Pergamon

PII: S0022-1910(96)00039-X

J. Insect Physiol. Vol. 42, Nos 11 12, pp. 1113–1119, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0022-1910/96 $15.00 + 0.00

Secretory Mechanisms for the Male Produced Aggregation Pheromone of the Palm Weevil *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae)

P. SÁNCHEZ,*†|| H. CERDA,* A. CABRERA,* F.H. CAETANO,‡ M. MATERÁN,* F. SÁNCHEZ,§ K. JAFFÉ*

Received 27 July 1994; revised 14 Feburary 1996

Histological and chemical analyses of different corporal segments of the palm weevil *Rhynchophorus palmarum*, revealed the presence of two symmetrical glands (modified salivary glands) in the prothorax of males, but absent in females. Using gas chromatography and mass sepectrometry, we demonstrated the presence of rhynchophorol (aggregation pheromone) in extracts of this gland, as well as in the rostrum and digestive tract (rectum) of males. Olfactometric experiments showed that the release of the aggregation pheromone starts approximately 10 min after the insect detects ethyl-acetate and continues for several hours. The pheromone is secreted through the feces and more concentrated through the mouth to a depression on the dorsal part of the rostrum where, via hairs and the surface tension of the secretion, it collects in a complex structure consisting of various types of hairs, which help disperse the pheromone. Copyright © 1996 Elsevier Science Ltd

Rhynchophorus palmarum Pheromone Gland Aggregation Secretory mechanism

INTRODUCTION

The palm weevil *Rhynchophorus palmarum* L. is a pest of African oil palm (*Elaeis guineensis*) and coconut (*Cocos nucifera*) plantations in Mexico, Brazil and the Caribbean (Bedford, 1980; Barreto, 1986; Hernández *et al.*, 1992; Sánchez and Cerdá, 1993). A population of 30 larvae in a single mature coconut plant is sufficient to cause its death (Fenwick, 1967; Griffith, 1968). *R. palmarum* is also a vector of the 'red ring disease' as the adults disperse the nematode *Bursaphelenchus cocophilus* (Cobb) which causes this disease.

R. palmarum, as other Coleoptera species, depends on chemical communication to coordinate its behavioral activities. The first evidence of *R. palmarum* producing semiochemicals was reported by Nadarajan (1986) and Rochat (1987). They demonstrated that males and females were attracted toward hexane extracts of males. Moura *et al.* (1989) and Rochat *et al.* (1991a,b) showed in field experiments that the attractiveness of traps baited with palm chunks increased when males of *R. palmarum* were present, even though neither males nor females alone were able to attract other weevils. This was interpreted to be due to the release of an aggregation pheromone when the males were feeding on palm chunks. The principal component of the pheromonal mixture was identified by Rochat *et al.* (1991b) and Oehlschlager *et al.* (1992) as (S)-6-methyl-2(E)-hepten-4-ol which was named rhynchophorol.

Males of *R. palmarum* search for potential host plants, which they locate via a complex odor mixture generated by the palm. Among these odors, ethanol, ethyl acetate, isoamyl acetate, pentane, isopentanol and hexanal have been identified (Jaffé *et al.*, 1993). When the insect locates an adequate host plant, it releases an aggregation pheromone in response to detection of ethyl acetate (Jaffé *et al.*, 1993). This causes a great number of individuals to aggregate on the plant, facilitating both mating behavior and the degradation of the plant. During this phase, the phyto-pathogenic nematode *B. cocophilus* is transmitted to the plant.

*Departmento de Biología de Organismos, Universidad Simón Bolívar, Apartado Postal 89000, 1080A Caracas, Venezuela.
†FONAIAP-Estación experimental, Miranda, Venezuela.
‡Instituto de Biociencias, Rio Claro, Brasil.
§Instituto de Estudios Avanzados (IDEA), Caracas, Venezuela.
||To whom all correspondence should be addressed.

