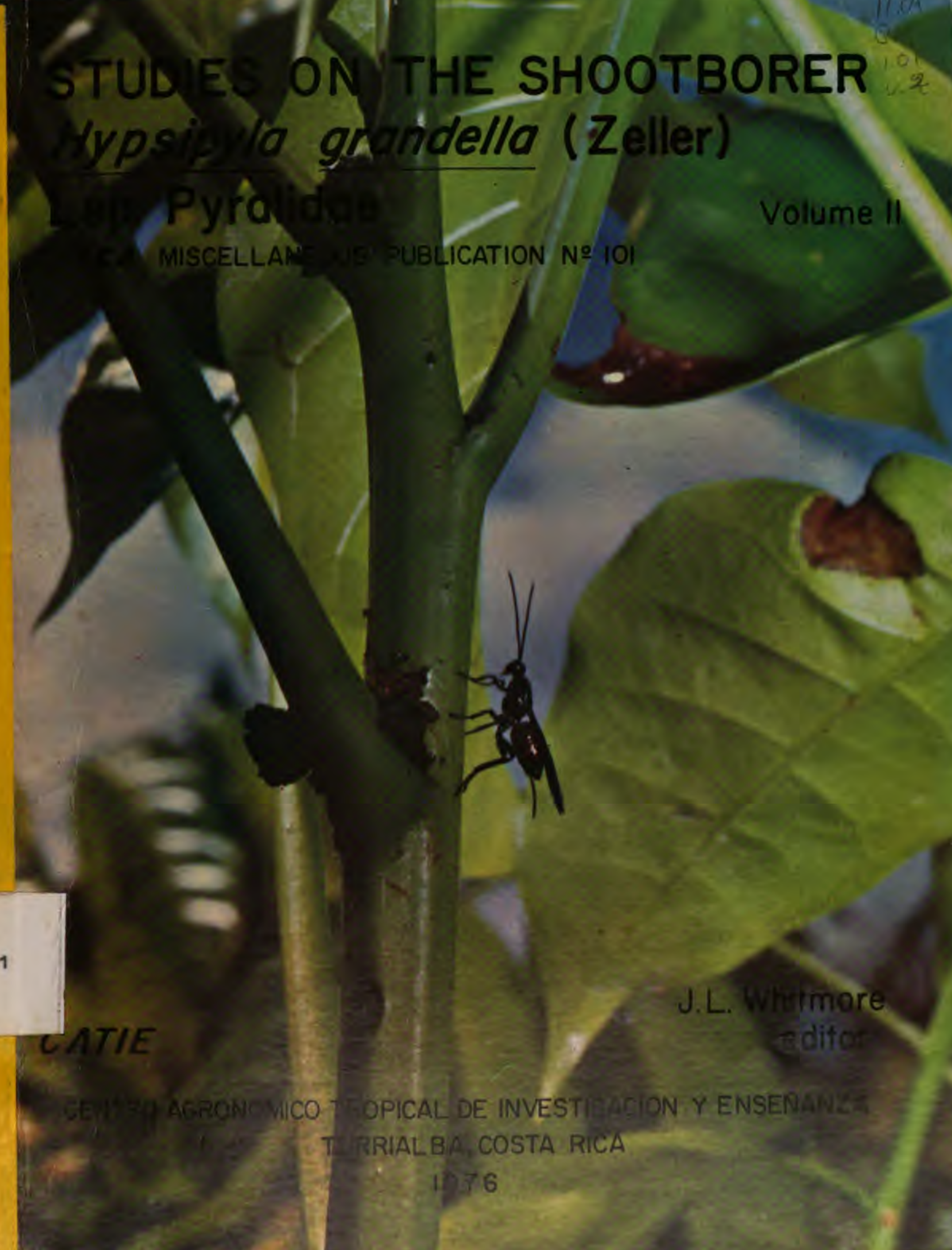

STUDIES ON THE SHOOTBORER *Hypsipyla grandella* (Zeller)

Lep.: Pyralidae

Volume II

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J.L. Whitmore
editor

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CENTRO AGRONOMICO TROPICAL DE INVESTIGACION Y ENSEÑANZA
TURRIALBA, COSTA RICA
1976

The parasite *Bracon chontalensis* locating a *Hypsipyla grandella* larva in a mahogany shoot. (Photo by P. Grijpma.)

El parásito *Bracon chontalensis* localizando una larva de *Hypsipyla grandella* en un tallo de caoba. (Foto por P. Grijpma.)

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* Travel report CIBC West Indian Station, 1968.
** From Proceedings of the First Symposium on Integrated Control of *Hypsipyla*. IICA–CTEI. Turrialba, Costa Rica. 1973.
*** Adapted from the author's M.S. Thesis, IICA–CTEI. Turrialba, Costa Rica. 1973.
☆ Translated from the original in Zeitschrift für angewandte Entomologie 72(3):259–266. 1973.
☆☆ Originally published in the Journal of Agriculture of the University of Puerto Rico 58(2):276–278. 1974.
☆☆☆ Originally published in Annals of the Entomological Society of America 68(2):319–320. 1975.

PREFACE

This collection of articles is the second such volume resulting from investigations carried out by members of the Inter-American Working Group on the Shootborer **Hypsipyla grandella** *(Zeller). Many of the articles were published previously in the journal "Turrialba"; those which have been published elsewhere are noted accordingly.*

The contents of this booklet reflect the growth of the Working Group's research on this tropical forest insect which affects some of the commercially important tree species of the Meliaceae. The articles are published in English or Spanish and abstracted. They present new information which eventually may result in control of this insect pest and more successful commercial establishment of the tree species involved.

The Inter–American Working Group on **Hypsipyla grandella**, *established at Turrialba in September, 1970, has over 100 members who represent 28 countries. The initial stimulus provided by, and the further participation of Dr. R. I. Gara, Dr. G. G. Allan and their students from the College of Forest Resources, University of Washington, Seattle, deserve special mention, as does Dr. Pieter Grijpma, who guided the Group during its first four years.*

Funds for this publication were made available by the Netherlands Bureau of International Technical Assistance which, together with the Department of Natural Resources and the Nuclear Energy Program of CATIE, also supported the research.*

J. L. Whitmore

* Centro Agronómico Tropical de Investigación y Enseñanza, formerly IICA–CTEI (Centro Tropical de Enseñanza e Investigación del Instituto Interamericano de Ciencias Agrícolas).

OBSERVACIONES SOBRE LA BIOLOGIA DE *H. FERREALIS* (HAMPSON), UNA ESPECIE AFIN*

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ABSTRACT

This investigation presents data on the life cycle, larval behavior and adult emergence of *Hypsipyla ferrealis* (Hampson), a species closely related to *H. grandella* (Zeller). Study material was obtained from attacked fruits of *Carapa guianensis* Aubl. *(Meliaceae)* collected in Siquirres, in the Atlantic coast region of Costa Rica, and from fecundated females of *H. ferrealis* collected in light traps in Turrialba. Subsequent rearing on *C. guianensis* seed took place under laboratory conditions. Of the 4,328 seeds collected 36.3 per cent were attacked.

The life cycle, from egg eclosion to adult emergence, lasted an average of 35 days. Seventy per cent of the larvae had three, and thirty per cent had four ecdyses.

A larval period of inactivity, possibly a diapause, was observed in twenty five per cent of the larvae. These larvae constructed a special chamber in a corner of the seed in which they remained for a period varying from one to five months before pupating. Their weight was twice as much as of the others. It is assumed that the period of larval inactivity is a survival mechanism for the period in which no adequate food source is available. The remaining seventy five per cent of the larvae pupated outside the seeds and emerged eleven days later.

Emergence occurred in the beginning of the evening. The maximum frequency was observed twenty minutes after zero lux.

The author

Introducción

Una plaga importante de las meliáceas en América Tropical es el barrenador de los brotes *Hypsipyla grandella* (Zeller). No obstante, existen otras especies de microlepidópteros que también causan daños a esta familia de árboles valiosos.

En este trabajo se describen algunas observaciones sobre la biología de *Hypsipyla ferrealis* (Hampson), barrenador de los frutos del cedro macho, *Carapa guianensis* Aubl. (Meliaceae). *H. ferrealis* es parecida y puede ser confudida con *H. grandella* (Zeller).

Revisión de literatura

El primer dato sobre el hospedante de *H. ferrealis* fue registrado por Heinrich (5), quien estudió algunos ejemplares criados por Ballou en semillas de *C. guianensis* en Venezuela.

Entwistle (2) indica que *C. guianensis*, *Spondias mombin* L. (Anacardiaceae) y *Rheedia* sp. (Guttiferae) en Trinidad son atacadas por esta especie. En el mismo país, Rao y Bennett (7) registraron *Chrysodoria* sp. (Tachinidae) como parásito de *H. ferrealis*.

Becker (1), estudiando larvas obtenidas de semillas de *C. guianensis*, provenientes de la región amazónica de Brasil, publicó algunos datos sobre su comportamiento y describió la larva y la pupa. Rego (8) describió los daños provocados en semillas de esta misma especie por *H. grandella*, en Río de Janeiro.

* Recibido para la publicación el 2 de abril de 1973.

** Actualmente con el Departamento de Zoología, Universidade Federal do Paraná, Brasil.

Cuadro 1. Ataques de *H. ferrealis* en semillas de *C. guianensis*.

Muestra	Fecha de recolección 1972	Arboles padres	Semillas sanas	Semillas atacadas	Semillas (Total)	Semillas atacadas (%)
1*	28–IX	1	211	12	223	5,4
2	10– X	1	43	48	91	52,7
3	10– X	5	845	232	1.077	22,7
4	13– X	10	1.657	1.280	2.837	43,5
Total		17	2.756	1.572	4.328	36,3

* 170 semillas sueltas y 9 frutos que contenían 53 semillas.

Materiales y métodos

El material utilizado en este estudio provino de cuatro muestras de semillas de *C. guianensis* (Cuadro 1). Estas semillas fueron recogidas del suelo bajo sus árboles padres, con excepción de la muestra 1, que estaba formada de 170 semillas y nueve frutos recolectados de un árbol volteado el día anterior a la recolecta.

Los árboles se encontraban en un bosque primario y medían entre 0,40 y 1 m de diámetro encima de las gambas, y entre 25 y 40 m de altura. El árbol de la muestra 1 se encontraba a 450 m y los demás a 300 m de altitud.

El sitio de recolección se ubica a 3 km al oeste de la carretera Turrialba–Siquirres, a unos 10 km antes de Siquirres (10°03'N, 83°34'O).

Según Tosi (10), el bosque pertenece a la zona de vida Bosque Muy Húmedo Tropical, Transición a Premontano, dentro del sistema de Holdridge.

Las larvas obtenidas en estas semillas, listas para empupar, fueron transferidas a cajas plásticas de 22 x 33 x 5,5 cm, divididas en 24 compartimientos de 5 x 5 x 5 cm. En el fondo de cada compartimiento se colocó papel toalla mojado para mantener la humedad relativa a 100 por ciento.

Para el estudio del ciclo de vida las larvas fueron obtenidas de huevos ovipositados en el laboratorio por dos hembras recolectadas en una trampa de luz en Turrialba, el 29 de diciembre de 1972, ovipositados sobre papel toalla húmedo en una caja plástica de 28 x 13 x 6 cm.

Después de la eclosión cincuenta larvas fueron transferidas a reglas de cría, construidas de Formica con 3 cm de ancho y 18 cm de largo, con una abertura en una de las extremidades (Fig. 1 a). La mitad de las reglas tenía 1 mm de espesor con abertura de 1 x 5 cm, en las cuales se criaron las larvas del primer y segundo instar. La otra mitad tenía 2,5 mm de espesor con abertura de 1,5 x 5 cm, en las cuales se criaron las larvas del tercer, cuarto y quinto instar. En el cuarto y quinto instar, en vista del desarrollo de las larvas, se usaron dos reglas sobrepuestas del último modelo.

La abertura dentro de la cual las larvas se desarrollaron sobre el alimento se cerraron mediante dos láminas de vidrio fijadas por ligas de hule (Fig. 1 b). Este sistema facilita la manipulación y la observación bajo microscopio del desarrollo y comportamiento de las larvas.

Fig. 1. Reglas de cría para el estudio del ciclo biológico de *H. ferrealis*: a) abierta; b) cerrada.

Para la alimentación de las larvas se usaron porciones de semilla de *C. guianensis* cambiadas diariamente. Para evitar la desecación del alimento, las reglas fueron colocadas dentro de cajas plásticas, iguales a las usadas en la oviposición, con papel toalla húmedo en el fondo de la caja.

Se siguió el desarrollo completo de estas larvas desde la eclosión hasta la empupación. Las cápsulas cefálicas fueron sacadas y medidas después de cada ecdisis, con el fin de construir una tabla para la determinación de los instar larvales.

En el momento de la recolecta, se sacaron todas las larvas de veinte semillas atacadas de la muestra 4 para determinar la estructura de la población larval. El instar larval fue determinado mediante la medición microscópica del ancho de las cápsulas cefálicas y comparadas con la tabla obtenida en el estudio del ciclo de vida. De la misma muestra 4 se sacaron al azar 45 semillas para determinar el número promedio de larvas por semilla atacada.

El estudio fue realizado en el laboratorio a una temperatura media de 23,9 ± 1,4°C y humedad relativa de 68,1 ± 10,4 por ciento.

La emergencia de los adultos se observó bajo condiciones de luz natural. La intensidad de luz fue medida con un fotómetro Gossen, Modelo 2.59–406.

Resultados y discusión

Comportamiento de la larva

Las larvas de *H. ferrealis* se alimentan del contenido de las semillas. Se observó que en las semillas atacadas, caídas al suelo, las larvas permanecieron en su interior hasta el momento de encapullar. En las semillas que aún se encontraban dentro del fruto, se notó que las larvas hicieron huecos en el tegumento y pudieron trasladarse de una semilla a otra.

Las larvas de esta especie son muy tolerantes a convivir en grupos. De las 45 semillas atacadas, sacadas al azar de la muestra 4, el promedio fue de 9,3 ± 7,7 larvas por semilla (Cuadro 2). En veinte de estas 45 semillas había 168 larvas, variando desde el segundo hasta el quinto instar. El mayor número, 116, que representaba el 69,1 por ciento, pertenecía al quinto instar, veintisiete al cuarto, ocho al tercer y diecisiete al segundo instar. Dos de estas semillas contenían treinta larvas cada una, en varios instar, y en una de ellas había veintitrés larvas listas para empupar. A pesar del gran número de larvas en algunas semillas, no se observó ningún caso de canibalismo, lo que es frecuente en *H. grandella.*

Al prepararse para empupar se notó que las larvas se comportaron de dos maneras distintas. La mayoría, aproximadamente el 75 por ciento, salió de la semilla y construyó un capullo en el fondo de las cajas. En condiciones naturales esto sucede en el suelo, entre las hojas muertas. Durante la recolección de las semillas se encontraron varios capullos en estas condiciones. Las demás larvas construyeron una cámara pupal en uno de los ángulos internos de las semillas, como ya fue registrado anteriormente (1) (Fig. 2 a). En las semillas atacadas fueron encontradas desde una hasta seis cámaras por semilla.

Las larvas que salieron de las semillas buscaron un sitio apropiado e inmediatamente empezaron a construir un capullo. Se empuparon, en promedio 2,9 ± 0,7 días después de iniciar la construcción del capullo (observación de 87 ejemplares). La emergencia ocurrió 11,2 ± 0,9 días después de la empupación (Fig. 3).

Cuadro 2. Número de larvas de *H. ferrealis* por semilla de *C. guianensis.*

Número de larvas	Número de semillas	Porcentaje
0– 5	16	35,6
6–10	14	31,2
11–15	5	11,1
16–20	6	13,3
21–25	1	2,2
26–30	3	6,6
Total	45	100,0

Las larvas que construyeron una cámara pupal dentro de las semillas permanecieron sin alimentarse en el interior de estas cámaras (Fig. 2 b) por un período de uno a cinco meses de inactividad. Se supone que este período de inactividad es un período de diapausa que evita que los adultos emerjan en el período en que *C. guianensis* no tiene semillas desarrolladas. Antes de empupar, estas larvas construyeron un capullo dentro de las cámaras (Fig. 2 c). Los adultos demoraron en promedio 90,1 ± 30,7 días para emerger, después del inicio de la construcción de la cámara (Fig. 4). El período pupal fue igual en ambos grupos.

El capullo (Fig. 2 c) en ambos grupos es blanco, más espeso que el de *H. grandella,* y contiene mucho aceite, elemento que evita la entrada de agua en el capullo, protegiendo de esta forma la pupa en los suelos pantanosos o muy húmedos, donde se encuentra *C. guianensis.*

Se pesaron veinte larvas de cada grupo, las empupadas dentro de las semillas pesaron en promedio más que el doble de las que se empuparon fuera, esto es 0,393 ± 0,084 g y 0,186 ± 0,063 g respectivamente.

Las veinte larvas que se sacaron de las cámaras pupales para pesarse fueron colocadas después dentro de una caja plástica con papel toalla húmedo. Estas larvas trataron de reconstruir una nueva cámara entre el papel y el fondo de la caja. Este cambio de ambiente hizo que las larvas rompiesen su inactividad, lo que las llevó a encapullarse, emergiendo aproximadamente once días después. Por esta razón no se observó detalladamente el período de inactividad de este grupo de larvas, ya que sería necesario abrir con frecuencia las cámaras pupales, lo que podría alterar su comportamiento normal.

Como las condiciones del laboratorio fueron distintas a las naturales, se colocó una muestra de treinta larvas con sus cámaras bajo un bosque, sobre el suelo. Se observó que el período de inactividad larval fue más largo que en el laboratorio. Mientras que todos los adultos ya habían emergido en el laboratorio, dieciséis larvas todavía permanecían sin encapullar en la muestra del bosque, el 5 de marzo de 1973.

Para construir la cámara, la larva limpia un espacio en uno de los ángulos internos de la semilla, de preferencia en uno de los vértices. Lo hace tomando con las mandíbulas las partículas de excremento y de la semilla, empujándolas luego hacia los lados. Después de haber preparado el espacio escogido, la larva corta interna-

Fig. 2. Cámaras pupales de *H. ferrealis:* a) cerrada; b) abierta y con larva; c) abierta y con capullo. La flecha indica el orificio de salida del adulto.

mente el tegumento de la semilla en la línea que delimita el espacio limpio del restante de ella. El pedazo de la semilla cortado queda fijo solamente por una capa fina del tegumento, de manera que con una pequeña presión se separa fácilmente. Como gran parte de las semillas es consumida por los roedores, este mecanismo evita la destrucción de la cámara pupal y por lo tanto la larva no es molestada. Al completar el corte en el pedazo de la semilla, la larva construye con hilos blancos de seda, una película espesa muy resistente para cerrar la cámara; esta película cambia gradualmente hacia un color café, semejante al color del tegumento de la semilla. Al terminar la película, la larva se mantiene aislada en el interior de la cámara pupal, entre la película y el tegumento de la semilla. Luego, la larva hace un orificio de 2–3 mm de diámetro en el tegumento (Fig. 2 b), dejando una pequeña capa de éste, lo que evita la entrada de agua y predatores al interior de la cámara, y permite la salida del adulto.

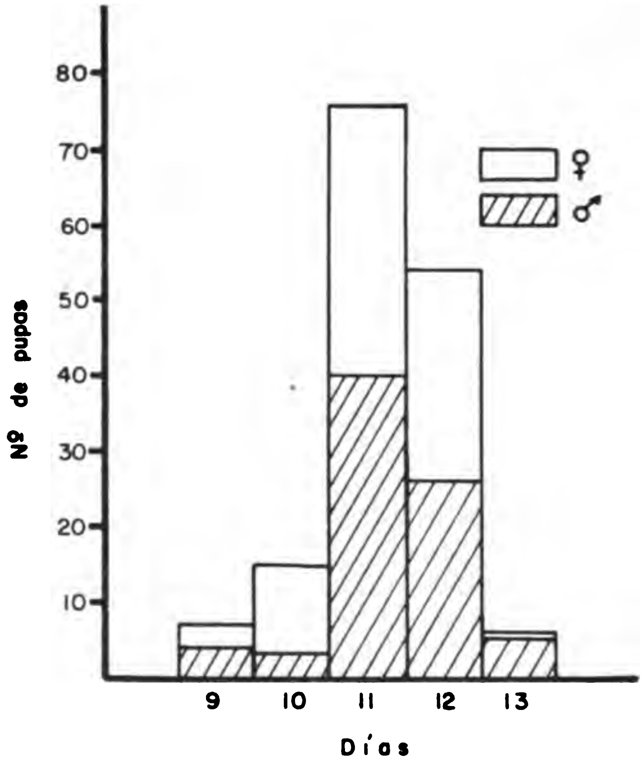

Fig. 3. Distribución de la frecuencia de las pupas de *H. ferrealis* en relación con la duración del estado pupal.

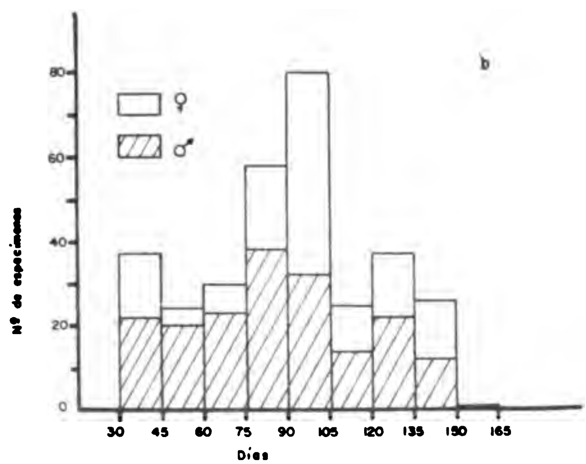

Fig. 4. Distribución frecuencial de la duración del período de inactividad larval de *H. ferrealis,* incluyendo el estado pupal, de las larvas que se empupan dentro de las semillas.

Cuadro 3. Período de desarrollo (días) de *H. ferrealis,* a partir de la oviposición, en condiciones de laboratorio.

	Eclosión	1a. ecdisis	2a. ecdisis	3a. ecdisis	4a. ecdisis	Encapullamiento	Empupación	Emergencia
Promedio	5	8,4	10,8	14,1	17,3	23,4	25,1	35,6
Variación	0	±0,4	±0,7	±1,0	±1,1	±1,8	±1,8	±1,8
Período observado	5	8–9	10–13	13–18	16–19	21–27	22–29	34–40
Número de ejemplares	50	47	40	30*	9	22	22	14
Número de ejemplares muertos	3	7	10	5	3	0	8	

* De éstos, 16 se empuparon enseguida.

Ciclo de vida

Las larvas de *H. ferrealis* tienen dos tipos de comportamiento distintos. Las larvas que salen de las semillas construyen de inmediato el capullo y se empupan, mientras que las que se quedan en el interior de las semillas permanecen inactivas por un período de por lo menos uno a cinco meses. El período pupal es igual en los dos grupos.

En el estudio del ciclo de vida, en las reglas de cría, todas las larvas se comportaron como las que se empupan fuera de las semillas.

El ciclo completo de *H. Ferrealis,* desde la oviposición hasta la emergencia, se completó en 35,6 ± 1,8 días en promedio (Cuadro 3). Grijpma (4) observó que en *H. grandella* el ciclo en condiciones de laboratorio es aproximadamente igual. El período de incubación de los huevos de *H. ferrealis* fue de cinco días, la fase larval requirió de veintidós a veintinueve días. El sesenta y cuatro por ciento de las larvas tuvieron tres ecdisis y el resto cuatro. En *H. grandella* el número de ecdisis fue desde cinco hasta seis (6).

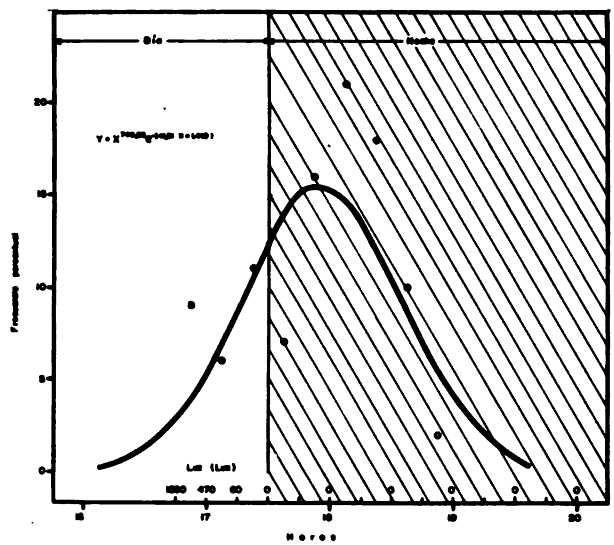

Fig. 5. Frecuencia esperada y observada de emergencia de *H. ferrealis* en relación con intensidad de luz y la hora del día.

Emergencia de los adultos

La emergencia ocurrió al anochecer y los primeros adultos empezaron a emerger mientras la intensidad de luz fue superior a 1.000 lux, aproximadamente a las 16:45 horas. La frecuencia aumentó rápidamente a medida que la intensidad de luz bajó a cero lux, aproximadamente a las 17:30 horas, y alcanzó un máximo aproximadamente a los veinte minutos, después de haber bajado a este nivel (Fig. 5). En *H. grandella* el máximo de frecuencia ocurrió antes de haber bajado la intensidad de luz hasta cero lux (9).

La distribución de la frecuencia se ajustó, con una confiabilidad de 45,5 por ciento a la distribución gama. No se encontró diferencia significativa entre la emergencia de los machos y de las hembras, a un nivel de cinco por ciento ($P \leq 0{,}05$).

Los adultos emergieron por la parte anterior de la pupa (Fig. 6 a); las rupturas ocurrieron en las regiones cefálica y toráxica. En el lado ventral, la pupa se rompió en las suturas que separan las maxilas de los demás apéndices, desde la base hasta la extremidad de las antenas. En algunos ejemplares, la ruptura en esta región ocurrió totalmente en uno de los lados y parcialmente en el otro. En el lado dorsal se verificaron dos rupturas, una longitudinal y otra transversal. La ruptura longitudinal ocurrió en el medio del tórax, desde la sutura entre el protórax y la cabeza hasta la sutura entre el metatórax y el primer segmento abdominal. La ruptura transversal se verificó entre el protórax y el mesotórax, desde una antena hasta la otra. En las partes laterales la ruptura sucedió en la sutura entre la antena y el primer par de alas, desde la base hasta el tercio distal. En la parte

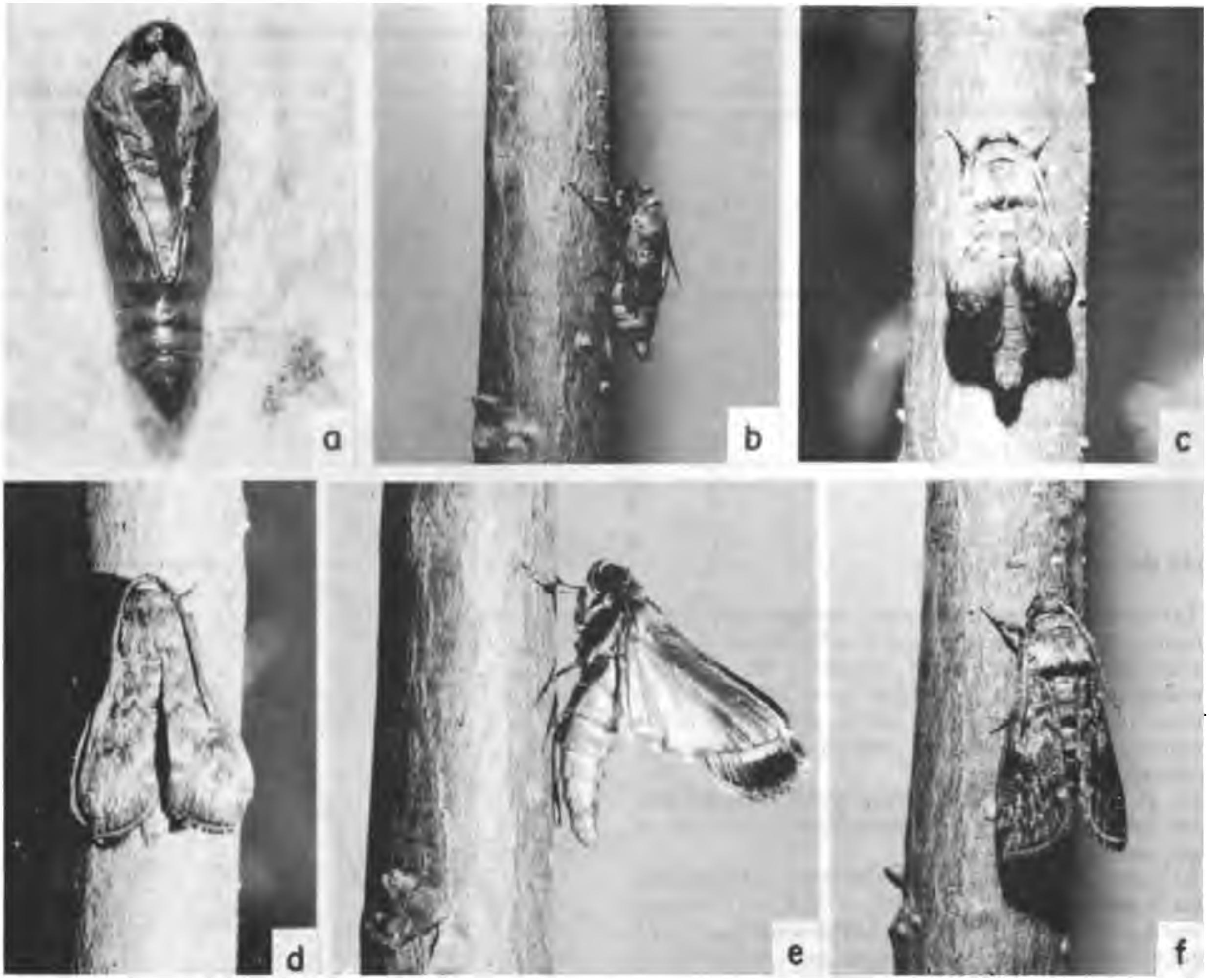

Fig. 6. Emergencia del adulto de *H. ferrealis*: a) emergiendo de la pupa; b) recién emergido; c) y d) alargando las alas; e) endureciendo las alas; f) en reposo.

anterior, la cabeza, comprendiendo las regiones del vértice y fronte, se destacó completamente de la pupa. La ruptura ocurrió en las suturas que separan estas partes de la cabeza del protórax, base de las antenas y maxilas.

El tiempo para alargar y endurecer las alas después de la emergencia (Fig. 6) fue de 3,5 ± 0,6 y 8,6 ± 1,6 minutos respectivamente. Los adultos se quedaron inmóviles en el mismo sitio (Fig. 6 f) hasta media hora después de bajar sus alas.

La proporción entre sexos fue de aproximadamente 6:5 (735 ♂♂ y 602 ♀♀) en las larvas que se empuparon fuera de las semillas y de 4:3 (183 ♂♂ y 135 ♀♀) en las larvas que se empuparon dentro de las semillas.

Hospedantes

La literatura cita como plantas alimenticias de las larvas de esta especie a *C. guianensis* (1, 2, 5), *Spondias mombin* L. y *Rheedia* sp. (2). En Costa Rica las larvas se encontraron solamente en los frutos y semillas del cedro macho. En dos muestras de frutos de dos árboles de *Spondias mombin* L., en el área del Centro Tropical de Enseñanza e Investigación del IICA, no fueron encontradas larvas de *H. ferrealis*.

Existe la posibilidad de que hay otro, u otros hospedantes. Una de las razones es que en Turrialba, situada en una región en donde no ocurre naturalmente el cedro macho, se pueden recolectar fácilmente los adultos de *H. ferrealis* a la luz durante todo el año, siendo más frecuente en agosto y setiembre. En el período entre el 17 de agosto y el 25 de setiembre de 1971 se recolectaron 238 adultos en una trampa de luz de mercurio y en las ventanas de los laboratorios del CTEI. En una sola noche, el 18 de setiembre, se recolectaron 52 ejemplares de *H. ferrealis*. Por otra parte vale mencionar que esta época del año coincide con el período en el cual los frutos del cedro macho caen al suelo.

Fig. 7. Semillas de *C. guianensis,* atacadas por *H. ferrealis;* a) con partículas de excremento larval expuestas; b) con huecos hechos por las larvas para trasladarse de una semilla hacia otra, mientras están en el fruto; c) recortada mostrando los daños internos.

Otra razón es que el 75 por ciento de los adultos de *H. ferrealis* emergen en una época en la cual *C. guianensis* no tiene frutos.

Síntomas del hospedante

El único fruto que se encontró atacado presentaba una lesión próxima al pedúnculo dejando expuestas dos de las semillas. Por esta lesión salían restos del fruto y de semillas, y partículas de excremento de las larvas (Fig. 7 a), como ya fue observado anteriormente por el autor (1).

Al abrirse el fruto se encontraron huecos de 2–3 mm de diámetro en la parte interna de la cáscara, que llegaban al exterior del fruto, quedando cubiertos por una fina película de la cáscara. Estos hoyos son hechos por las larvas que se quedan en el interior de las semillas antes de empuparse y permiten la salida del adulto.

Las semillas atacadas presentaron galerías irregulares en la parte interna (Fig. 7 c), y orificios en el tegumento (Fig. 7 b). En las semillas que fueron totalmente destruidas, el espacio anteriormente ocupado por los cotiledones estaba lleno de excremento, en general unidos entre sí por hilos de seda blanca.

No se encontraron larvas de esta especie atacando otras partes del hospedante. Becker (1) indica que de las ramas atacadas de *C. guianensis* de la región amazónica de donde provino el fruto emergieron solamente *H. grandella.*

A pesar del alto porcentaje de semillas atacadas (Cuadro 1), los daños ocasionados por el ataque no parecen ser un factor muy limitante para la germinación del cedro macho. De las 280 semillas atacadas de las muestras 2 y 3 (Cuadro 1), 141 (50 por ciento) estaban germinando en el día de la recolección. Al contrario, pareció que el ataque había adelantado la germinación, ya que de las 888 semillas sanas solamente 96 (11 por ciento) estaban germinando. El ataque podría ser favorable a la regeneración ya que las semillas que demoran mucho en germinar tienen una probabilidad mayor de ser consumidas por roedores.

