agriculture and Consumer Services in Gainesville, FL. It was thought to be a little known Neotropical species, *P. rugosalis* Moschler. Balciunas & Center (1981) attempted to study *P. rugosalis* in Panama before its introduction into our quarantine and reported results of tests with larvae of *Parapoynx* sp. The species tested by them was probably not *P. rugosalis* but rather a third, as yet unidentified species. Three subsequent attempts by researchers, including one of us (CAB), to collect and carry to our quarantine laboratory the unidentified *Parapoynx* sp. yielded only *P. diminutalis*. We continued our studies after determining the correct identity of our material in order to better document the laboratory biology and host range of this interesting species. We report here the results of our biological studies. Our host range studies have been reported elsewhere (Buckingham & Bennett 1989).

MATERIALS AND METHODS

All experiments were conducted in the quarantine facility at the Florida Biological Control Laboratory from 1980 to 1982. Hydrilla, the laboratory host plant, was collected periodically from several sites in Levy and Alachua counties or was grown in outdoor pools at the laboratory. The majority of test insects were from a colony established from eggs and larvae collected in November 1980 in the Rio Chagres near Gamboa, Panama, by J. K. Balciunas. Additional insects were collected at Orange and Lochloosa Lakes near Gainesville. Adult and larval specimens have been deposited in the Florida State Collection of Arthropods, DPI, Gainesville and in the National Museum of Natural History, Washington, D.C. The rearing colony was maintained in a quarantine greenhouse in a large wooden cage described by Buckingham & Bennett (1989). Larvae were also reared in 3.8 liter jars filled with water and hydrilla and capped with nylon organdy. The jars were held either in a greenhouse (12.2-31.1°C, 50-90% RH) or in a temperature- and humidity-controlled room (25 ± 1°C, 50-60% RH). Both had fluorescent lights on 16 h photophase. Plants and water were added when necessary. The rearing material was transferred at least weekly to new jars sterilized with bleach.

Biological Studies

All studies were conducted in environmental chambers at 26.7°C, 16 h photophase, unless otherwise indicated. Individual larvae on hydrilla were confined after hatching

in small plastic cups, 29.6 ml, with plastic lids. These small cups were used in many of the studies. The cups were examined daily for shed head capsules to determine the number and the durations of the stadia. Head capsule measurements were made with larvae preserved in 75% isopropyl alcohol. All measurements were made with a stereomicroscope and an ocular micrometer and are reported as follows: mean \pm standard deviation (range, number).

Neonate mortality outside of water on both dry and moist filter paper was tested by placing single larvae (n=30) without hydrilla in small plastic cups with lids. Neonate mortality in aerated water was tested in small plastic cups which had organdy glued over holes in the bottoms (n=20). The cups sat partially submerged on gravel in water-filled, aerated pans. These were compared with water-filled cups without holes (n=50). The neonates were tested at $22.2 \pm 1^\circ\text{C}$, and those in water were provided with hydrilla.

Most larval observations were made in small plastic cups, in 266 ml styrofoam cups, or in 0.95 liter or 3.8 liter jars. Hydrilla and water were added as needed. Larval and larval-pupal development times were determined at various temperatures in environmental chambers with 16 h photophases.

Pupal development times were determined by observing pupae removed from cocoons and held on moist cotton in small plastic cups or in plastic petri dishes.

Newly emerged females were confined individually with 2 newly emerged males in plexiglass cylinders (42 cm high, 14.5 cm ID) to determine longevity and realized fecundity, the number of eggs laid. We did not record matings, and deaths were recorded daily. Small amounts of water and hydrilla were placed in the bottom of each cylinder, which was capped with nylon organdy. Some females were dissected at death to determine the number of eggs remaining in the ovaries.

RESULTS AND DISCUSSION

Egg Stage

Description. Dorsoventrally compressed; outline circular at deposition, elliptical at maturity; chorion appears smooth at 50 \times ; bright yellow at deposition changing to whitish and then transparent at hatching; length and width of mature egg presented in Table 1. The height of one detached mature egg was 0.26 mm.

Development. Eggs were deposited in various size masses on leaves or stems of plants lying at the water surface, on moist filter paper in petri dishes, and on the sides of plexiglass cylinders at the waterline. The egg masses on hydrilla (Table 1) were generally smaller than masses placed on filter paper. Eggs were arranged in uneven rows within a mass. Most of the developing larvae were oriented in the same direction; however, often the larvae in one row, or portions of a row, were oriented in the opposite direction. Larval head position was initially indicated by two small dark eyespots; later the head was light brown with dark mandible tips. The curled embryo developed lying on its side, but within a few hours prior to hatching it actively moved within the egg. The development time is presented in Table 1. Usually all or most eggs in a mass hatched on the same day. Normal egg development occurred both underwater and outside of water on moist filter paper.