In contrast to other insect groups (Lepidoptera: Percy and Weatherston, 1971; Hymenoptera: Billen, 1986; Isoptera: Kaib and Ziesmann, 1992; for example), little is known about exocrine glands in Coleoptera. The few exocrine glands known are abdominal glands associated with the digestive tract (Tumlinson *et al.*, 1968; Levinson *et al.*, 1978; Borden, 1984; Dowd and Bartelt, 1993). Regarding *R. palmarum*, the identification of rhynchophorol was accomplished through chemical analysis and electroantennography of odors released by male weevils (Rochat *et al.*, 1991b; Oehlschlager *et al.*, 1992), but no information about the pheromone-producing gland, nor the pheromone releasing mechanisms was provided.

In this work we report on the function of structures related to the synthesis and release of the aggregation pheromone and describe a pheromone release mechanism in which no previous storage of chemicals occurs.

MATERIALS AND METHODS

Adult weevils were collected in Laguna de Tacarigua National Park using retention traps (Hernández *et al.*, 1992) baited with coco-palm tissue.

The insects were maintained in the laboratory in plastic containers at 23–27°C and 70–90% RH, with a 12h light/dark cycle and were fed two times a week with a piece of sugar cane weighing 80–100 g.

Behavioral responses were quantified in an olfactometer as described by Cerdá *et al.* (1994) and Jaffé *et al.* (1993). These assays consisted of presenting two odor sources by blowing a stream of air (collected in a tube) over the sources toward either side of the insect. The insects were suspended dorsally and were able to walk on a foam ball. We measured the insistence of an insect to walk or fly toward one of the odor sources during a 5 min period. Individuals were previously selected, sexed and coded in order to assess each individual's activity. Nine to 39 insects were assayed individually for each experimental situation.

During the bioassay testing odor preferences of the weevils, we recorded any movements by the weevil and in particular the orientation of the head, the movements of the rostrum and of the antenna. We registered a definitive choice (DC) when the insect walked for three or more minutes (maximum 5 min) toward only one of the odor sources. When the insect's movements showed variable choice, the test was considered as inconclusive and noted as 'no preference'. When the insect did not walk it was considered as non-responsive or 'inactive'. The odor sources were the rostrum, head, prothorax and rest of the body [Fig. 1(1)].

For bioassays determining when weevils start and end secreting the aggregation pheromone, we placed 5 male weevils in an Erlenmeyer flask and applied an air stream saturated in ethyl-acetate for 1 min. Then the flask with the 'activated' males was used to test the response of other 'test' weevils from both sexes as described above. The response of 'test' weevils to the 'activated' males was quantified using the following index: insect at rest (0), insect walking (1), walking fast (2), moving wings (3) and flying (4). The 'test' males were monitored each 3 min for 30 min. In this bioassay 'test' weevils remained for over 30 min suspended in the olfactometer which somehow seemed to affect their activity at the end of the assay. Thus, in another bioassay, we repeated this experiment but tested the response of 'test' weevils only once for 5 min, using the same index as described above.

The results were analyzed using a binomial test (Siegel, 1982) by comparing the frequency of individuals showing a DC response toward a specific source versus those orientating to both sources.

For morphological studies of the gland, the insect was anesthetized by cooling (−5°C for 15 min), its rostrum and head were then detached and the prothoracic dorsal sclerites were withdrawn. The dissection was accomplished with the aid of a stereoscopic microscope, and with the insect immersed in phosphate buffer, pH 7.2 and 360 mOsm/1. Tissue was fixed in aqueous Bouin's solution for 24h and then dehydrated using a graded series of acetone. Thereinafter it underwent critical point drying in CO_2 using a BALZERS CPD 020. This preparation and whole rostrums were then gold-plated in the Balzers sputter-coating device for 1 min. Observations and photographs of the gland and of the external structures of the rostrum were done in a MEB philips SEM 505.