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A NEW SPECIES OF *TRICHOGRAMMA* (HYMENOPTERA, TRICHOGRAMMATIDAE) FROM COSTA RICA*

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COMPENDIO

Se describe una nueva especie del género *Trichogramma*, parásito de los huevos de *Hypsipyla grandella*. Los huevos parasitados fueron encontrados en semillas de *Cedrela tonduzzi* C. DC. obtenidos de un bosquete natural cerca de Santa Cruz que se localiza en las faldas del volcán Turrialba a 1500 m.s.n.m. Es posible que esta especie de *Trichogramma* no sólo parasite los huevos de *H. grandella* sino también los huevos de *Sematoneura* sp. encontrados en las semillas de *C. tonduzzi*.

The New World appears to have a fair abundance of *Trichogramma* species, many of which are so similar morphologically as to make identification difficult. It is therefore gratifying to find an occasional species that is markedly different from others occurring in this region and other parts of the world.

Since 1970, P. Grijpma, Coordinator, Inter–American Working Group on *Hypsipyla*, Inter–American Institute of Agricultural Sciences of the OAS, Turrialba, Costa Rica, has been sending specimens of *Trichogramma* reared from *Hypsipyla grandella* (Zeller) (Lepidoptera: Phycitinae) to the author for determination. In April 1972, the author had found *T. semifumatum* (Perkins), *T. pretiosum* Riley and a species near *T. pretiosum* in material received from Mr. Grijpma. The species described below was received in October 1972 and was reared from *H. grandella* eggs collected by a graduate student. V. O. Becker at Santa Cruz, 1500 m.a.s.l., Turrialba, Costa Rica. The eggs of the hosts were found on green fruits of *Cedrela tonduzzi* C. D.C., hanging at a height of approximately 15 m on branches of a mature tree. The biology and ecology of this *Trichogramma* species is being studied by Mr. Grijpma and his colleagues.

Trichogramma beckeri sp. n.

Adults extremely small (0.5 mm long and 0.16 mm wide across head). Males reared from natural hosts under field conditions in Costa Rica, with nearly black pronotum and mesopleurae, yellow mesothorax and metathorax and black abdomen. Hind coxae smoky grey, otherwise legs pale yellow. Antennal flagellum (Fig. 1) unsegmented with short, tapering hairs, the longest being twice the maximum width of flagellum. Fringe on tornus of forewing (Fig. 2) about one–tenth the width of wing. Forewing with markedly smoky area under stigmal vein. Trichiation on remigium in regular rows on anterior half of disk, becoming somewhat irregular towards posterior half. Genitalia (Fig. 3) with very prominent highly chitinized dorsal expansion of gonobase (degb) having broad sides, which extended slightly beyond gonoforcepts laterally, posterior extremity being smoothly and broadly rounded, extending beyond chelate structures. A median ventral chitinized ridge (cr) extends from anterior margin of gonobase (gb) through entire length of genitalia. Apodemes of penis valve (apv) of same length as aedeagus (Fig. 3a). Chelate structures (cs, Fig. 3b) small, bilobed, highly chitinized and located fairly close to tips of gonoforceps. Gonoforceps (gf, Fig. 3b) narrow and tapering. Median ventral projection (mvp, Figs. 3 and 3b) peg–like, small but distinct, extending up to base of chelate structures.

Females more yellow than males when reared from natural host under identical field condictions, with yellow pronotum, mesopleurae and hind coxae. Antennal flagellum typically clubbed with a few short hairs. Ovipositor slightly longer than hind tibia (Fig. 4) and latter nearly twice the length of aedeagus.

Holotype: Male from Costa Rica: Santa Cruz, Turrialba; ex. eggs of *Hypsipyla grandella* on *Cedrela tonduzzi* C. DC.; 3rd October 1972 (V. O. Becker coll.) in the U. S. National Museum, Washington, D.C. Registered USNM No. 72494. Male and female genitalia mounted on slide. Paratypes in the British Museum of Natural History, London, England, Academy of Sciences, Leningrad, U.S.S.R., California Insect Survey, Berkeley, California, U.S.A., Indian Agricultural Research Institute, New Delhi, India and University of Costa Rica at San José, Costa Rica.

Remarks: The dorsal expansion of gonobase in this species is so distinct from all other known *Trichogramma* species that it justifies its being described as a new species. It also differs markedly from another newly described *Trichogramma* species, *T. bennetti* (1) reared from *H. ferrealis* Hmps. eggs in Trinidad (West Indies) by Dr. F. D. Bennett of the CIBC West Indian Station, both with regard to male genitalia as well as pigmentation. In some specimens examined, the dorsal expansion of gonobase was seen to extend to the tips of the gonoforceps, appearing to almost enclose the latter. The small and highly chitinized chelate structures and the

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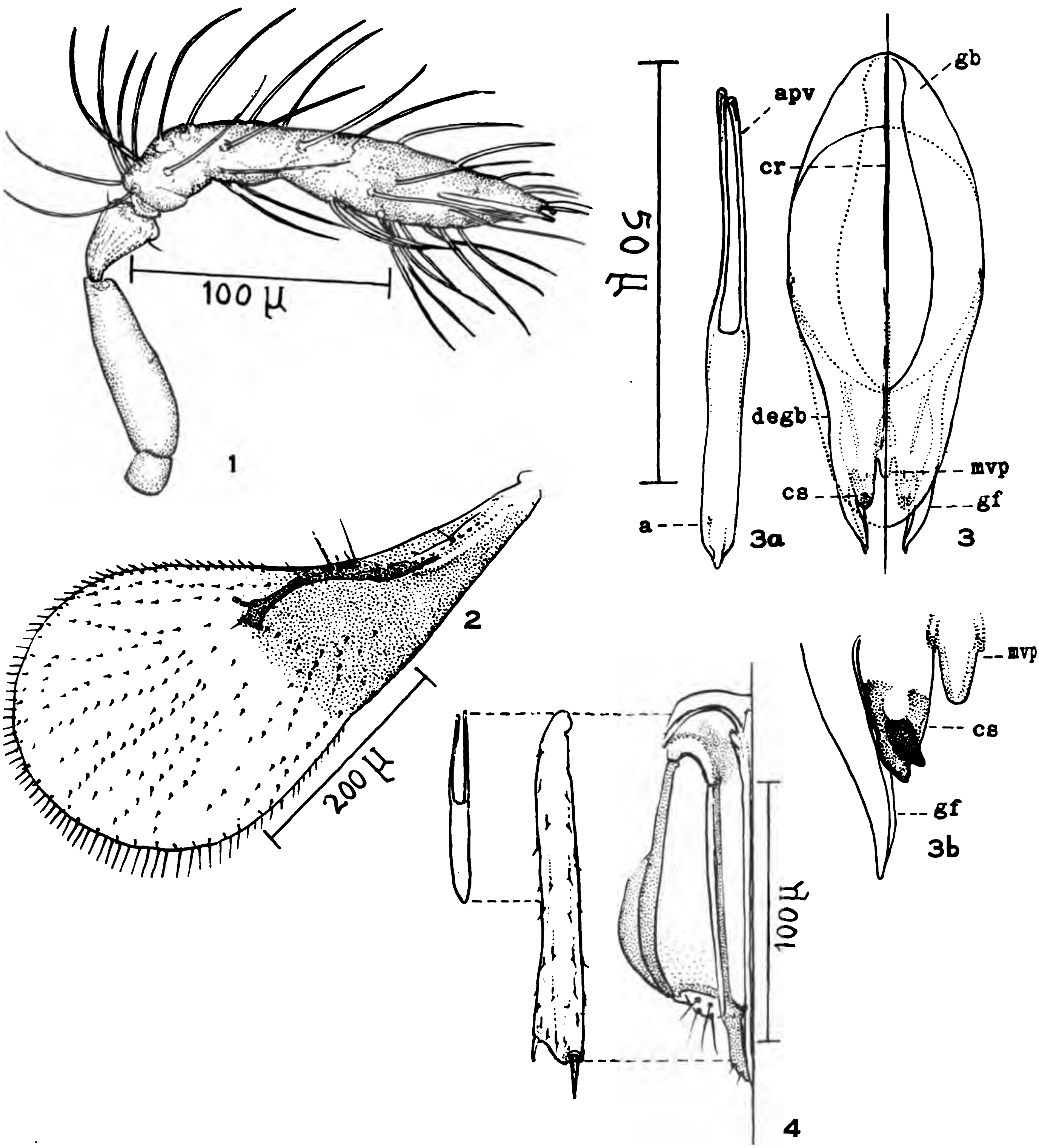

Fig. 1–4. *T. beckeri:* 1) Antennal flagellum; 2) Forewing; 3) male genitalia; 3a) aedeagus; 3b) enlarged view of median ventral projection, chelate structure and gonoforceps; 4) relative lengths of aedeagus, hind tibia and ovipositor.

thin pincerlike gonoforceps which barely potrude beyond the dorsal expansion of gonobase are also quite characteristic of this species.

According to P. Grijpma and V. O. Becker it seems possible that *T. beckeri* also attacks eggs of a *Sematoneura* sp. which are found in close association with those of *H. grandella* on *C. tonduzzi* seeds. Eggs of these two Lepidoptera are almost indistinguishable, but in the initial collection the parasitized eggs appeared to comprise only *H. grandella* although from subsequent collections of eggs *Sematoneura* larvae and moths have been reared.

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RECORDS OF TWO PARASITES NEW TO COSTA RICA*

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COMPENDIO

Se registran dos parásitos del barrenador de las meliáceas, *Hypsipyla grandella* (Zeller), nuevos para Costa Rica. Adultos de *Trichogramma fasciatum* (Perkins) (Hym., Trichogrammatidae) fueron obtenidos de huevos de *H. grandella* recolectados sobre frutos de *Cedrela odorata* L. y de ramas próximas a los frutos, en un bosquete ubicado en Santa Cruz, Guanacaste a 200 m.s.n.m. Adultos de *Brachymeria conica* (Ashmead) fueron criados de pupas de *H. grandella* recolectadas en frutos de *C. odorata* en el mismo lugar mencionado y en tallos tiernos de *C. tonduzzi* C. DC., *C.odorata* y *Swietenia macrophylla* King en plantaciones jóvenes de estas meliáceas, establecidas en los terrenos del Departamento de Ciencias Forestales Tropicales del IICA–CTEI, Turrialba, Costa Rica, a 650 m.s.n.m.

Any biological control program on a specific insect should preferably be preceded by an exhaustive inventory of the biocontrol agents already present in the country. With respect to the shootborer *Hypsipyla grandella* (Zeller) such surveys have been carried out repeatedly in Costa Rica since the establishment of the Inter–American Working Group on this forest insect pest in September 1970. In previous surveys by the author, the larval parasite *Hypomicrogaster hypsipylae* De Santis (4) and three egg parasites, *Trichogramma semifumatum* (Perkins), *T. pretiosum* Riley and a *Trichogramma* sp. near *pretiosum* were found parasitizing *H. grandella* in Turrialba, Costa Rica, at 650 m elevation (2). During more recent inventories two additional parasites were obtained.

The egg parasite *Trichogramma fasciatum* (Perkins) was reared from *H. grandella* eggs which were collected on fruits and small branches adjacent to fruits of *Cedrela odorata* L. trees growing at 200 m elevation in Santa Cruz, Guanacaste. The seed capsules were located at a height of 12 m on trees in open pasture land. Collection took place on January 23, 1973 during the dry season.

Nagarkatti and Nagaraja (3) indicate that *T. fasciatum* attacks *Diatraea saccharalis* (F.) (Pyralidae) in the West Indies, Peru and the USA. This *Trichogramma* species has also been collected in Mexico from eggs of *Heliothis zea* (Boddie) and from an unknown host in Argentina.

The parasite *Brachymeria conica* (Ashmead) was reared from *H. grandella* pupae collected on January 23, 1973 in fruits of *C. odorata* at Santa Cruz, Guanacaste. Adults of this parasite were also reared from *H. grandella* pupae found in young shoots of *C. tonduzzi* C. DC., *C. odorata* and *Swietenia macrophylla* King in a two years old mixed forest plantation of IICA–CTEI at Turrialba, Costa Rica.

In addition, *B. conica* adults were obtained from pupae of *Paramyelois transitella* (Walker) (Lep., Phycitinae) found in fruits of a *Forchhammeria* sp. (Capparidaceae) on the beach of Playa Hermosa, Guanacaste. They were also reared from pupae of *Anadasmus porinodes* (Meyrick) (Stenomidae) found in shoots of *Ochroma lagopus* (Bombacaceae) in a forest plantation at IICA–CTEI at Turrialba, Costa Rica.*

It is of interest to note that *B. conica* apparently has a wide ecological range. The Guanacaste area of Costa Rica has a dry period of 4–6 months, whereas the Turrialba region generally has only a one month dry season. Burks (1) indicates that *B. conica* has a distribution from Texas to Brazil. It has also been collected in Trinidad.

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* Personal communication, V. O. Becker, January 1973.

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A NOTE ON THE PARASITES AND ON THE SEASONAL ABUNDANCE OF *HYPSIPYLA GRANDELLA* IN BRITISH HONDURAS

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COMPENDIO

Como parte de un proyecto de introducción de parásitos para el control de *Hypsipyla grandella* (Zeller) en las Antillas y Belice, financiado por el Overseas Development Administration (ODA) de Londres, se hicieron reconocimientos rápidos durante tres ocasiones en el Bosque Chiquibul, Distrito de Cayo y en el Valle de Stann Creek, Belice, con el propósito de determinar: la abundancia relativa de esta plaga, el complejo de enemigos naturales presentes y liberación de parásitos exóticos.

Las siguientes observaciones, aunque limitadas, demuestran marcadas diferencias estacionales en los niveles de infestación de *H. grandella*, enfatizan el valor de estudios e indican que se puede obtener información preliminar sobre la ocurrencia de enemigos naturales durante visitas cortas si éstas están correctamente planeadas.

Seasonal Fluctuations in *H. grandella* populations

On visits to the Chiquibul Forest in July 1968, July 1969 and November 1969, the following procedures were adopted. Travelling along logging trails in a Land Rover accompanied by personnel from the Forestry Department, stops were made wherever one or more young cedar or mahogany trees were sighted. These were examined visually for evidence of attack, and damaged shoots suspected to be infested were cut and either examined in the field or later at the Forestry Rest House at St. Augustine. Infested shoots were split carefully and any *Hypsipyla* and accompanying natural enemies were placed in 5/8 x 3" glass vials and provided with sections of young shoots. During the two day visit, July 1–2, 1968 over 250 infested shoots were collected and on July 3, 1969 over 200 infested shoots were collected during a four–hour survey along the same route. By contrast a survey in which over 300 young trees were examined along the same route on November 3, 1969 only three recently attacked twigs were encountered and none of these contained *Hypsipyla* larvae. An analysis of a portion of the two earlier collections is given in Table 1, for comparison with that of the third collection.

Table 1. Analysis of damaged *Swietenia* and *Cedrela* shoots collected in the Chiquibul Forest, British Honduras, during three brief visits.

Data collected	July 1968	July 1969	November 1969
Trees examined	300*	300*	300*
Attacked shoots observed	250*	200*	3
N° of damaged twigs examined in detail	100	180	3
N° of *Hypsipyla**	123	237	–
Live larvae – Small	31	67	–
Live larvae – Medium	19	69	–
Live larvae – Large	56	42	–
Pupae	–	1	–
Dead larvae, causes	11	24	–
unknown	6	34	–
Parasitised			

* Includes parasitised and dead larvae.

Natural Enemies of *Hypsipyla* Encountered in British Honduras

Unfortunately because of unavoidable neglect concommitant with other travel, most of the *Hypsipyla* larvae died before they pupated or yielded parasites so that no quantitative data are available on the levels of parasitism apart from those recorded in Table 1. If it is assumed that the larvae dead from unexplained causes at the time of collection were killed by *Bracon chontalensis* (which paralyses its host prior to oviposition and was the most abundant parasite) or to pathogens then approximately 13.8 per cent were accounted for by natural enemies in the 1968 collections and 24.5 per cent in the 1969 collections. The levels of total parasitism would have been higher because additional hymenopterous parasites were obtained from some of the larvae, others died from nematode attack (? *Hexamermis*) and two died from a fungal infection caused by ? *Beauveria bassiana.*

As added evidence of an appreciable level of parasitism, seven of a collection of 23 larvae from Canada Hall, Stann Creek, were parasitised. Notes on the parasites encountered based on the 1968 collections are given in Table 2.

Table 2. Notes on parasites reared or collected in association with *Hypsipyla* collections in British Honduras, July 1968.

Family and Species	Biological data
Braconidae *Apanteles* sp. (*Laevigatus* group)	Gregarious endoparasite reared from two larvae.
Apanteles sp. (*ater* group)	Solidary endoparasite. One adult reared from half-grown larva. Two empty cocoons seen.
Bracon chontalensis Cam.	Solitary ectoparasite of medium-sized larvae. Nine adults reared. Additional empty cocoons seen.
Agathis sp.	Solitary endoparasite of medium-sized larvae. One specimen reared.
Ichneumonidae *Eiphosoma* sp.	Solitary endoparasite emerging from medium-sized larvae. Scarce.
Chalcidoidea Indet. sp.	Ectoparasite of small larvae. Two attacked hosts collected but adults nor reared.
Tachinidae *Metopiops mirabilis* Tns.	Larval-pupal parasite. One specimen reared.

In addition to the parasites evidence of other natural enemies was also obtained. Predaceous Coleopterous larvae were collected from two *Hypsipyla* tunnels. While they fed readily on small *Hypsipyla* larvae they died (possibly of starvation) en route to Trinidad. Numerous small dipterous larvae were encountered on dead *Hypsipyla* larvae. These were not reared but they were probably scavengers rather than predators.

In the 1969 collections two larvae alive at the time of collection sucumbed to fungal attack ? *Beauveria bassiana* and two others from nematode attack ? *Hexamermis* sp.

Parasite Releases in British Honduras

Partial details of shipments of parasites of an allied species *H. robusta* to British Honduras have been given elsewhere (Bennett and Yaseen 1972). Further details are given in Table 3.

Table 3. Shipments of *Hypsipyla* parasites from Trinidad to British Honduras, 1969–1972.

Species	Number of shipments	Number of individuals
Anthocephalus renalis	2	44
Tetrastichus spirabilis	8	6,350
Phanerotoma sp.	6	2,480
Trichogrammatoidea nana	5	3,500
Total	21	12,374

The first two consignments were timed to coincide with the November 1969 visit. Both contained adults of the solitary pupal parasite *Anthocephalus renalis* Wtstn. and the gregarious pupal parasite *Tetrastichus spirabilis* Wtstn. The first shipment was delayed in transit and the live adults of *A. renalis* were released in the Chiquibul Forest; all adults of *T. spirabilis* and half of those of *A. renalis* were dead. The condition of the second shipment was much better, releases of both species being made in the Mayflower Plots near Melinda. In neither area was there sufficient evidence of recent attack to offer a reasonable likelihood that establishment occurred.

Discussion

The differences in levels of attack by *H. grandella* between July 1968 and 1969, and November 1969 were very marked and as no releases of exotic parasites had been made prior to November 1969, these even if established later could not have influenced *Hypsipyla* populations at that time. In Trinidad and the Lesser Antilles there is a marked reduction in the production of new shoots in young plantations of cedar and mahogany and a coincident reduction of attack by *H. grandella.* The same sequence may account for the seasonal scarcity in British Honduras.

From the number of species of parasites and the level of parasitism encountered it is likely that the native natural enemies keep *H. grandella* in partial check and that if even a relatively small increase in the mortality levels could be achieved by the establishment of an exotic parasite it is possible that *Hypsipyla* populations would drop to a more acceptable level.

The condition at the time of the parasite releases reported above were far from ideal, i.e. due to delays in transit many of the parasites were in a weakened condition and as there was very little evidence of recent attack it is likely that most of the released adults perished before they found suitable hosts. Some of the later shipments were also delayed so that even if field conditions were more favorable the chances that permanent establish occurred are probably very low. However, releases of all three species were made in the Silkgrass and Sibun. Forest Reserves (H. C. Flowers, personal communication, July 1971).

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EVALUATION OF THE EFFICIENCY OF PARASITES*

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COMPENDIO

El control biológico de plagas ha demostrado en algunos casos resultados tan obvios que su evaluación con métodos estadísticos es completamente innecesaria. Sin embargo, en otros casos el control biológico necesita ser cuidadosamente evaluado, con métodos y técnicas que se describen brevemente en este artículo, para poder demostrar su éxito.

Se discute también aquí, algunos puntos sobre la selección de parásitos para su introducción y la problemática de poder predecir con seguridad, entre varias especies de parásitos a introducir, el mayor o menor grado de efectividad de cada parásito en el control de la plaga; además se mencionan algunas de las posibles causas en la falla del establecimiento adecuado de éstos.

En síntesis, varios puntos en el campo del control biológico y sus posibles aplicaciones, son brevemente analizados para ayudar a resolver el problema del *Hypsipyla grandella* (Zeller) en plantaciones de Meliáceas.

Introduction

In order to evaluate the efficiency of a parasite in the field it is essential that estimates of the host population or the level of damage that the pest causes be established. Information on the mortality factors already operating on the pest prior to the introduction is also useful and should be obtained if a critical assessment of the effect of the introduced natural enemy is to be carried out.

In instances where spectacular biological control has occurred entomologists as well as others who have witnessed the "before" and "after" populations of the pest –or its effect on the crop– are left with very little doubt that the control was brought about by the introduced agent. For example the introduction of the ladybird beetle *Rodolia cardinalis* (Mulsant) following the establishment of the cottony cushion scale *Icerya purchasi* Maskell, first in California in 1888 and later into several other areas of the world, has usually effected spectacular control. Despite the absence of quantitative data on populations of the pest, or on other mortality factors already in operation prior to the introduction, or the absence of absolute population counts after the releases, there is widespread acceptance that successful control has been due to the introduction of *Rodolia*. It has been demonstrated subsequently by eliminating *Rodolia* with selective pesticides that the populations of cottony cushion scale will return to their former level providing excellent proof that this Coccinellid is responsible for control. Similarly in Barbados the control of the recently introduced citrus blackfly *Aleurocanthus woglumi* Ashby by the release of the parasites *Prospaltella opulenta* Silvestri and *Eretmocerus serius* Silvestri was so spectacular that the condition of the trees had changed "miraculously" a few months later. Also the numbers of the parasite adults and the numbers of nymphs with parasite emergence holes were so abundant that need for statistical proof to attribute the control to the action of the parasites seemed unnecessary. Attempts to obtain detailed data on the same pest undertaken at the time of the introduction of *Prospaltella opulenta* into Jamaica were terminated when the size of sample, restricted initially because of the arduous task of counting hundreds of individuals per leaf, was later often too small even to detect the presence of the pest (van Whervin 1968). In a brief survey which I carried out in 1972 the pest was still scarce and only one blackfly of a sample of 301 was unparasitised. There are numerous other similar instances e.g. the control of coconut scale *Aspidiotus destructor* Signoret in Principe (Simmonds 1960) where individual counts of scales per frond were superfluous but the decrease of yields following the accidental introduction of the pest followed by a rise and return to former levels after the introduction of the coccinellid *Cryptognatha nodiceps* Marshall was ample proof that successful control had occurred.

* Lecture prepared for IICA Symposium on the Integrated Control of *Hypsipyla*. Turrialba, Costa Rica, March 5–12, 1973.

There are, however, many other instances where, while some level of control has been exerted, shifts in the pest population have been more gradual and where the role of the introduced natural enemy or enemies is less evident. It is in these instances that the need for pre–release data on normal fluctuations of the pest population, including mortality factors already in operation are more obvious.

Hence as the outcome of parasite introductions can seldom be predicted in advance i.e. whether they will give a spectacular, partial or insignificant level of control, it is recommended that where possible estimates of the host population and/or the level of damage it causes as well as data on the mortality factors already in operation be obtained to enable a more critical future assessment of the effect of the introduced agent or agents. However, frequently, populations of recently introduced pests build up with such incredible rapidity there is not time to wait to accumulate several years of data before releasing natural enemies. Hence in Hawaii from economic consideration the urgent need to take immediate action in the form of obtaining and releasing natural enemies has often outweighed any suggestion that importations be delayed until prerelease data is accumulated (C. J. Davis, pers. comm., 1972). This was certainly also the situation with the citrus blackfly in Barbados to which I have referred. A delay of a year or two to study the pest before parasite releases would have resulted in the loss of most of the fruit trees.

Sampling Techniques for Pest Populations

Sampling techniques have been developed to estimate population size of many types of insects. In discussing these, Southwood (1969) has pointed out that estimates of insect populations are of two types, absolute and relative. Absolute estimates express the population size in numbers per unit of habitat whereas relative estimates are measures of population for comparative purposes. He considered that absolute estimates are essential for the construction of life tables, for comparing populations of different stages, or between different areas or at different times. If a standard sampling unit e.g. number of insects per leaf or per 100 leaves is adopted and if the number of leaves per plant and plant population per unit area are known then absolute estimates of the pest population can be determined.

Still referring to Southwood (1969) there are four ways of estimating an absolute population. These are: a) counting the number of insects in sampling units of the habitat; b) the Lincoln Index or capture/recapture estimation; c) removal sampling i.e. collecting or trapping repeatedly in the same area until the catch becomes negligible; d) measuring the distances between individuals and if the distance is random converting the data to numbers per unit area. From our experience the estimation of absolute populations of *Hypsipyla* is difficult and only the first method would appear to be applicable.

Relative estimates are useful for comparative purposes. They are unknown units in relation to the entire population, are affected by many variables and cannot be readily incorporated into life tables. They may be based on catch per unit effort or on trapping. The first involves searching or collecting for a fixed distance or time. Sweeping with a net for a fixed distance is a widely used method but is inapplicable for sampling for *Hypsipyla* or its parasites. Sticky traps have also proved useful and this technique has already been employed here in Costa Rica when studying host preferences of the *Hypsipyla* adults (Grijpma and Gara 1970). Light traps have also been employed. Many factors influence light trap catches but consistent operation may give a measure of the magnitude of fluctuations from generation to generation.

Size of Pest Populations and Damage Tolerance Levels

The difficulties in obtaining absolute estimates of *Hypsipyla* populations in the Neotropics in forests where natural regeneration occurs are, I am sure, self evident to most of us who have attempted to study any aspect of this pest. Even in special plots or plantations the task is great, and apart from taking a census of attacked or unattacked trees the problem of obtaining meaningful data, while not insurmountable, requires a sizable budget and work force. Few if any attempts have been carried out over a sufficiently long period to ascertain the levels of attack that can be tolerated to permit or encourage the large scale planting of meliaceous trees or as far as I am aware the budget that can be spent on control operations which, if successful, would make it an economic venture. For example what percentage of trees will produce millable lumber if attacked only once during the first three or four years –or if attacked once per year during that period or twice a year and so on? Even for pests of agricultural crops which have been under intensive investigations for many years economic thresholds have not been established. Hence Allen *et al.* (1972) referring to the cotton boll worm state that no economic threshold has been established and there is no significant correlation between yield and total seasonal pest counts. This is attributed to the interplay of several other factors and there is little doubt that attempts to establish economic thresholds for attack by *Hypsipyla* spp. would also be a difficult excercise, would vary for each tree species, and from area to area.

With some crops a very low threshold of damage can be tolerated whereas for others there is a very high threshold. The nature of attack and extent of injury affects this and also affects sampling techniques and tolerance levels. By way of an example I shall refer to the assessment of damage to sugarcane caused by *Diatraea* spp., lepidopterous shoot borers of sugarcane. If a shoot is attacked when quite young a "dead heart" is formed i.e. the central whorl of leaves dies first and the shoot dies. Unless a high proportion of shoots is attacked there is "compensation" i.e. other shoots are rapidly produced or the energies of the root system, available soil nutrients etc., are directed to other unattacked shoots. Once the canes have produced several nodes the stalks are less likely to be killed outright and may continue to grow if attacked by a single larva. However, it has been determined, on the average, that if an internode is attacked the amount of sugar from that particular internode is halved. In other words if at harvest time 16% of the internodes show evidence of attack there is a drop in yield of approximately 8%. It has generally been accepted that if an average level of internode (or joint) damage greater than 4–5% at harvest time occurs from year to year some form of control operation should be considered. While it may still be economic to commercially grow sugarcane when the level of attack (joint infestation) is in the region of 15–20% the margin of profit is greatly reduced. Hence in sugar producing areas where the level of joint infestation is in the order of 4–5%, large scale control programmes may not be warranted but when the level is in the order of 10–20% efforts are decidedly justified. Even if the level of attack is in the region of 5% in terms of joint infestation the number of canes attacked may be quite high. A relatively high level of attack can be sustained when attack over the entire growing season is considered. From data presented by Fernández (1960) on attack by *Diatraea* spp. (Fig. 1), it will be noted in the category, 2–4% attacked internodes, that 37% of the canes were attacked yet this level of attack is considered insignificant. In the next category, 5–10% attacked internodes, 57% of the canes were attacked yet sugarcane is successfully and profitably grown where the average level of attack falls in the category. Sugarcane is of course harvested annually whereas meliaceous species seldom are before 30 or 40 years, even though attack by *Hypsipyla* occurring after the first 4–7 years is unlikely to have serious consequences except possibly on seed production. In order to determine the levels of infestation that can be tolerated and hence the level of control which is required to prevent economic loss long term studies are necessary.

Possibilities of the Use of Life–Tables

I do not intend to dwell in much detail on the use of predictive models and life–tables in assessing the efficiency of parasites. Varley and Gradwell (1970) have discussed their uses and also the difficulties of utilizing this approach.

The purpose of using life–tables in biological control is to attempt to deduce which are the most important of the causes of mortality the so called "key–factors" –and from these it may be possible to assess the value of individual species of natural enemies in restricting host numbers, and the effect on the numbers of the next generation of host that the elimination of any one particular mortality factor or the introduction of another mortality factor would have, and to predict the opportune time for the release of natural enemies.

With an introduced pest attempts may be made to compare life–tables in the area of introduction with those in the country of origin and to assess the potential of the various species of natural enemies prior to their introduction into the new area. When the pest is native over most of its range as is *H. grandella* Zeller, and if the natural enemies of a related species are to be introduced meaningful comparisons are seldom possible.

Some workers suggest that until such life–tables have been produced and evidence obtained that one or more natural enemies are in fact "key factors" exerting a critical degree of mortality on the host population at a given stage, it is pointless, or at least of very problematical value, to introduce any parasite or predator regardless of however "important" this enemy may appear superficially. For a given species life–tables vary considerably from one area to another in the range of the target or pest species –and even from field to field and from one year to another– marked differences between the mortality caused by different natural enemies (and other factors) may occur. In other words the key factors regulating populations in different areas may not be the same. I quote the following remarks on the value of life–tables in planning biological control introductions from Simmonds (1972): "Some claim that it is quite useless to make introductions of natural enemies against a pest until in their area(s) of origin they have been studied quantitatively to the extent that fairly complete life–tables can be drawn up indicating at each stage of the pest life–cycle the extent to which individual factors contribute to mortality, and from such tables it can be deduced whether in fact a natural enemy is a limiting factor in population control. In my view, whilst life–tables may be useful in indicating key factors most important in controlling a pest population, there are so many varying factors involved –variations of the populations within very small geographical limits, influence of their own natural enemies etc. on the actual effect of parasites and predators, the importance of which would alter if these checks were removed, and, of course wide variations in both pest and natural enemy populations from year to year –that life–table studies tend to become an end in themselves without too much practical bearing on the possible value of a natural enemy when introduced into a new area. Inherent difficulties in producing such tables, normal natural variation, etc. make them, except possibly in unusually stable conditions, of very doubtful value in assessing accurately the potentialities of a natural enemy introduced into a new environment. I fully appreciate that this is a rather controversial subject with strong proponents of the essential nature of life–table studies. I can only put a point of view based on very widespread and varied experience."

My views are quite similar. Moreover, most life–tables studies on insects have been with species with more–or–less discrete generations. Difficulties in applying them to species with overlapping generations are even greater. Also it seems virtually impossible to predict exactly how effective a parasite may be when it is transferred from its original habitat into another regardless of however similar these appear to be ecologically. Nor is it possible to predict the efficiency of a parasite in a new area, in the absence of its own natural enemies or the effect of new natural enemies which may attack it there.

In biological control the objective is to predict the long–term consequences of the introduction of an additional mortality factor and in pest management programmes to determine the optimum time and numbers of natural enemies required to achieve control. Biological control methods might either be aimed at damping the injurious upper range of an insect's population fluctuations (and this need not necessarily greatly alter the average level because if the upper limits of the oscillation are damped the troughs also may not be so pronounced) or alternatively lowering the average level of the population and altering its status from that of a pest to a rare insect. This need not necessarily affect the amplitude of its fluctuations i.e., if the average abundance of *Hypsipyla* per acre is 20 with the populations fluctuating between 1 and 100 while after the introduction of a mortality factor the average abundance drops to 2 per acre with fluctuations between 0.1 and 10 a similar range of fluctuations occurs but whereas the former might be responsible for severe loses the latter populations might only rarely cause significant damage.

With the programme of biological control of sugarcane borer in Barbados it can be observed from the simplified hypothetical "life–table data" on *Diatraea saccharalis* in Table 1 that introduced parasites may account for a relatively small proportion of the initial population of each generation and yet in practice reduce an insect from the status of a major pest to a minor one. Because of the relative ease in obtaining adequate samples it would be less difficult to obtain adequate data for the construction of actual life–tables for *D. saccharalis* (and this is being attempted in Barbados), than for *Hypsipyla*. Nevertheless an introduced parasite, if it is sufficiently abundant to exert a controlling effect on the host population, will be encountered fairly regularly at least seasonally if a reasonably sized random sample is obtained.

The selection of parasites for introduction

So far I have barely mentioned parasites of *Hypsipyla*, their relative efficiency and methods of assessing it.

The intent of the organizers of this Symposium when selecting the title "The evaluation of the efficiency of parasites" was almost certainly to define methods of evaluating the results of parasite releases in the field. However, some attempts to evaluate their efficiency may commence even before a search for parasites for introduction has begun. While this aspect and continuing investigations up to the time of field release is more appropriately dealt with under the topic of "Criteria for the selection of natural enemies" I think that as they have direct bearing on the results, i.e. the release and attempted establishment, I am justified in making a few relevant remarks. Later I shall be somewhat critical of our own investigations on *Hypsipyla* to show that even the initial selection of the most promising species is not a straightforward or predictable excercise. Many attempts have been made to list the desirable traits or the attributes of an effective natural enemy. High searching capacity or ability to find its host at low population levels ranks high on the list. In some instances a highly specific natural enemy appears to be desirable but polyphagous species at times offer certain advantages. A high potential rate of increase is important particularly in a variable environment. It should have the ability to occupy all of the host–inhabited niches and to survive periods of host scarcity. It is useful if it will culture easily in the laboratory but as I pointed out this morning this should not be the overriding factor in selecting a natural enemy. Doutt and DeBach (1964) have summed up the essence of these attributes as follows "these characters merely mean that the natural enemy is well adapted biologically, physiologically and ecologically to the host."

