

Mass Rearing of *Haplaxius crudus* (Hemiptera: Cixiidae), an Important Insect Pest in the Palm Oil Industry of Colombia

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Abstract: The palm leafhopper, *Haplaxius crudus* (Van Duzee) (Hemiptera: Cixiidae), is a vector of the pathogen that causes the lethal wilt disease in oil palm in Colombia. The development of a methodology for mass rearing of this insect is required to study its biology, behavior, population control, and to determine the causal agent of the lethal wilt disease. The experiment was carried out in Colombia, at the Research Experimental Center "Palmar de las Corocoras" of Cenipalma. The environmental conditions of the rearing unit during the study were 25.7±3.4°C, and 85±13% R. H. The mass production of *H. crudus* required the performance of five processes: 1) Planting and maintenance of *Panicum maximum* Jacq. cv. Mombasa grass, which hosts *H. crudus* nymphs, and of coconut palms (*Cocos nucifera* L.) var. dwarf yellow, to hold the adult insects; 2) Collecting nymphs in fields to create a breeding stock; 3) Infesting clumps of *P. maximum* grass with *H. crudus* eggs; 4) Maintaining the infested clumps, and 5) Trapping of emerging adults in cages. The time from the infestation of the clumps of grass, to the emergence of adults took 56.2±4.1 days. The rearing unit reached an average production of 3,145 adults per week (52.8% female and 47.2% male). The development of this rearing enables the production of enough *H. crudus* individuals to research lethal wilt, to mitigate the impact of this disease on the Colombian oil palm growing industry.

Keywords: Mass Production of Insects, Lethal Wilt, Palm Leafhopper

1. Introduction

The palm leafhopper, *Haplaxius crudus* (Van Duzee) (Hemiptera: Cixiidae), was first described in 1907 in Jamaica. It is present in southern Florida and has been reported in Cuba, Cayman Islands, Trinidad, and in the meridional area of tropical America, from Mexico to northern South America [1], including Brazil [2]. The adult of *H. crudus* is the only insect considered to be a vector for the lethal yellowing of coconut palms (*Candidatus Phytoplasma palmae*), a highly destructive disease for coconut and ornamental palms in Florida and several countries in the Caribbean Basin [1].

In Colombia, *H. crudus* is responsible for the dissemination of the pathogen that causes the lethal wilt (L. W.) disease in oil palm [3]. This disease has spread to plantations of the

departments of Meta and Casanare and is the main plant health issue for oil palm in the Eastern Palm Growing Region of Colombia. The first cases of L. W. were reported in Bajo Upia (Casanare) in 1994 [4]. Between 1997 and 2018, a total of 911,738 palms infested with lethal wilt were eradicated [5, 6].

The life cycle of *H. crudus* includes the egg, nymph, and adult stages. *Haplaxius crudus* females lay eggs in the soil, close to the base of stalks of grasses and under drying leaf sheaths [7]. When the nymphs emerge, they move towards the roots of grasses to feed and remain in nests built with secreted silk from abdominal glands [7, 8]. When *H. crudus* reach the adult stage, they fly towards palm leaves and stay on the underside of the leaves to mate and feed by sucking the palm's sap. Females later return to grassy areas to lay their eggs and continue their reproductive cycle [2, 9]. The total life cycle of *H. crudus* under shaded roof conditions (26.5±4.1°C; R. H.:

58.1±14.6%) takes 63.7±3.6 days [10], with the egg stage lasting 15.4±0.9 days, the nymph stage (including five instars) lasting 48.3±2.7 days, and total life span of adults lasting 19.7±10.9 days.

Research previously conducted at Cenipalma reported a higher proportion of males than females (7:1) in the palm's leaves. The lower abundance of females on palm's leaves is explained because they remain part of the day in clumps of grass to lay their eggs near the base of the grass stalks [9]. In these investigations, they also mentioned that it is possible to find more adults between 07:00 - 9:00 and 16:00 - 18:00, sampling with an entomological net.