Samples of the glands and sections of the rostrum were prepared for light microscopy observation by fixing the tissue in 2.5% glutaraldehide and 1% paraformaldehyde, in isotonic PBS. Fixation started immediately after the tissue was exposed during the dissection. Pieces of tissue were immersed in the aldehyde fixative to complete prefixation after washing them in buffer solution. Samples were then fixed in 1% osmium tetroxide in PBS, pH = 7.2 / 360 mOsm/1 and dehydrated with a graded series of ethanol ending with propylene oxide. Subsequently, samples were placed in propylene oxide/epoxy mixture 1:1 resin (Polybed) for 45 min at 4°C and finally, they were fixed in resin mixture for 48h at 60°C. Thick and semi-thin sections were obtained with a Reichert ultramicrotone and then stained with 4% toluidine blue. Observations and photos were done with a POLIVAR (Reichert) light microscope.

For the chemical analysis, extracts in dicloromethane were obtained. The insect body was dissected so as to obtain in the thoraxic glands, rostrum, rectum and the rest of the body as described above. Chemical analysis of glands were made in both normal condition (no stimulation) and in insects stimulated with ethyl acetate during one minute. The parts were placed into a vial containing the solvent (CH_2Cl_2). Solvent extracts were concentrated by blowing nitrogen to reduce the volume to 100 μl. Identification of rhynchophorol was achieved by monitoring selected ions in a gas chromatograph PERKIN-ELMER Autosystem 900 coupled with a mass detector PERKIN-ELMER QMASS-900, and by compar-

ture. In the rostrum of the males we observed two types of hairs (Fig. 2), both showing at their bases small granules possibly formed by waxes secreted at the base of the hairs. The hairs on the depression of the dorsal part of the rostrum of males form a kind of channel which lead up the apical extreme of the rostrum [Fig. 2(2)]. In effect, no direct communication between the pheromone producing prothoraxic gland and the hairs in the rostrum could be found. However, we observed though ducts leading from the trichogen cells through the cuticle to the base of the hairs [Fig. 2(4) and (5)]

The results of scanning electron microscopy of the parietal gland showed a glandular structure surrounded by numerous fat bodies, and alveolar microtubules that connected to larger efferent ducts [Fig. 1(2)]. In the alveolus, the cells had a typical 'acinus' arrangement, including lacunar spaces in the basal and distal acuminal region, with several adjacent cells through which the synthesized substances could flow. No reservoir could be found, but rather, the secretion could flow through the efferent ducts to even wider ducts, eventually being released in the digestive tract. The connection to the digestive tube could not be observed but was implied by circumstantial evidence from the chemical analysis and behavioral observations (see below).