Ideally when considering the selection of the most effective parasites, the complex of species occurring elsewhere is compared with that already present in the area where control is required. Priority is given to the introduction of those species attacking stages of the host which are not attacked by closely related parasites i.e. an attempt to fill unoccupied niches. Attention is also paid to species which cause a significant level of mortality in their country of origin, their host range and geographic distribution. Other factors, similarity of general habitat, microhabitats, climate, etc. are also taken into consideration when selecting areas where a search for natural enemies is to be initiated. If the pest for which control is desired is an introduced one search for effective natural enemies is usually centered initially in the country or area where is originated.

Our programme aimed at the biological control of *Hypsipyla* in the West Indies was drawn up to coincide with investigations at our Indian Station where a survey of the natural enemies of *Hypsipyla robusta* Moore financed by other sources was underway. Due to the time lapse in obtaining funds for our investigations the studies in India were almost completed and hence instead of obtaining "surplus" material of several species from those studied at minimal cost we were obliged to start work with the few species of parasites which were readily available. In this instance it meant species which were readily cultured in the laboratory or species which withstood the vagaries of airmail shipment and could be maintained with a minimum of effort and expenditure. Fortunately studies in India indicated that these "readily available species" contributed significantly to the total mortality due to parasitism and also attacked stages of *Hypsipyla* for which at that time there were no known closely related counterparts in the Neotropics. For example in parts of India *Antrocephalus renalis* Wtrst. parasitises 14.1–19.2% of the pupae and *Tetrastichus spirabilis* Wtrst. attacks 37–66% of the same stage whereas in Trinidad only one Chalcidoid pupal parasite *Brachymeria*, is known and it is only rarely encountered. Similarily *Phanerotoma* sp., an egg–larval parasite attacks up to 26.8% of its host in India while in Trinidad no egg–larval parasites have been recorded (Rao 1969; and Rao and Bennett 1969). It was recognized that the main toona areas of India are quite dissimilar ecologically to the Neotropics and the climates are markedly different. Even today and possibly still largely by default (i.e. lack of investigations elsewhere), India would still appear to be the best source for natural enemies. There are more than 50 species of parasites of *H. robusta* in India (Rao and Bennett 1969) whereas records from West Africa, which climatically is more similar to the Neotropics, suggest that fewer parasites occur, none of which on the basis of information at hand (Roberts 1966) appears to be a significant mortality factor. These remarks do not suggest that consideration should not be given to further investigations in West Africa or that parasites from that area should not be tried. It is very probable that intensive investigations will reveal the presence of additional parasites and predators.

Most criteria laid down for selecting natural enemies are not absolute and it is seldom if ever possible to predict accurately whether an introduced parasite will become established and if so whether it will provide effective control. The possibility that "rare" parasites should be introduced first has been considered. Also the introduction of a species closely related to one already present has been successful in a number of instances. In California one species of *Aphytis* has displaced a closely related species and provided efficient control of its diaspine host. Also in the West Indies those species which appeared to be the best candidates for introduction for the control of *Diatraea* have not always provided the expected level of control whereas other "unpromising" species have provided satisfactory control. Specifically the Tachinids *Metagonistylum minense* Tns. and *Paratheresia claripalpis* (Van der Wulp), two "logical" parasites for the control of *D. saccharalis* did not become established in Barbados despite several trials whereas *Apanteles flavipes* (Cam.), an Asian parasite, which on the basis of initial laboratory trials was not considered promising, has become an important parasite in the field (Alam, Bennett and Carl 1971).

In summary there are two schools of thought regarding the selection of parasites for introduction. One point of view is that only the "best" parasite should be released, the selection being made only after long–term field and laboratory studies in the country of origin of the whole complex of natural enemies, their inter–relationships etc. On the other hand P. DeBach and F. J. Simmonds (see Simmonds 1972), two noted leaders in biological control, share the view that the outcome of an introduction cannot be predicted in advance with sufficient accuracy to preclude the introduction of any primary parasite and while "priorities" should be listed, the introduction of additional species or parasites should continue as long as the pest remains a problem. A single species of parasite or predator is unlikely to control the pest over its entire range and hence several species, if available, should be released. Those species best adapted for a particular area or for a certain season will become dominant. As an example of the value of this approach the sugarcane borer has been controlled successfully in some sections of the West Indies by the establishment of one or more of the following parasites *Lixophaga diatraeae, Metagonistylum minense, Paratheresia claripalpis* and *Apanteles flavipes.* A species successful in one island e.g. *Metagonistylum* in St. Lucia failed to even become established in a neighbouring one (Barbados).

On the other hand some workers in the field of biological control consider that the interactions of a large complex of natural enemies which are competing for the same resource (or host) in some instances could reduce the overall level of control. This aspect has been discussed fully by Zwölfer (1970). Nevertheless there are no recorded instances where the introduction of additional species of natural enemies has resulted in an increase in the pest population. (These remarks refer to obligatory primary parasites and predators not to species which may develop in a secondary as well as a primary role).

Price (1972) has proposed methods aimed at formalizing the approach to the collection, liberation and recovery of entomophagous insects. There are, however, few cardinal rules to follow as each problem presents a complicated network of inter–related factors such that decisions have to be taken as to method of operation compatible with the circumstances involved. While there is a strong case for taking into account as many factors as possible and to document the steps taken as fully as possible each problem presents a different picture and procedures have to be adopted accordingly.

Post Release Evaluation

As in most areas in the Neotropics where attempts to grow meliaceous species on a plantation scale the level of damage is usually so high, at least seasonally, that the level of reduction to permit a reasonable percentage of the trees to escape attack is so great that should such a reduction occur following the establishment of parasites it would be patently obvious. Similarly if such a reduction occurred after the release of several species of parasites the field collection of only a few hundred *Hypsipyla* eggs, larvae and pupae might indicate with fair probability which species was responsible for the greatest measure of control. For example in Barbados following the establishment of *Lixophaga diatraeae* and *Apanteles flavipes* where the levels of parasitism are quite high, the chances of finding evidence of one or both parasites by examining as few as 5 to 10 infested canes are also very high. I have been present in Barbados on several occasions with visiting entomologists and it has been quite amusing to watch a rather sceptical expression change to one of satisfaction when the visitor himself locates parasite cocoons or puparia usually on his first, second or third try.

Recovery surveys are generally of two types –quantitative and qualitative. Unless regular pre–release population studies have been made the first recovery surveys are usually qualitative. Dependent on the circumstances these are made within a few weeks or a few months of the parasite releases. The main object is to attempt to determine whether or not there is any evidence that the parasite is breeding in the field and if so how far it has spread. Parasite release programmes may be adjusted dependent on whether or not recoveries are made. If evidence of parasitism is readily observed then rather than continue the liberation of parasites in that particular site releases may be shifted to new areas. In certain situations on the basis of evidence of early establishment parasite importations may be terminated. For example following the initial release of citrus blackfly parasites in Barbados, arrangements for additional shipments of the same parasites as well as material of other species were cancelled because the parasite *Prospaltella opulenta* built up rapidly and ample material could be obtained from the initial release sites for liberations in new areas. Similarily when *Metagonistylum minense* was released against *Diatraea saccharalis* in St. Lucia in 1934, significant field recoveries were made in the first generation when collecting *Diatraea* larvae for the parasite breeding programme. The buildup of this parasite was so rapid that the numbers which could be produced in the laboratory were insignificant compared to the field populations and the breeding programme was terminated (Box 1939).

Early qualitative surveys may also give an indication as to which species should be emphasized in future breeding programmes if several parasites have been released.

To carry out quantitative surveys on parasites of *Hypsipyla* I shall discuss in broad terms some of the difficulties encountered and refer to work on *Diatraea* or other insects which may be relevant. Simmonds (1948) has discussed some of the difficulties in determining the true value of parasitic control by means of field samples and some of these factors e.g. the effect of removal of the host from the field while it would otherwise still be susceptible to attack will be mentioned under the various categories. As in my talk this morning I shall discuss survey methods for the different categories of parasites.

Methods of assessing the abundance of egg parasites

The collection of *Hypsipyla* eggs in the field is very time consuming particularly if a search has to be made on larger trees. Grijpma (1972) has described his experience in carrying out a survey of the native parasite *Trichogramma semifumatum* in plots of Australian cedar in Costa Rica. His statement "No further count was made of these hatched eggs in view of the great difficulties in locating them" points to the painstaking efforts required when attempting to obtain quantitative data on egg parasitism (or data on this stage to include in life–table studies). Comparative data on the abundance of eggs can be based on units of foliage searched or on a time basis rather than the 100% survey described by Grijpma in which all foliage is inspected carefully. However, as parasitised eggs may be more readily noticed than unparasitised ones these methods also should be used with caution.

Another approach is to expose cards of host eggs obtained from laboratory cultures in the field for 24 or 48 hours and then hold them in the laboratory for possible parasite emergence. This method has been used successfully in India following the release of *Trichogramma fasciatum* Perks. against the sugarcane borer *Chilo infuscatellus* Snellen to determine that establishment had occurred but it is questionable whether reliable quantitative data can be obtained in this manner.

However, when assessing the effectiveness of the introduced species *Trichogramma evanescens* Westwood as well as a local species for the control of *Pieris rapae* (L.) and *Trichoplusia ni* (Hubner), Parker *et al* (1972) added additional eggs of *T. ni* and recollected these as well as naturally deposited eggs 24–72 hours later. They claimed that with this method of applying host eggs an adequate sampling of rates of parasitism can be undertaken when normal host populations are too low to obtain accurate estimates. Attempts by my colleague Dr. M. Yaseen to obtain evidence of the establishment of *Trichogrammatoidea nana* by exposing *Corcyra* eggs in the field during short visits to the Lesser Antilles were not successful.

Grijpma (1972) in his survey on *Trichogramma* spp. attacking *H. grandella* in Costa Rica removed all eggs and while in this sample all intact eggs were parasitised the removal of eggs from the field excludes any possibility that they might have been attacked later. Hence to obtain critical data, a search should be made at daily intervals, those eggs which are discovered should be marked by attaching a coloured thread or string to a nearby leaf and left until no longer susceptible to parasitism or predation. This method has been used successfully for marking eggs of *Diatraea* and other graminaceous stalk borers (Metcalfe and Brenniere 1969), a different coloured thread or string being used for each day to indicate the newly encountered eggs. This method is time consuming even for studies on *Diatraea* when the eggs are laid in clusters and is indeed even more tedious for studies on *Hypsipyla*. However, a similar method has been utilized in studies on egg mortality of *H. grandella* in Venezuela (Roovers 1971).

In their studies on egg parasitism in Barbados, Metcalfe and van Whervin (1967) demonstrated that mortality due to *Trichogramma fasciatum* fluctuated widely from season to season. They concluded that fluctuations in parasitism are bound to occur partially as a response to host density and partially as a result of competition from predators as well as variations in the microclimate associated with general climatic changes and the stages of growth of the cane.

It can safely be said that if an exotic egg parasite did become established and became effective the results would more likely be noted from a decline in larval population rather than on quantitative studies of egg parasites, unless long term sampling has been in operation prior to the releases. However, as I have indicated earlier, relatively small egg samples should suffice to indicate that the parasite was well established.

Methods of assessing the abundance of larval parasites

As with other types of parasites data on percentage parasitism without parallel data on host mortality from other causes and size of host populations can only indicate the abundance or otherwise of the parasites in their host. Unsupported figures of percentage parasitism unless they can be associated with conclusive evidence of decreased host populations and crop damage after introduction of a new parasite into a new area should be regarded circumspectly (Bennett 1969). Nevertheless the initial step in assessing the effectiveness of a larval parasite is to obtain data on the abundance of the parasite. Dependent on the time and technical assistance available visits are made to several release sites and as much host and parasite material as possible is obtained. In the Neotropics there are generally overlapping generations so that all stages of the pest, and probably also of the natural enemy, are present during most of the year. Hence, as with the *Diatraea* parasites already mentioned, evidence of establishment may be obtained quickly. If not, large samples of host larvae are carried back to the laboratory where either by dissections or by rearing until parasites emerge, or the hosts pupate (or all too frequently die) parasitism is assessed. If evidence of parasitism is found then collections along transects from the release site are made to determine how far the parasite has spread, where possible enough material being collected to obtain quantitative as well qualitative data on the relative abundance of the parasite at increasing distances from the release sites and in different microhabitats.

Sampling stations, preferably the same ones utilized for pre–release population counts, should be visited at regular intervals, fortnightly or monthly, to obtain as much data as possible on parasite buildup and, hopefully, pest decline. It is not unlikely, particularly if several local parasites already occur, that the populations of the introduced species which may initially build up very rapidly, may subside and be little more abundant, or perhaps may be even scarcer, than some of the native species. Despite this, satisfactory control may result provided the mortality caused by the introduced species is **superimposed**, at least in part (or seasonally), on top of that caused by the natural enemies previously present rather than merely displacing part of the mortality caused by one of these species. I will refer again to the parasites of *Diatraea saccharalis* in Barbados. Fig. 2 shows the relative abundance of the larval parasites of *D. saccharalis* during 1971. In some seasons *L. diatraeae* is more abundant whereas in others *A. flavipes* is commoner. Data from other years also suggests that, seasonally, conditions may be better suited to the increase of one or the other. From laboratory studies we know that hosts attacked by both species only rarely produce progeny of both parasites.

L. diatraeae is intrinsically superior to *A. flavipes*, i.e. if attacked simultaneously or up to four or five days after being stung by *A. flavipes*, *Lixophaga* is invariably successful. Hence larvae that are successfully parasitised by *A. flavipes* are ones that escape attack by *L. diatraeae* prior to or in the five days after attack by *A. flavipes*. It is possible, or even probable, that *L. diatraeae* restricts the population buildup of *A. flavipes* for part of the year but when, due to unfavourable conditions, it becomes seasonally scarce, *A. flavipes* then becomes the more abundant species. While both are introduced species a similar relationship could be expected to occur between native and introduced *Hypsipyla* parasites, i.e. the native species seasonally masking the presence of an introduced species with the latter demonstrating its potential at seasons unfavourable to the native species. Many of us with practical experience in biological control will accept a good "cause and effect" relationship as ample evidence that successful biological control has occurred, i.e. if a reduction of damage is achieved for most years and there is a correlated high level of parasitism by an introduced parasite.

To sample for larval parasites of *Hypsipyla* the general method is to make field collections of infested twigs and to carry them to the laboratory for examination. If data on the relative abundance of infested twigs is recorded at the same time then a correlation between incidence of damage and parasitism can be worked out. In the laboratory the infested twigs are carefully split, live larvae are transferred to vials supplied with food until they pupate or are killed by internal parasites. If paralyzed larvae are encountered a careful search should be made for parasite eggs in the tunnel or adhering to the larva. Larvae with parasite larvae can either be left "in situ" or transferred to small vials with a limited amount of plant material to maintain the correct relative humidity until the parasites complete their development.

Alternatively if the parasites can be identified from the immature stages all host larvae may be dissected. This method has the following advantages: a) the results of the collection can be ascertained more rapidly; b) there is less loss of material than experienced with the alternate method of rearing the field collected host larvae wherein a few to several of the larvae die before pupation or the emergence of parasites. The latter method i.e. rearing to the adult stage is generally more widely used because less skilled personnel can attend to the collections and because more accurate determinations can be obtained on parasite adults. Both methods are frequently employed when assessing parasitism of the sugarcane borers *Diatraea* spp. For collections made during short visits to other areas I usually resort to dissections because it is not practicable to leave collections to be reared. Also the transport of the material to Trinidad contravenes the Plant Quarantine Regulations. Furthermore as there is usually only one or two parasite species involved the problems of identifying the parasites in their immature forms are minimal.

Based on the relative numbers of parasitised and unparasitised larvae of the different host stages the percentage parasitism can be worked out. Again the steps outlined by Simmonds (1948) to allow for the premature removal of the material from the sphere of parasite activity should be borne in mind. Of particular note is the fact that some larval parasites, e.g. *Phanerotoma*, cause their hosts to seek a "pupation" site earlier than do unparasitised hosts and unless these sites are known the species may be under–represented in a sample.

Attempts to assess the abundance of the adults of an introduced parasite has also been made but these usually give only a crude index of population size. Miskimen (1962) reported the establishment and relative abundance of *Lixophaga diatraeae* in different areas in St. Croix on the capture of adults in a sweep net, in contrast to the traditional methods of collecting host larvae. This method would be suitable for another *Diatraea* parasite, *Jaynesleskia jaynesi*, as the adults regularly frequent flowering weeds at the edges of canefields.

The use of sticky panels (14 cm x 14 cm square panels of clear plastic coated with tangle foot) hung at different heights and in different habitats were used by Weseloh (1972) to study the spatial distribution of the gypsy moth and its parasites, (egg and pupal as well as larval parasites). His results indicate that all parasites are not randomly distributed among all of the niches occupied by the host populations reinforcing Simmonds (1948) earlier findings. He also emphasizes the danger of relying on only a single sampling method for assessing the parasite populations.

Methods of assessing the abundance of larval–pupal and pupal parasites

The fact that the pupation sites for *Hypsipyla* spp. vary considerably, –i.e. pupation may occur within an attacked shoot, in a seed capsule, under loose bark or other irregularities of the tree trunk, or on the ground,– makes difficult an accurate assessment of pupal parasitism. In India where larvae of certain generations of *H. robusta* regularly pupate under the bark large samples have been collected by scraping off the rough bark of a section of the tree trunk and banding the tree with corrugated cardboard or loosely tied jute sacking. Larvae searching for pupation sites encounter these shelters and pupate beneath them. These are left for several days to permit attack by pupal parasites. They are then removed to the laboratory and held for parasite emergence or examined for evidence of predation. Individuals which pupate in twigs are obtained and handled in the same manner described for larval parasites.

Discussion

To assess the efficiency of natural enemies, as I have outlined, is not always easy. To account for the failure of establishment is often even more difficult and attempts to explain why a parasite does not become established may be of equal or greater fundamental value than the evaluation of a successful introduction. Among the apparent causes of failure of establishment may be:

a) **Unsuitability of the host.** This may apply in instances where the parasite was obtained from a different host. While straightforward nonsuitability of the host can often be determined in host acceptance and host suitability studied in the laboratory, there are instances where hosts accepted in the laboratory are not attacked in the field. Differences in the biology of the original and the "intended" host may account for this. For example certain parasites of *Diatraea* developed well on the larvae of certain Old World graminaceous stem borers when tested in the laboratory. However, in the field, larvae of some species of stem borers close the entrance holes to their tunnels by means of a silken cap and the parasite cannot successfully penetrate this barrier.

b) **Different climatic conditions.** Climatic conditions while suitable for part of the year are unsuitable for the balance of the year and hence when moving a parasite to a different climatic zone only temporary establishment may occur, the parasite dying out or seasonally becoming too scarce to provide effective control.

c) **Release of inadequate numbers.** The possibility that the numbers of parasites or predators released are not adequate to afford a reasonable chance of establishment is sometimes offered as the reason for failure to obtain establishment. Unfortunately there is no hard and fast rule about this. Some parasites have become established from releases of 50 or fewer adults, others have failed despite the release of hundreds of thousands. Generally releases of large numbers at several sites should be continued for a year or longer to ensure that a parasite has had a reasonable chance of establishment in all seasons.

Table 1. Hypothetical life–table for *Diatraea saccharalis* (Fab.) before (A) and immediately after (B) the introduction of larval parasites assuming a population of 100 ♀ per acre each producing 100 eggs.

	A[1] – No larval parasites			B – With larval parasites		
Eggs laid – 10,000[2]	**% mortality**	**No. of survivors**	**Mortality expressed as % of original eggs**	**% mortality**	**No. of survivors**	**Mortality expressed as % of original eggs**
Egg parasitism[3]	50	5,000	50.00	50	5,000	50.00
Early larval mortality[4]	90	500	45.00	90	500	45.00
Larval parasitism[5]	–	500	–	40	300	2.00
Pupal predation[6]	20	400	0.10	20	240	0.62
Adult mortality[7]	20	320	0.80	20	192	0.48

1 A represents an increasing population and B a declining one.

2 Populations of 23,000 per acre have been reported.

3 Egg parasitism by *Trichogramma* and *Telenomus* is often higher than this figure.

4 Mortality in this order has been reported by several workers.

5 Prior to the introduction of *Lixophaga diatraeae* (Towns.) and *Apanteles flavites* (Cameron) into Barbados, there were no larval parasites present. Limited predation by ants and earwigs occurred.

6 Based on limited data from Barbados.

7 Hypothetical.

d) Inadequate genetic base to stocks. The possibility that adequate genetic variation has not been included in the releases should also be considered. Frequently mass cultures are derived from a small nucleus resulting in inbreeding. Also time to allow for the development of a locally adapted strain may be necessary. For example in Barbados *Lixophaga diatraeae* was released periodically for more than 30 years without evidence of permanent establishment. A programme was then initiated in which stocks from four separate islands were crossed and massreleased. From small pockets where initial establishment occurred new cultures and mass–releases based on the progeny of those few individuals of these crosses that survived one or two generations in the field in Barbados were made. Thus apparently after a preliminary "screening" under field conditions, a stock adapted to Barbados conditions developed.

The purpose of mentioning these is not to explore in full the reasons for failure but to point out that attempts to establish parasites should not be abandoned too prematurely if success is not obtained following the initial releases. Despite all that has been said, the fact remains that we cannot accurately predict the outcome of an introduction or to assess in advance the effectiveness of any of the *Hypsipyla* parasites with sufficient accuracy to categorically state that the introduction of any particular species should be given priority over another species. Accordingly, the introduction of natural enemies should be continued in as logical an order as possible, as long as *H. grandella* continues to be a major limiting factor to the production of mahogany and cedar in the Neotropics.

In conclusion, I fully endorse the opinion expressed by DeBach (1972) that biological control by the importation of natural enemies has not reached a point of diminishing returns but rather needs increased emphasis. The attempts to utilize this method for the control of *Hypsipyla* to date have only begun to be explored. Adequate releases to afford a reasonable chance of establishment of any species have only been made in Trinidad and possibly certain of the Lesser Antilles. Even in these areas only four species i.e. less than 10% of the species of parasites known from India alone have been tried and for the vast areas of Central and South America no releases or only token releases have been made.

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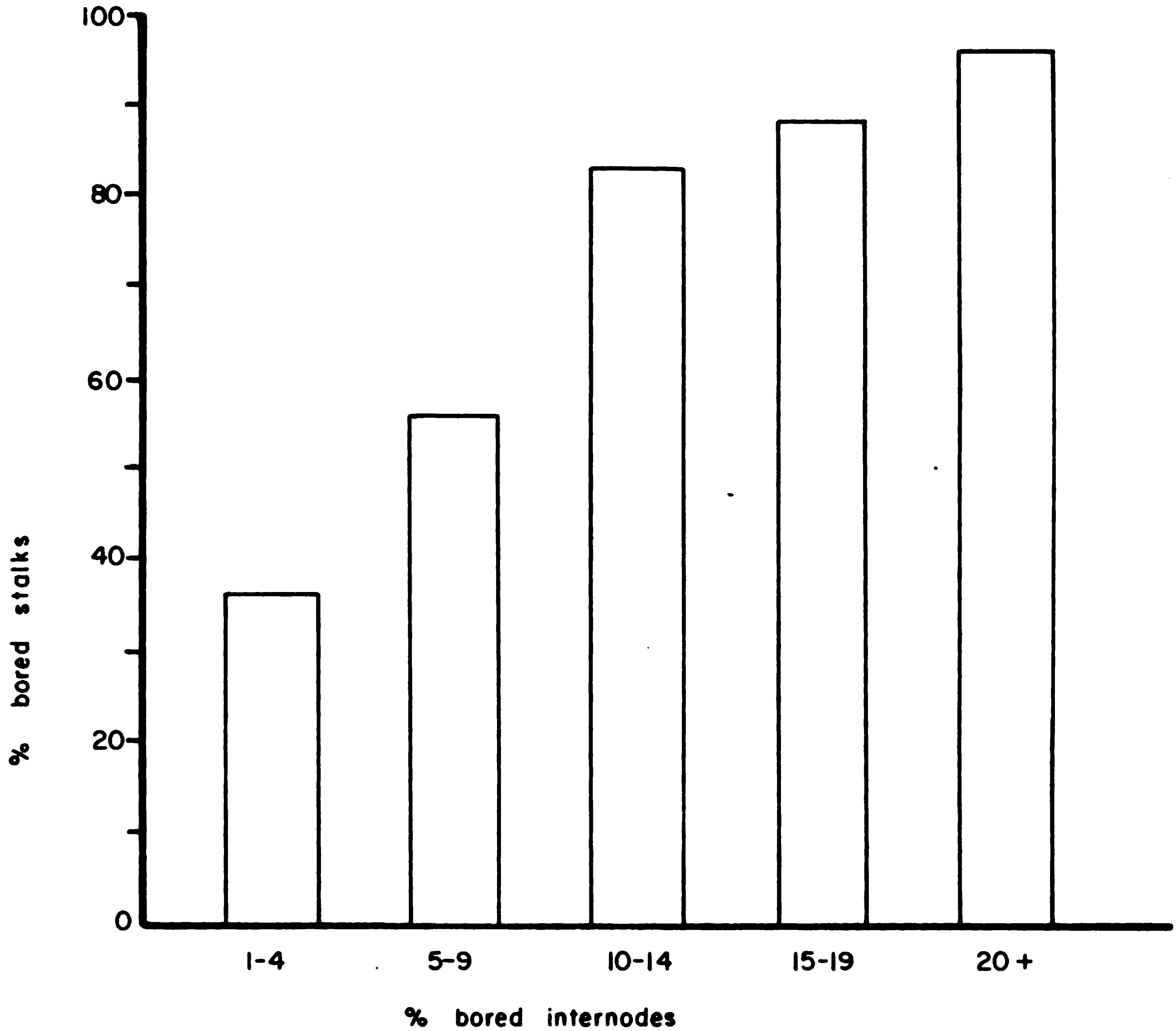

FIG. 1. Relationship between joint and stalk infestation by *Diatraea* spp. in Costa Rica (based on data in Fernández 1960).

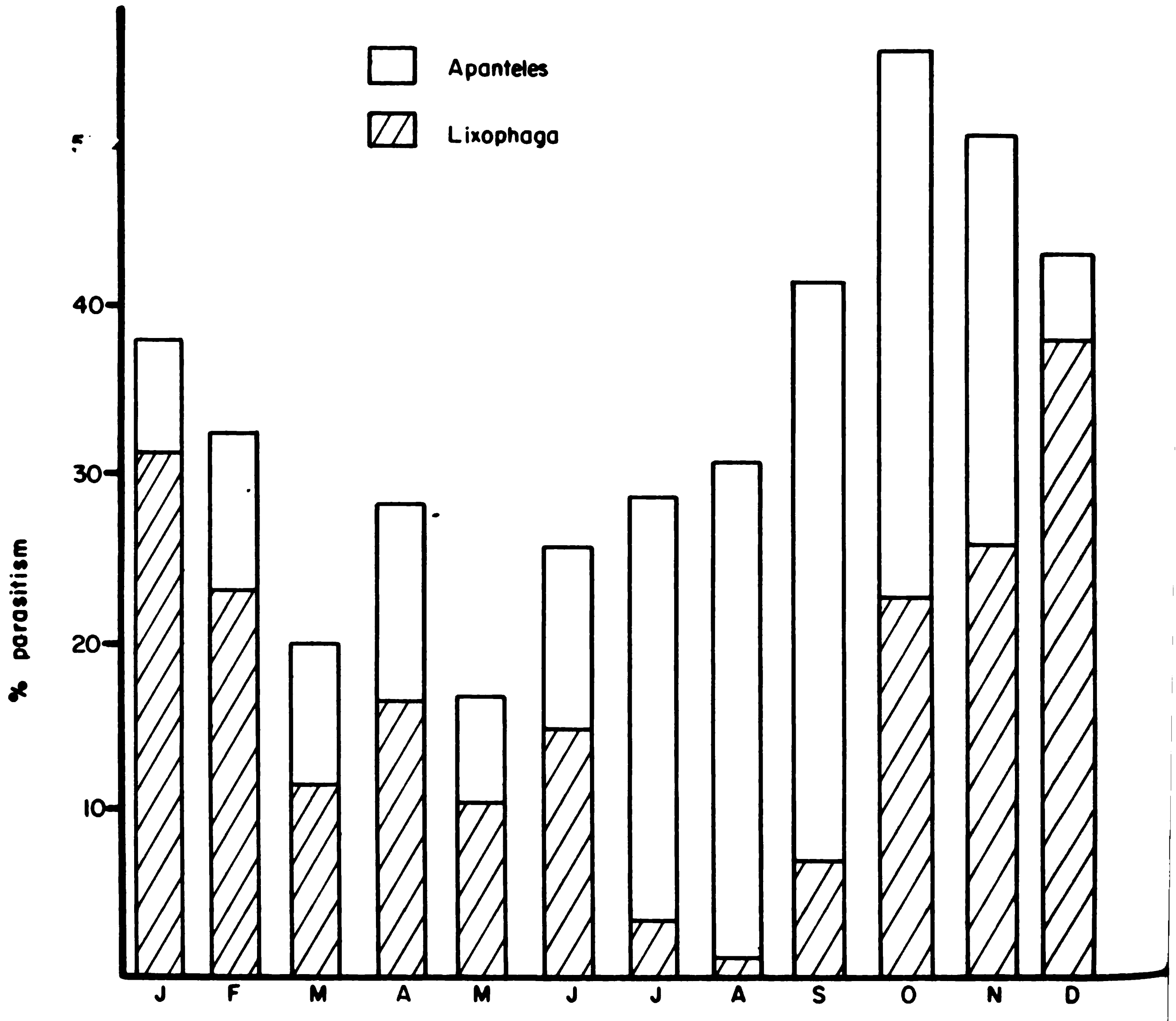

FIG. 2. Monthly levels of parasitism of *Diatraea saccharalis* by the introduced parasites *Lixophaga diatraeae* and *Apanteles flavipes* in the high rainfall areas in Barbados during 1971 (unpubl. data supplied by M. M. Alam).

THE ANTENNA OF INSECTS AS AN ELECTROMAGNETIC SENSORY ORGAN*

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COMPENDIO

Las polillas emiten una señal infrarroja de 10 μm que es modulada por las alas. Los registros con el osciloscopio demuestran que la señal tiene vectores azimutal y de trayectoria de vuelo. Una vela encendida imita por azar la producción de radiación infrarroja vectoriada de muchas polillas. Se discute el papel que juega el sensor de barrera ("picket–fence", celocónico) como detector–antena para la señal infrarroja. Se presenta un espectro de la radiación infrarroja autoemitida por una feromona en estado gaseoso y se discute el papel que juegan las antenas como un sistema dieléctrico piezopiroeléctrico para la detección de la radiación molecular autoemitida por las feromonas y por los olores de las plantas hospedantes.

El autor

Introduction

Morphological characteristics of moth antennae especially of *Hypsipyla grandella* (Zeller), as well as theoretical and experimental evidences have given me reasons to believe that the insect antennae functions as an electromagnetic detector, particularly in the infrared region of the spectrum.

The idea that Lepidoptera might utilize some portion of the electromagnetic spectrum other than visible light is not a new one. Fabre (18) first speculated on the attraction of the male great peacock moth, to the female and also to a burning candle. He postulated some unknown form of radiation common to both, and was mystified by the fact that the males were more attracted to the candle than to the female.

Duane and Tayler (15) compared the total emission spectrophometric curve of a saturnid, *Hyalophora cecropia* (L.), with that of a black body at the same temperature. Since everything in nature radiates in the infrared region, they reasoned that the male might "see" the female against the cooler background. Laithwaite (25) postulated that the insect antennae might be an infrared detector; he based his idea on the similarity of the antennae to certain radar antennae designed by man.

It was unfortunate that the popular press sensationalized these speculations and presented them as evidence for some mysterious form of attractant radiation. Such blownup reports did not do justice to the ideas of these competent amateur entomologists. Indeed, the journalistic speculations led Dethier (11), in his discussion of the electromagnetic hypothesis, to state "It is a curious and rather discouraging reflection that those who investigate desultorily prefer to solve apparent dilemmas by advancing radical hypotheses rather than by assuming the less ostentatious drudgery of critically reexamining data which have led to the dilemma and, when necessary, by undertaking to perform missing, crucial experiments".

The first definitive work on the antennae as a receptor of infrared radiation was of the mathematical nature. Grant (20) searched the literature for measurements of insect sensory pits. Although he gave no data on Lepidoptera, he showed by comparisons of configurations how sensory pits of certain species of insects could be considered as dielectric wave guides. Callahan (3) analyzed the infrared output of the corn earworm moth, *Heliothis zea,* and calculated peak radiation for several night flying moths. He pointed out the importance of the atmospheric windows to reception of infrared radiation by insects and presented a mathematical model of antennae sensilla as polytubular dielectric arrays for infrared detection. Later, Callahan (6) demonstrated that the antennae had the proper configuration and dielectric constant to function as a dielectric antennae array and he postulated that such energy might come from the total infrared emission of the moth body or from the inter– and intramolecular vibrations of pheromones or other attractants. Callahan

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(4) modified an infrared bolometer and succeeded in recording the infrared emission from the thorax of flying moths. He showed that the maximum radiation comes from approximately 90° off the side of the moth and speculated that the moth chopped or modulated the infrared radiation with the wings. He demonstrated that the corn earworm moth could detect an infrared emitter in the 8– to 13 μm range in a totally dark room. The emitter put out a directional pattern, and the moths approached it from the vectors of maximum radiation. Callahan (7) measured the infrared emission of earworm moths and superimposed the signal on an electronic infrared pulser with a duty cycle of 40 beats/sec (the wing beat frequency of the corn earworm moth). The bolometer response (500 ms) was not fast enough to observe the wing beat chopping frequency, but the superimposed signal gave definite indications that such a chopped frequency did occur. Callahan (6, 8, 9) showed that the insect exoskeleton has thermoelectret and pyroelectric properties and predicted that critical analysis of insect infrared emissions would depend on the development of fast response (few milliseconds) infrared pyroelectric detectors. He also postulated that the insect exoskeleton has all the properties of such detectors. Callahan (2, 5, 8, 10), Evans (17), Bruce (1), and Eldumiati and Levengood (16) demonstrated, contrary to previous belief (21), that insects and other arthropods can detect infrared radiation. The recent development of efficient room temperature pyroelectric detectors such as the triglycine sulfate (TGS) detector should encourage critical research into the phenomemon of infrared emission and detection by insects.