Haplaxius crudus is an important insect pest for Colombian oil palm cultivars because it is a vector of the pathogen that produces the lethal wilt disease [3]. For the Colombian palm oil industry, extensive research is required to understand the etiology of lethal wilt and the role of *H. crudus* in the infection and dispersal process and to develop an early detection method to minimize oil palm losses in the plantation. These studies also include biology, phenology, and population dynamics of *H. crudus* to develop an integrated pest management program. However, previous attempts to mass-produce these insects have not been successful due to the lack of adequate facilities, efficient and standardize control processes, and operators trained in the daily activities involved in managing rearing insects of this nature [11, 12]. Therefore, efforts are needed to develop an effective and continuous process for mass rearing *H. crudus* to reach an adequate scale of the population to perform the research aimed at reducing the impact of lethal wilt on Colombian oil palm plantations.

2. Materials and Methods

2.1. Location

The *H. crudus* rearing unit is located at Cenipalma's Experimental Field "Palmar de Las Corocoras" (CEPC, for the name Spanish) in Paratebuena, Cundinamarca, at an altitude of 227 masl, the latitude of 4°22'04" N and longitude of 73°10'16" W. The average temperature at the unit is 25.7±3.4°C, the relative humidity of 85±13% and an average annual rainfall of 2,454 mm.



Figure 1. Rearing unit de *Haplaxius crudus*. A. The module of infestation with coconut palms in drums and egg-laying cages in the foliage. B. The module of maturation and emergence with cube-shaped cages covered with anti-aphid mesh to handle the infested clumps of grass and the emergence of *H. crudus* adults.

The rearing unit consisted of two modules of shading roofs, each equipped with an automated mist system for temperature and humidity control. The first module used for infestation contained coconut palms (*Cocos nucifera* L.) and egg-laying cages. (Figure 1A). The second module used for maturation and emergence included cages to manage the grass clumps for early development and adult stage of *H. crudus* (Figure 1B).

2.2. Host Plants

Based on early evidence for host preference, the plant material to host the nymphs of *H. crudus* was obtained from commercial seeds of *Panicum maximum* Jacq. cv. Mombasa (Pastos y Leguminosas S. A, Villavicencio, Colombia [2, 8, 13]. The protocol from Moya and Bustillo 2015 was used for this study [11]. In brief, the seeds were planted in plastic pots of 0.75 l (HDienes S. A. S, Bogotá, Colombia, plastic plant pots # 12), by uniformly distributing 0.15 g of seeds over a substrate consisting of two parts of previously solarized soil, one part of peat, and one part of coconut fiber. Five weeks after planting the grass, this was transplanted by removing the clump of grass from the germination pots (Figure 2A) and placing it in a larger plant pot of 5 l (HDienes, Bogotá, Colombia, plastic plant pots # 23). The pots were filled with soil to 2/3 of its capacity (Figure 2B). The clump of grass was fertilized with 2 g of NPK plus Phytohormones (Rebrote®, Kibutzim Ltda, Bogotá, Colombia) and 2 g of urea (Yara Colombia S. A, Bogotá, Colombia). The plant pot was covered with an aluminized thermal insulator, with a thickness of 5 mm (Thermolon, PlastiTek S. A. S, Bogotá, Colombia); in the center of the cover, a hole was made through which the leaves of the grass came out (Figure 2C). In this way, a dark and wet chamber was created to favor the development of rootlets, which are essential factors for the survivorship of the nymphs [14]. After four weeks of growth, the plant material was ready to begin infestation with *H. crudus* adults (Figure 2D).