Larval Stage

Description. Seven (I-VII) instars: I - whitish almost transparent; no tracheal gills; orange malpighian tubules noticeable; one pair of long anal setae (0.20-0.22mm);

smaller setae in longitudinal rows dorsally and along each side of abdomen; head capsule light brown with dark brown ocelli and epicranial suture; unspotted; mandibles reddish; light brown pronotal shield about equal to width of head capsule (Table 1); length of neonates about 1.00 mm. II - VII - whitish, mature larva turns yellowish just before pupation; tracheal gills present along each side of abdomen, number increasing on successive instars; head capsule light brown with scattered small dark brown spots at base of setae (Fig. 1A.), length increasing with each instar to 7.4-14.1 mm for last instar; instars best separated by widths of head capsules (Table 1). Head capsule widths were not compared between sexes. A detailed description and an illustration of the head capsule of the fourth instar was presented by Yoshiyasu (1985) for Japanese specimens.

Development. The duration of the larval stage and pupal stage at 26.7°C is presented in Table 1, along with the approximate duration of each stadium. The days required for development from neonate to adult at 4 constant temperatures is presented in Table 2. A plot of the development rates (1/days to develop) against temperatures revealed that 36.1°C was near the upper threshold. Although adults were produced at that temperature, the development rate slowed. The estimated lower threshold for development obtained by the "linear approximation" method (Wilson & Barnett 1983) was 12.7°C. The threshold was identical with linear regression of the 3 lower temperatures ($y = -0.0308 + (0.00243x)$, $r = 0.99$). The estimated degree-days for mean development times and for the ranges calculated using 12.7°C were 424 (351-551), 388 (294-490), 418 (340-483), and 494 (397-608), respectively, for the four temperatures listed in Table 2. Although these are approximations, they should be useful when planning additional field or laboratory studies with this species.

Behavior. Neonates were active crawlers. Although some began feeding immediately, most wandered about the containers before settling on plants. It was not unusual to find that some had crawled from the water and out of containers that were not covered. Neonates fed on leaves, either by scraping the surface and rendering the leaf transparent, or by completely eating portions of the leaf. Most fed without a shelter but some made simple shelters by cutting small (about 2mm long) pieces of leaf and attaching them to the leaf surface. Most second instars made these simple shelters, and all later instars constructed tubular cases by tying together pieces of leaves or stems. Their cases might be mistaken for those of some caddisflies. Balciunas & Minno (1985) included *P. diminutalis* in a key to the larval cases of insects on hydrilla in the United States. Larvae generally fed on leaves by partially exiting from the cases they carried with them. At night, however, larvae were observed crawling without cases. On one occasion, many naked larvae were clustered together near the surface in an aerated jar that had a fluorescent light lying across the mouth. This suggested an attraction to light, but we did not test this hypothesis. Leaves were eaten most readily, but portions of the stem were also eaten during heavy feeding.

Mortality. Neonates often crawled from their containers, but they were unable to develop outside water. All died within 1 h in dry cups, whereas in cups with moist filter paper all survived for 23.5 h. Survival in moist cups without hydrilla was 83% at 32 h, 57% at 46.5 h, 30% at 55 h, and 0 at 72 h. Two of an additional three neonates tested in moist cups with hydrilla were still alive at 72 h but both were dead at 6 d. These relatively long periods of survival out of water suggest that neonates might successfully disperse with hydrilla carried on boat trailers, boat propellers, etc. When neonates were held in water without food, forty-two percent were alive after 6 d in the greenhouse at 14.3-26.7°C, but none was alive after 14 d.

Early instar mortality (within the first 2 weeks) ranged from 30 to 100% and was greater than, or equal to, 50% in ten of 21 diverse experiments where individual lar-

TABLE 1. BIOLOGICAL STATISTICS FOR *P. DIMINUTALIS*.