Direct observations of the rostrum of males being

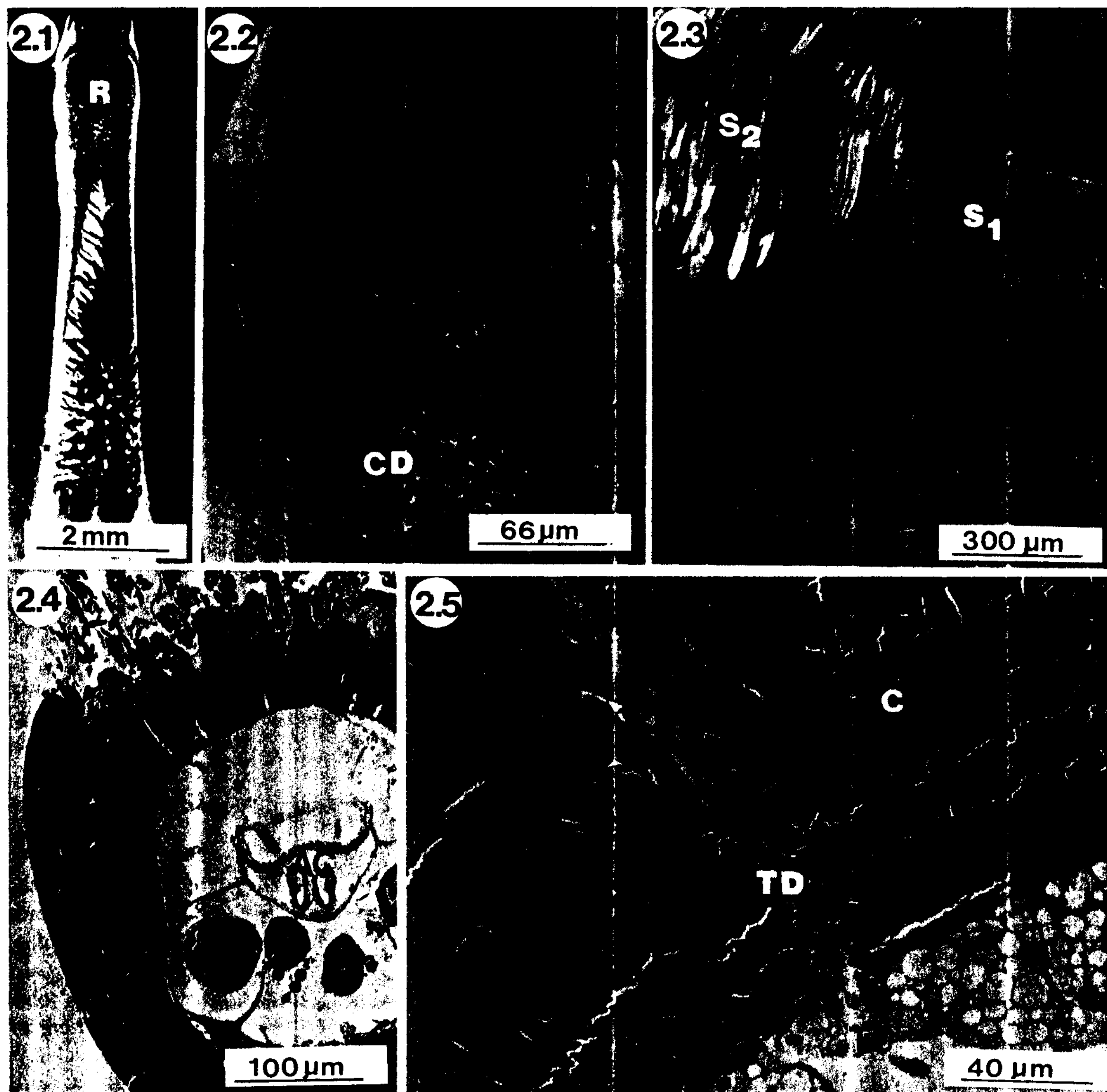

FIGURE 2. 2(1) Scanning microscope view of the rostrum of male weevils where the apex is indicated with R; 2(2) further amplification of the apex of the rostrum showing the central depression (CD); 2(3) scanning micrograph of two types of hairs (S1 and S2) on the dorsal part of the rostrum; 2(4) light micrographic view of a transversal section of the rostrum showing cutting of the hairs (H) and the alimentary canal (AC); 2(5) further amplification showing transversal ducts (TD), cuticle (C) and trichogen cells (TC).

stimulated with ethyl-acetate odors showed that the insects produced a clear droplet which was extruded from the mouth while the mandibles remained closed, so that the droplet was secreted dorsally, contacting the apical part of the dorsal depression of the rostrum filled with hairs. On three occasions, we could observe the droplet dispersing rapidly after contacting the hairs in the depression and immediately afterward all hairs on the dorsal part of the rostrum were moist. Unfortunately, insects placed under a stereoscope did not emit the secretion, making close observation and photography impossible. A simulation of this phenomenon using dead insects and droplets of ethanol revealed a curious capillary effect: the width of the depression and the position of the hairs were such that a droplet, placed at the apical end of the depression, would immediately spread to all hairs on the rostrum of the male.