The insect antennae

It is perhaps redundant to point out that size alone imposes certain restrictions on the functioning of the insect nervous system. The distance over which a nerve impulse must travel, when comparing an insect to a mammal, is short but at the same time size imposes a reduction on the number of neurons contained within the system. Reduction in numbers of neurons implies a reduction in the informational capacity of the system (12). In other words, insects, because of their size, have sacrificed informational capacity for speed and neural transmission. Insects, however, have complex arrays of dielectric sensilla on their antennae. The complex arrangement and the intringuing forms that various sensilla take are indicative of multiplicative or correlation arrays. The multiplicative array is a signal processing antennae in which the wave form is difficult to predict but which is capable of both coherent and incoherent detection. Frequencies would be assumed to have wavelength dimensions of the dielectric spines and to emanate from partially coherent or incoherent sources (e.g. from plant or insect molecules). Such an array would allow for long postdetection integration time, thus, compensating for loss in predection signal to noise ratio. It would be a particularly suitable array configuration for insects, as a certain amount of signal processing would be accomplished by the antennae itself, thus, "making up", so to speak, for the loss of information capacity brought about by the reduction of neurons in the insect nervous system. Dielectric waveguides, e.g. rods or tubes of dielectric material in free space, are considered as open waveguides and are by nature periodically spaced. They may be ring–shaped, helical, corrugated, tapered, or they may have walls with slits or partially transparent walls.

Every known shape may be found in some insect or other arthropod

In order to increase the gain of a dielectric tube antennae of a given material, one must increase the length and decrease the tube wall thickness. This has been demonstrated both theoretically and experimentally by antenna design engineers. The ultimate result of such a method of increasing gain in a dielectric tube type antenna is that the walls become so thin that they have no structural integrity; however, less radical techniques are available for reducing the effective dielectric constant of tube type antennae. The technique consists of drilling numerous fine perforations in the tube of the antenna. Such an antenna is called a perforated dielectric tubular antenna. It exhibits extremely high gain. Among insects many of the sensory sensilla are perforated.

Weinstein (30) has noted that dielectric waveguides can support the propagation of electromagnetic modes which have no radiation losses at all. He says:

> "At a given frequency, there may be a finite number of such modes, whilst all other modes are attenuated by radiation. In other periodic structures there may also be modes that can propagate without attenuation if the period of these structures is smaller than one half of the wavelength in the medium surrounding the structure. The existence of electromagnetic waves whose attenuation is exactly zero is characteristic property of open waveguides".

By definition, open waveguides may be ring–shaped, helical, or corrugated, or may have almost any ordinary configuration whose walls are partially transparent. Dielectric rods and tapered dielectric tubes in free space are also open waveguides.

Electron scanning microscope picture of the antennae of *Hypsipyla grandella* (Figs. 1, 2) show every type of antennae sensilla described by Callahan (9) for the corn earworm moth *Heliothis zea* (Boddie). Of particular interest are the picket–fence sensors (coeloconica), shoe horn sensors (auricular) sensilla and long tapered spines (long trichodea). In my opinion studies on the sensilla of insect antennae have not emphasized the specific shapes of these sensors. If shape has meaning, and it usually does in living organisms, (just as it does in dielectric antennae engineering) then we must look to a frequency system to lead us to an understanding of how they work).

IR emission from a vibrating moth

The picket fence sensor of moths is the best example of what is known in dielectric antennae engineering as an array configuration. I believe that this antennae sensor "tunes into" and detects the first signal that I mentioned in the introduction, the total infrared emission from a vibrating moth. This IR signal should not be confused with the emission and absorption IR bands from molecules of pheromones or host scents. Emissions and

Fig. 1. Long curved trichodea on the antennae segment of a male mahogany shootborer *Hypsipyla grandella* (1300X). These long curved trichodea are lined up in evenly spaced rows.
Such accurate aligment is typical of polytubular dielectric antennae arrays.

absorption bands of gaseous molecules are low energy, narrow bandwidth frequencies. The IR emission of a vibrating or flying moth is broadband and is chopped by the moving opaque wings of the moth.

The oscilloscope recordings show a definite vector indicating pattern that is dependent on the angle of the TGS detector (receiver) in relation to the flying insect (Figure 3). The system is analogous to what in aviation communication jargon is known as an ILS (instrument landing system). An ILS system consists of a runway transmitter that transmits azimuth (horizontal) vectors and a glide path (vertical) vector. The aircraft receiver locks onto an azimuth and glide path signal and homes in on the transmitter. Such a system is ideally suited for directional orientation to a point source of electromagnetic energy. Because of the similarity of the aircraft system to the moth system, it will be referred to as an ILS system in this paper.

Figures 4 (A to E) demonstrate the azimuth indicating signal of the corn earworm moth. A fast sweep analysis (20 to 200 ms) shows the wave detail of the generated infrared frequency. From 0 to 80° (Figs. 4A and C), the wave shows a maximum primary (left) and minimum secondary (right) peak or double wave. The minimum side secondary peak (right) increases in amplitude in relation to the primary (left) peak as the moth is rotated from 0 through 95°. At approximately 95°, both peaks are of equal amplitude from the baseline up. At 50° (Fig. 4B) for instance, the secondary side peak is about 1/3 the height of the primary peak, and the total amplitude is approximately 1/2 that at 90°. It is slightly less than 1/2 the amplitude of that at 80° (Fig. 4A top). The side peak (right) can be seen as increasing in amplitude in relation to the main peak from 20 to 80° (Figs. 4A and C). The peaks become equal in amplitude at 95°. Recording from the opposite side (270°) show

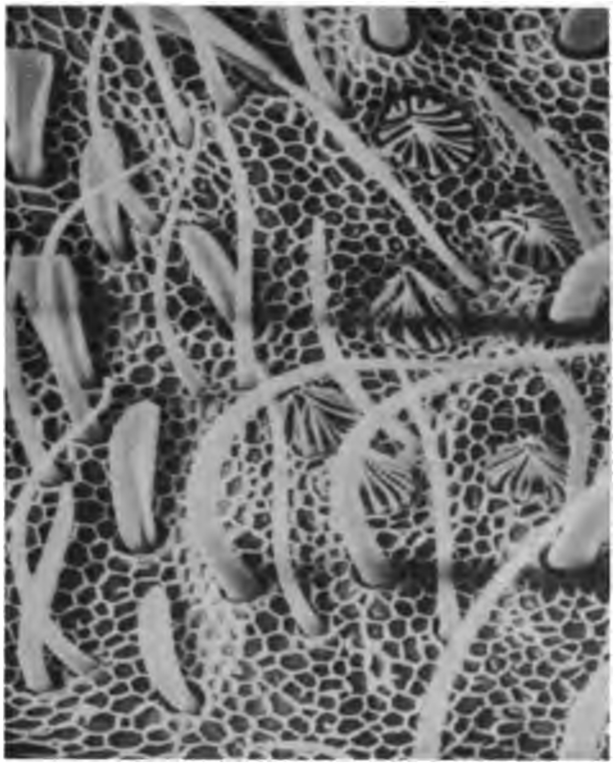

Fig. 2. Shoe horn and picket fence sensors on an antennae segment of a male *Hypsipyla grandella* (1500X). The picket fence sensors are probably thermal pyroelectric–dielectric antennae arrays for detecting the vectored blackbody radiation from vibrating moths. The show horn sensor may be a specialized sensor which resonates by shape to some IR line or lines from attractant or host plant scents.

that the two peaks reverse position, the small secondary peak moving to the opposite side (left) of the primary peak.

Figure 4D demonstrates the glide path vector at 30° and 2 o'clock above the radiating moth. The second peak (right lobe) shifts higher and higher from the base line as the glide path angle is increased from 3 o'clock (horizontal) to 1 o'clock. At between 1 and 12 o'clock, the secondary side peak begins to disappear and the primary peak spreads at the base. From below at 4 and 5 o'clock the primary and secondary peaks are spread much wider at the base and approach each other in amplitude as the vector drops below and down toward 5 o'clock.

After a little experience, it is quite simple, while rotating the moth and moving it up and down, to predict both the aximuth and glide path angles by watching the oscilloscope. The height and amplitude of the secondary peak obtained as the moth rotates and the vertical position of the secondary peak on the primary peak tells one exactly where the receiver is in relation to the transmitting moth.

If the sweep is slowed up to 200 ms (Figs. 4E and F), the secondary small (right) peaks fill in between the primary peaks and appear as a solid bar across the recording. The inverse square law of radiation is also shown in Figs. 4E and F. At 1–cm distance, the signal strength is approximately 4 times that at 2 cm.

An interesting observation from these measurements is that old moths, even though the wing beat frequency and shape may be normal, lose the signal. The only apparent physical difference of such a moth is the loss of scales, especially wing scales. Infrared transmission spectrum of moth wings show them to be essentially transparent from 22 to 222 μm*. There are many absorption bands between 2 and 22 μm, especially in the 7– to 14–μm window. For this reason, the wing as an opaque chopper in the near infrared region but not in the far infrared past 22 μm. Whether or not the loss of

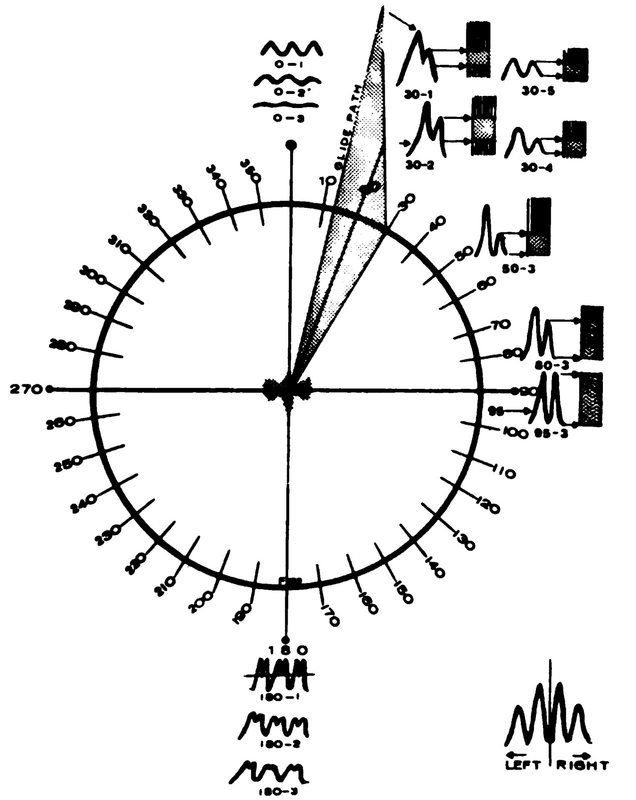

Fig. 3. Azimuth and glide path vectors of an infrared emitting corn earworm moth. 0° (head) and 180° (tail are shown at top and bottom for 1, 2 and 3 o'clock approaches. The oscilloscope approach signal (shaded) and signal form 30° at 1 o'clock (30–1) and 30° at 2 o'clock (30–2) are given on the right. Representative signal forms at the indicated vectors are also shown from a below approach, 4 and 5 o'clock at 30° (30–4 and 30–5) and from 50°, 80° and 95° at 3 o'clock wave is shown for a 20-ms sweep, and the up and down bar form for a 200-ms sweep. The secondary lobe (peak) moves up and down on the primary peak according to the glide path angle (30-1, 30-2, etc.) and moves from the base line up according to the azimuth at 3 o'clock, horizontal, (50-3, 80-3, 95-3, etc.).

* Callahan, P. S. and Turner, K. (1972) Second Quarterly Report. Insect Attractant. Behav. Res. Lab., Gainesville, Fla. 2, 4-5.

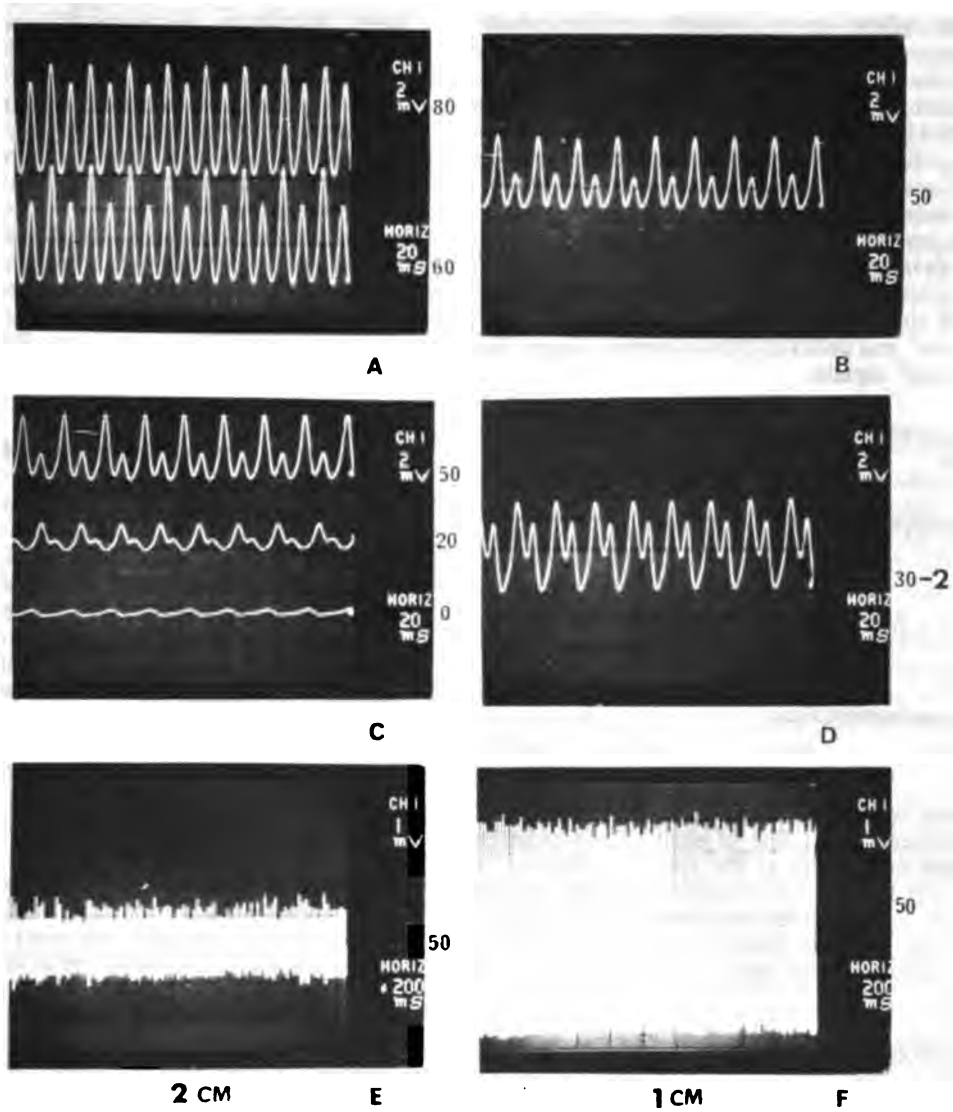

Fig. 4. Oscilloscope recording of the vectore IR radiation from the corn earworm moth *(Heliothis zea)*. A and C–vectored radiation from 0° (head on) to 80° (side of thorax); note that the smaller side lobe of the radiation wave increases in amplitude as the signal amplitude (large peak) increases in height from 0 to 80°. B–vectored radiation al 50° off the side of the thorax of the moth. D–vectored radiation at 30° off the side of the moth and from 2 o'clock high above moth (30-2). Note that the smaller side lobe has risen above the base line on the main signal lobe. The side lobe rises higher and higher above, the base line as the glide path (detector angle) rises toward 5 o'clock high. E and F–recordings at 200 ms sweep bring the peaks close together. The side lobe peaks, then make a filled in bar across the signal display. Spacings can be seen at the top of the sweep where the bar (tips of small lobes) stop. These recordings were taken at 50° off the thorax and demonstrate the inverse square law as the detector signal is increased four times by moving the moth closer to the detector by one–half the distance (2 cm to 1 cm).

scales affects the chopping efficiency of the wings by making them more transparent has not been determined. A second possibility, of course, is that olds moths simply lose too much metabolic (infrared) heat. Both these ideas are being studied at present.

Lepidoptera have a high emissivity. Callahan (3) approximated the emissivity of the corn earworm moth as being 0,95, close to 1. Hudseph (22) verified the findings of Callahan and further pointed out that the emissivity was not only close to unity but also generally uniform across the blackbody curve. He also calculated the maximum radiant energy across the moth blackbody as being highest in the 7– to 14–μm, atmospheric window. He found maximum radiation across the peak blackbody from 25,6 x 10^{-4} (w cm^{-2} μm^{-1}) at 6 μm to a maximum of 35,6 x 10^{-4} (w cm^{-2} μm^{-1}) at 8 μm. From 8 to 11 μm, the radiant energy remained above 24 x 10^{-4} (w cm^{-2} μm^{-1}).

If we consider the area of the side of a corn earworm moth as one cm^2 of radiating surface, then an earworm moth at a flying temperature of 30° would have an absolute temperature of 30° + 270° = 300° K.

At 300° K the radiation of a m^2 source area equals 6 x 10^2 watts/m^2 and peaks at approximately 10 μm. The radiation per cm^2 equals.

$$\frac{6 \times 10^2 \text{ watts m}^2}{10^4} = 0.06 \text{ watts/cm}^2$$

since total radiation = $\epsilon W / 2\pi$ steradians

Then:

$$\frac{\epsilon W}{2\pi} = \frac{0.95 \times 0.06 \text{ watts/cm}^2}{2\pi} = \frac{0.0570}{6.28}$$

$$= 0.0076 \text{ watts/steradian.}$$

The radiant intensity emitted varies as the cosine of the angle between direct line of sight to the radiating object and the normal line to the surface: therefore, if we consider the normal line to a plane diffusing source as a straight line to the center (shoulder) of the side of a flying earworm, then the radiation 40° off on a line of sight vector drawn to the center of the earworm's side would be:

$$0.0076 \cos 40° = 0.0058 \text{ watts/steradian}$$

or a drop of 0.0018 watts 40° off maximum, which is 95° for moths. This would be a drop of approximately 25 per cent at a 40° vector. Thus, the experimental results (Figs. 4A to E) verify Callahan's (3) theoretical predictions for the intensity of emission of a corn earworm moth.

Levengood and Limperis (26) calculated the maximum detection ranges, based on the theoretical limits, of a thermal and a phototype detector. A TGS pyroelectric detector, though it does not operate exactly like the usual thermal detector, nevertheless, theoretically, fits that category. They calculated that there would have to be at least 10^{-11} watts arriving at the receptors of the moth's antennae to reach the limit of detectivity at 300° K. This amount of energy would produce an observable signal. Their calculations assumed, however, that the moth emits radiation equally in all directions which it does not.

The maximum detection range for a searching moth, assuming it possessed a thermal–type detector* on its antennae, would be approximately 260 feet. However, this would be only 95° off the shoulder of a transmitting moth.

It is this drop–off in signal strength (plus the wave form) that makes the moth ILS system ideal for point source navigation. A searching moth could identify the azimuth and glide path by wave form while simultaneously steering toward the maximum directional signal. The infrared electromagnetic energy contains not only detailed azimuth and glide path information but also generalized directional information based on the intensity of the signal.

It is obvious from recording of damaged moths that the infrared signal depends on the shape of the wing and wing beat pattern of the moth.

The picket fence detector

If we assume that the complex ILS output of moths is being utilized, then the receiving antennae must have a sensor that has a directional configuration. It should preferably be an antennae–detector that is arranged as an array for pattern analysis or that has multiplicative array–type characteristics. Such an antenna would, by proper placing of array detectors, phasing bars, etc., be able to extract and analyze a patterned source such as the moth ILS output. The picket–fence sensor (coeloconica) ideally fits this picture (Fig. 2).

Callahan (8, 9) pointed out that the coeloconica of the noctuid moth is, in all probability, a thermal sensor. This is based on the fact that regardless of size of the moth, from the smallest to the giant witch moth, *Ascalapha odorata* (L.) the dimensions of the picket–fence coeloconica are the same. This also holds true for the tiger moths, pyralids, and most other moth families. One parameter that would seem to be standard for all moths is the blackbody thermal (infrared) radiation in the 7– to 11–μm infrared window.

If one assumes that the dimensions of the antenna–detector are the same order of magnitude as the wavelengths involved, an assumption of all antennae design, then the diameter of the sensor must be within the range of 7 to 14 μm. This is certainly true of the picket–fence sensor. Not only are the dimensions correct, but an omnidirectional configuration is also evident. The central peg is fluted, and each flute points toward a solid dielectric picket. The angle around 360° for each of the 14 flutes is approximately 26°. Such a spacing would be expected if each dielectric picket were to act as a reflector or phasing bar for incoming directional radiation.

There is a general theorem of antennae engineering to the effect that any individual source of electromagnetic radiation may represent an antenna of any complexity provided that the amplitude and phase of its field (output) can be expressed as a function of angle, that is, that the field pattern and phase pattern with respect to the phase center is known (24). The reciprocal law makes this applicable to a receiving antenna.

* This is an assumption based on the fact that the insect waxes and the exoskeleton are thermoelectret, piezoelectric and pyroelectric substances (6).

Any electromagnetic wave propagating in a given direction has four characteristics: amplitude, phase, frequency, and polarization. It is not inconceivable that a directionally vectored receiving antenna, by processing the scalar quantity of the amplitude of the transmitted wave form, could decode the signal before it was even passed on to the brain. This has the definite advantage in a small animal such as a moth of reducing the need for high internal informational capacity. Dethier (12) as pointed out before stated that insects, because of their small size, have a reduced number of nerves and have sacrificed informational capacity for speed of neural transmission. If this is so, then one way to compensate for low internal information capacity is to decode the incoming information before it reaches the nervous sytem for integration.

An antenna, of course, is not a detector. The primary function of an antenna is to pick up (lock into), amplify, and in the case of arrays, analyze a signal. However, we have used the term antenna–detector because it is not inconceivable that a detector system might be built right into the antenna (sensilla). Man is now using a few crystals of an organic pyroelectric substance (triglycine sulphate) to detect low infrared energies. Callahan (6, 8, 9, 10) has pointed out numerous times that the thin–layered strata of the insect exoskeleton makes it an ideal solid–state mechanism. There has been little study of insect waxes, polar–hydrocarbons, esters, and alcohols from a solid–state viewpoint. All are excellent thermoelectret, pyroelectric, and piezoelectric substances. Glycine, an amino acid, and sulphates are known to be present in the exoskeleton (28, 29). It is entirely possible that such an amino acid "solid–state" detector exists in insects. The study of the insect exoskeleton from a solid–state approach is certainly long overdue.

One last fascinating phenomenon was discovered with the TGS detector. Scans of an ordinary wax candle flame in the 7– to 14–μm window (8.5– to 11–μm filter) showed wave forms very similar to that of the moths. Moreover, a series of twenty (200 ms) scans of the flame demonstrated that the flame produced random wave forms that at times were very stable. The frequency, amplitude, and phase of the infrared signal was dependent on the air currents and breezes blowing in the room. Almost every characteristic type of moth wave form could be duplicated by blowing the flame and randomly storing the sweeps on the oscilloscope phosphor. Some of the sweeps actually showed large and small peaks just as the moth ILS output. In some cases there was a gradual damping of the frequency output.

Since the days of Aristotle man has speculated on the peculiar affinity of a moth for a candle flame. It is not attraction to light alone. Why does a moth fly to a candle flame but not to the steady flame of a Bunsen burner or to the massive output of a campfire? Also, the behavior of a moth with a candle is unpredictable. I have many times seen certain species of moth suddenly attempt to mate with the flame. It is therefore of extreme interest that the infrared output of a moth is mimicked again and again by the random oscillation of a flame and that the frequency, amplitude and phase of moth and candle signal can exactly duplicate one another. Until we can further analyze the picket–fence detector we can only speculate.

We have discussed the IR blackbody emission of night flying moths and the picket fence sensor (coeleconica) as a possible detector for this vectored radiation, but what about all of the other types of sensilla on moth antennae? You will note in Figures 1 and 2 that the long trichodea sensillae are lined up like soldiers in a neat row. There are also quite a few unique shoe horn sensors (auricular) on each antennae segment of *H. grandella.* In most moths that have been studied, the trichodea are presumed to detect pheromone molecules. The function of the shoe horn sensor which was first described by Callahan (9) is unknown.

Radiating molecules

Everything in nature exchanges radiant energy and the sun is the primary source of the earth's input radiant energy. There has been a vast amount of entomological research devoted to the high energy UV and visible spectral regions of the radiation environment. Both UV and visible light profoundly influence the behavior of arthropods.The effect of the radiation (IR) on the insect has been largely overlooked, precisely for the reason that it is low energy radiation, and is extremely difficult to detect. Historically speaking, the infrared region was studied by physicists because it is the region of the spectrum most suited to the experimental verification of Maxwell's theory of electromagnetic radiation and also of Plank's quantum hypothesis. The relationship between the refractive index η and dielectric constant ϵ ($\eta = \sqrt{\epsilon}$) holds to a greater extent in the infrared region of the spectrum than the visible. The large range of wavelengths between visible and millimeter radiation allowed for a high degree of accuracy in the experimental verification of Plank's radiation law. Later advances in the field of infrared generation and detection were accelerated by the use of IR spectroscopy in the determination of the structure of molecules. It is these emitting and absorbing molecules that I am considering when I speak of the radiation environment and the insect in this paper.

All molecules emit radiation. Spectra made up of low frequency lines that are widely separated are associated with atoms; spectra made up of a continuum of multiple lines, grouped as bands, are associated with molecular vibrations: it was soon realized that these molecular signature lines were a key to understanding the structure of the molecule. Since molecules emit and absorb photons of specific frequencies, it follows that the detection of these frequencies can be used to identify the molecules. It also follows that within certain regions only certain energy changes are possible (Table 1).

Chemists usually measure absorption spectra; however, in certain cases emission spectra are more meaningful. Drexhage (14) measured the emission of fluorescent organic dyes and found that such excited dye molecules emit light (visible) by a process that is remarkably analogous to that of an antenna emitting radio waves. Since light is electromagnetic radiation, Drexhage asked the question: "Is there any relationship between radio–wave emitting antennas and light–emitting antennas (the excited molecules)? ". The answer is yes, as his experiments so uniquely demonstrated. He coated a

TABLE 1. Spectral regions and principal types of energy changes. After Dixon (13).

Wavelength		'Frequency'		Radiation	Energy change
cm	µg	sec^{-1}	cm_{-1}		
10					
10^4		3×10^6		Radiowaves	Magnetic resonance
10		3×10^9	10^{-1}	Microwaves	Magnetic resonance Rotation
0.1	1000	3×10^{11}	10		
	50		2×10^2	Far infrared	Rotation
	2.5		4×10^3	Infrared	Vibration
Å				Near infrared	Vibration Electronic excitation
8000	0.8		1.25×10^4	Visible	Electronic excitation
4000			2.5×10^4	Near ultraviolet	Electronic excitation
2000			5×10^4	Far ultraviolet	Electronic excitation and ionization
100			10^6		

1 micron (µm) = 10^{-4} cm. 1 Angstrom (Å) = 10^{-8} cm.

front surfaced gold mirror with a monomolecular layer and excited the molecules with UV. His experiment showed that the fluorescence emission closely resembled the corresponding patterns of a radio antenna. Figure 5 is a polar diagram of the emitting molecules of an organic dye.

The beam width of the major directional lobes of both a dye and a dielectric antennae are about 20°. Beam widths narrower than approximately 20° are not possible with a single dielectric rod, and arrays of rods are utilized to obtain narrower beams. Directional antennae, both transmitter (the molecules) and receiver (the dielectric tapered tubes) show characteristic side lobes. The dielectric antennae sensilla of insects are tapered.

Many long chain organic molecules absorb and emit in the intermediate and far infrared, from 2.5 to 400 µm. Antennae sensillae of insects fall within this range. This would be expected since a receiving antennae should be of the dimensions of the transmitted frequency. There is an excellent fit between the length of insect sensilla (2.5 to 300 µm) and the major absorption–emittance bands of organic molecules (2.5 to 300 µm).

Callahan (3, 4, 6) discusses the "frequency fit" of molecules, atmospheric windows, and dielectric antennae design to this region of the intermediate and far infrared spectrum.

In the literature covering the infrared spectrum of known pheromones there is a fundamental misconception as to the physical state of the "messenger" chemical as it is utilized by the insect. All such IR frequencies are discussed from the standpoint of IR spectrum taken in the liquid state, yet it is doubtful if a single entomologist working on pheromones would deny that they are decoded by the insect in the gaseous state. It is well known that in the "finger print" IR identification region 600 to 3800 cm^{-1} that gas phase frequencies are usually, but not always, higher by some 2 to 10 cm^{-1}, or more, than the corresponding liquid phase frequencies (19). It also is apparent to me that researchers attempting to correlate insect "smell" with IR vibrational frequencies of pheromones are working at slightly to long wavelengths (31). As Callahan (3, 4, 5) pointed out, a dielectric antennae system should consist of tapered spines of the same dimensions (lengths) as the molecular IR wavelengths (2 to 80 µm). Furthermore in order to

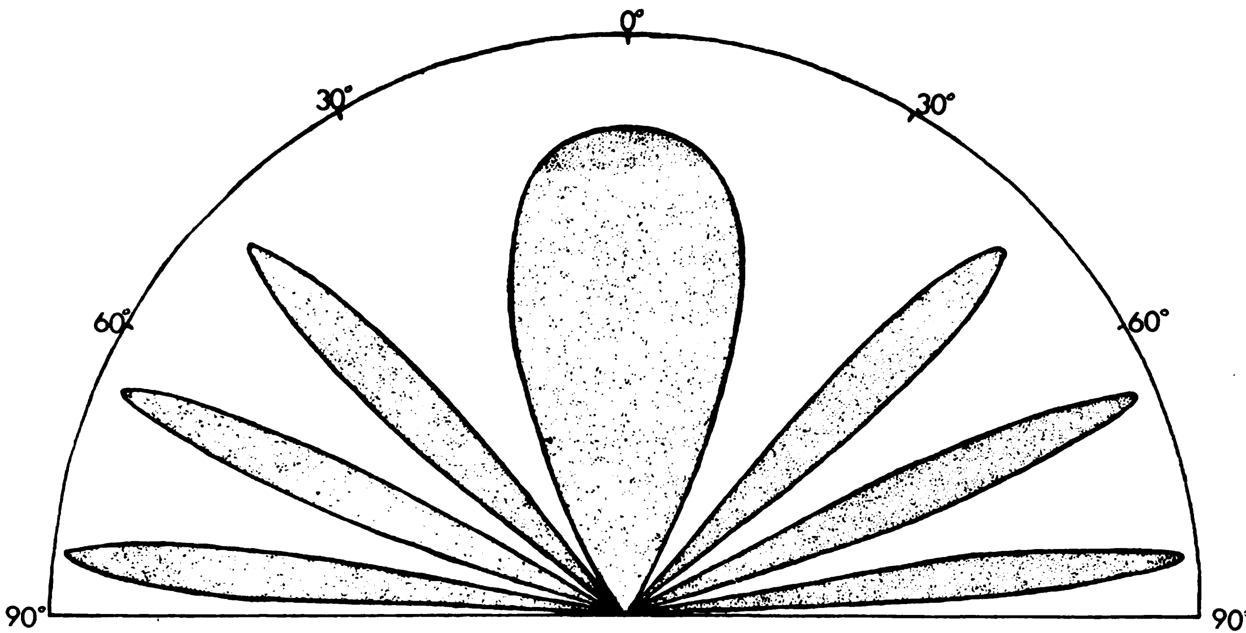

Fig. 5. Polar radiation output from the fluorescense of a monomolecular layer of organic dye. This type of self–emitted radiation is analogous to a radio station putting out vectored coherent radiation from an antennal array (after Drexbage, 14).

avoid unnecessary molecular background noise from water vapor the frequencies would probably lay in IR windows of the atmosphere, particularly the 7 to 14 μm IR window.

Another characteristic of the gaseous state, as distinguished from the liquid state, is that the IR spectral lines have much more narrow bandwidths in the gaseous state.

As early as 1965, physical chemists were utilizing multiple–scan interferometry to characterize organic compounds by means of their self–emitted infrared radiation (27). They invariably showed good correspondence between the emission and absorption bands.

This author is the first researcher to obtain high resolution IR emission bands from the gaseous phase of an insect pheromone. Figure 6 is an example of one such emission from the pheromone of the cabbage looper *(Trichoplusia ni)*. The top (Fig. 6) is the liquid emission spectrum at high resolution (2 wavenumber) of the region from 1100 to 1000 cm^{-1} (9.09 to 10 μm). Below is the gas spectrum of the same region showing a strong emission line at 1053 cm^{-1}. The astonishing characteristic of the gas spectrum is the fact that not only does it have extremely narrow band–width (less than 2 wavenumbers) but that the noise level on either side is almost flat. There are several such strong absorption self–emitting IR lines in the cabbage looper pheromone.

These spectrum were obtained with a Fourier transform infrared interferometer (high resolution instrument). The lower gas spectrum was obtained over a short path length of 10 cm utilizing a system developed by the author. It is known that low frequency piezoelectric resonance can be utilized to give an enhanced responsivity of a pyroelectric detector over a narrow bandwith if the incident radiation is modulated at a mechanical resonance frequency of the detector element. I therefore modulated the detector at the wing beat frequency of the cabbage looper and obtained the unique noise free gas emission spectrum (Fig. 6 bottom). It should be observed that the emitted line is shifted about 2 wavenumbers from 1055 (top) liquid spectrum, to 1053 (9.5 μm, bottom) gas spectrum.

Since the moth detects the pheromone in nature while it is vibrating, it is obvious that the piezoelectric properties of the exoskeleton of arthropods (6, 32) might be utilized to enhance the signal, or suppress the noise of the self–emitted IR radiation of pheromones. Two ro three self–emitted frequencies coded to two or three dielectric array sensilla, of different average lenths, make an almost perfect coded identification system. The Air Force would call it an IFF system (identification friend or foe).

The point to be made is that a long range orientation signal (ILS), produced by the chopped blackbody moth radiation and coupled with a shorter range (IFF) molecular identification system makes for a highly sophisticated and efficient communication system. The air driven molecule not only serves to identify the species but also becomes a short range directional and releaser stimulant as the moth approaches its mate.