Statistic	n	Mean	SD	Range
Measurements				
Mature egg (mm)				
Length	10	0.44	0.02	0.42-0.48
Width	10	0.34	0.02	0.32-0.34
Egg mass size (Nos.)	112	29.7	19.4	2-99
Larval head capsule (mm)				
Instar I	18	0.21	0.01	0.20-0.24
II	5	0.29	0.02	0.26-0.30
III	1	0.42		
IV	8	0.62	0.03	0.60-0.66
V	16	0.81	0.05	0.75-0.90
VI	30	1.02	0.02	0.98-1.06
VII	18	1.15	0.04	1.10-1.20
Female pupa (mm)				
Length	11	7.82	0.34	7.14-8.33
Width	11	2.09	0.10	1.96-2.30
Width at spiracles	11	2.31	0.09	2.21-2.47
Male pupa (mm)				
Length	10	6.63	0.20	6.14-6.80
Width	10	1.67	0.11	1.45-1.79
Width at spiracles	10	1.84	0.11	1.62-1.96
Wingspan (mm)				
Female	10	18.12	0.98	16.50-19.67
Male	10	13.40	0.93	12.34-14.84
Development Times (Days @ 26.7°C)				
Egg				4-6
Larva + Pupa	17	27.1	3.8	21-35 ¹
Prepupa				1-2
Pupa	10			6-7
Total (Estimated)				25-41
Longevity (Days)				
22.2-25.6°C				
Female	33	5.2	2.1	1-10
Male	26	5.9	3.2	1-17

¹Instar I=4, II=3, III=3-5, IV=2-3, V=2-5, VI=3-8, VIII=3-9 days.

TABLE 1. (CONTINUED) BIOLOGICAL STATISTICS FOR *P. DIMINUTALIS*.

Statistic	n	Mean	SD	Range
24.4°C				
Female	3	4.3	0.6	4-5
Male	6	4.0	1.4	3-5

¹Instar I=4, II=3, III=3-5, IV=2-3, V=2-5, VI=3-8, VIII=3-9 days.

vae were monitored. There were no obvious causes for this; however, initial mortality (4-9 d) was generally less [$41 \pm 30\%$ (0-100, 18 experiments)] when eggs containing active larvae were transferred to the test containers rather than neonates [$61 \pm 20\%$ (30-90, 6)]. In various experiments (Buckingham & Bennett 1989), more adults were generally produced in the greenhouse than in the laboratory.

Aeration of the water did not significantly improve survival in the small containers with individual neonates and hydrilla. After 5 days, 16 of 20 neonates were alive in aerated cups versus 34 of 50 in non-aerated cups (X^2 , $p=.48$). After 13 days, 15 larvae were still alive in the aerated cups versus 30 in the non-aerated cups ($p=.36$). There was no apparent increase in survival in various other experiments in which the small cups were aerated versus those experiments in which they were not aerated. Aeration, however, did decrease mortality in rearing jars containing older larvae with too high a density of hydrilla stems. Several jars were found with moribund larvae that recovered when the water was aerated. This mortality with densely packed hydrilla was probably due to the decrease in oxygen at night (measured at 1.4 ppm) caused by plant respiration. Chantaraprapha & Litsinger (1986) also reported that larval mortality was high (50-60%) during their experiments with this species. Mortality was generally higher when large numbers of larvae, especially the early instars, were present. Because there was an abundance of leaves for the early instars, the

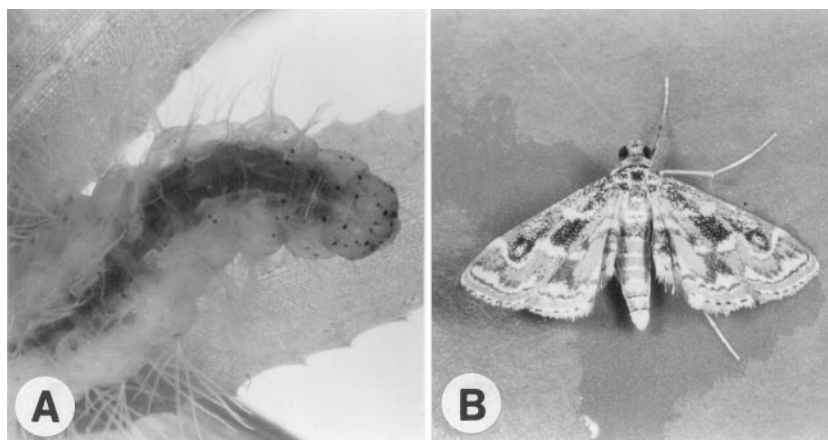


Fig. 1. *Parapoynx diminutalis* Snellen: A) mature larva with characteristic dark spots on the head capsule, B) male.

TABLE 2. PERCENTAGE SURVIVAL AND TIME (DAYS) REQUIRED OF *P. DIMINUTALIS* FOR DEVELOPMENT FROM NEONATE TO ADULT AT FOUR CONSTANT TEMPERATURES.