Our behavioral assays testing the body part of the insect which produced the aggregation pheromone are summarized in Table 1. When stimulating the insects simultaneously with odors released by the head vs. those released by the prothorax, both sexes preferred odors from male prothorax. In other tests, odors released by the rostrum were more attractive than those from the rest of the body. When odors from the rostrum were tested against those from the prothorax, individuals preferred the prothorax.

TABLE 1. Response of *R. palmarum* to odors of different body parts from conspecific weevils. The number of weevils of each sex is indicated for each response category

	Males	Females	Total
Rostrum male	16	14	30 *
Rostrum female	5	5	10
No preference	9	8	17
Inactive	1	1	2
Rostrum male	3	4	7 *
Air	0	0	0
No preference	2	1	3
Inactive	0	0	0
Rostrum female	1	1	2
Air	3	3	6
No preference	1	1	2
Inactive	0	0	0
Rest of the body male	0	1	1
Air	2	2	4
No preference	2	2	4
Inactive	1	1	2
Rest of the body female	4	4	8 *
Air	1	0	1
No preference	0	1	1
Inactive	0	0	0
Rest of the body male	5	2	7
Rest of the body female	15	13	28 *
No preference	9	10	19
Inactive	1	2	3
Proboscis male	12	18	30 *
Rest of the body male	6	9	15
No preference	9	14	23
Inactive	9	6	15
Head male	0	1	1
Prothorax male	9	7	16 *
No preference	6	5	11
Inactive	1	1	2
Rostrum male	0	0	0
Prothorax male	1	2	3
No preference	4	1	5
Inactive	0	0	1

* indicates statistically significant values ($P < 0.05$, binomial test)

Selected ion monitoring analysis by gas chromatography-mass spectrometry (m/e = 128, 95, 71, 57 and 43), showed the presence of rhynchophorol from two pools of 8 insects gave values of rhynchophorol of 940 and 1300 ng/insect in the glands of stimulated insects, 2.1 and 2 ng/insect in glands from insects under normal condition; and 0.2 to 0.3 ng/rostrum, 0.05 to 0.13 ng/rectum, 0.0 ng/remains of the thorax and 0.0 ng/remains of the abdomen of stimulated insects.

Experiments in which groups of 5 males were stimulated ('activated') for 1 min with ethyl-acetate odors, and then used to stimulate 'test' weevils fixed in the olfactometer, showed that male weevils activated 'test' weevils approximately 10 min after smelling ethyl-acetate (Fig. 3), suggesting that the production of aggregation pheromone by 'activated' males is initiated some minutes after being stimulated by ethyl-acetate. When we observed the response of fresh 'test' weevils for only 5 min in front of groups of 'activated' males at various times after 'activating' the males (Fig. 4), the 'activated' males stimulated the 'test' weevils (thus, probably emitting the aggregation pheromone) for several hours.

DISCUSSION

Abundant information about the chemistry of the aggregation pheromones released by Coleoptera exists.

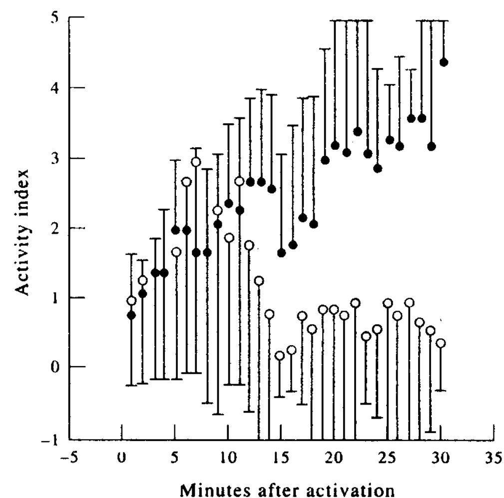

FIGURE 3. Temporal pattern of the release of the aggregation pheromone produced by males after being stimulated with ethyl-acetate odors. The mean of the activity index of performance of weevils in the olfactometer, submitted to odors from 5 activated males, is plotted at different time intervals from the moment the males at the source were activated (filled circles). Controls are indicated in open circles. Standard deviation is indicated in one direction only, N = 10.