With what we now understand from the sciences of solid state physics and antennae design, it should be possible to decode these frequencies in order to duplicate them by physical means. This, of course, will not be a simple task, because of the complex shapes and arrays of the antennae sensors and molecular emittors.

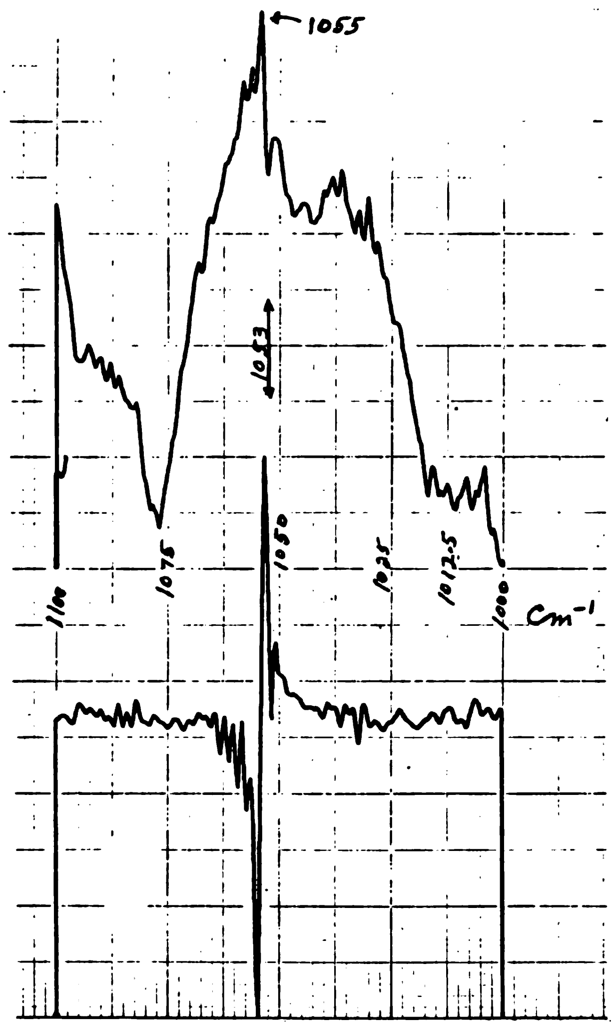

Fig. 6. Liquid and gaseous self–emitted infrared radiation from the cabbage looper pheromone. Top emission from the liquid spectrum has a broad band IR peak at 1055 cm^{-1}. Bottom gaseous spectrum shows a very narrow band (1 wavenumber) IR emission at 1053 cm^{-1}. In this region gas frequencies would be expected to shift higher (higher wavenumber) but do not in the vapor phase. The gas emission was modulated at the wing beat frequency of the cabbage looper. Note how this technique "cleaned up" the noise on either side of the narrow band emission. Such a signal is ideal for a coded frequency communication system. Two or three such narrow band self–emitted frequencies could be detected by two or three different sensilla of various sizes arranged in array patterns. There are millions of coded possibilities in such a frequency identification system.

The arrangement of the sensilla on insect antennae so far studied in our laboratory have been in array–like patterns. Sensilla arrays are the norm, not the exception, and are indicative of multiplicative signal processing dielectric antennas as pointed out before. As more and more insects are scanned, new configurations of dielectric antenna shapes will be discovered. Configurations will include not only thin–layered tubular protuberances, such as the sensilla but also slits and holes and other types of dielectric antennae. Kieley (23) has pointed out that a hole in a block of dielectric material is also a dielectric aerial. In the light of conclusive experimental evidence, that insect sensilla can function as dielectric waveguides and arthropods can detect infrared frequencies (1, 8, 9) the prevailing belief that these unique configurations are meaningless is untenable. If they are meaningless, then dielectric antenna engineers and solid state physicists are designing systems and using formulas that accurately describe what insects have developed on their own antennae with no significance to the insects' communications system.

Summary

Moths emit a 10 μm infrared signal which is chopped by the wings. Oscilloscope recording demonstrate that the signal has an azimuth and glide path vector. A burning candle randomly mimics the vectored IR output of many moths. There is a discussion of the picket–fence sensor (coeloconica) as in IR antennae–detector for the signal. A spectrum of the self–emitted infrared radiation from a moth pheromone in the gaseous state is given and the antennae discussed as a piezoelectric–pyroelectric dielectric array for the detection of self–emitted molecular radiation from pheromones and host plant scents.

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OBSERVATIONS ON EMERGENCE AND MATING OF ADULTS IN CAPTIVITY*

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COMPENDIO

Este trabajo presenta observaciones sobre la emergencia y apareamiento de *Hypsipyla grandella* en cautividad. La duración promedio del estado pupal fue de ocho días. El tamaño de las pupas mostró un alargamiento de 12 por ciento poco antes de la emergencia. La emergencia ocurrió más frecuentemente en las horas del anochecer cuando la luz ambiental alcanzó una intensidad entre 15 y cero lux. Los adultos emergieron por la parte anterior de la pupa y generalmente se demoraron aproximadamente un minuto en salir. La expansión y secamiento de las alas duró aproximadamente 11 minutos. Los adultos se quedaron casi inmóviles durante el día. La actividad en general, incluyendo vuelo, caminata y el movimiento rotatorio de las antenas por adultos recién emergidos estacionados, se inició cuando la luz ambiental se aproximó a una intensidad de 10 lux. Las hembras empezaban a ponerse sexualmente atractivas cinco horas después de cero lux y después de poco comportamiento de cortejo, aparearon entre 6,5 y 10 horas después de cero lux. Las parejas quedaron juntas aproximadamente dos horas.

The shootborer, *H. grandella* feeds on meliaceous hosts throughout the American tropics. Studies on the biology and behavior of this insect include the partial life history by Ramírez–Sánchez (8) and Roovers (9); host preference of the larva and host selection by Grijpma (3) and Grijpma and Gara (4, 5). This paper presents observations on emergence and mating of adults in captivity.

Materials and methods

Insects used in this study were obtained from the culture maintained by the Inter–American Working Group on *Hypsipyla* at Turrialba, Costa Rica, and from natural field populations in the Turrialba region. Measurement of pupae and observations at the time of emergence were conducted under laboratory conditions with an average temperature of about 25°C (range 20–31.5°C). Observations on post emergence and mating behavior were made in a screened insectary where the average temperature was about 22°C (range 15–37°C).

Sexed pupae were kept in compartments (5 x 5 x 5 cm) of large plastic boxes, or in small plastic boxes (9 x 6.5 x 3 cm). Light intensity measurements were made using a Gossen 2.59–406 photometer.

Night time observations were facilitated by indirect light of 0.4 lux from incandescent light bulbs located on the external walls of the insectary or by using a two–cell flashlight.

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Results

Development of the pupa

Thirty pupae (15 ♂, 15 ♀) without cocoons were observed from pupation to adult emergence, average duration of the pupal stage was 8.2 ± 0.3 days*.

During development the pupae seemed to undergo a series of minute elongations and contractions. During the first six days the change in size of the pupae was slight. One day prior to emergence, when the eyes, antennae, proboscis, legs, and wings of the adult became visible, elongation was greatest. It was most pronounced during the hour prior to emergence when the pupae increased 1.5 ± 0.2 mm in length.

Time of emergence

The frequency of emergence and corresponding ambiental light conditions are presented in Fig. 1. Peak emergence occurred just before dark between 15–0 lux light intensity. No significant difference ($t_{.05}$) existed between the times emergence of sexes.

Activity in general, including flying, walking, or simply rotating of antennae by stationary adults started between 10–0 lux intensity.

* For all time and numerical measurements given throughout this paper, $x \pm t_{.05}\, s_x$

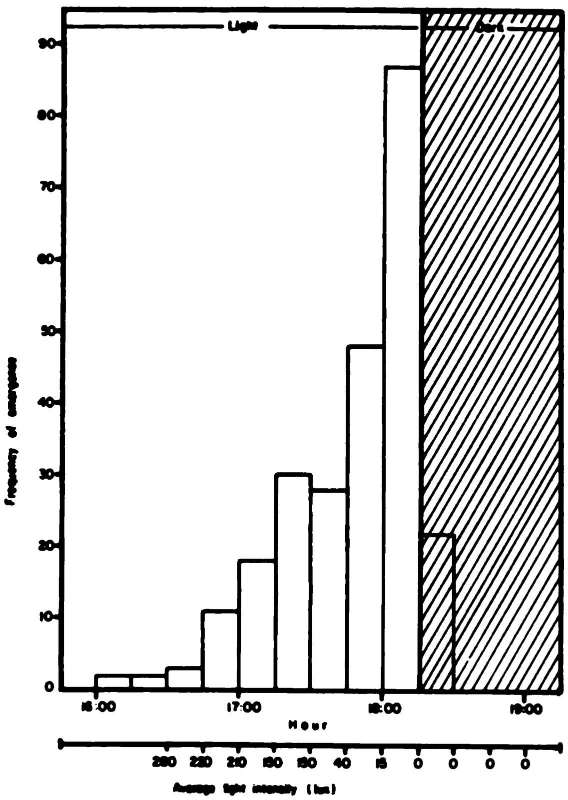

Fig. 1. Emergence of *H. grandella* and corresponding ambient light intensity as related to time.

Adult emergence from the pupal case

Just prior to emergence, movement of the adult within the pupae case was very noticeable. It was manifested principally by the twisting of the tip of the abdomen.

At emergence the ventral side of the pupal case split along one, and sometimes both, of the sutures between the maxillae and the legs, extending from the base of the maxillae to the base of the fourth abdominal segment. Laterally, the pupal case split along one–third of the suture between the antennae and the wings. The dorsal side of the pupal case split longitudinally from the junction of the pronotum and the head to the junction of the metanotum and the first abdominal segment, and transversally along the suture between the pronotum and mesonotum from one antenna to the other (Fig. 2). The top of the pupal case was carried by the emerging adult and usually fell off after it was completely out of the pupal case. Adult emergence usually lasted less than one minute.

Post emergence behavior

Thirty seven moths (13 ♂, 24 ♀) were observed immediately after leaving the cocoon. Following emergence, 55 per cent of the adults walked less than 0.5 minutes and became stationary with folded wing pads exposing about one half of the abdomen (Fig. 3a). The rest of the moths walked about one meter in 2.9 ± 0.7 minutes before becoming stationary. Short duration post–emergence walking behavior is also reported for *Dioryctria abietella* (1).

Shortly after becoming stationary, the wing pads began to swell and total wing expansion was completed in 3.7 ± 0.6 minutes (Fig. 3 b, c). The moths remained motionless with expanded wings in a horizontal position for 1.2 ± 0.2 minutes. Forewings and hindwings were then raised vertically over the abdomen and thorax for 4.9 ± 0.4 minutes (Fig. 3 d). The wings were then lowered to the normal horizontal position, (Fig. 3 e).

Total time from emergence to completion of wing expansion and drying was 11.0 ± 0.9 minutes. No significant differences ($t_{.05}$) were observed between sexes for all of the post–emergence activities.

Female calling behavior

Eight pairs of adults were observed during mating activity beginning with the female calling behavior through copulation. Copulation behavior of nine additional pairs was also observed.

Readiness of the females for pairing is indicated by bending the abdomen dorsally, up between the wings, and may be accompanied by an alternate protrusion and retraction of the ovipositor (7). *H. grandella* females exhibited such calling behavior without ovipositor movement (Fig. 1), but even in such a posture were not always receptive to mating (Fig. 4 a). Fatzinger and Asher (2) described the same calling behavior for *D.*

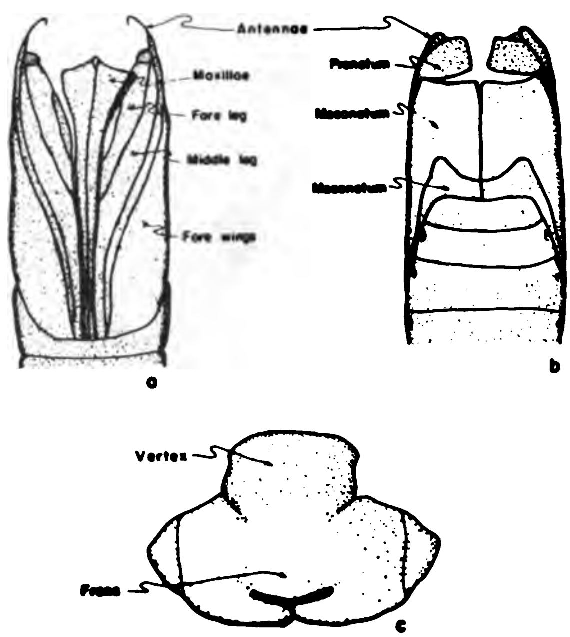

Fig. 2. Pupal case of *H. grandella* showing the ruptures after emergence of the adult; a) ventral view; b) dorsal view; c) cephalic capsule.

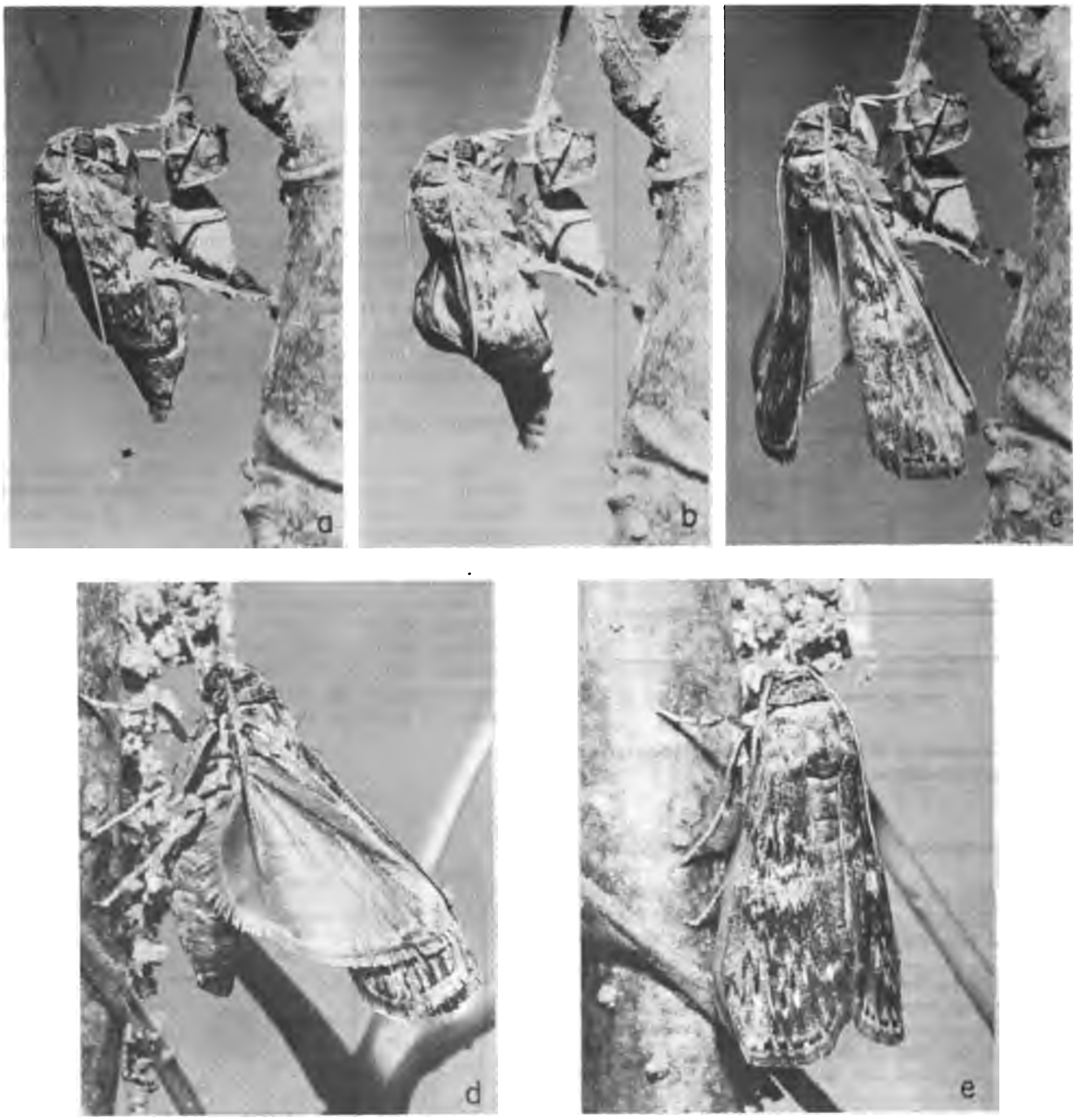

Fig. 3. **Adult of *H. grandella* immediately after the emergence; a) recently emerged from the pupa; b. c) expanding wings; d) drying wings; e) in resting position.**

abietella, and a characteristic antennal movement at a frequency of a 4.5 beats/antenna per second. They also indicated that occasionally females would assume a partial calling posture without antennal movement, and that such females were generally nonreceptive to mating. *H. grandella* females did not characteristically rotate their antennae during calling, and appeared to be equally receptive or nonreceptive when the antennae were rotated or motionless. Norris (7) points out that calling does not seem to be related to the condition of the ovaries. In the case of *Plodia* for example, the calling posture was assumed before ripe eggs were present, when they were full of ripe eggs, or when they were empty after oviposition. The same author indicates that it was a shortage of sperm in the receptaculum seminis which caused calling. If, in fact, calling is an automatic response triggered by lack of sperm, it could be manifested by females physiologically not ready for mating and therefore partially explain nonreceptivity.

The calling of *H. grandella* females started about six hours after zero lux, peaked about three hours thereafter (Fig. 5) and lasted 95.4 ± 10.5 minutes.

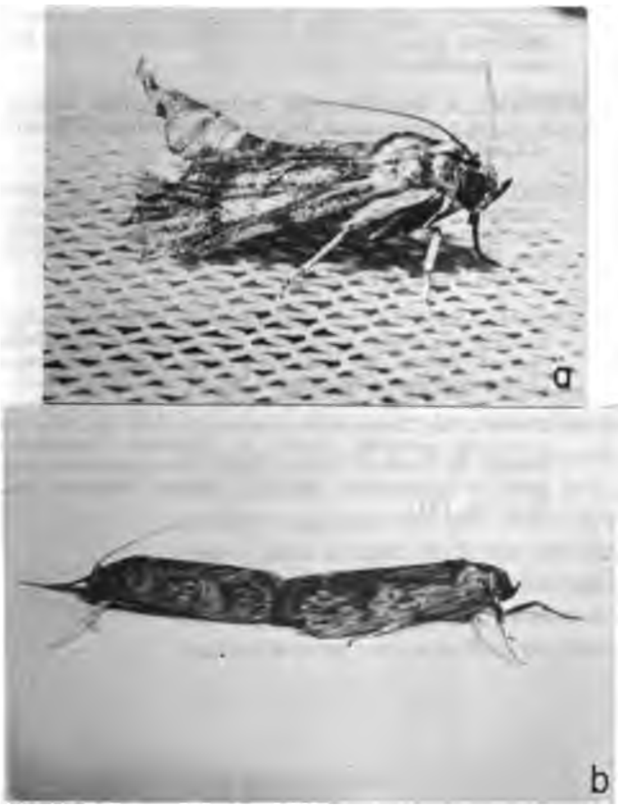

Fig. 4. *H. grandella;* a) female in calling position; b) adults in copulating position.

Courtship

Insects require a minimum of sexual behavior for approaching and identifying each other before copulation. In some insects this pre–mating or courtship behavior is very elaborate whereas in other it is so minimal as to be seemingly lacking (6). Courtship behavior of *H. grandella* approaches the latter situation. Male moths responding to attractive females flew toward and landed about 10–15 cm from females. Males then walked to the rear of the females and brushed their antennae on the ventral surface of the females' abdomen. Following this short activity the males moved into a position orthogonal to the females and moved their abdomen at a 45° angle to clasp the females' abdomen. All of the activity corresponding to courtship terminated in about 30 seconds.

Copulation

Couples remained attached for an average of 123 ± 33.7 minutes in a posterior–to–posterior mating position (Fig. 4 b). The minimum and maximum copulation times were 90 and 170 minutes respectively. The average total time of pairing in *Anagasta* (Ephestia) *kübniella* is 180–240 minutes, in *Plodia* 60–90 minutes (7); and in *D. abietella* 99 ± 4.0 minutes (2). *H. grandella* moths spent most of this time exhibiting little activity. Antennal positions and activity noted for both sexes included rotating antennae, holding antennae extended anteriorly but motionless, and antennae held back over the thorax, sometimes crossed. Generally the moths remained in one place while coupled. However, two females were observed walking, actually pulling the clasped–on males with them. Another female rubbed its right leg on its abdomen and against the male as if attempting to free itself from copulation. One pair did interrupt its attachment for about four minutes, and then recoupled.

Acknowledgements

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Thanks are also due to Edward Holsten, graduate student of the University of Washington, for several of the photographs which illustrate this communication.

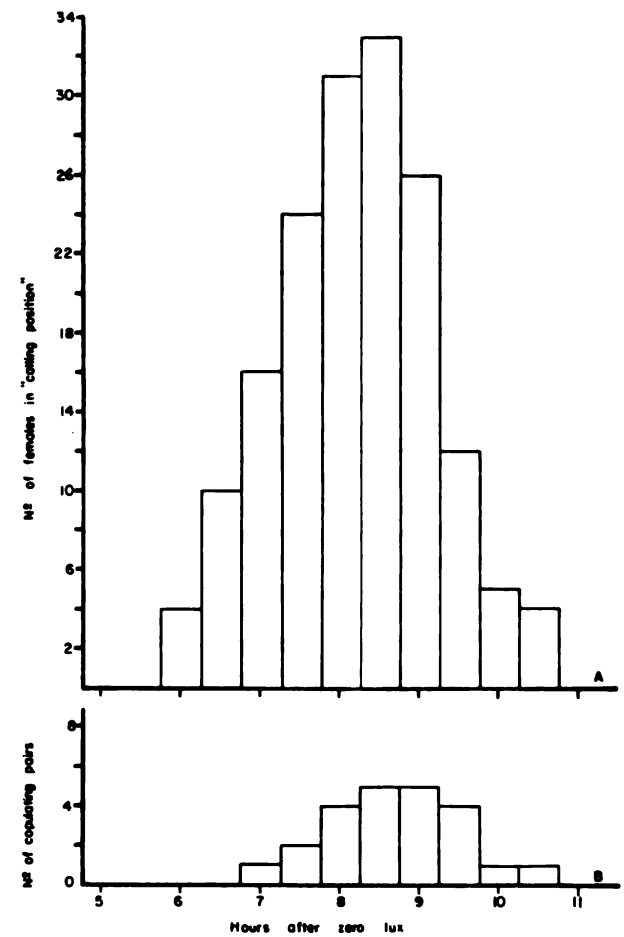

Fig. 5. Number of *H. grandella* females in calling position (A) and copulating pairs (B) in relation to time after zero lux.

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UN NUEVO METODO PARA OBTENER OVIPOSICION EN CAUTIVIDAD*

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ABSTRACT

A new method to obtain oviposition in captivity of *Hypsipyla grandella* (Zeller) is described. This method employs mated females placed in small plastic boxes in the laboratory instead of obtaining oviposition on towel paper in big screened cages in open air. In a trial comparing the new and previously used method, the average number of fertilized eggs obtained per female in plastic boxes was 210.7 as compared to 109.8 in cages placed in open air. It was also determined that if males and females (sex ratio 1:1) remain 72 hours in the copulation cages, this period in comparison with a permanence of 24, 48, 96 and 120 hours, resulted in the highest number of fertilized eggs. The new method is more economic than the previous method employed.

Introducción

Con el fin de estudiar eficazmente los posibles métodos para el control del barrenador de las Meliáceas *Hypsipyla grandella* (Zeller), es indispensable obtener una producción masiva de este insecto. Grijpma (1) obtuvo buenos resultados en ensayos de copulación y oviposición, con adultos de esta especie criados en una dieta artificial desarrollada por Hidalgo–Salvatierra (3). En este método los adultos son colocados en jaulas, que en su interior tienen bastidores de madera con bandas de papel toalla, para oviposición. Posteriormente, los huevos son recortados del papel toalla. Este sistema resulta muy oneroso, ya que los huevos son depositados en forma dispersa, o localizados solamente en ciertas áreas de las bandas de papel toalla, lo cual demanda mucha mano de obra para su recolección y se consume gran cantidad de papel. Los huevos obtenidos de esta manera son colocados en cajas de cría que contienen dieta artificial.

El objetivo principal de esta investigación fue probar un nuevo método de laboratorio en el cual las hembras copuladas ovipositen en pequeñas cajas de plástico, recubiertas en su interior con papel toalla. Así se lograría que los huevos sean depositados en forma concentrada sobre dicho papel y después podrían ser trasladados a recipientes hasta que eclosionen las larvas, las cuales, posteriormente, por medio de un pincel, pueden ser colocadas individualmente en frascos con dieta artificial (5).

También se determinó el tiempo óptimo que las hembras deben permanecer en las jaulas de copulación, para obtener la máxima producción de huevos fértiles en estas cajas.

Materiales y Métodos

El presente estudio se realizó en el Centro Tropical de Enseñanza e Investigación del Instituto Interamericano de Ciencias Agrícolas de la OEA, Turrialba, Costa Rica, el cual está ubicado a 600 m s.n.m. con una temperatura promedio anual de 22,5°C y 2547 mm de precipitación.

Se utilizaron adultos de *H. grandella* recién emergidos, criados en dieta artificial (5) y sexados en estado pupal (2).

La copulación de los adultos se obtuvo en jaulas de 90 x 90 x 90 cm, construidas de madera, recubiertas externamente por un cedazo plástico. Las jaulas estuvieron localizadas en el campo cubierto de césped a 50 cm de altura (Figura 1).

Para los ensayos de oviposición se usaron dos tipos de recipientes: a) jaulas del tipo anteriormente descritas, que en su interior tenían bastidores de madera recubiertos con papel toalla y b) cajas de plástico de 12 x 9 x 3,5 cm revestidas en su interior con papel toalla (Figura 2), bajo condiciones de laboratorio.

A fin de determinar en cual tipo de recipiente se obtiene mayor número de huevos fértiles, se colocaron 15 machos y 15 hembras en una jaula de copulación, donde permanecieron 72 horas. De los resultados de ensayos preliminares ya se sabía que tal período de 72 horas es suficiente para obtener un porcentaje relativamente alto de hembras copuladas, y también que el apareamiento ocurre desde la primera noche (4). Luego se sacaron 10 hembras al azar, de las cuales 5 se colocaron en jaulas de oviposición y las otras 5 se pusieron individualmente en cajas plásticas. Este tratamiento se repitió cinco veces.

Para determinar el tiempo óptimo que las hembras deben permanecer en las jaulas de copulación, con el fin

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* Recibido para su publicación, 22 de junio de 1972.

Fig. 1. Jaulas utilizadas para copulación y oviposición de *H. grandella.*

de obtener el mayor número de huevos fértiles, se colocaron 25 machos y 25 hembras en las jaulas de copulación. Con intervalos de 24 horas se extrajeron al azar 3 hembras y 3 machos para mantener la proporción de sexos. Esta labor se repitió hasta el quinto día. Las hembras que fueron sacadas de las jaulas de copulación, se las colocó en cajas de oviposición.

En ambos ensayos, cada 24 horas se notó el número de huevos depositados por las hembras en el papel toalla; no se tomaron en cuenta los huevos que no podrían ser aprovechados. Se consideraron como huevos fértiles todos aquellos que a las 24 horas se tornaron rojos. Las hembras permanecieron en las cajas y jaulas de oviposición hasta que murieron. Posteriormente se las disecó para comprobar si habían sido copuladas.

A los datos obtenidos en el primer ensayo se les hizo una prueba de "t" para determinar cuál de los dos métodos es el mejor. En la segunda parte del ensayo se hizo un análisis de variancia para ver si había diferencia entre tratamientos y con la prueba de rangos múltiples de Duncan se determinó el tiempo óptimo de permanencia en la jaula de copulación.

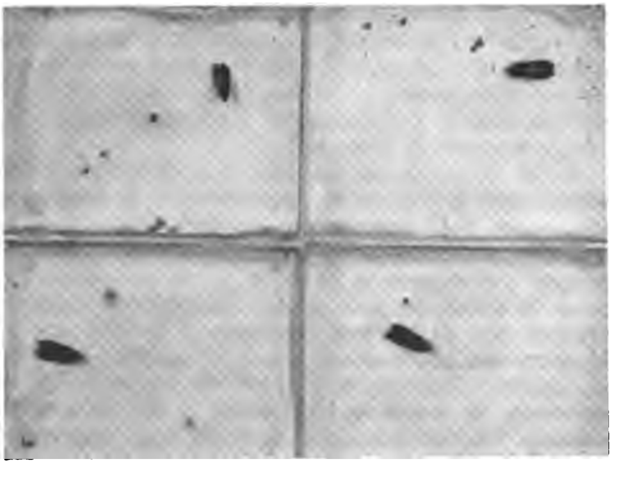

Fig. 2. Cajitas plásticas utilizadas para oviposición de *H. grandella.*

CUADRO 1. Oviposición de *H. grandella* sobre papel toalla en cajas y jaulas (Promedio de 5 hembras por repetición).

Repeticiones	Cajas		Jaulas	
	Oviposición total	Huevos fértiles	Oviposición total	Huevos fértiles
I	282,8	270,0	131,4	124,6
II	149,0	86,6	94,6	71,6
III	260,2	251,6	141,6	132,8
IV	262,0	256,2	183,0	169,4
V	204,6	189,0	60,6	50,6
Total	1158,6	1053,4	611,2	549,6
$\bar{X}$	231,7	210,7	122,2	109,8

CUADRO 2. Número promedio de huevos fértiles depositados por tres hembras sobre papel toalla en relación con el período de permanencia en las jaulas de copulación.

Repetición	Período de permanencia (horas)				
	24	48	72	96	120
1	158,3	147,0	158,0	114,3	14,3
2	198,0	271,0	132,0	127,0	41,6
3	115,0	171,6	246,6	58,0	39,0a/
4	74,0	85,6	236,0	72,0	– b/
5	0,0	110,7	288,3	77,0	77,5c/
Total	545,3	785,9	1060,9	448,3	172,4
$\bar{X}$	109,7	157,1	212,1	89,7	40,2

a/ Oviposición de una hembra.
b/ No hubo hembras disponibles para esta repetición, debido a que murieron en las jaulas de copulación.
c/ Oviposición de dos hembras.

CUADRO 3. Análisis de variancia para los períodos de permanencia en las jaulas de copulación.

FV	GL	SC	CM	Fc
Períodos	4	77.476,59	19.369,14	5,519*
Error	19	66.672,38	3.509,17	–
Total	23	–	–	–

* Significativo al nivel de 1 por ciento.

Resultados y discusión

En el Cuadro 1 se encuentran los promedios de oviposición total y huevos fértiles por hembras de *H. grandella* en cajas y jaulas de oviposición. Es notorio que el promedio de huevos fértiles en cajas es de 210,7, lo que representa un 95,9 por ciento más que el promedio de 109,8 huevos obtenidos en jaulas. Se comprobó que existe diferencia significativa al nivel del 5 por ciento. El método de las cajas resultó mejor que el de las jaulas, ya que en éstas las hembras depositan parte de los huevos en el cedazo y en los bastidores de madera, los cuales no pueden ser fácilmente localizados ni ser aprovechados.

En el Cuadro 2 se consigna el número de huevos fértiles en relación con los días de permanencia de las hembras en las jaulas de copulación. Se encontró que hay diferencia significativa al nivel de 1 por ciento entre los tratamientos (Cuadro 3). Al realizar la prueba de rangos múltiples de Duncan se encontró que el mejor período de permanencia de las hembras en las jaulas de copulación es de 72 horas después de la emergencia al nivel del 5 por ciento, siguiéndole el período de 48 horas.

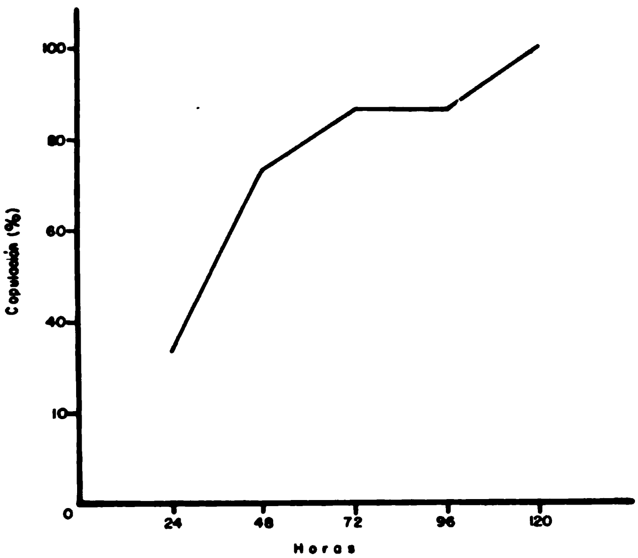

Fig. 3. Porcentajes de hembras copuladas en los diferentes períodos de permanencia en las jaulas de copulación.

En la Figura 3, se muestran los porcentajes de copulación con intervalos de 24 horas, notándose que con la permanencia de 72 y 96 horas se obtiene un igual porcentaje de copulación de 86,7 por ciento, elevándose éste hasta el 100 por ciento en las 120 horas.

Para obtener el máximo de huevos fértiles se deben mantener las hembras por 72 horas en las jaulas de copulación, ya que con este período se obtiene el mayor número de huevos fértiles por hembra de 212,6 huevos con un 86,7 por ciento de hembras copuladas. Aunque el porcentaje de copulación sea mayor después del tercer día, sin embargo, no compensa mantener a las hembras más de 72 horas en la jaula de copulación, ya que después de este período se pierden muchos huevos fértiles.

Conclusiones

1. El método de oviposición en cajas es superior al de las jaulas; además el trabajo es más sencillo por lo cual hay economía de mano de obra y consumo de papel.
2. No es aconsejable dejar a las hembras más de 72 horas en las jaulas de copulación, ya que después de este período se pierden gran cantidad de huevos.

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AN IMPROVED METHOD FOR ARTIFICIAL REARING*

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COMPENDIO

Se hace una evaluación de un método modificado de crianza artificial de *Hypsipyla grandella* (Zeller). Se compararon cuatro tratamientos de las larvas: a) tratamiento A_1: una larva por frasco, frascos sin esterilizar; b) tratamiento A_2: dos larvas por frasco, frascos sin esterilizar; c) tratamiento B_1: una larva por frasco, frascos esterilizados; y d) tratamiento B_2: dos larvas por frasco, frascos esterilizados.