Temp (°C)	% Survival	n	Mean	SD	Range
22.2	70	21	44.6	5.1	37-58
26.7	40	20	27.7	3.8	21-35
30.6	60	24	23.4	2.6	19-27
36.1	40	20	21.1	2.8	17-26

higher mortality suggests that larvae killed each other rather than competed for resources. However, no observations were made to confirm that.

Larvae are often quite damaging in research plantings of hydrilla in pools or other small protected containers in Florida. We immersed larvae with hydrilla in a commercial preparation of *Bacillus thuringiensis*, Dipel Hg, (Sunnyland Corp., Sanford FL) at 10% of the dosage recommended as a garden spray and produced 80% mortality within 4 days compared to no mortality in the controls. The surviving larvae in the Dipel Hg did not feed and were dead when examined after 10 days. Larvae were found relatively often on other plant species associated with hydrilla in our research plantings, but in addition to hydrilla, we have found immatures in the field only on coontail, *Ceratophyllum demersum* L. (1 larva, 1 pupa), southern naiad, *Najas guadalupensis* (Sprengel) Magnus (2 larvae), and Illinois pondweed, *Potamogeton illinoensis* Morong (5 larvae, 3 pupae). This is in sharp contrast to the 14 plant species in 13 genera that produced adults in our host range tests (Buckingham & Bennett 1989).

Pupal Stage

Description. Similar to those of other species of *Parapoynx* (Lekic 1971, Virakamath et al. 1974, Yoshiyasu 1985). Narrow, elongate, with 3 distinct spiracular tubercles along each side and 2 strong dark setae on top of the head. Female differentiated most easily from male by the length of the antennae. Female antennae extending to abdominal segment A4 just anterior to the wing tips; male antennae exceeding the wing tips and extending to A5. Females generally larger than males (Table 1). The abdomen expands in length when the pupa darkens prior to emergence. Lengths of expanded darkened pupae: female - 8.97 ± 0.05 mm (8.93-9.01, 4); male - 7.9 mm (n=1).

The pupa was enclosed in an air-filled white silken cocoon. The cocoon was firmly attached along one side to the submersed stem or occasionally was attached at one end and perpendicular to the stem. The cocoons were covered with leaves or bare stem sections and were similar to the larval cases. Pupae obtained air through the cocoon from 1-4 excavations of various sizes made in the stems by the larvae. Air was held between the layers of the multilayered silk cocoon in a broad silvery band surrounding the spiracles. Lekic (1971) illustrated similar holes in the plant associated with cocoons of *P. stratiotata* L., and Yoshiyasu (1985) illustrated a silvery band in the cocoon of *Parapoynx vittalis* (Bremer). The length of female cocoons was generally greater than that of male cocoons (Table 1).

Development. Prepupae remained immobile in the cocoon for 1-2 d before pupating. Pupal development times are presented in Table 1. Distinct color changes were associated with pupal development. Body color changed from white to yellow while

the eyes darkened to red and then to dark brown. The wing pattern was visible shortly before emergence. The pupa actively moved its abdomen when disturbed during the first few days but was immobile during the last few days. Pupae died in the cocoons if the stems were only a few cms long or if the stem became waterlogged. Pupae developed normally when removed from cocoons in water to moist sphagnum or to filter paper in closed containers. All cocoons were constructed on submersed stems. This contrasts with the observation of Varghese & Singh (1976) that the anterior end of the pupa often remained above the water surface and of Chantaraprapha & Litsinger (1986) that they pupate out of water on plant vegetation. These differences might be due to water level changes exposing the cocoons or to a complex of species rather than one widely distributed species.

Adults

Description. Adults are white with sinuous light-brown or tan bands on the wings (Fig. 1B). Variable amounts of black scales mask portions of the bands. The body has transverse tan stripes. Females had a wider wingspan (Table 1), more pointed forewings, a more robust abdomen, and relatively shorter antennae than the males. The tip of the male abdomen has a tuft of white setae that is more noticeable than that of the female. With experience, we could easily distinguish males by this character. Detailed taxonomic descriptions along with illustrations of the genitalia can be found in Agassiz (1978), Speidel (1984), and Yoshiyasu (1985). The North American species most easily confused with *P. diminutalis*, especially the male, is *Parapoynx allionealis* (Walker). However, the light-brown or tan middle (postmedial) band on the forewing of *P. diminutalis* is much more sinuous than that on *P. allionealis*, which was illustrated by Monroe (1973). The wing patterns of both sexes of *P. diminutalis* are similar although the male generally has more black scales on the wings. The amount of black tended to vary inversely in both sexes with the temperature at which the larva was reared and with the type of host plant. Both sexes generally held the wings outspread and pressed to the substrate while resting. When disturbed, however, they often landed with the forewings folded over the hindwings but not overlapping the body.