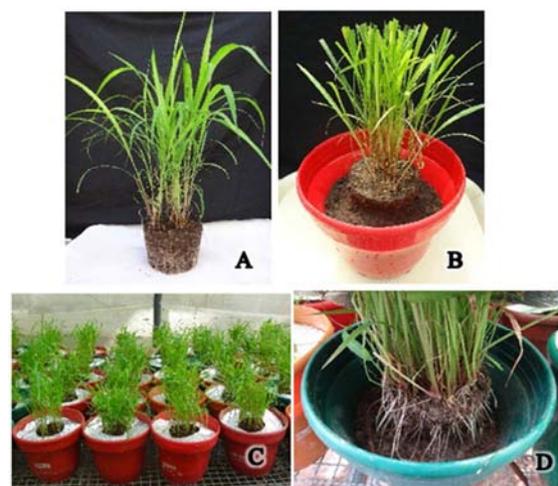


Figure 2. Transplantation of *Panicum maximum*. A. Picture of a clump of grass five weeks after planting. B. Clump took from a # 12 plant pot placed in a # 23 plant pot. C. Clumps in # 23 plant pots covered with Thermolon. D. Picture of a clump four weeks after transplanting, observe the side rootlets.

Coconut palm, dwarf yellow variety, between 12-18 months old, were used as a food resource for *H. crudus* adults [11]. Palm trees were acquired from a commercial nursery (Don Juan Viviero, Meta, Colombia) and were transplanted in plastic drums with a diameter of 60 cm and a depth of 50 cm, to facilitate handling [11]. Palms were watered once a week, and fertilization was conducted every three months with 40 g of an edaphic fertilizer grade 19-4-19-3 (YaraMila Hydran, Yara Colombia, Bogotá, Colombia). In addition, frequent scouting was conducted to evaluate the presence of spiders and ants, considered predators of *H. crudus*.

2.3. Initial Population

The population of *H. crudus* to start the mass rearing was collected from oil palm plantations. Clumps of *P. maximum* and *Paspalum virgatum* L. infested with immature insects were collected [3, 11] (Figure 3).



Figure 3. A clump of *Panicum maximum* grass with *Haplaxius crudus* nymphs.

The clumps were extracted from the soil, and the roots were placed in black plastic sheets to facilitate the collection of the nymphs [13]. The nymphs were carefully collected using soft tweezers and small plastic spoons. Groups of approximately 100-150 nymphs were placed in containers with roots of the host grass [12]. Later, these containers were placed inside a polystyrene cooler, with a cooling gel pad to maintain the viability of the nymphs during their transfer to the rearing unit.

2.4. The Infestation of Clumps of Grass

The oviposition cages were formed using the clumps of *P. maximum* grass grown at the rearing unit, and the coconut palms planted in drums. These conditions simulate the process that takes place in the field: the adults fly to the palms, feed on them, copulate, and then the females fly down to lay the eggs on the neck of the root of the grass stalks, to enable the emerging nymphs to feed off the grass rootlets.

The oviposition cage consisted of a frame of rectangular-shaped iron rods (20 cm wide x 28 cm long x 60 cm tall), placed above the clump of *P. maximum* grass (Figure 4A), and covered with a nylon cloth liner (Figure 4B). This cloth was attached to the plant pot and the tip of the coconut palm leaf with an elastic band (Figure 4C). The purpose of this was to arrange in the nylon liner (Figure 4D) to enable the introduction of between 10 and 20 pairs of *H. crudus* adults from the rearing unit. The *H. crudus* adults were held for eight

days to ensure that they coupled and laid eggs.

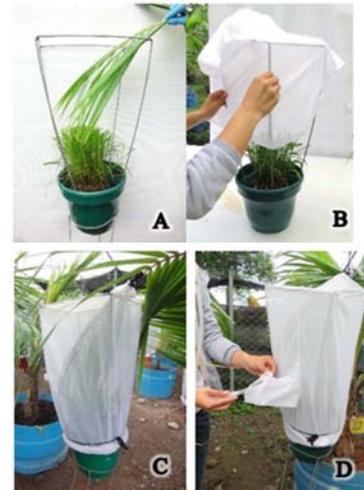


Figure 4. Setting up the *Haplaxius crudus* egg-laying cage. A. The metal frame over the plant pot with a clump of *Panicum maximum* and location of the coconut palm leaf. B. Placing the nylon cloth liner over the metal frame. C. Nylon cloth liner is attached to the plant pot and the coconut palm leaf using elastic textile. D. Introduction of *Haplaxius crudus* adults in the egg-laying cage.