La omisión de la esterilización de los frascos en los cuales se crían las larvas en dieta artificial resultó en un retraso de cuatro días en la pupación máxima.

En los tratamientos A_1, A_2, B_1 y B_2, 500 frascos con dieta artificial produjeron 330, 387, 313 y 342 pupas respectivamente. Las pupas femeninas en promedio pesaron más que las masculinas (166 vs. 151 mg). Las pupas correspondientes a los tratamientos A_1 y B_1 pesaron en promedio más que las de A_2 y B_2 (164 vs. 152,5 mg).

La duración media del estado de pupa en los cuatro tratamientos A_1, A_2, B_1 y B_2 ocurrió a los 40, 43, 37 y 37 días después de la oviposición respectivamente.

Las 330, 387, 313 y 342 pupas de los tratamientos A_1, A_2, B_1 y B_2 produjeron 244, 310, 277 y 294 adultos respectivamente.

Las hembras vivieron por más tiempo (189 vs. 150 horas) y fueron más grandes 17,3 vs. 16,4 mm) que los machos.

El porcentaje medio de eclosión de huevos fértiles por hembra fue 87,0, 88,0, 92,5 y 97,9 por ciento en los tratamientos A_1, A_2, B_1 y B_2 respectivamente. Los huevos fértiles eclosionaron entre 83 y 118 horas después de la oviposición.

Los ingredientes más caros de la dieta artificial fueron vitaminas y agar. Una cantidad de dieta suficiente para 150 frascos costó ₡5,49 (colones de Costa Rica, US$0,64). El costo total de materiales utilizados en la crianza fue ₡0,18, ₡0,15, ₡0,16 y ₡0,15 por adulto en los tratamientos A_1, A_2, B_1 y B_2 respectivamente. El costo total de labor en relación con la crianza fue ₡0,32, ₡0,30, ₡0,30 y ₡0,31 por adulto en los tratamientos A_1, A_2, B_1 y B_2 respectivamente.

El autor

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Introduction

A good rearing system of *Hypsipyla grandella* (Zeller) is essential for intensive investigations on the biology and control of this insect.

A useful artificial rearing technique has been described earlier by Grijpma (2). At the Tropical Training and Research Center of the Inter–American Institute of Agricultural Sciences (IICA–CTEI), Turrialba, Costa Rica, this technique was used with minor changes for about one and a half year.

Since February 1972, however, two basic modifications were introduced. Larvae are now reared individually or in pairs in 1 oz glass jars, instead of up to 100 individuals together in polystyrene containers 28 x 13 x 4 cm in size. Oviposition now takes place in small polystyrene boxes (11 x 3 x 3 cm) in an air conditioned laboratory, replacing wire screen cages (90 x 90 x 90 cm, 50 cm above ground) in the open air (3).

The modified method is evaluated in this study. The experiment was conducted between September 22 and December 20, 1972.

Materials and methods

At present, 3000 adults of *H. grandella* are reared per month in the laboratories at IICA–CTEI in Turrialba.

Eggs are kept in glass jars (12,5 x 12,5 x 12,5 cm) in an air conditioned laboratory until hatching. First instar larvae are put individually with a fine, soft brush onto approximately 6,5 g of artificial diet in 1 oz glass jars (cylindrical, height 4 cm, diameter 3 cm) with metalic covers. After pupation, the cocoons are dissolved in a 2,5 per cent sodium hypochlorite solution. The pupae are sexed and put onto towel paper in polystyrene boxes (28 x 13 x 4 cm). At 16:00 hours, these boxes are placed into a wire screen cage (90 x 90 x 90 cm on 50 cm high legs) in open air. In the cage the boxes are opened so that the adults can emerge freely. Towel paper attached to wooden frames, which cover three of the four walls of the cages, serves as a resting place for emerged adults and as a protection against sun and rain. At 7:00 hours, the boxes with pupae are taken out of the cages. Pupae are otherwise killed by direct exposition to sunshine and/or high temperatures during the day. The roofs of the cages are covered with plastic to keep the interior more or less dry and to prevent the towel paper from tearing.

After three nights the female *H. grandella* are taken out of the cages to insure oviposition of a maximum number of eggs per female (3). Subsequently, they are placed in polystyrene boxes (12 x 9 x 3,5 cm) and glass Petri dishes (diameter 9 cm, height 1,5 cm), covered inside with moistened towel paper. These boxes and dishes are kept in the rearing laboratory.

Eggs hatch about three and a half days after oviposition. The towel paper on which oviposition took place is removed every three days, shortly before hatching and replaced by new paper, if the female is still alive. The towel paper containing the eggs is air dried and kept in glass jars and a new rearing cycle begins.

In the experiment described in this paper a comparison was made between four treatments of the larvae.

Treatment A_1: One larva per jar, non–sterilized jars.
Treatment A_2: Two larvae per jar, non–sterilized jars.
Treatment B_1: One larva per jar, sterilized jars.
Treatment B_2: Two larvae per jar, sterilized jars.

The experiment was carried out with ten repetitions of 50 jars per treatment.

During the experiment, temperature and relative humidity in the rearing laboratory were recorded continuously. Temperature and humidity conditions in the copulation cages were measured by placing a hygrothermograph in one of the cages during the period January 12–February 12, 1973.

The artificial diet used in the experiment was developed by Katiyar* and is a modification of the diet described by Hidalgo–Salvatierra (1). This diet consists of the following ingredients:

50	g	Carrot powder
75	g	Wheat chaffs
35	g	Soya flour
60	g	Sugar
10	g	Agar
15	g	Alpha Cel
5	g	Wesson's Salt Mixture
10	g	Vitamin Diet Fortification Mixture
1	g	Sorbic Acid
1	g	Parahydroxy Benzoic Acid
0.5	g	Ascorbic Acid
1	g	Benzoic Acid Na–Salt
10	ml	Acetic Acid (25 per cent)
5	ml	KOH (4M)
5	ml	Formaldehyde Solution (10 per cent)
5.5	ml	Aureomycine (45.8 mg)
700	ml	Distilled water

This quantity of diet was evenly distributed over 150 jars without weighing, each jar receiving an average of 6,5 g. Of each 150 jars 100 were taken for one repetition of A_1 and A_2 or B_1 and B_2, leaving the rest of the jars for normal ongoing rearing.

Pupation and emergence of each individual was recorded using the same criteria as Grijpma (2) in order to obtain comparable results. Each pupa was weighed four or five days after pupation. Poisson and binomial distributions were tested for best fit to the observed frequency distributions of pupation and emergence.

To determine the quality of the adults obtained from the four treatments, the following characteristics of the insects were compared: a) longevity; b) size; c) number of eggs produced per female; d) percentage of eggs hatched per female; and e) time needed for egg hatching per female. For these purpose insects were taken randomly from the populations obtained from the four treatments, during the whole period of the experiment.

Longevity under laboratory conditions was determined by placing 24 males and 24 females of treatments A_1 and A_2, and 30 males and 30 females of treatments B_1 and B_2 in compartmented polystyrene boxes with 6 x 4 compartments, each of 5 x 5 x 5 cm. No food or water was provided. Mortality was recorded at least twice a day.

* K. Katiyar, personal communication, 1972. IICA–CTEI, Turrialba, Costa Rica.

Size of adults was determined by measuring the distance between the fringe of the wings and the tip of the head with a vernier caliper, which measured to an accuracy of 0.01 cm. Only adults with undamaged wings were taken.

Table 1 presents the number of adults taken into consideration for the determination of egg production per female. The females which were still alive after the three–day period in the copulation cages, were put into oviposition boxes. Their egg production was counted every three days until death occurred. To determine the percentage of eggs hatched and the time needed for egg hatching per female, up to 20 eggs were taken from the oviposition boxes of several females from each treatment. On several days, at about 19:00 hours, the boxes of the females, taken out of the copulation cages the same day, were checked and, if possible, 20 eggs were separated for observations of the time needed for hatching. All eggs oviposited between 18:00 hours and 19:00 hours were considered as oviposited at 19:00 hours.

For determination of the percentage of eggs hatched per female, the following numbers of females and eggs (in parenthesis) were taken into consideration in treatments A_1, A_2 and B_1 and B_2 respectively: 15 (298), 10(200), 10(199) and 14(280). Data about the time needed for hatching were obtained from the following numbers of females and eggs (in parenthesis) in treatments A_1, A_2, B_1 and B_2 respectively: 13(239), 7(138), 10(184) and 14(274).

Production costs of adults in this rearing system were calculated for all stages of the rearing cycle. The time of labor needed was recorded or, in a few cases, estimated. The cost of materials and diet ingredients used was calculated taking into account the quantity of material needed to maintain a production of 3122 adults per month. A year was considered as consisting of 365.25 days and one month as 365.25/12 days = 30.4 days = 30.4/7 weeks = 4.34 weeks.

Example: Cost calculation of towel paper.

Estimated use of towel paper (for copulation cages, oviposition, cleaning tables etc.) three rolls weekly, *i.e.* 3 x ₡9.10 weekly, *i.e.* ₡27.30 x 4.34 monthly = ₡118.42 monthly, *i.e* ₡118.42/3122 per adult = ₡0.03795 per adult (US$1.00 = Costa Rican ₡8.57).

Costs which varied between the four treatments were calculated separately for each treatment.

Example:

- Artificial diet ingredients cost ₡5.48929 per quantity used for 150 jars.
- In treatment A_1 150 jars with larvae resulted in 150 x 0.488 = 73.2 adults.
- Thus the cost per adult from treatment A_1 was ₡5.48929/73.2 = ₡0.074990.
- In treatments A_2, B_1 and B_2 150 jars with larvae resulted in different numbers of adults; consequently the cost per adult also differed.

TABLE 1. Determination of egg production per female of *H. grandella.*

Period in copulation cages	Number of adults present originally			
	Treatment A_1		Treatment A_2	
	Males	Females	Males	Females
3- 6/XI/72*	2	4	2	4
10-13/XI/72*	12	9	12	9
13-16/XI/72	8	8	8	8
16-19/XI/72	8	(8)**7	8	(8)**7
19-22/XI/72	4	6	5	5
Total	34	(35)**34	35	(34)**33
	Treatment B_1		Treatment B_2	
23-26/XI/72	3	3	3	3
29/XI-2/XII/72	2	2	2	2
2- 5/XII/72	4	4	4	4
5- 8/XII/72	7	7	7	7
8-11/XII/72	7	7	7	7
11-14/XII/72	2	2	2	2
Total	25	25	25	25

* Some adults stayed longer, and others stayed less in the copulation cages than the period indicated.

** One female of A_1 and one of A_2 were excluded from the calculations because of possibility of confusion.

Overhead and the following equipment and materials were not included in the cost calculations:

- Equipment used for the preparation of the artificial diet like balance, blender and thermometer.
- Distilled water, used in preparation of artificial diet.
- Use of autoclave, in which diet jars are sterilized.
- Microscope and lamp, used for sexing pupae.

Transportation costs of imported materials were not included because they vary from place to place. However, it should be pointed out that, in developing countries, the cost of air freight and additional expenses for imported materials and diet ingredients can easily amount to 40 per cent or even more of the catalogue price of materials.

Results and discussion

In the rearing laboratory, temperature and relative humidity varied between 21.0 and 30.5°C and 51 and 76 per cent respectively during the period of the experiment.

In the copulation cages, temperature and relative humidity varied between 15.5 and 33.0°C and 43 and 100 per cent respectively in the period January 12–February 12, 1973.

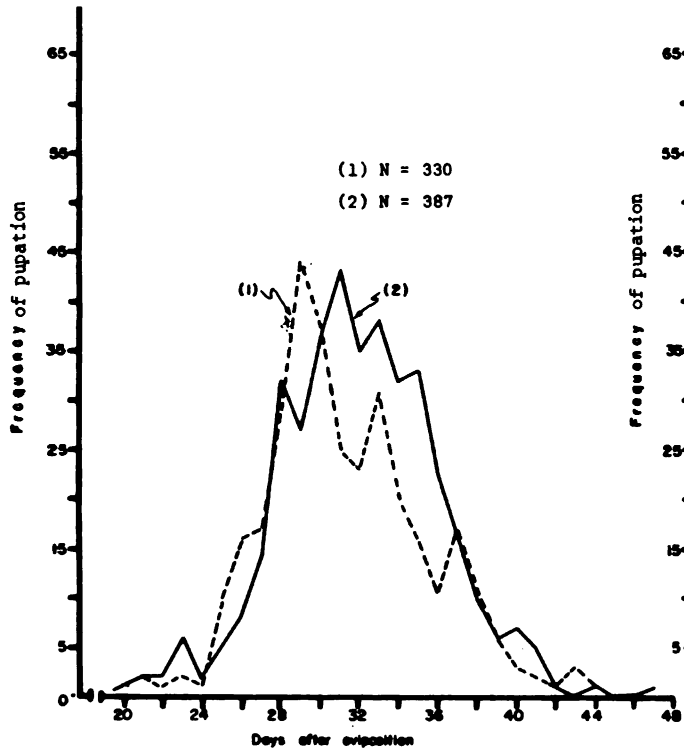

Fig. 1. Frequency of pupating larvae of *Hypsipyla grandella* in relation to time. Non–sterilized jars, one larva per jar (1) and two larvae per jar (2).

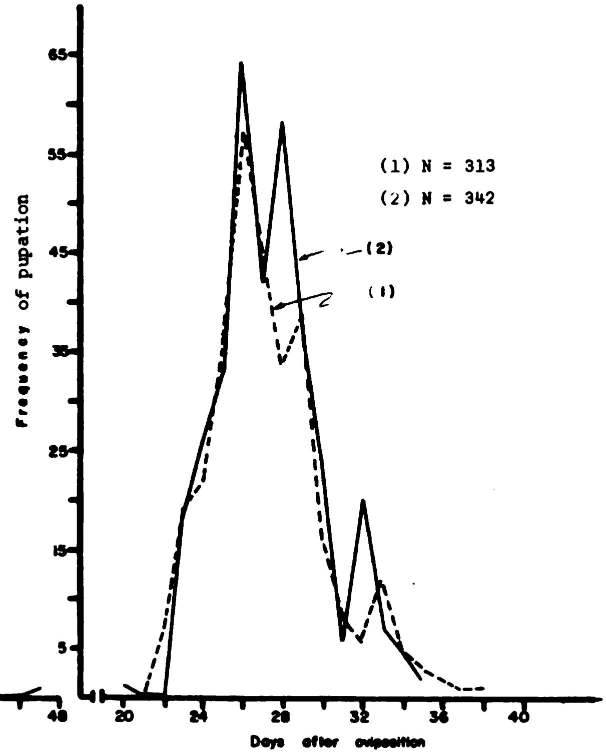

Fig. 2. Frequency of pupating larvae of *Hypsipyla grandella* in relation to time. Sterilized jars, one larva per jar (1) and two larvae per jar (2).

Figures 1 and 2 present data on the frequency of pupating larvae. The maximum numbers are reached in A_1 at 29 days, A_2 at 31 days, B_1 at 26 days and B_2 at 26 days after oviposition. Grijpma (2) obtained maximum pupation of larvae on a somewhat different artificial diet at 26 days and on natural diet at 25 days.

These results seem to indicate that omitting sterilization of the jars results in a delaying effect on the pupation of *H. grandella*. Almost all non–sterilized jars show contamination by fungi, which did not kill the larvae but apparently influenced the duration of the larval stage. Of two distributions (Poisson and binomial) tested for best fit to the observed frequencies of pupating larvae, Poisson distribution fitted best in all four treatments.

The data with respect to number of pupae obtained per sex and treatment, are presented in Table 2. The 1000 non–sterilized jars (A_1 + A_2) produced more pupae than the 1000 sterilized jars (B_1 + B_2). None of the observed differences between non–sterilized and sterilized jars (A vs. B), between one and two larvae per jar (A_1 + B_1 vs A_2 + B_2) and between males and females, however, proved to be significant at the 10 per cent level. Nor were significant differences found at the 10 per cent level between treatments A_1, A_2, B_1 and B_2.

The observed weight of the pupae is presented in Table 3. No statistically significant differences existed between the weight of pupae obtained in the four treatments A_1, A_2, B_1 and B_2, nor between A and B (10 per cent level). Observed differences in weight of pupae between males and females, however, were significant at the five per cent level. Female pupae were generally heavier than males (166 vs. 151 mg). Differences between treatments 1 and 2, one and two larvae per jar respectively, were significant at the 0.5 per cent level. The individually reared larvae of treatment 1 resulted in clearly heavier pupae than those of treatment 2 (164 vs. 152.5 mg). This could be explained by the fact that in treatment 1 twice as much diet was available per larva. Another factor could be that two larvae in a jar may hinder each other, so that they have less time to feed on the diet.

The frequency of duration of the pupal stage in treatments A_1 and A_2 had its maximum at 11 days for both males and females, while in treatments B_1 and B_2 this was 10 days for both males and females. The average duration of the pupal stage in treatments A_1, A_2, B_1 and B_2 were 10.67, 10.56, 10.02 and 9.86 days respectively. Pupae from contaminated jars (A_1 and A_2) needed more time to complete the metamorphosis from larva to adult; 10.61 vs. 9.94 days, significant at the 0.5

TABLE 2. Number of *H. grandella* pupae obtained per sex and treatment in artificial rearing.

Treatment	A_1			A_2			B_1			B_2		
Sex	♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂+♀
No. of pupae	173	157	330	178	209	387	158	155	313	183	159	342

per cent level (Fig. 3). The difference between $A_1 + B_1$ vs. $A_2 + B_2$ (10.34 vs. 10.22 days) was also significant (2.5 per cent level).

The data on emergence are presented in Figures 4 and 5. The maxima of emerging adults of treatments A_1, A_2, B_1 and B_2 were observed at 40, 43, 37 and 37 days after oviposition respectively. Grijpma (2) obtained maximum emergence of *H. grandella* reared on artificial diet at 37 days after oviposition and of *H. grandella* reared on natural diet at 35 days after oviposition. Apparently the modification of the artificial diet did not influence the occurrence of maximum emergence of the moths (the moths produced from treatments B_1 and B_2 as well as those reared by Grijpma on artificial diet in sterilized jars had their maximum emergence at the age of 37 days). The delayed pupation and the longer duration of the pupal stage in the non–sterilized jars resulted consequently in later maximum emergence of *H. grandella*.

Poisson distribution fitted the observed frequencies better than the binomial distribution in all four treatments (Figures 4 and 5).

Table 4 presents the results with respect to the number of adults obtained per treatment and per repetition. Observed differences between the four treatments A_1, A_2, B_1 and B_2 were not significant, nor the differences between males and females, between A and B, non–sterilized vs. sterilized jars and between 1 and 2, at the 10 per cent level.

In the longevity test (Figures 6 and 7, Table 5) a significant difference at the 1 per cent level was found between sexes. Females lived longer than males (averages 1888.8 vs. 150.0 hours). This is in agreement with the results of Grijpma (2). In treatment A_1, females lived only slightly longer than males (differences of the averages 7.8 hours). In treatments A_2, B_1 and B_2 these differences were 43.9, 58.1 and 40.4 hours respectively.

With respect to longevity, the differences between treatments 1 and 2, one and two larvae per jar (172.3 vs. 166.6 hours), and between treatments A and B, without and with sterilization of diet jars (160.1 vs. 176.9 hours) were not significant at the 5 per cent level.

The data obtained with respect to size of adults are presented in Table 6. At the 5 per cent level there was

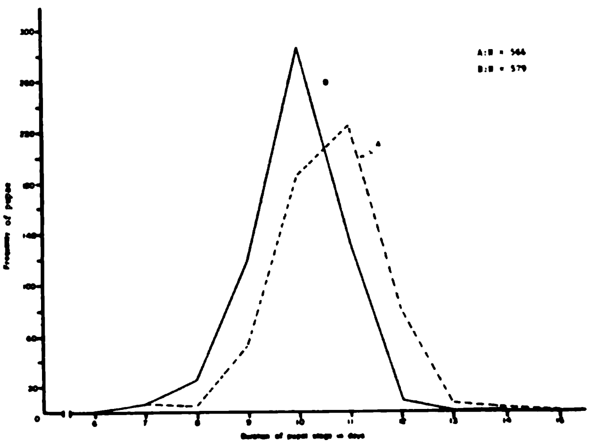

Fig. 3. Frequency distribution of duration of pupal stage of *Hypsipyla grandella* reared on artificial diet in non–sterilized (A) and in sterilized (B) jars.

TABLE 3. Average weight of *H. grandella* pupae obtained per sex and treatment in artificial rearing.

Treatment	A_1			A_2			B_1			B_2		
Sex	♂		♀	♂		♀	♂		♀	♂		♀
Average weight per sex (mg)	157	–	172	149	–	164	154	–	175	144	–	152
Average weight per treatment (mg)	–	164	–	–	157	–	–	164	–	–	148	–

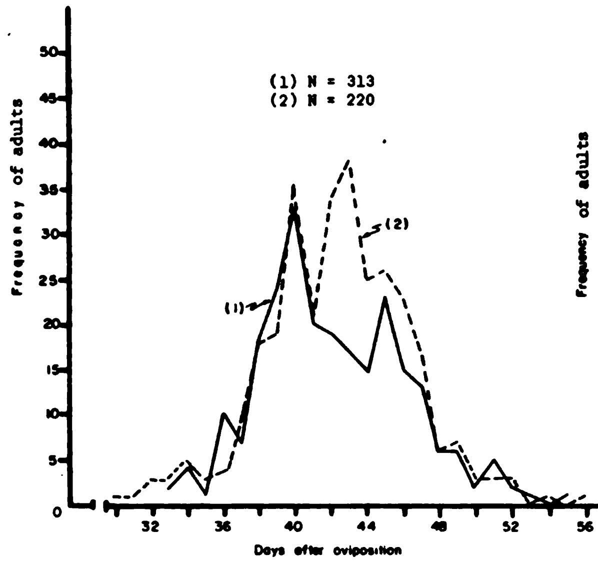

Fig. 4. Frequency of emerging adults of *Hypsipyla grandella* in relation to time. Non–sterilized jars, one larva per jar (1) and two larvae per jar (2).

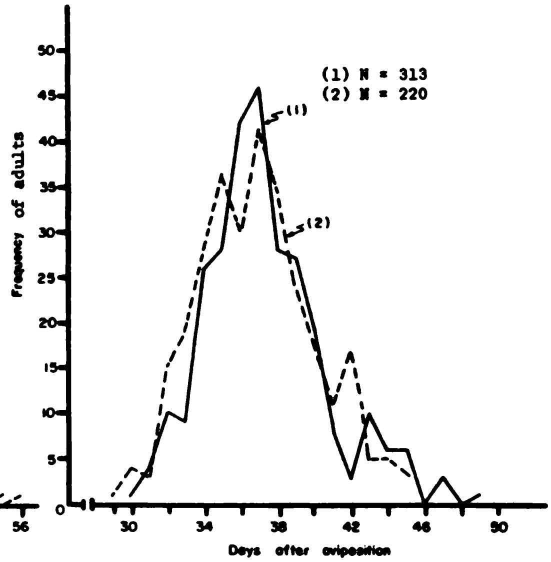

Fig. 5. Frequency of emerging adults of *Hypsipyla grandella* in relation to time. Sterilized jars, one larva per jar (1) and two larvae per jar (2).

no significant difference between the males of the four treatments A_1, A_2, B_1 and B_2, nor between the females of the four treatments A_1, A_2, B_1 and B_2. The only difference which proved to be significant at the 1 per cent level was that between males and females. The average size of males was 16.4 mm and of females 17.3 mm. These results are about the same as were obtained by Grijpma (2) with adults reared on a somewhat different artificial diet. His results were for males 16.7 ± 0.8 mm (range 13.0 – 19.4 mm) and for females 17.9 ± 1.1 mm (range 13.0 – 22.1 mm), based on 35 measurements for each sex.

The data of egg production per female per treatment are presented in Table 7. As the number of adults considered per treatment differed considerably for the four treatments A_1, A_2, B_1 and B_2, only the following statistical comparisons were made: A_1 vs. A_2 and B_1 vs. B_2. None of these comparisons, however, resulted in a significant difference at the 5 per cent level.

A comparison between fertilized egg production per female of treatments A and that of treatments B has not been made, as the densities of adults in the copulation cages were not the same and because oviposition of B_1 and B_2 females was determined several weeks after that of A_1 and A_2 females. Consequently the data presented above can only be considered as an indication of the possibility that females from sterilized jars (treatments B_1 and B_2) oviposit more fertilized eggs than females from non–sterilized jars (treatments A_1 and A_2).

The average hatching percentage of *H. grandella* eggs per female was highest in treatment B_2 (97.9 per cent). For the other treatments this value was 92.5 per cent (B_1), 88.0 per cent (A_2) and 87.0 per cent (A_1) (Table 8). The range in treatment A_1 was 10 – 100 per cent, in A_2, 0 – 100 per cent, in B_1, 70 – 100 per cent and in B_2, 75 – 100 per cent. None of the observed differences, however, was significant at the 10 per cent level.

Time needed for egg hatching (averages per female) was lowest in treatment B_2 with 85.0 hours, second lowest in B_1 with 85.8 hours, while in A_1 and A_2 these figures were 87.2 and 89.4 hours respectively (Table 9).

In treatment A_1 the range of variation was 84.3–89.4 hours, in treatment A_2 87.1–93.7 hours, in treatment B_1 83.2–90.6 hours and in treatment B_2 83.1–88.3 hours.

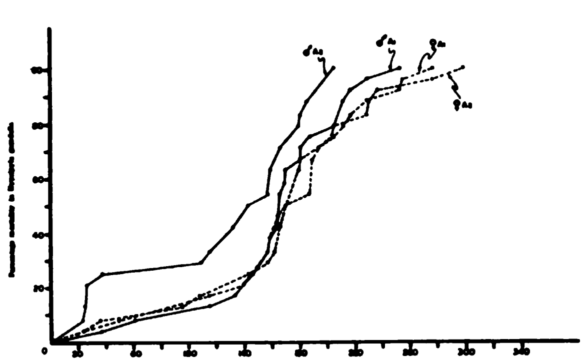

Fig. 6. Comparison of longevity of *Hypsipyla grandella* adults (males and females) obtained from artificial rearing in non–sterilized glass jars, one larva per jar (A_1) and two larvae per jar (A_2). Each graph is based on 24 adults.

TABLE 4. Number of *H. grandella* adults obtained per repetition and per treatment resulting from artificial rearing of larvae in glass jars.

Repetition	Treatments and Sexes											
	A_1			A_2			B_1			B_2		
	♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂+♀
I	9	15	24	12	16	28	3	6	9	2	6	8
II	15	10	25	19	19	38	13	15	28	4	8	12
III	13	15	28	20	21	41	13	7	20	20	10	30
IV	12	12	24	19	17	36	8	10	18	17	10	27
V	9	9	18	15	18	33	20	23	43	16	17	33
VI	17	15	32	16	22	38	17	17	34	22	16	38
VII	7	11	18	18	20	38	21	16	37	23	19	42
VIII	1	0	1	4	2	6	15	18	33	26	19	45
IX	21	15	36	21	27	48	16	13	29	17	18	35
X	23	15	38	1	3	4	19	7	26	11	13	24
Total	127	117	244	145	165	310	145	132	277	158	136	294

The absolute minimum time required for hatching was 83 hours in all four treatments, the absolute maxima were in A_1 118 hours, in A_2 107 hours, in B_1 107 hours and in B_2 96 hours. These values possibly could have been lower if the eggs were checked more frequently. Many times it was observed that at 6:00 hours many eggs had already hatched. The eggs were checked twice at 4:00 hours, but at that time no egg had hatched.

Observed differences in time needed for egg hatching varied significantly at the 0.5 per cent level for the four treatments A_1, A_2, B_1 and B_2.

Separate comparisons of treatments showed that the differences A vs. B, A_1 vs. A_2 and A_2 vs. B_2 were significant at the 0.5 per cent, 2.5 per cent and 0.5 per cent level respectively. The differences one vs. two larvae per jar, B_1 vs B_2 and A_1 vs. B_1 were not significant at the 10 per cent level.

However the observed differences are so small, that they are of little importance in the artificial rearing.

Cost per adult produced in the artificial rearing

The cost of materials and labor involved in the artificial rearing is presented in Table 10.

The total cost as presented in Table 10, points out that in order to maintain the production of *H. grandella* adults at the level of the period July–December 1972, treatment A_2 (two larvae per jar, non–sterilized jars) would be cheapest to reach a production of 3122 adults

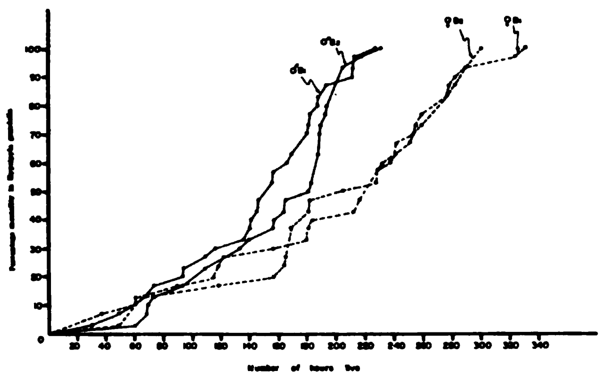

Fig. 7. Comparison of longevity of *Hypsipyla grandella* adults (males and females) obtained from artificial rearing in sterilized glass jars, one larvae per (B_1) and two larvae per jar (B_2). Each graph is based in 30 adults.

TABLE 5. Average longevity of *H. grandella* adults obtained from artificial rearing.

Treatment	Sex	Longevity in hours $x \pm t_{0.01} \cdot s_x$	Number of adults considered
A_1	♂	165.9 ± 27.6	24
	♀	173.7 ± 33.8	24
A_2	♂	128.5 ± 37.4	24
	♀	172.4 ± 36.6	24
B_1	♂	145.2 ± 26.9	30
	♀	203.3 ± 41.0	30
B_2	♂	159.4 ± 24.8	30
	♀	199.8 ± 40.7	30

per month. However, the cost differences between treatments are small; only treatment A_1 seems somewhat more expensive.

TABLE 6. Size of recently emerged *H. grandella* adults from artificial rearing system.

Treatment and sex		No. of adults measured	Average size (mm*)	Observed max. (mm)	Observed min. (mm)
A_1	♂	37	16.7 ± 0.3	18.0	15.0
	♀	24	17.2 ± 0.7	18.6	12.6
A_2	♂	38	16.4 ± 0.5	18.0	12.0
	♀	20	17.1 ± 0.7	18.8	14.7
B_1	♂	62	16.4 ± 0.5	19.3	12.7
	♀	38	17.7 ± 0.7	21.0	14.0
B_2	♂	69	16.3 ± 0.4	19.2	13.1
	♀	46	17.1 ± 0.7	20.7	13.9

* $x \pm t_{0.01}\, s_{\bar{x}}$

Cost analysis and possible improvements

Most expensive materials used in the rearing are artificial diet, towel paper and metalic covers for glass jars, which formed 40, 24 and 14 per cent respectively of total material cost. Most expensive ingredients of the artificial diet are vitamins and agar, both of which form about 30 per cent of the diet cost. It is worth trying to substitute the vitamins by a cheaper product like yeast. It seems that agar is unnecessary as a diet ingredient, since a preliminary trial employing a diet with and without agar resulted in about the same number of adults. A formal experiment to substantiate these results seems to be indicated.

The amount of towel paper used as oviposition substrate, in the copulation cages for protection and to clean tables, is substantial.

TABLE 7. Average eggs production per *H. grandella* female in the four treatments.

Treatment	Number of adults put in cages		Average egg production per female	
	♂	♀	Fertile	Sterile
A_1	34	34	179	12
A_2	35	33	128	24
B_1	25	25	252	32
B_2	25	25	239	7

TABLE 8. Average hatching (%) of fertilized *H. grandella* eggs.

Treatment	A_1	A_2	B_1	B_2
Number of females taken into consideration	15.	10	10	14
Average hatching of eggs (%)	87.00	88.00	92.50	97.86

TABLE 9. Average time (hours) required for hatching of *H. grandella* eggs.

Treatment	A_1	A_2	B_1	B_2
Number of females taken into consideration	13	7	10	14
Average time required for hatching (hours)	87.15	89.43	85.80	84.93

The metalic covers for glass jars do not last very long because of oxidation. Six months of duration were taken for the cost calculation. It may be worth painting oxidized covers in order to prolong their use.

It is possible to replace oviposition boxes and dishes by carton or plastic containers, which are cheaper and readily available. The cost of the presently used boxes and dishes forms about 9 per cent of total material cost.

Labor cost turned out to form the greatest part of the cost per adult (Table 10). Most labor consuming steps in the rearing procedure involve the preparation of boxes and Petri dishes for oviposition (14 per cent of total labor cost), the preparation of artificial diet (12 per cent of total labor cost), cleaning of boxes and Petri dishes (10 per cent of total labor cost), checking and renewing boxes and Petri dishes (10 per cent of total labor cost), checking diet jars with larvae, separating blue (last instar) larvae (8 per cent of total labor cost) and putting larvae onto artificial diet (6.5 per cent of total labor cost). Efforts to bring down in artificial rearing of *H. grandella*, labor cost should be concentrated on the above mentioned, most expensive steps in the rearing procedure.

In a mass rearing program, the cost per adult would probably be considerably lower. The earlier suggested improvements, the elimination of sexing pupae and counting of emerged adults are estimated to result in a cost reduction of at least 30 per cent.

TABLE 10. Total cost of materials and labor for artificial rearing per 1000 *H. grandella* adults (in Costa Rican colones*).

Treatment	A_1	A_2	B_1	B_2
Materials	176.226	153.916	159.231	154.122
Labor	316.538	303.400	298.256	310.105
Total	492.764	457.316	457.487	464.227

* US$1.00 = CR₡8.57

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ELECTROANTENNOGRAMS (EAG)
AS A TOOL IN THE ANALYSIS OF INSECT ATTRACTANTS*

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COMPENDIO

Las respuestas olfatorias en los insectos, tales como las representadas en un electroantenograma (EAG), rinden información sobre la capacidad sensoria de oler sustancias que sirven para reconocer parejas, alimentos, o sitios de oviposición. El EAG puede servir como un método conveniente y rápido para detectar estímulos naturales importantes. Sin embargo, el método EAG debe ir acompañado siempre por pruebas de comportamiento.