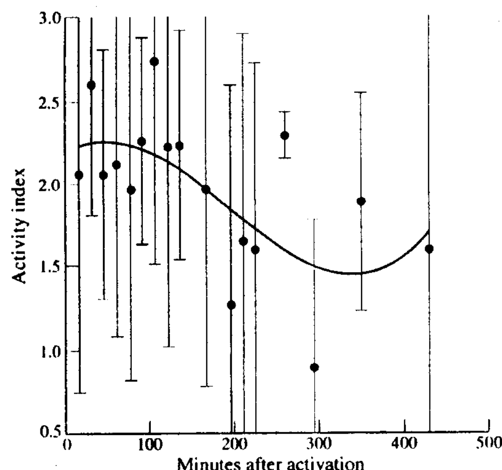

FIGURE 4. Duration of males aggregation pheromone production. The mean of the activity index of performance of weevils in the olfactometer, submitted to odors from 5 males which were activated with ethyl-acetate odors, is plotted at different time intervals from the moment the males at the source were activated. Standard deviation is indicated, N = 12.

However, less is known about the exocrine glands and the biosynthetic mechanisms of pheromones. In the case of the Rhynchophorinae, the aggregation pheromones of six species of the genus *Rhynchophorus* have been identified (Oehlschlager *et al.*, 1992, 1993; Gries *et al.*, 1994; Hallett *et al.*, 1993; Rochat *et al.*, 1993; Weissling *et al.*, 1994). However, nothing is known about either the glands of origin, nor the release mechanisms of these aggregation pheromones.

Our chemical, behavioral and morphological evidence suggest that the lateral gland in the prothorax is responsible for the synthesis of the aggregation pheromone. This gland, due to its location, can be considered as a modified salivary gland, similar to the one reported for *Shedorhinotermes lamanianus* (Kaib and Ziesmann, 1992), as both glands probably release their secretions into the digestive tract.

Our results suggest that the aggregation pheromone is produced and continuously released once the exploring male is stimulated with appropriate odors, i.e. ethyl-acetate. Clearly males do not release the aggregation pheromone in the absence of plant volatiles (Moura *et al.*, 1989; Rochat *et al.*, 1991a; Jaffé *et al.*, 1993). Thus, the insects synthesize and release the pheromone only after smelling ethyl acetate (see also Jaffé *et al.*, 1993).

The morphological examination of the gland and the behavioral observations suggest that the insect does not possess a reservoir to store the pheromone, indicating that the pheromone must be synthesized and released in direct response to the stimulus. This may also occur in other species in which there is no accumulation of pheromone (Raina *et al.*, 1989; Teal *et al.*, 1989).

Our results with *R. palmarum* present the first detailed description of a pheromone releasing mechanism in Coleoptera. Here, the prothoracic glands (probably modified salivary glands) are the source of the aggregation pheromone. The pheromone is synthesized as a response to the presence of a stimuli (ethyl-acetate) and secreted through the feces and/or directly released through the oesophagus to the mouth, where it is channeled via capillary forces to the depression on the dorsal surface of the rostrum to a conglomerate of hairs, which aid in the evaporation of the volatiles.

Given the close phylogenetic relationship between *R. palmarum* and other species of Rhynchophorini (Wattanapongsiri, 1966), it is probable that males of other species of the same genus also possess the parietal gland which would produce the aggregation pheromone. Similar pheromone releasing mechanisms could also be expected in insects of the same tribe and even among Curculionidae in general.

REFERENCES

Barreto J. M. (1986) Principales plagas y enfermedades del cocotero en Venezuela. *Coco y Palma* **38,** 14–20.