2.5. Maintenance of the Infested Clumps of Grass

The infested clumps in the initial egg-laying cages were transferred to cube-shaped baskets made from aluminum angle bars (1.0 m wide x 1.0 m long x 0.8 m tall), which were covered with an anti-aphid mesh, and closed with Velcro strips placed on the opposite sides. Each can host 16 plant pots with clumps of grass [11] (Figure 5A). These clumps remained in the baskets until the emergence of the *H. crudus* adults was completed.

The plant pots with the clumps were moistened using a manual sprayer, aiming the water at the base of the clump. Manual pest control was performed, and they were trimmed once a week. Fertilizer was applied monthly using the Tottal® (Colinagro S. A, Bogotá, Colombia) leaf fertilizer, with a dilution of 15 cc/liter of water. Whenever low growth of roots in clumps was observed, the Hormonagro 1® (Colinagro S. A, Bogotá, Colombia) physiological regulator fertilizer was added in a dosage of 1 mg / l.



Figure 5. Development and recovery of *Haplaxius crudus* adults in the breeding unit. A. Clumps of *Panicum maximum* grass infested with eggs to obtain adults. B. Emerging *Haplaxius crudus* adults in the cage.

2.6. The Emergence of *H. crudus* Adults

Newly emerged adults were collected daily. The clumps were spraying with water to facilitate the collection; this made

them fly towards the cage walls (Figure 5B), where they are collected with a mouth aspirator. All the trapped *H. crudus* adults were counted, sexed (Figure 6), and used for research purposes while maintaining 50% of the population to develop the mass rearing.

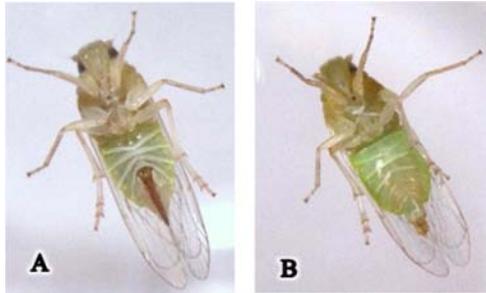


Figure 6. Details of the sexual dimorphism of *Haplaxius crudus* adults. A. Female. B. Male.

3. Results and Discussion

3.1. Host Plants

Every week, an average of 100 clumps of *P. maximum* were planted to maintain enough amounts for infestation with *H. crudus*. The coconut palms were replaced every six months, once they had surpassed the required age and height for the mass rearing, or whenever they displayed severe attacks from the red mite, *Raoiella indica* Hirst. (Acari: Tenuipalpidae) [15]. Tsai et al. [16] and Eden-Green [17] in Florida, made colonies de *H. crudus* on a small scale, under laboratory conditions using a different grass variety, *Stenotaphrum secundatum* Walt., to studies the transmission by *H. crudus* of the agent that causes lethal yellowing of coconut palm. This type of grass was not used in this study because it was not

present in the oil palm ecosystems of the eastern Colombian region [13]. *P. maximum* was used to obtain *H. crudus* eggs because it is common in Colombian oil palm regions, and this insect prefers laying eggs in this grass variety.

3.2. Initial Population

A total of 21,335 *H. crudus* nymphs were collected between instar III and V to establish and maintain the breeding stock during the two-years cycle of this study. A total of 10,390 adults emerged (48.7%), and 56% of the adults were females. 71% of nymphs of instar V reached the adult stage, which makes this instar the most appropriate for build the mass rearing. Instead, a lower proportion of nymphs in instar III and IV reached the adult stage: 34.3% and 45.2%, respectively.