Una respuesta de EAG requiere la actividad simultánea de un número de células sensorias. Como los insectos machos poseen a menudo muchos receptores idénticos de feromona, se pueden registrar reacciones mensurables a las feromonas. En algunos casos, el método parece ser aplicable también a insectos que localizan por el olfato al alimento o a plantas en las que ovipositan. Los resultados preliminares con *Hypsipyla grandella* revelan que los extractos crudos de *Cedrela odorata* son percibidos por receptores de la antena, aunque los extractos de un árbol no hospedante, *Anthocephalus cadamba,* también indujeron una reacción algo más débil. Se presentan algunos ejemplos de insectos que muestran reacciones claras en la antena a los olores de las plantas nectíferas. En procedimientos de identificación y estudios de especificidad de feromonas, el EAG puede dar información esencial. Así, el EAG puede servir como herramienta útil en el análisis de varios aspectos del comportamiento de los insectos, en el que el sentido del olfato cumpla una función crucial.

El autor

Introduction

Insects depend to a large extent on their sense of smell to locate their food sources, oviposition sites and mating partners. In 1956 Schneider and Hecker (24) showed that when an isolated antenna of the male silkworm is exposed to an air puff containing pheromone, an electrical signal can be measured between the tip and the base of the antenna. This signal was termed electroantennogram (EAG). Because of its close correlation with generator potentials recorded from the antennal olfactory receptors at various concentrations, the EAG is believed to reflect the summated generator potentials (26). EAG's have been obtained from various insects and with different types of odorous stimuli in addition to pheromones. Consequently the EAG may be used as a bioassay in the identification of not only pheromones, but also food odours and attractants of oviposition plants. In the latter cases, however, we are possibly not dealing with a single receptor type responding to one highly specific stimulus. When the EAG is used in a more complex stimulus/receptor situation, it may be appropriate to reconsider the interpretation of an EAG and to evaluate its advantages and its limitations. After a brief review of the literature some preliminary data will be presented on the analysis of hostplant attractants to the shootborer *Hypsipyla grandella* (Zeller) (Phycitidae).

Recording of EAG's

EAG's are slow electrical potentials and consequently should be recorded with a DC recording system. Although some authors (18, 29) have met no difficulties when using metal electrodes, preferably non–polarizable electrodes (e.g. Ag–AgCl) are employed. The combination of pipette electrodes and the high electrical resistance of the delicate insect antennae makes it essential to use a high–impedance DC–preamplifier. Usually the recording electrode penetrates the distal end of the antenna or is slipped over the entire tip. The removal of the ultimate antennal segment may facilitate electrode placement and reduce electrical resistance. Roelofs and Comeau (21) obtained optimal results after cutting the three distal segments in the case of the small antenna (3 mm) of *Argyrotaenia velutinana.* When isolated antennae are used (8, 24), the indifferent electrode is fixed in an identical way at the proximal part of the antenna.

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Isolation of the antenna has the advantage that manipulation is simplified and that no signals arising in the insect head and in the muscles of scapus and pedicellus or movements of the antenna interfere with the recordings. On the other hand small antennae especially may deteriorate rapidly and intact insects or heads may provide a better preparation. The indifferent electrode is now placed in one of the basal antennal segment or sometimes in the head (19, 29). Under such conditions the preparation may last for several hours or even days (20). Some authors report a consistently lower amplitude of responses in isolated antennae as compared to intact insects or head preparations (6, 19). When in the recording system fluid surfaces are exposed to the odorous stimulus, electrical artifacts may arise when some natural compounds are used, as we experienced with, for instance, geraniol. Using a dead antenna as a control will bring to light unnatural responses. Terpenes also induced EAG–like artefacts in dead antennae when insulated tungsten electrodes were used (9).

For most stimuli the tip of the antenna becomes negative relative to the antenna base. Occasionally a positive deflection is observed. Schneider (25) reports a positive reaction to sub–lethal doses of ether and chloroform. In accordance with the afore mentioned explanation of the EAG a positive electrical signal must be attributed to the hyperpolarization of a number of receptors. Positive reactions to methylene cloride, methyl cyclohexanepropionate and some related compounds were obtained in the Japanese beetle whereas attractive substances like eugenol and phenethyl propionate elicited negative EAG's (4). The negative EAG does not necessarily represent, however, "an 'attractive' response", as erroneously concluded (4), but merely a reaction to "excitatory odor stimuli" (7) which in certain cases could be interpreted by the animal as a deterrent substance, depending on the type of receptors stimulated. Using another insect *(Porthetria dispar)* Adler, Beroza and Sarmiento (3) noticed several exceptions to the conception that EAG response intensities to various compounds are correlated with their attraction in the field.

EAG shape

The shape of an EAG may vary considerably. When the odour is offered as a square pulse the EAG rarely follows exactly the stimulus. In the case of olfactory pit responses in the fly *Lucilia sericata* (15), however, a high degree of congruency was obtained for some compounds. Usually the EAG shows a tendency to level off after the initial top value has been reached. When stimulation is terminated, the evoked potential will gradually return to its basic level. The rate of this disadaptation has sometimes been correlated with the type of behavioural reaction induced by the chemical. In a number of lepidopterous species the EAG produced by the attractant has a much slower recovery time than other test chemicals. Because of several exceptions to this rule, however, no general conclusion is allowed as to the correlation of EAG shape and behavioural activity (21). In some cases more complex EAG shapes have been described. Schneider (25) recognized four phases in certain reactions of silkworm antennae and Abushama

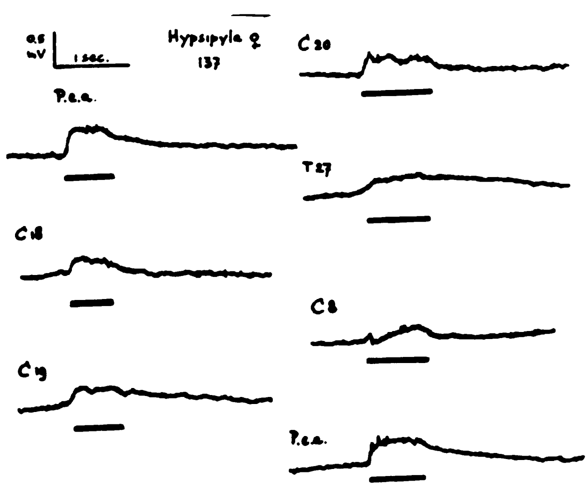

Fig. 1. EAG of *Hypsipyla grandella.* P. e.a.: phenylethylacetate; leaf extracts of Cedar in acetone (C18, C8), ethylether (C19), ethylacetate (C20) and leaf extract of *Toona ciliata* var. *australis* in hexane (T27). Horizontal lines indicate periods of stimulation.

(1), working with termites, also observed multiphasic potentials. In the latter study a relation with behavioural reactions is suggested: "Numerous oscillations (in the intermediate phase of the EAG) were recorded in the case of repellent odours while none of these are shown towards attractive biological extracts" (1). It would be interesting to obtain more details of this phenomenon and to translate it into receptor activities. The EAG of the American cockroach also shows some fluctuations when tested with amyl acetate, whereas the sex attractant induces a "smooth" reaction (8). No simple physiological explanation seems available to account for the observed irregularities. The finding that some olfactory receptors possess a longer latency than others (10) could bear some relevance to the shape of an EAG.

EAG size

The amplitude of an EAG varies according to insect species and stimulus. At optimal stimulus concentration the response signal usually does not exceed 5 mV. Occasionally values up to about 8 mV (22) or even 11 mV (21) have been observed, but often they remain considerably smaller. In the Japanese beetle, for instance, maximal reactions of only 0,2 mV were encountered (4). Undoubtedly the number of sensory cells reacting influences the size of the response. A relatively small number of olfactory cells may explain why EAG's seem to be absent in certain insects. Pheromone receptors are usually present in large numbers and therefore reactions to these attractants can be obtained in many cases, not only in nocturnal insects, but also in, for instance, the diurnal Danaids (28).

When the recording electrode was placed near the middle of the flagellum of the moth *Trichoplusia ni* the EAG's obtained were not significantly smaller than when it was localized at the tip of the antenna (19). In the red–banded leafroller, on the contrary, the EAG decreased gradually when more and more segments were removed (21).

In contrast to behavioural reactions, EAG responses are in general independent of a number of environmental and physiological conditions. Payne *et al.* (19) mention explicitly a remarkably constant reaction, with no significant variation at the various times of day, light intensities, or male ages tested. Generally complete responsiveness of the antenna after stimulation did come back within one minute. A slight decrease of sensitivity in aged males has been noticed by Roelofs and Comeau (21), and Adler (2) warns about possible effects of circadian rhythms on the receptiveness of antennae.

Oviposition attractants

Few studies are available in which EAG's have been employed in an analysis of the volatile factors which direct insects to their oviposition sites. Asher (6) reports strong responses in *Dioryctria abietella* to basal parts of first–year cones of its host trees, *Pinus elliottii*. Plant extracts as well as pinene evoke an EAG in both sexes too. Possibly the host plant odour also serves to bring both sexes together as in the case of bark beetles of the genus *Dendroctonus*. Males and females of *D. brevicomis* show EAG responses to host terpenes, like α–pinene and 3–carene and also to the aggregation hormones brevicomin and frontalin. The reactions in males were consistently of lower amplitude than in females (18).

The shootborer *Hypsipyla grandella* is a destructive insect in Spanish cedar and mahogany trees. There is strong evidence that volatile principles of the host, possibly complemented by other factors, play a crucial role in the attraction of the females to oviposit (14). In an attempt to identify the attractants we have used the EAG to determine the stimulating capacity of a series of leaf extracts, both from the host plant *Cedrela odorata* and from the resistant species *Toona ciliata* var. *australis*. Preliminary results indicate that especially crude acetone extracts of *C. odorata* give distinct EAG's (Fig. 1). The acetone extract of *Anthocephalus cadamba*, however, also induced an EAG, though of slightly smaller amplitude. *T. ciliata* var. *australis* extracts were also stimulating. All extracts appeared to be highly volatile. One should bear in mind that the volatiles which are emitted by a plant may occur in only small amounts inside the plant. Furthermore, it is conceivable that the crude plant extracts contain volatile substances which are normally not released by the plant or only in small quantities. Therefore, crude plant extracts can probably not be considered to represent natural stimuli.

In addition to the extracts, EAG responses were also obtained from some pure chemicals. A strong reaction occurred with phenylethylacetate, whereas geraniol and hex–2–en–1–al induced somewhat weaker reactions.

A sudden increase of the temperature elicited a positive EAG in *H. grandella*. This indicates the presence of inhibitory processes in a number of receptors.

Food attractants

In some cases, the EAG has been applied to the analysis of food specific odours. Moorhouse and Nesbitt (1966, personal communication) observed that when volatile substances extracted from grass are separated in a gas chromatograph and subsequently passed over the antenna of a locust only a restricted number of the grass components elicit an EAG. The same experimental set–up has been used to analyse crude pheromone extracts (17).

Nocturnal insects whi. ' feed on nectar exemplify another conspicuous situ. n in which the sense of smell fulfils a vital role. .ie floral scent of *Abelia grandiflora* is highly attractive to both sexes of the moth *Trichoplusia ni*. This corresponds with the fact that this odour induces a clear EAG, whereas the odour of the leaf of the same plant evokes only weak reactions (12). The sphingid *Manduca sexta* also clearly orients itself on odours, and, conformably, EAG responses have been obtained to salicylamylacetate and ethylacetate (Schoonhoven and Brantjes, unpublished results). Adults of *Hadena bicruris* likewise show good responses to the odour of *Phlox* flowers. Fragrances collected from *Phlox* flowers give higher EAG values than those from *Melandrium album* and *Nicotiana*. Several flowery substances, such as geraniol, ethylacetate, emylacetate, also elicit an EAG response (Brantjes and Schoonhoven, unpublished results).

Sex attractants

The EAG method has been applied in the analysis of both fundamental as well as practical aspects of olfaction in relation to insect sex attractants. Schneider and his collaborators introduced this convenient method and have used it in their detailed studies on the fundamental basis of pheromone perception (27). Several investigators (17, 23) have employed this technique in isolation and identification procedures of female sex attractants. Other insect pheromones are also demonstrable with this method, such as aggregation pheromones in bark beetles (18), male sex pheromones in Lepidoptera (11, 13, 28) and the effects of queen substance on queen, drone and worker honey bees (5).

In several cases the EAG method has yielded basic information, but for some questions other techniques, such as single unit recordings, are needed. Using EAG responses, it was shown that the female sex attractant is only perceived by the males (an exception to this principle is mentioned by Grant (11)). In the above mentioned other examples of pheromones both sexes appear to be responsive which could be expected on behavioural grounds, in bark beetles and honey bee workers, but which was unforseen in the case of the pheromones secreted by male hairpencils in Lepidoptera. Also, in the elucidation of pheromone specificity and the interspecific relationships of insects the EAG has been proven to open a potential source of unique information. Priesner (20) in a meritorious study offered new evidence which is relevant for the taxonomy of Saturnids. When EAG data are combined with the analysis of chemical structures of the pheromones involved, new insights into the phylogeny of insect taxa may arise, as has been shown for the Lepidoptera (22).

In the analysis of pheromone mixtures and the interactions with synergists and inhibitors the EAG method has also provided important information. Synergists and inhibitors of the attractant, when tested alone, evoke antennal responses in the red–banded leaf roller moth, though no behavioural responses result (21). *Adoxophyes orana* reacts in field experiments only to the combination to the two components, which together constitute the female sex attractant. The two components also stimulate olfactory cells when applied singly, as is indicated by their resulting EAG's (16). Discrepancies between behavioural and EAG reactions can probably only be explained by additional information derived from other methods.

Conclusion and summary

Olfactory responses in insects, as represented in an EAG, yield information of the sensory capacity to smell chemicals which serve to recognize mating partners, suitable foods or oviposition sites. The EAG is of little value in the analysis of the insect's capacity to discriminate between different odours. On the other hand, due to the possibility to use the EAG routinely, it may serve as a convenient and rapid method to detect important natural stimuli. Behavioural tests, however, should always accompany the EAG method, because the latter provides only information of certain receptory processes, but gives no insight into the central processes which turn peripheral information into behavioural activities.

An EAG response requires the simultaneous activity of a number of sensory cells. Since male insects often possess many identical pheromone receptors usually measurable reactions to pheromones can be recorded. In some cases the method appears also applicable to insects localizing food or oviposition plants by smell. Preliminary results on *H. grandella* reveal that crude plant extracts of *Cedrela odorata* are perceived by antennal receptors, although extracts from a non–host tree *Anthocephalus cadamba,* also induced a somewhat weaker reaction.

Some examples are presented of insects which show clear antennal reactions to the odours of nectar flowers.

In identification procedures and specificity studies of pheromones the EAG may provide essential information.

Thus, the EAG may serve as a useful tool in the analysis of various aspects of insect behaviour, in which the sense of smell fulfils a crucial function.

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EFECTOS DE RADIACION GAMMA EN LARVAS, PUPAS y ADULTOS*

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ABSTRACT

In this study the effect of gamma radiation on last instar *H. grandella* larvae, pupae of early and advanced age, and adults was evaluated with reference to mortality, deformation, fecundity and longevity. Complete sterilization of male and female adults was obtained in all treated life stages. However, irradiation of the adult stage was most effective. Applying doses of 35 kr to male and 30 kr to female *H. grandella*, no influence of radiation on mortality and deformation was observed. In addition, longevity and copulation of adults were less influenced by these treatments, than longevity and copulation of adults obtained from any of the other life stages treated.

In the control with untreated *H. grandella*, fertility and fecundity of normal females mated with irradiated males (35 kr) were respectively 1.5 and 47.9 percent. Normal males mated with irradiated females in the above mentioned treatment (30 kr) resulted in 0.0 percent fertility and 34,4 percent fecundity.

The authors

Introducción

El barrenador de las meliáceas, *Hypsipyla grandella* (Zeller) (Lepidoptera:Pyralidae), constituye un factor limitante para el establecimiento de plantaciones de cedro (*Cedrela* spp.) y caoba (*Swietenia* spp.) en los trópicos americanos. Ataca a los brotes tiernos, produce una deformación del tallo lo cual reduce el crecimiento o puede llegar a matar a la planta. Además daña frutos y semillas, afectando la regeneración natural de estas especies valiosas. Este insecto ha ocasionado grandes pérdidas en Perú, Puerto Rico, Trinidad y Guatemala en plantaciones de cedro y caoba, maderas de alto valor económico en el mercado mundial (2, 7, 14).

Para el combate de esta plaga, se ha probado el control químico, biológico y silvicultultural, pero hasta el momento no se conoce un método adecuado para eliminar los ataques de este barrenador. Un posible método dentro del control integrado podría ser la 'técnica de los machos estériles'; teniendo en cuenta las tendencias actuales de evitar el uso de insecticidas químicos para el control de las plagas, debido a la resistencia que adquieren los insectos a estos productos o el peligro de contaminación ambiental.

Esta técnica ha sido estudiada en muchos insectos dañinos a la agricultura y ganadería. Entre los éxitos que se han logrado con este método, podemos citar la eliminación del 'gusano tornillo' *Cochiliomya hominivorax* (Coq.) de la Isla de Curaçao y de la región suroriental de los Estados Unidos (8).

La presente investigación tuvo como objetivo principal estudiar la posibilidad de aplicar la 'técnica de los machos estériles' en el control de *H. grandella*. Los objetivos específicos fueron los siguientes:

1. Determinar las dosis de esterilización con radiación gamma para machos y hembras tratados como larvas del último instar, pupas de temprana y avanzada edad y adultos recién emergidos.
2. Estudiar los efectos de la radiación en la mortalidad, deformaciones físicas, fecundidad y longevidad, en todos los estados de desarrollo anteriormente indicados.
3. Determinar el estado de desarrollo más apropiado para hacer esterilización del insecto.

Materiales y métodos

El presente estudio se efectuó en el laboratorio de Entomología del Programa de Energía Nuclear, localizado en el Centro Tropical de Enseñanza e Investigación del Instituto Interamericano de Ciencias Agrícolas de la OEA (IICA–CTEI) en Turrialba, Costa Rica, que está

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** Ahora en el Servicio Forestal del Ecuador.

*** Ahora en la Universidad del Zulia, Maracaibo, Venezuela.

CUADRO 1. Efectos de la radiación gamma sobre larvas* de *H. grandella.*

Dosis (kr)	Mortalidad (%) Larval**	Pupal	Adultos deformados (%)	Fertilidad☆ (%)	Fecundidad☆☆ (%)	Longevidad ($\bar{X}$)
			Machos irradiados			
0,0	–	–	–	84,9	100,0	5,9
4,0	0	2,7	3,1	62,6	97,2	5,7
5,0	2	3,8	20,9	20,5	73,6	3,7
5,5	4	12,1	51,9	3,6	23,8	3,7
6,0	4	12,9	63,3	0,4	32,8	3,3
8,0	13	32,8	84,5	–	–	–
			Hembras irradiadas			
0,0	–	–	–	84,8	100,0	8,1
4,0	0	10,5	21,8	47,8	37,9	7,1
5,0	2	10,3	34,3	19,5	5,9	6,9
5,5	4	12,0	52,3	0,0	0,2	6,0
6,0	4	21,7	66,7	0,0	0,1	5,5
8,0	13	46,5	100,0	–	–	–

* Larvas de 22 a 30 días, 100 irradiadas por tratamiento.
** Ambos sexos.
☆ Oviposición de tres días
☆☆ Oviposición total

ubicado a 620 m s.n.m., con una precipitación promedio anual de 2.682 mm y temperaturas medias mensuales, máximas y mínimas de 27,1° y 16,9°C, respectivamente.

Las larvas, pupas y adultos de *H. grandella* necesarias para este estudio fueron criados en dieta artificial bajo condiciones de laboratorio, con temperatura de 25 ± 3°C y humedad relativa de 73 ± 6 por ciento. Para la cría se empleó la técnica utilizada por Grijpma (5) y modificada por Katiyar (9).

Se determinó el efecto de la radiación gamma, en larvas del último instar (22 a 30 días de edad), pupas de temprana edad (1 a 3 días de edad), pupas de avanzada edad (7 a 9 días de edad) y adultos de 15 a 20 horas después de la emergencia.

Como fuente de radiación gamma se empleó una pila de ^{60}Co, que emite aproximadamente 1 kr/54 seg.

Las dosis de radiación a aplicarse en cada uno de los estados de desarrollo del insecto se las determinó en base a ensayos preliminares.

A las larvas se les aplicó las siguientes dosis de radiación: 0,0, 4,0, 5,0, 5,5, 6,0 y 8,0 kr. En cada tratamiento se irradió 25 larvas con cuatro repeticiones.

A los machos y hembras en estado de pupa de temprana edad se les expuso a las siguientes dosis de radiación: 0, 6, 9, 12, 15 y 18 kr. Cada tratamiento estuvo constituido entre 17 y 25 pupas por sexo con cuatro repeticiones.

A ambos sexos de pupas de avanzada edad se les sometió a las siguientes dosis de radiación: 0, 10, 15, 20, 25 y 30 kr. En cada tratamiento se utilizó de 18 a 20 pupas, por sexo, con cuatro repeticiones.

A los adultos (machos y hembras) se les irradió con las siguientes dosis: 0, 5, 10, 15, 20, 30 y 35 kr. En cada tratamiento se empleó cinco adultos por sexo, con cuatro repeticiones.

La irradiación fue simultánea para ambos sexos. A las larvas se les irradió individualmente en cajas de plástico de 1 x 1 x 1 cm para evitar el canibalismo. A las pupas se las expuso a la radiación en cilindros de cartón de 10 cm de largo por 2 cm de diámetro. Los adultos fueron irradiados en cilindros metálicos.

Después que las larvas fueron expuestas a la radiación se les proporcionó alimento hasta que puparen. A las pupas de las larvas irradiadas, como a las pupas irradiadas, se las mantuvo separadas por sexos, hasta la emergencia de los adultos.

Luego de la emergencia los machos y las hembras fueron colocados en jaulas de cedazo plástico de 31 x 23 x 18 cm para que copulen. En cada jaula se colocó de 4 a 10 parejas. Las jaulas de copulación fueron mantenidas por tres noches consecutivas en un invernadero, tiempo en el cual se obtiene el 86 por ciento de copulación (13). Luego las jaulas de copulación fueron trasladadas al laboratorio. De dichas jaulas se sacaron las hembras para que ovipositaran y se dejó a los machos para los ensayos de longevidad.

Las hembras fueron colocadas individualmente en vasos de cartón de 4 oz, recubiertos internamente con papel toalla rugoso y tapado con el mismo material, para oviposición. Después de permanecer las hembras tres días en dichos vasos se trasladaron a un nuevo vaso de las mismas características que el anterior. En este vaso se las

CUADRO 2. Efecto de radiación gamma sobre pupas de temprana edad* de *H. grandella*.

Dosis (Kr)	Mortalidad pupal (%)	Adultos deformados (%)	Fertilidad ** (%)	Fecundidad *** (%)	Longevidad días ($\bar{X}$)
			Machos irradiados		
0	–	–	72,6	100,0	6,4
6	11,5	7,8	28,6	88,0	5,0
9	16,7	21,1	14,9	53,1	3,6
12	28,2	43,2	4,7	42,2	3,8
15	35,9	52,8	0,9	34,1	3,4
18	51,3	78,4	0,0	20,5	2,7
			Hembras irradiadas		
0	–	–	72,6	100,0	9,6
6	9,5	15,3	15,7	38,1	8,0
9	21,6	40,6	3,2	28,6	6,1
12	28,4	61,7	0,0	5,8	4,8
15	39,2	88,7	0,0	5,6	4,2
18	55,4	93,8	–	–	3,2

* Pupas de 1 a 3 días de edad.
** Oviposición de 3 días.
*** Oviposición total.

CUADRO 3. Efecto de la radiación gamma sobre pupas de avanzada edad* de *H. grandella*.

Dosis (kr)	Mortalidad pupal (%)	Adultos deformados (%)	Fertilidad ** (%)	Fecundidad *** (%)	Longevidad días ($\bar{X}$)
			Machos irradiados		
0	–	–	79,6	100,0	6,7
10	7,8	10,5	63,1	86,2	6,0
15	15,6	11,6	32,7	62,2	4,8
20	20,3	20,3	15,7	83,8	5,9
25	23,4	27,4	6,3	60,4	5,7
30	21,9	30,9	2,7	43,4	4,6
			Hembras irradiadas		
0	–	–	79,6	100,0	10,5
10	18,3	10,0	47,9	57,9	8,7
15	15,0	10,8	18,5	62,7	7,7
20	30,0	18,7	1,9	53,5	7,4
25	31,7	24,3	0,8	47,0	7,3
30	35,1	38,9	0,1	33,0	7,1

* Pupas de 7 a 9 días de edad.
** Oviposición de 3 días.
*** Oviposición total.

mantuvo hasta la muerte. Con los huevos depositados en los papeles del primer vaso se efectuó los ensayos de fertilidad y con los huevos depositados en los papeles de ambos vasos se realizaron los estudios de fecundidad.

A los vasos con los huevos depositados en los papeles hasta los tres días se les dejó destapados para que las larvas eclosionadas de los huevos pudieran escapar libremente y de esta forma evitar el canibalismo.

Todas las hembras muertas fueron disectadas para determinar si habían sido copuladas. Esto se comprueba por la presencia de espermatoforos en la bursa copulatrix.

A los adultos en todo el período de vida se los alimentó con una solución de agua azucarada al 10 por ciento. En las jaulas de copulación se les suministró este alimento en Erlenmeyers de 125 cc con una mecha de papel. En los vasos de oviposición fue suministrado en pequeños recipientes plásticos de 3 cc con algodón.

La mortalidad en estado larval, se ha determinado por el número de larvas que murieron antes de pupar. La mortalidad en estado pupal fue determinada por la diferencia entre el número de pupas tratadas y el número de adultos que emergieron. En ambos casos se utilizó mortalidad corregida.

Las deformaciones físicas que produce la radiación, solo fueron determinadas en los adultos. Se tomó en cuenta la configuración de las alas y deformaciones visibles en el cuerpo o apéndices. Tanto la mortalidad como la deformación de adultos fue corregida por la fórmula de Abbott (10).

La fertilidad fue determinada por el número de huevos depositados por las hembras copuladas durante los tres días de oviposición. Se consideraron como huevos viables aquellos que eclosionaron hasta los ocho días después de la oviposición.

La fecundidad se calculó en base al número total de huevos depositados por las hembras en los vasos de oviposición hasta la muerte. Para calcular la fecundidad solo se tomó en cuenta las hembras que habían sido copuladas.

La longevidad de machos y hembras fue calculada con los adultos utilizados para los ensayos de fertilidad y fecundidad. Diariamente se retiró de las jaulas y de los vasos de oviposición los machos y hembras muertos. Esta labor se la efectuó hasta que murieron todos los adultos.

Resultados y discusión

En el Cuadro 1 se puede observar que el efecto de la radiación no es inmediato cuando se irradian larvas de *H. grandella*. Este se manifiesta en mayor grado con el aumento del desarrollo del insecto, así al aplicarles a las larvas 8 kr, la mortalidad larval es de 13 por ciento, la mortalidad pupal en las hembras es de 46,5 por ciento y de los adultos que emergieron el 100 por ciento presentaron deformaciones físicas. Al irradiar a los machos en estado larval con 6,0 kr se puede notar que la fertilidad se reduce al 0,4 por ciento, la mortalidad pupal de 12,9 por ciento, el 63,3 por ciento emergen deformados y la longevidad se reduce de 5,9 días en el testigo a 3,3 días. En cambio al irradiar larvas con 5,5 kr, se obtiene la esterilidad total de las hembras, notándose que con esta dosis la mortalidad pupal 12,0, el 52,3 de las hembras emergen deformadas y la longevidad se reduce de 8,1 días del testigo a 6,0 días.

CUADRO 4. Efecto de radiación gamma sobre adultos* de *H. grandella*.

Dosis (kr)	Fertilidad ** (%)	Fecundidad *** (%)	Longevidad días ($\bar{X}$)
	Machos irradiados		
0	75,7	100,0	6,5
5	65,0	95,9	6,1
10	59,0	90,5	5,1
15	37,0	79,0	4,2
20	14,2	78,7	5,3
25	6,9	66,9	4,7
30	3,0	66,7	5,5
35	1,5	47,9	4,4
	Hembras irradiadas		
0	75,7	100,0	10,3
5	54,9	74,1	8,8
10	47,7	64,8	8,1
15	18,4	66,5	8,6
20	3,4	50,0	8,1
25	1,5	31,6	7,4
30	0,0	34,4	6,8
35	0,0	28,4	6,5

* Adultos de 8 a 22 horas de edad, 20 adultos por tratamiento.
** Oviposición de 3 días.
*** Oviposición total.

En el Cuadro 2 se nota que las hembras son más susceptibles a la radiación que los machos cuando se exponen en estado de pupas de temprana edad. Al aplicar 15 kr a los machos en estados de pupa de temprana edad, se obtiene 0,9 por ciento de fertilidad. Con esta dosis la mortalidad pupal es de 35,9 por ciento. El 52,8 por ciento de los adultos emergen deformados y la longevidad de adultos se reduce de 6,4 días del testigo a 3,4 días. En cambio al aplicar 12 kr a las hembras en este estado de desarrollo, se obtiene la esterilidad total, la mortalidad pupal es de 28,4 por ciento. El 61,7 por ciento de los adultos emergen deformados y la longevidad de adultos se reduce de 9,6 del testigo a 4,8 días.

Igual como en el caso del estado larval, se puede observar en el Cuadro 3 que las hembras son más susceptibles que los machos irradiados en el estado pupal avanzado, la fertilidad es de 2,7 por ciento y la mortalidad pupal de 21,9 por ciento. El 30,0 por ciento de los adultos emergidos presentan deformaciones físicas y la longevidad se reduce de 6,7 días del testigo a 4,6 días en los adultos. Mientras que al irradiar a las hembras con la misma dosis, la fertilidad se reduce a 0,1 por ciento y la mortalidad pupal es de 35,1 por ciento. El 38,9 por ciento de los adultos nacieron deformados y la longevidad de los adultos se redujo de 10,5 del testigo a 7,1 días.

Irradiando los adultos recién emergidos de *H. grandella* se nota que las hembras son más susceptibles en cuanto a la fertilidad. En el Cuadro 4, se aprecia que al exponer a 35 kg a los machos en estado adultos, la fertilidad se decrece a 1,5 por ciento y la longevidad disminuye de 6,5 días del testigo a 4,4 días. En cambio las hembras irradiadas con 30 kg quedan totalmente estériles y la longevidad decrece de 10,3 días del testigo a 6,8 días.

Según lo anterior la susceptibilidad en cuanto a la fertilidad de *H. grandella* a ser esterilizada decrece, conforme aumenta el desarrollo del insecto (de larva a adulto). Esta diferencia de sensibilidad puede deberse al diferente desarrollo de la espermatogénesis y oogénesis en las larvas, pupas y adultos (7).

El efecto de la radiación en la mortalidad y deformación de adultos, es más marcado en las larvas y pupas de temprana edad, que en las pupas de avanzada edad. Proverbs y Newton (11) indican que la alta radiosensibilidad que presentan las pupas de avanzada edad puede deberse al distinto grado de diferenciación y multiplicación celular que presenta este estado de desarrollo.

La longevidad de los machos y hembras irradiados decrece en todos los estados de desarrollo estudiados, notándose que al aplicar las dosis esterilizantes, la longevidad se reduce en mayor grado en las larvas y pupas de temprana edad.

Según lo anterior parece que el mejor estado de desarrollo de *H. grandella* para ser utilizado en la 'técnica de machos estériles' es el estado adulto, a pesar de que al aplicarles las dosis esterilizantes a ambos sexos el promedio de longevidad es menor que el que se obtiene al irradiar con las dosis esterilizantes a las pupas de avanzada edad; pero al irradiar adultos no se observa mortalidad ni deformaciones físicas.

La fecundidad de las hembras normales que copularon con machos irradiados disminuyó de acuerdo al aumento de la radiación en los estados de desarrollo estudiados. Parece que el esperma y/o líquido seminal de los machos irradiados, inhibe en alguna forma la normal oviposición de las hembras. En otros lepidópteros también se encontró que la fecundidad se reduce cuando hembras normales copulan con machos irradiados (3, 4). En cambio Husseiny y Madsen (6), y Reyes (12) indican que la fecundidad de las hembras *Paramyelois transitella* y *Leucoptera coffeella* no se afecta cuando copulan con machos irradiados.

La fecundidad de las hembras irradiadas y apareadas con machos normales disminuyó fuertemente por el efecto de radiación y la susceptibilidad decrece según el estado de desarrollo en que son irradiadas. La mayor o menor sensibilidad de las hembras, en cada una de las fases estudiadas puede deberse a las diferentes etapas de desarrollo de la oogénesis en las células germinales (1).

Conclusiones

1. Se puede inducir esterilidad en ambos sexos de *H. grandella* al irradiarlos como larvas del último instar, pupas de temprana edad, pupas de avanzada edad o adultos recién emergidos.
2. La susceptibilidad de *H. grandella* a la radiación gamma, en relación a la mortalidad y deformación de adultos decrece a medida que aumenta el estado de desarrollo del insecto.
3. La longevidad de los adultos de ambos sexos decreció con el aumento de la radiación en todos los estados del insecto estudiado.
4. La fecundidad de las hembras normales que copularon con machos irradiados y la de las hembras irradiadas que se aparearon con machos normales disminuyó con el incremento de la radiación en todas las fases del insecto que se estudió.
5. Parece que el estado de desarrollo más apropiado para la 'técnica de machos estériles' es el adulto, ya que al irradiar con las dosis esterilizantes de 35 kr para los machos y 30 kr para las hembras se reduce menos la longevidad y el porcentaje de copulación en comparación con los otros estados de desarrollo estudiados. Además, en el estado adulto no hay efecto de la radiación en la mortalidad y deformaciones físicas.

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HEXAMERMIS ALBICANS (SIEBOLD) (NEMATODA: MERMITHIDAE) A PARASITE OF THE LARVA*

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COMPENDIO

Se registra una nueva asociación parasítica entre el nemátodo *Hexamermis albicans* y el barrenador de las Meliáceas *(Hypsipyla grandella)*. Entre 5 y 25 por ciento de las larvas del barrenador, muestreadas en una plantación de Meliáceas del Departamento de Ciencias Forestales Tropicales del CATIE en Turrialba, Costa Rica, murieron debido al parasitismo por el nemátodo. El nivel más alto de parasitismo se encontró en la época de más lluvia y el nivel más bajo al final de la época seca. Las larvas parasitadas fueron recolectadas de ramas de *Cedrela* spp. y de *Swietenia macrophylla* King, encontradas entre 1 y 2 metros encima del suelo. Se incluye una lista de lepidópteros parasitados por miembros del género *Hexamermis*.