Billen J. (1986) Morphology and ultrastructure of the abdominal gland in dolichoderine ants (Hymenoptera:Formicidae). *Ins. Soc.* **33,** 278–295.

Bedford G. O. (1980) Biology, ecology, and control of palm rhinoceros beetles. *Ann. Rev. Entomol.* **25,** 309–339.

Borden J. H. (1984) Semiochemical-mediated aggregation and dispersion in the coleoptera. In *Insect Comunication* (Ed. Lewis T.), pp. 123–149. Academic Press, New York.

Cerdá H., Hernández J. V., Martinez R., Sánchez P. and Jaffé K. (1994) Estudio olfactométrico de la atracción del picudo del cocotero *Rhynchophorus palmarum* L. a volátiles de tejidos vegetales. *Agron. Trop.* **44,** 203–215.

Dowd P. F. and Bartelt R. (1993) Aggregation pheromone gland of *Carpophilus freemani* (Coleoptera: Nitidulidae) and gland distribution among other sap beetles. *Ann. Entomol. Soc. American.* **86,** 464–469.

Fenwick D. W. (1967) The effect of weevil control on the incidence of red ring deesease. *J. Agric. Soc. Trinidad.* **67,** 224–231.

Gries G., Gries R., Perez A., Gonzales L., Pierce H., Oehlschlager A., Rhainds M., Zebeyou M. and Kouame B. (1994) Ethyl propionate: synergistic kairomone for african palm weevil Rhynchophorus phoenicis L. (Coleoptera:Curculationidae). *J. Chem. Ecol.* **20,** 889–896.

Griffith R. (1968) The relationship between the red ring nematode and the palm weevil. *J. Agric. Soc. of Trinidad and Tobago* **68,** 342–356.

Hallett R. H., Gries G., Gries R., Borden J. H., Czyzewska E., Oehlschlager A. C., Pierce H., Angerilli N. P. D. and Ranf N. (1993) Aggregation pheromone of two asian palm weevils, *Rhynchophorus ferrugineus* (Oliv) and *R. vulneratus* (Panz). *Naturwissenschaften* **80,** 328–331.

Hernández J. V., Cerdá H., Jaffé K. and Sánchez P. (1992) Localización hospedera, actividad diaria y optimización de la captura del picudo del cocotero *Rhychophorus palmarum* L. (Coleoptera: Curculionidae), mediante trampas inócuas. *Agron. Trop.* **42** (3–4).

Jaffé K., Sánchez P., Cerdá H., Urdaneta N., Hernández J. V., Guerra G., Jaffé R., Martinez R. and Miras B. (1993) Chemical ecology of the palm weevil *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae): Attraction to host plants and to a male produced aggregation pheromone. *J. Chem. Ecol.* **19,** 1703–1720.

Kaib M. and Ziesmann J. (1992) The labial gland in the termite *Schedorhinotermes lamanianus* (Isoptera:Rhinotermitidae): Morphology and function during communal food exploitation. *Ins. Soc.* **39,** 373–384.

Levinson H. Z., Levinson A. R., Jen T. J., Williams J. and Kahn G.

(1978) Production site, partial composition and olfactory perception of a pheromone in male hide beetle. *Naturwissenschaften* **65,** 543–545.

Moura J., Vilela E., Sgrillo R., Aguilar M. and Resende M. (1989) A behavioral olfactory study of *Rhynchophorus palmarum* (L) (Coleoptera: curculionidae) in the field. *Ann. Soc. Entomol. Bras.* **18,** 267–274.

Nadarajan L. (1986) Investigations on the pheromone communication in the palm weevil *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae). Francia, Tese de pos-doutoramento, 38pp. Laboratoire des Mediateurs chimiques Brouessy. INRA.