Even though the percentage of *H. crudus* nymphs that reached the adult stage was low, and in other studies they used adults collected in the field to start up the mass rearing [11, 17], here we found that nymphs collected in the field are the safest way to obtain the initial population of *H. crudus*, and not having the risk of introducing adults to the rearing unit that have possibly acquired the L. W. pathogen in the field.

3.3. The Infestation of Clumps of *P. maximum*

The *H. crudus* adults from the initial breeding stock and their offspring were used for reproduction. A total of 115,232 females and 105,248 males of *H. crudus*, were used to infest 6,490 clumps of *P. maximum*. 57% of the adults used for infestation have newly emerged youths, and the remaining 43% were older adults that were removed from the egg-laying cages after the infestation period (30% 8-day-old adults, 11% 16-day-old adults, and 2% 21-day-old adults since emergence).

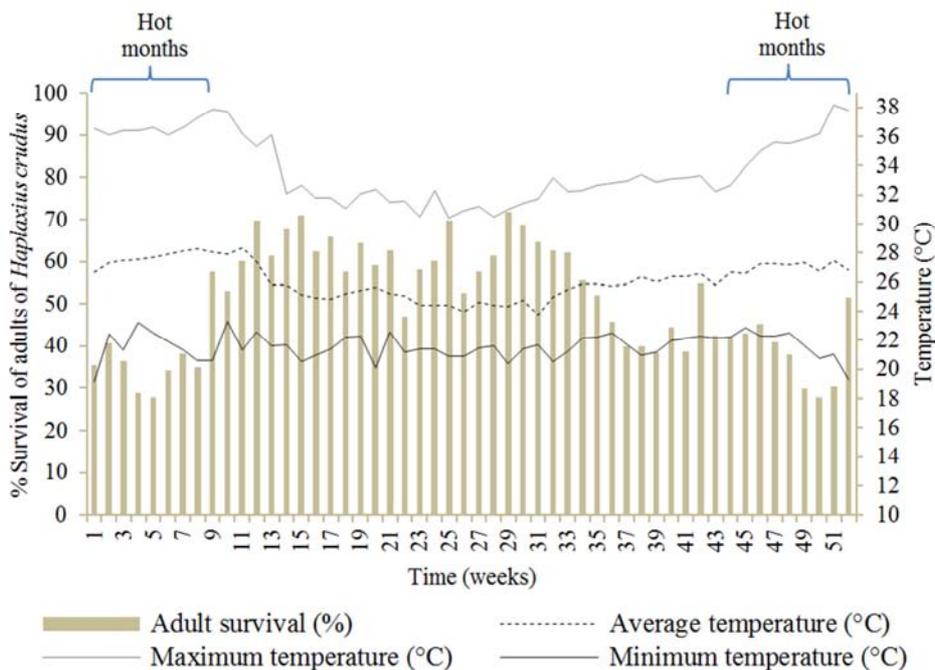


Figure 7. Percentage of survival of *Haplaxius crudus* adults inside the egg-laying cages. Observe the reduction in the warmer months of the year in the area where the breeding unit is located.

This activity must be carried out by trained personnel, because trapping the *H. crudus* adults abruptly using the mouth aspirator can cause the premature death of the adults, then causes a reduction in the number of laid eggs, leading therefore to lower production of *H. crudus* adults. Also, the infestation of the clumps seems to be affected by high temperatures, reducing adult survival rates in the egg-laying cages at the rearing unit located at Palmar de las Corocoras. High temperatures happen mainly in the periods of January-February and November-December (Figure 7). This limiting factor was mitigated at the rearing unit by installing a mist system, with temperature and humidity control, to maintain the most suitable temperature for insect development.