Development and Longevity. The development time from neonate to adult at 26.7°C is presented in Table 1. No cohorts were followed from egg deposition to adult; however, total development time at 26.7°C should be 25-41 days based upon an egg stage of 4-6 d and the larval/pupal times.

Mean longevities of males and females were the same (Table 1). In a greenhouse at fluctuating temperatures, mean longevities were slightly longer: females 5.2 ± 2.1 days ($n=33$, max 8 days), males 5.9 ± 3.2 days ($n=26$, max 17 days). However, deaths were not recorded on the weekends in that experiment.

Fecundity. In oviposition experiments females that had been reared at various temperatures from 21.1°C to 26.7°C laid a mean of 222.9 ± 140.6 eggs (3-524, 26). The mean without four females who laid less than 22 eggs each was 261.2 ± 116.3 eggs (108-524, 22). Fourteen of the preceding 22 females were dissected at death. Eleven had no eggs in their ovaries and three had only 24.7 ± 20.5 eggs (4-45). Differences among individuals in egg deposition apparently reflected true differences in the egg complements except for individuals that laid very few eggs. For example, three females that laid 0, 3, and 19 eggs had 26, 199, and 196 eggs, respectively, in their ovaries at death.

Behavior. Adults began emerging in the large greenhouse cage approximately 30 min after dusk and began flying about 30 min later. After emergence, they sat above or on the water surface while their wings expanded. If disturbed before their wings expanded, they ran quickly across the hydrilla or the water surface, their long legs providing speed as they remained high above the substrate. Even though unable to fly

during these first few minutes, they would not be easy prey for most predators. We observed a few adults emerge shortly before dusk in India in 1985 while we were conducting another project, although the majority of adults emerged after dusk. One of us (CAB) also observed pre-dusk emergence in Panama in 1982.

Mating was not observed in the greenhouse cage until about 3 h after dusk. Pairs *in copula* rested facing in opposite directions for at least 30 min. The maximum time *in copula* was not determined. We did not test females for multiple matings. No females oviposited the night they emerged. In the cage, they oviposited within the first hour after dusk. The female sat above the water surface and usually inserted her ovipositor into the water to oviposit on leaves or stems. Eggs were placed occasionally, just above the water surface.

Because of the 1-day preoviposition period, there is probably heavy preovipositional mortality in the large water bodies in Panama and Florida. Hydrilla mats extend considerable distances from shore, thus many moths rest during the day exposed on the mats. Balciunas & Habeck (1981) reported heavy bird predation on moths at a large Florida lake. We have also observed large numbers of detached moth wings on the water surface above hydrilla mats.

Although the adult proboscis was greatly reduced, it was apparently functional. Adults that had been held in a refrigerator for several days without water and adults collected at outdoor lights extended their probosci to the water when presented wet leaves and wet paper toweling. We did not test longevity without water, but adults provided sugar water lived no longer than those with water.

Adults in the greenhouse cage were attracted to both incandescent white lights and UV blacklights. They did not respond to incandescent red light which was used to observe them without disturbance.

The sizes of both the egg and the last instar head capsule of our population were considerably smaller than those reported by Varghese & Singh (1976) in Malaysia. Although these differences might be merely populational or technique differences, they suggest that two different species might have been studied. Our 4th instar head capsule width was equal to that of a Japanese 4th instar illustrated by Yoshiyasu (1985). The number of larval instars that we found (7), was different from that reported by Varghese & Singh (1976) (6) and by Chantaraprapha & Litsinger (1986) (4). Fecundity and development times also varied, but those are often greatly influenced by techniques. Taxonomic studies of these populations and others of this widely distributed species appear to be warranted.

Parapoynx diminutalis is apparently well established in both Panama and Florida, but its potential to extensively damage or control hydrilla is probably greater in Panama because of the milder climate. In Florida, at least in north-central Florida, near Gainesville, the larval populations are reduced to almost undetectable levels during late winter and early spring probably by the cool water temperatures in autumn and winter and the annual late winter decline in hydrilla. However, some populations near Gainesville rebounded quickly in some years, so that by late summer the defoliation of surface hydrilla stems was extensive. Early summer augmentation of moth populations by the release of immatures or adults might allow the populations to increase fast enough to at least slow the growth of hydrilla. Extensive defoliation might also increase the plants sensitivity to herbicides or pathogens. If plants attacked were more susceptible, an integrated program might be feasible.

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