Oehlschlager A. C., Pierce H. D., Morgan B., Wimalaratne P. D. C., Slessor K. N., King G. G. S., Gries G., Gries R., Borden J. H., Jiron L. F., Chinchilla C. M. and Mexan R. (1992) Chirality and field testing of Rhynchophorol, the aggregation pheromone of the American palm weevil. *Naturwissenschaften* **79,** 134–135.

Oehlschlager A. C., Chinchilla C., Gonzalez L. M., Jiron L. F., Mexon R. and Morgan B. (1993) Development of pheromone-based trapping system for the American palm weevil, *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae). *J. Econ. Entomol.* **86,** 1381–1392.

Percy J. and Weatherston J. (1971) Studies of physiologically active arthropod secretions. IX. Morphology and hystology of the pheromone-producing gland of some female Lepidoptera. *Can. Entomol.* **103,** 1733–1739.

Raina A. K., Jaffe H., Kemple T. G., Keim P., Blacher R. W., Fales H. M., Riley C. R., Klum J. A., Riggway R. and Hayes D. (1989) Identification of a neuropeptide hormone that regulates sex pheromone production in female moth. *Science* **224,** 196–798.

Rochat D. (1987) Etude de la comunication chimique chez un Coleoptere Curculionidae: *Rhynchophorus palmarum* L. Disertation of Ms. University of Paris. VI. 30 pp.

Rochat D., Gonzales A., Marian D., Villanueva A. and Zagatti P. (1991a) Evidence for male produced aggregation pheromone in American palm weevil *Rhynchophorus palmarum* L. *J. Chem. Ecol.* **17,** 1221–1227.

Rochat D., Malosse C., Lettere M., Ducrot P., Zagatti P. H., Renou M. and Descoins C. (1991b) Male-produced aggregation pheromone of the american palm weevil *Rhynchophorus palmarum* L.: Collection, identification, electrophysiological activity and laboratory bioassay. *J. Chem. Ecol.* **17,** 2127–2141.

Rochat D., Descoins C., Malosse C., Nagnan P., Zagatti P., Akamou F. and Mariau D. (1993) Ecologie chimique des charancons des palmiers Rhynchophorus spp. (Coleoptera). *Oléagineux* **48,** 225–236.

Sánchez P. and Cerdá H. (1993) Revisión bibliográfica: El complejo *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae)-*Bursaphelenchus cocophilus* (Cobb.) (Tylenchida: Aphelenchoididae) en palmeras. *Bol. Entomol. Venez.* **8,** 1–18.

Siegel S. (1982) Estadística no paramétrica. Trillas México.

Teal P., Tulinson J. H. and Oberlander H. (1989) Neural regulation of sex pheromone biosyntesis in *Heliothis* moths. *Proc. Nat. Acad. Sci. U.S.A.* **68,** 2488–2492.

Tumlinson J. H., Hardee D., Minyard J. P., Tompson A. C., Gast R. T. and Hedin P. A. (1968) Boll weevil sex attractant: Isolation studies. *J. Econ. Entom.* **61,** 470–474.

Wattanapongsiri A. (1966) A resision of the genera Rhynchophorus and Dynamis. Depart. Agric. Sc. Bul. Bangkok (Trinidad). 1: 185p.

Weissling T. J., Giblin-davis R. M., Gries G., Gries R., Perez A. L., Pierce H. D. and Oehlschlager A. C. (1994) Aggregation pheromone of Palmetto weevil, *Rhynchophorus cruentatus* (F) (Coleoptera: Curculionidae). *J. Chem. Ecol.* **20,** 505–515.

Acknowledgements—The authors thank the technical assistance of Leonardo Caraballo, Carlos Cárdenas of USB and of Gerónimo Guerra of FONAIAP-Miranda. We profited from discussions with José V. Hernández and critical readings of the manuscript by M. Whitehouse. Morphological work profited from logistic help by G. Villegas at IDEA. The work was financed by the project QF-36 of BID-CONICIT.