3.4. Maintenance of the Infested Clumps of Grass

Haplaxius crudus colony was maintained free of predator arthropods and grass pests. The most frequent predators at the *H. crudus* breeder were spiders, mainly of the genus *Theridion* Walckenaer (Araneae: Theridiidae), which were found inside the cages from which the *H. crudus* adults emerged. This spider genus has been found in Florida (USA) as a predator of

H. crudus adults present in coconut palms [18]. In the host grasses, the most common pests were nymphs of grass spittlebugs, *Aeneolamia varia* Fabricius (Hemiptera: Cercopidae), and defoliating larvae of different types of Lepidoptera.

3.5. The Emergence of *H. crudus* Adults

The emergence of adults displayed a variable pattern (Figure 8). We suggest maybe one of the causes was an uneven infestation of *P. maximum* clumps each week. The availability of adults for infestation depended on the number of insects required for the research studies. The time elapsed, from the infestation of the clump with *H. crudus* eggs to the emergence of the adults lasted on average 56.2 ± 4.1 days ($25.7 \pm 3.4^\circ\text{C}$ and $85 \pm 13\%$ R. H.). This result is similar to the one reported by Tsai and Kirsch [19], who found the duration of the egg and the nymph stage of 52.6 ± 12 days at 30°C . However, the length of these stages seems to be related to the variability of temperatures at the location, which produces different growth rates of individuals within the same niche [19]. The variation is associated with the fact that *H. crudus* is a heterovoltin, i.e., the number of generations is affected by temperature [20].

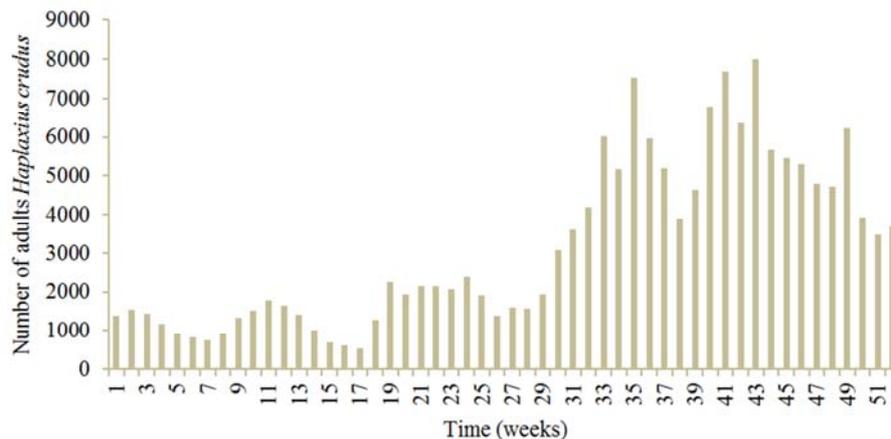


Figure 8. Weekly production of *Haplaxius crudus* adults at the breeding unit.

Based on the records of the emergence of *H. crudus* at the rearing unit, the average production of adults per week was 3,145, 13,679 per month, and overall production over two years of 328,294 adults. Of this total, 52.8% were females and 47.2% males, obtaining an average of 63.5 adults per infested clump of grass. However, during weeks 34 and 37, emergence rates increased to 174 adults per clump. Under these favorable environmental and infrastructure conditions, this rearing unit has a maximum infestation capacity of 360 clumps per month, with potential production of 27,000 individuals, which is sufficient to support the research studies carried out by Cenipalma.

4. Conclusions

Parameters were established for the mass rearing of *Haplaxius crudus* under shaded roof and semi-controlled

environmental conditions. Furthermore, results from this research can help in the optimization of *H. crudus* mass production. With a well established mass rearing protocol, other studies can be conducted to understand aspects of this insect biology and ecology. In addition, studies for the molecular characterization of the pathogen that causes the lethal wilt disease in oil palm and on IPM approaches on biological control agents and insecticide efficacy would be more easily conducted having a well establish protocol and colonies. In the near future, more studies will also.

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