Research on the control of the mahogany shootborer *Hypsipyla grandella* (Zeller) in Costa Rica, has uncovered an interesting parasitic association between the mermithid nematode *Hexamermis albicans* and this pest insect.

Although a mermithid, probably of the genus *Hexamermis*, had been collected earlier in 1969 from larvae of *H. grandella* in Costa Rica (8), it had never been identified properly, possibly because no adult stage could be obtained.

Specimens of *Hexamermis albicans* were reared from *H. grandella* larvae collected during surveys for biocontrol agents in a meliaceous plantation located in the Florencia Sur forest of the Tropical Agricultural Research and Training Centre at Turrialba, Costa Rica. These insect larvae were obtained from branches of *Cedrela* spp. and *Swietenia macrophylla*, located between 1 and 2 m above the ground. The adult stage of the nematodes was obtained by placing the nematodes for approximately six weeks on moist sand after they had emerged from the *H. grandella* larvae. Figure 1 shows the relative size of the parasite after emergence, as related to the insect larva. The larvae die after the parasite exits from the body cavity.

Some adult nematodes were also collected from the sandy clay loam soil beneath the trees. The percentage of living *H. grandella* larvae which proved to be parasitized by *H. albicans* varied between 5 and 25 per cent. The highest level of parasitism was found towards the end of the rainy season and the lowest at the end of the dry season.

Fig. 1. Relative size of nematode parasite compared with larvae of mahogany shootborer. (Courtesy of Edward Holsten).

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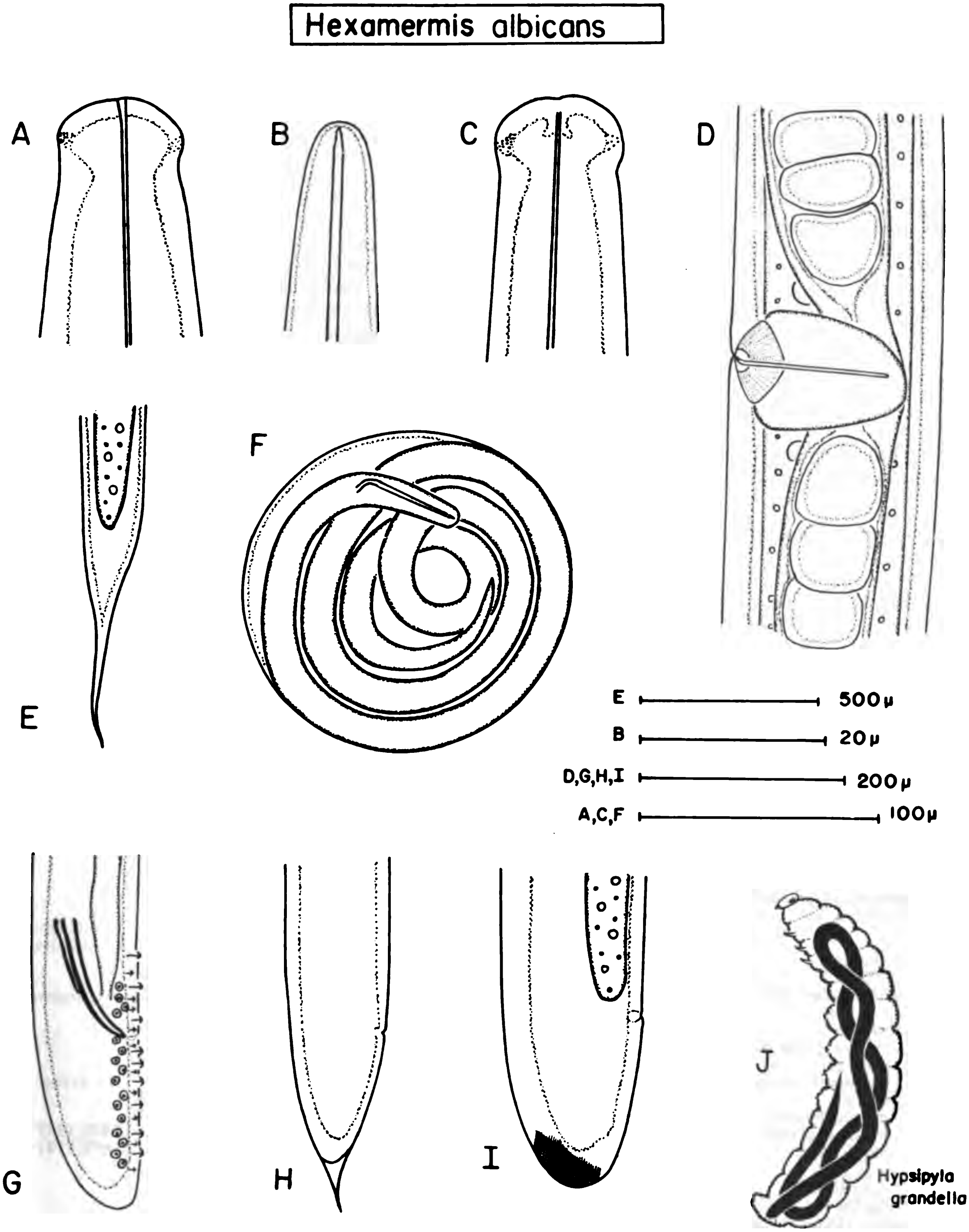

Fig. 2. ***Hexamermis albicans.*** **A Female head. B. Head of a parasitic larva, showing tooth. C. Male head. D. Vulva and vagina. E. Third stage larval tail tip. F. Unhatched egg containing preparasitic larva. G. Male tail. H. Juvenile male tail tip. I. Female tail. J. Mahogany shootborer larva *Hypsipyla*, host.**

Measurements of *Hexamermis albicans* from Costa Rica (Fig. 2).

Five males: L = 25.5 mm (11.0 – 33.2 mm). W = 129.0 microns (114.0 – 136.8 microns). Spicule L = 113.3 microns (92.6 – 130.0 microns). Four females: L = 57.7 mm (42.0 – 83.8 mm). W = 187.4 microns (156.8 – 220.4 microns). V = 47.4 per cent (40.2 – 53.7 per cent). Egg = 127.3 = 88.7 microns.

The identification of this nematode as the ubiquitous *H. albicans* follows the redescriptions of this species by recent Soviet workers. It appears to have a very wide host range. Comparison of the Costa Rican specimens with specimens of European *H. albicans* collected by Steined in the early 1920's shows little difference. *Hexamermis* spp. are often found parasitic in lepidopterans; some of these are listed in Table 1.

A review of the literature of *Hypsipyla* shows that a *Hexamermis* sp. has also been collected from *H. grandella* in Venezuela (20) and British Honduras (3). In India, a *Hexamermis* sp. is reported to parasitize larvae of *Hypsipyla robusta* Moore in Kalimpong, Top–Slip (Madras) and Dehra Dun. The percentages of parasitism during the rainy season at Dehra Dun and Kalimpong were 5 and 9 per cent respectively (20).

Roberts (21) reports a *Hexamermis* sp. parasitizing *Hypsipyla robusta* in Nigeria and indicates that this nematode was responsible for the death of as much as 40 per cent of some of the larval samples collected during the wet season at Ibadan. The same author found that of all parasites encountered in *H. robusta* the nematode has the highest level of parasitism. This information points at the possibility of employing *Hexamermis* in a successful biocontrol program of *Hypsipyla*. The most urgent needs with respect to future investigations on insect parasitic nematodes in such biocontrol program are activation of mass production, field release and the establishment of successful field trials (15).

TABLE 1. List of lepidopterans reported parasitized by *Hexamermis*.

Host	Region	Authority, year and reference
	MACROLEPIDOPTERA	
	Pieridae	
Aporia crataege (L.)	Europe	Assmuss, 1958, (2)
	USSR	Artyukhovsky, 1955, (1)
	Nymphalidae	
Inachis io (L.)	Europe	Siebold, 1853, (24)
	Europe, Asia	Linstow, 1898, (14); Schultz, 1900, (22)
Mellicta athalla (Rott.)	Europe, Asia	Schultz, 1900, (22)
Nymphalis antiopa (L.)	Europe, Asia, N. Amer.	Linstow, 1898, (14)
Polygonia c–album (L.)	Europe	Linstow, 1898, (14)
Vanessa sp.	Europe	Sievold, 1853, (24)
	Satyridae	
Pyronia tithonus (L.)	Europe, Asia	Linstow, 1898, (14)
	Sphingidae	
Mimas tiliae (L.)	Europe, Asia	Hagmeier, 1912, (10)
	Arctiidae	
Arctia caja (L.)	Europe, N. America	Siebold, 1855, (25)
Diacrisia (Spilosoma) lubricipeda (L.)	Europe, N. Asia	Schultz, 1900, (22)
Endrosa aurita (Esp.)	Europe	Linstow, 1898, (14)
	Noctuidae	
Agrotis infusa (Boids.)	Australia	Common, 1954, (7); Welch, 1963, (27)
Agrotis ipsilon (Hufn.)	USA	Puttler *et al*, 1973, (19)
Aletia (Heliophila) pallens (L.)	Europe	Assmuss, 1958, (2);
	N. America	Schultz, 1900, (22)
Apamea monoglypha (L.)	Europe, Asia	Linstow, 1898, (14)
Astoides (Mormonia) sponsa (L.)	Asia	Linstow, 1878, (11)
Autographa gamma (L.)	Europe, Asia, Africa	Assmuss, 1958, (2)
Callierges (Lithocampa) ramosa (Esp.)	Europe	Schultz, 1900, (22)
Catocala nupta (L.)	Europe, Asia to India	Siebold, 1853, (24); Linstow, 1898, (14)
Cerapteryx (Episema) graminis (L.)	Europe, N. America	Siebold, 1853, (24); Linstow, 1898, (14)
Cucullia scrophulariae (Schiff.)	Europe	Linstow, 1878, (11); 1898, (14); Schultz, 1900, (22)
Cucullia tanaceti (Schiff.)	Europe, Asia	Siebold, 1853, (24); Linstow, 1878, (11); 1898, (14)
Cucullia verbasi (L.)	Europe, Asia	Linstow, 1898, (14)
Diloba caerulocephala (L.)	Europe, Asia	Linstow, 1898, (14)
Ephesia fulminea (Scop.)	Europe	Linstow, 1878, (11)
Epilecta linogrisea (Denis & Schiff.)	Europe, Asia	Schultz, 1900, (22)
Euxoa burnnea (Hufn.)	W. Asia, Europe	Siebold, 1853, (24); Linstow, 1898, (14)
Mythimna (Leucania) 1–album (L.)	C & S Europe	Assmuss, 1858, (2)
Noctua orbona (Hufn.)	Europe, Asia	Siebold, 1853, (24); Linstow, 1898, (14)
Polia persicaridae (L.)	Siberia	Assmuss, 1858, (2); Schultz, 1900, (22)
	Europe	Linstow, 1898, (14)
Polia (Melanchra pisi (L.)	Europe	Siebold, 1850, (23)
Scollopteryx libatrix (L.)	Europe, Asia, N. America	Assmuss, 1858, (2); Linstow, 1898, (14)

TABLE 1. (Continuation)

Host	Region	Authority, year and reference
	Geometridae	
Biston betularia (L.)	N. Asia, Europe	Siebold, 1853, (24); Linstow, 1898, (14); Schultz, 1900, (22)
Cidaria berberata (Schiff.)	Europe	Linstow, 1898, (14)
Deilinia exanthemata (Scop.)	Europe, Asia, Canada	Siebold, 1858, (26); Linstow, 1898, (14)
Ennomos alniaria (L.)	Europe	Assmuss, 1858, (2)
Hydriomena coerulata (F.)	Europe, Asia	Schultz (Kriechbaumer), 1900, (22)
Hydriomena furcata (Thun.)	Europe, N. America	Schultz, 1900, (22)
Lomaspilis marginata (L.)	Europe, Japan, Asia	Siebold, 1858, (26)
Operophtera brumata (L.)	USSR, Europe, N. America	Artyukhovsky, 1955, (1); Linstow, 1898, (14); Schultz, 1900, (22); Polozhentsev, 1955, (17)
Oporinia dilutata (Schiff.)	Europe	Linstow, 1898, (14)
Selenia (Ennomos) bilunaria Esp.	N. Asia, Europe	Linstow, 1889, (13)
Thera juniperata (L.)	Europe	Linstow, 1898, (14)
	Lymantriidae	
Dasychira sp.	USSR	Artyukhovsky, 1955, (1)
Euproctis chrysorrhea (L.)	Europe, N. America	Linstow, 1898, (14); 1853, (24)
Lymantria dispar (L.)	USSR	Polozhentsev, & Artyukhovsky, 1953, (16); Polozhentsev, 1955, (17); Polozhentsev, Artyukhovsky, & Kharchenko, 1964, (18)
	Europe	Linstow, 1898, (14)
Stilpnotia salicis (L.)	Europe, N. Asia	Linstow, 1898, (14)
	Lasiocampidae	
Gastropacha sp. (Eriogaster)	Europe, Asia	Linstow, 1898, (14)
Malacosoma neustria (L.)	Europe, N. & W. Asia	Linstow, 1878, (11); Linstow, 1898, (14)
Odonestis pruni (L.)	Italy	Siebold, 1853, (24)
Philudoria potatoria (L.)	Europe	Assmuss, 1858, (2)
Poecilocampa populi (L.)	Europe	Linstow, 1879, (12); Linstow, 1889, (13)
	Notodonitidae	
Notodonta dromedarius (L.)	Europe	Linstow, 1898, (14)
Notodonta ziczac (L.)	Europe	Siebold, 1853, (24)
Phalera bucephala (L.)	Europe, Asia	Siebold, 1853, (24)
	USSR	Artyukhovsky, 1955, (1)
Ptilophora plumigera (Esp.)	Europe	Linstow, 1898, (14)
	MICROLEPIDOPTERA *Zygaenidae*	
Zygaena purpuralis Brünnich	Europe, W & N. Asia	Siebold, 1853, (24); Siebold, 1858, (26)
	Tortricidae	
Heyda salicella (L.)	Siberia Europe	Siebold, 1853, (24) Linstow, 1898, (14)
Laspeyresia pomonella (L.)	Europe, USA, Africa	Siebold, 1853, (24); Linstow, 1898, (14); Schultz (Goeze), 1900, (22); Chittenden, 1905, (6)
Pandemis corylana (F.)	Europe	Linstow, 1898, (14)
Pandemis heparana (D & S)	Europe	Linstow, 1898, (14); Siebold, 1853, (24)
	Tineidae	
Tinea sp.	Europe	Linstow, 1879, (12)
	Pyralidae	
Diatraea saccharalis (F.)	Cuba, Venezuela	Guagliumi, 1962, (9)
Hypsipyla grandella (Zeller)	British Honduras	Bennett, 1968, (3)
	Venezuela	Rao & Bennett, 1969, (20)
	Costa Rica	Nickle, & Grijpma, 1974, (present paper)
Hypsipyla robusta Moore	India	Chatterjee & Singh, 1965, (5)
	Cossidae	
Cossus cossus L.	Europe	Assmuss, 1858, (2)
	Hepialidae	
Hepialus humuli (L.)	Europe	Assmuss, 1858, (2)
	Yponomeutidae	
Yponomeuta evonymellus (L.)	Europe, Asia	Linstow, 1878, (11); Linstow, 1898, (14)
Yponomeuta padellus (L.)	Europe, Asia	Bugnion, 1878, (4); Linstow, 1878, 1898, (11, 14); Siebold, 1853, 1858 (24, 26)

Summary

A new parasitic association between the mermithid nematode, *Hexamermis albicans* and the mahogany shootborer *Hypsipyla grandella* from Costa Rica is reported. From 5 – 25 per cent of the insect larvae sampled were parasitized and killed by this parasite. The parasitized larvae were found in branches of *Cedrela* spp. and *Swietenia macrophylla,* located between 1 to 2 m above the ground. Highest level of parasitism was found towards the end of the wet season and lowest level at the end of the dry season. A review of the literature on lepidopterans parasitized by *Hexamermis* is included.

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MICROLEPIDOPTEROS ASOCIADOS CON *CARAPA, CEDRELA* Y *SWIETENIA* EN COSTA RICA*

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COMPENDIO

This study comprises the Microlepidoptera associated with the following Meliaceae in Costa Rica: *Carapa guianensis* Aublet, *Cedrela odorata* Linnaeus, *C. salvadorensis* Standley, *C. tonduzii* C. de Candolle, y *Swietenia macrophylla* King.

Associated with these five tree species the following seven species of Microlepidoptera were found: *Hypsipyla grandella* (Zeller), *H. ferrealis* (Hampson), *Sematoneura atrovenosella* Ragonot, *S. grijpmai* Becker, new species, *Humiphila paleolivacea* Becker, new genus and species, *Antaeotricha ribbei* Zeller and *Phyllocnistis meliacella* Becker, new species.

H. grandella, the most harmful species, is associated with all these tree species. *H. ferrealis* is associated with *C. guianensis*. The larvae of *S. grijpmai* were found in fruits of *C. odorata*. *Humiphila paleolivacea* feeds on dead bark of the trunk of *C. guianensis* and can also be found in organic matter in the soil near of the base of the trunk. The larvae of *A. ribbei* were found feeding on leaflets of *C. odorata* and *S. macrophyla*. *P. meliacella* is a leafminer on leaflets of all these tree species with the exception of *C. salvadorensis*.

Introducción

Las especies de la familia Meliaceae en las Américas que pertenecen a los géneros *Carapa, Cedrela* y *Swietenia* ofrecen madera de gran valor comercial. En los últimos años las reservas naturales de estas especies disminuyeron rápidamente debido a la explotación intensiva y a la tala de los bosques para la agricultura y la ganadería. En vista de esto, se intentó establecer plantaciones de tales especies en varios países latinoamericanos. La mayoría de estos intentos fracasaron debido a los daños provocados por las larvas de un microlepidóptero, *Hypsipyla grandella* (Zeller), en los brotes jóvenes de las plantas.

Debido a los problemas causados por las larvas de esta especie, la literatura en general atribuye los daños solamente a *H. grandella*.

Con base en material recolectado por miembros del Grupo Interamericano de Trabajo sobre *Hypsipyla*, en Turrialba, se verificó que varias especies de microlepidópteros estaban asociados con este grupo de plantas, y no solamente *H. grandella*.

El objetivo de este trabajo fue el de relacionar y describir cuáles especies están asociadas con este grupo de plantas en Costa Rica y qué tipo de daños hacen. Además, se hace mención de su biología, comportamiento, distribución, hospedantes y parásitos, con la excepción de los parásitos de *H. grandella* que serán estudiados en un trabajo posterior.

Revisión de Literatura

La literatura indica que hay tres especies de microlepidópteros asociados con *Carapa, Cedrela* y *Swietenia* en la América Latina. Dos de estas, *Hypsipyla grandella* (Zeller) y *H. ferrealis* (Hampson), pertenecen a la familia Pyralidae (Phycitinae) y la otra, *Antaeotricha dissimilis* (Kearfott) a la familia Stenomidae.

Desde que fue registrada por Hutson, citado en Lamb (48), en 1918, causando daños en plantaciones de *Cedrela odorata* L. y *Swietenia macrophylla* King en Barbados, *H. grandella* fue encontrada causando los mismos daños en los siguientes países: Argentina (23,

* Este trabajo fue presentado como parte de los requisitos para obtener el título de *Magister Scientiae* en la Escuela para graduados del Instituto Interamericano de Ciencias Agrícolas de la OEA.

40, 41); Barbados (23); Belice (Honduras Británica) (12, 64, 72); Brasil (3, 10, 12, 14, 21, 25, 30, 36, 37, 41, 42, 48, 50, 51, 58, 59, 60, 61, 70, 71, 72, 78); Colombia (12, 41); Cuba (12, 26, 27, 62, 68, 72); Estados Unidos (12, 41, 48); Granada (Pequeñas Antillas) (23); Guatemala (12, 23, 41, 72); Guyana (12, 41); Haití (41, 44); Honduras (12, 16, 41); Jamaica (12, 48); Martinica (3, 23); México (39, 48, 72); Panamá (12, 21, 41); Paraguay (12, 41); Perú (3, 12, 13, 20, 21, 23, 72, 75); Puerto Rico (3, 12, 18, 41, 43, 48, 49, 52, 53, 56, 57, 72, 76, 77); Surinam (3, 12, 19, 23, 29, 31, 72); Trinidad (4, 15, 23, 54, 55, 72); Tobago (54, 55); Venezuela (1, 12, 35, 41, 66, 69, 72). En Costa Rica fue registrada por primera vez en 1935 por Ballou (2) y después por otros autores (12, 28, 32, 33, 41).

La mayor parte de estos trabajos son muy superficiales, citan solamente la asociación del insecto con los hospedantes y repiten observaciones citadas en trabajos anteriores.

El primer dato sobre el hospedante de *H. ferrealis* fue registrado por Heinrich (41), quien estudió algunos ejemplares de esta especie criados por Ballow en semillas de *C. guianensis* en Venezuela. Entwistle (23) indica que en Trinidad *H. ferrealis* ataca también a *Spondias mombin* L. (Anacardiaceae) y *Rheedia* sp. (Guttiferae). El autor (6) estudiando larvas de *H. ferrealis* obtenidas de semillas de *C. guianensis* provenientes de la región amazónica del Brasil, publicó algunos datos sobre su comportamiento y describió la larva y la pupa. Recientemente (7) se publicaron datos más detallados sobre la biología de esta especie.

Se encontró en Brasil (5) larvas de *Antaeotricha dissimilis* (Kearfott) alimentándose de hojuelas de *Cedrela fissilis* Vel.

Fletcher (24) encontró en India larvas de *Acrocercops auricilla* (Stainton) (Gracillariidae) minando las hojuelas de *Swietenia* sp.

Materiales y Métodos

Las especies de hospedantes incluidas en este estudio son: *Carapa guianensis* Aublet, *Cedrela odorata* Linnaeus, *C. salvadorensis* Standley, *C. tonduzii* C. de Candolle y *Swietenia macrophylla* King. Además de estas especies fueron recolectadas muestras de *C. angustifolia* Sessé & Moc. y *S. mahagoni* Linnaeus en las plantaciones y en el vivero forestal del CATIE, en Turrialba. Las semillas de *C. angustifolia* procedieron de Venezuela y las de *S. mahagoni* de la República Dominicana.

Como estas especies están distribuidas en regiones ecológicas distintas, raramente habiendo más de dos especies en una misma región, la selección de los locales fue efectuada de tal manera que cada especie se quedó representada por lo menos en una región.

La distribución de los hospedantes se basó en el sistema de zonas de vida de Holdridge (45), según el mapa ecológico de Costa Rica hecho por Tosi (73). Se consideró como áreas de distribución de una especie a todas las áreas delimitadas por una zona de vida en la cual se recolectó alguna muestra de dicha especie.

El muestreo fue hecho en forma sistemática, buscándose todas las partes atacadas de los árboles encontrados. Se consideró como muestra todo el material atacado recolectado de un árbol adulto o, en los casos de plantaciones jóvenes, de todo el material recolectado en una plantación. Las muestras de frutos de las especies de *Cedrela* fueron tomadas cosechándose todos los frutos en varios árboles. Estas muestras fueron llevadas al laboratorio para determinar la incidencia de infestación. Los huevos, larvas y pupas sacados de estas muestras fueron criados en el laboratorio para obtener datos biológicos, adultos para identificación y parásitos.

Para la identificación dendrológica se tomó muestras botánicas de los árboles en que fueron sacadas las muestras de material infestado.

La recolección de las muestras fue efectuada entre octubre de 1972 y junio de 1973.

Las larvas obtenidas de las muestras fueron transferidas a cajas plásticas de 22 x 13 x 5,5 cm, divididas en 24 compartimientos de 5 x 5 x 5 cm, y a cajas de 28 x 13 x 6 cm. El fondo de cada caja fue cubierto con papel toalla mojado para mantener la humedad relativa a 100 por ciento. Las larvas fueron alimentadas regularmente con material de las mismas especies en que fueron encontradas. La temperatura en el laboratorio fue de 23,9 ± 1,4°C y la humedad relativa de 68,1 ± 10,4 por ciento.

Las muestras botánicas fueron identificadas por el Dr. L. R. Holdridge, del Centro Científico Tropical, San José, Costa Rica, por el Dr. L. Fournier, de la Universidad de Costa Rica y por el Dr. B. T. Styles del Instituto Forestal de la Comunidad Británica, Oxford, Inglaterra.

Los microlepidópteros obtenidos fueron identificados por el autor hasta el nivel de especie, con excepción de *Antaeotricha ribbei* Zeller, cuyo género fue identificado por el autor y la especie por el Dr. W. D. Duckworth, de la Institución Smithsoniana, Washington. La identificación y descripción del Chrysauginae fue revisada por el Dr. E. Cashatt, del Museo del Estado de Illinois y la Gracillariidae por el Dr. D. R. Davis de la Institución Smithsoniana.

La terminología usada en las descripciones fue de acuerdo a la seguida por Heinrich (41), traducida al español según el Diccionario Chambers (17). Los términos usados que no aparecen en este diccionario están en el texto como se usan en inglés y grifados.

Resultados y Discusión

Los hospedantes y su distribución:

Las especies de Meliáceas de los tres géneros estudiados ocupan zonas de vida específica. Con excepción de *C. odorata*, que puede aparecer en zonas que son características de otras especies, cada zona de vida tiene su especie propia. En conjunto, los tres géneros estudiados están distribuidos por todo el país, con excepción del Bosque Húmedo Montano Tropical. Esta zona de vida está ubicada en una faja altitudinal encima de 2.000 m de altitud.

Carapa guianensis Aublet

En Costa Rica *C. guianensis* se encuentra distribuida en los llanos muy húmedos del Atlántico y del Pacífico Sur (Fig. 1), en el Bosque Muy Húmedo Tropical. Puede subir en las laderas en las fajas transicionales entre esta zona de vida y el Bosque Muy Húmedo Premontano Tropical, hasta 800 m de altitud.

Cedrela odorata Linnaeus

En Costa Rica *C. odorata* es la especie que tiene mayor distribución de las especies estudiadas. Está distribuida por todo el país, con excepción de los pisos altitudinales Montano Bajo Tropical y Montano Tropical (Fig. 2). Fue observada desde el nivel del mar hasta 1.200 m de altitud.

Cedrela tonduzii C. de Candolle

De las especies estudiadas en este trabajo, *C. tonduzii* es la que tiene su distribución en las regiones de mayor altitud, entre 1.500 y 2.000 m aproximadamente (Fig. 1). Estas regiones están clasificadas en las zonas del Bosque Húmedo y Muy Húmedo Montano Bajo Tropical. Se encontró árboles de esta especie en las faldas de los volcanes Poás, Irazú y Turrialba.

Cedrela salvadorensis Standley

C. salvadorensis es una especie rara en Costa Rica y de distribución restringida. Se encontró solamente seis árboles, cuatro cerca de San Pedro de Poás y dos en Villa Colón (Fig. 1). Su distribución se sitúa entre 800 y 1.200 m de altitud, en el Bosque Húmedo Premontano Tropical.

Swietenia macrophylla King

S. macrophyla tiene su distribución restringida a la parte más seca de Costa Rica, desde el norte de Puntarenas hasta la frontera con Nicaragua, en el lado del Pacífico (Fig. 1). Ocurre desde el nivel del mar hasta 500 m de altitud aproximadamente, en el Bosque Húmedo Premontano Tropical, en las asociaciones monsónicas con estación seca fuerte, y penetra al Bosque Seco Tropical cuando hay disponibilidad de agua en el suelo.

Los insectos

En las especies de hospedantes estudiadas se encontraron siete especies de microlepidópteros pertenecientes a tres familias: Pyralidae, Stenomidae y Gracillariidae. Tres de las siete especies encontradas son nuevas y dos de las cuatro descritas anteriormente no se conocían los hospedantes.

PYRALIDAE
Phycitinae

Hypsipyla grandella (Zeller)

Nephoteryx grandella Zeller (79); *Hypsipyla grandella* (Zeller) Ragonot (65); *Hypsipyla cnabella* Dyar (22); *Hypsipyla grandella* (Zeller) Heinrich (41).

Adulto. (Fig. 12a). Largo del ala anterior del macho 14,2 ± 1,6 mm (11,1 a 18,3 mm; n = 40), de la hembra 15,2 ± 2,3 mm (11,1 a 20,0 mm; n = 33).

Palpos labiales con el primer segmento blanquecino, el segundo gris claro y el tercero gris oscuro. Antena blanquecina, ciliada en el macho, débilmente ciliada en la hembra. Cabeza gris fusca. Tórax gris fusco con escamas gris oscuro en la base de las tégulas. Alas anteriores gris fuscas con brillo violáceo leve, sombreadas con escamas castaño rojizo especialmente posterior a Cus; venas R_5, Ms y Cus marcadas con escamas negras; una mancha clara en el centro del ala, desde Rs hasta 1A; una banda poco marcada y sinuosa, paralela al **termen** desde la costa hasta M_3; seis o siete puntos negros a lo largo del **termen** entre las venas; cílios gris, con una línea oscura en la base. Patas blanquecinas, mezclado con escamas gris del lado externo, blanquecino iridescente del lado interno. Abdomen gris del lado dorsal, gris fusco anteriormente; blanquecino iridescente en el lado ventral.

Organo genital masculino (Fig. 3), conforme preparación microscópica VOB 120. Uncus estrechado posteriormente a su base amplia, apicalmente redondeado. **Gnathos** más corto que el uncus; proceso central angosto con ápice en forma de V. **Transtilla** completa, con dos expansiones posteriores en forma de cuernos y dos anteriores en forma digital. **Anellus** aproximadamente en forma de V. Vinculum más ancho que largo. Valvas más largas que el uncus, pilosas internamente; **clasper** redondeados, pilosos en el ápice. Edeago cilíndrico, estrechado hacia el ápice; vesica armada con un diente quitinizado de forma helicoidal, mitad del largo del edeago.

Organo genital femenino (Fig. 4c), conforme preparación microscópica VOB 121. Ostium amplio. Antro cónico, membranoso. Conducto de la bolsa angosto, membranoso. Inserción del conducto seminal en la bolsa cerca de su unión con el conducto de la bolsa. Bolsa copulativa amplia, membranosa, estrechada abruptamente hacia la parte posterior. **Signum** presente en forma de una área con puntación fuerte.

Tipos. Heinrich (41) afirma que desconocía la ubicación del tipo de *H. grandella* y que el de *H. cnabella* se encuentra en el Museo Nacional de los Estados Unidos, Institución Smithsoniana, Washington, D.C.

Localidad de los tipos. Según Heinrich (41) el tipo de *H. grandella* era procedente de Brasil y el de *H. cnabella* procedente de Córdoba, México.

Distribución (Fig. 13). En Costa Rica se encontró por todo el país, en las áreas de distribución de las especies de los géneros *Carapa, Cedrela* y *Swietenia.* En el Continente Americano se encuentra desde la Florida en el sur de los Estados Unidos hasta Argentina, incluyendo las islas del Caribe (ver Revisión de Literatura).

Observaciones. El tamaño de los adultos de esta especie es muy variable. Esta característica probablemente está condicionada a la disponibilidad y calidad del alimento. Los ejemplares obtenidos de larvas criadas en tallos eran generalmente menores que los obtenidos de larvas criadas en frutos. Algunos ejemplares obtenidos de larvas criadas en tallos tenían entre 11 y 12 mm de largo de la ala anterior, mientras que otros obtenidos de larvas criadas en frutos de *C. tonduzii* tenían casi el doble, aproximadamente 20 mm de largo del ala anterior. Heinrich (41) indica que los ejemplares por él estudiados medían entre 20 y 45 mm de envergadura. Grijpma (34) indica que los ejemplares obtenidos de larvas criadas en una dieta artificial eran mayores que los obtenidos de larvas criadas en dieta natural formada por tallos y hojas.

Se observó, también, una pequeña variación en el color. Algunos ejemplares tenían las alas poco marcadas y con dibujos menos nítidos que los ejemplares típicos.

Heinrich (41) presenta en su trabajo buenas ilustraciones de los órganos genitales de ambos sexos, pero no presenta ilustración del adulto. Las ilustraciones del órgano genital masculino presentadas en este trabajo (Fig. 3a) difieren un poco de la presentada por Heinrich (41). El contorno de la valva es menos curvada en la ilustración de este autor (41).

Como la apariencia del órgano puede variar conforme al grado de compresión del cubre objetos sobre el porta objetos, en la preparación microscópica, probablemente el montaje usado en el trabajo de Heinrich estaba menos comprimido que el usado en este trabajo. El órgano genital femenino difiere un poco más. La ilustración de este órgano presentada en este trabajo (Fig. 4c) es un poco más plegada que la presentada por Heinrich (41). Como la mayor parte de este órgano es membranoso, principalmente la bolsa copulativa y el conducto de la bolsa, al hacerse la preparación microscópica estas partes tienden a plegarse debido a la acción del xilol. Dependiendo del tiempo en que permanecen en este líquido pueden presentarse más plegadas o menos plegadas.

Según Heinrich (41), *H. grandella* es similar a *H. robusta* de India y Africa, especie que causa daños a las especies de *Meliaceae* de aquellos continentes. Según este autor (41) los órganos genitales de esta especie son diferentes de los de *H. grandella*.

Pupa. Castaño en el lado ventral, castaño oscuro, casi negro en el lado dorsal; fusiforme; superficie con puntuación esparcida con excepción del lado central del tórax que es liso. Antenas exteriores a las patas medianas. Proboscis y patas medias alcanzan el ápice de las alas; patas posteriores por debajo de la proboscis, a veces un poco visible en la extremidad. Coxas anteriores parcialmente delimitadas. Larvópodos visibles en el 5° y 6° segmentos. Espiráculos desarrollados y salientes. Setas pequeñas y poco visibles. Cremaster poco saliente con ocho setas expesadas, con extremidad en forma de gancho, dispuestas en arco.

Larva. En el primer instar la larva es de color castaño rojizo; cabeza castaña oscura, el eclosionar más ancha que el diámetro del cuerpo; área ocelar negra. El color cambia gradualmente hacia el castaño en el penúltimo instar. En el último instar las larvas pueden tener dos colores distintos, azul claro o rosa claro. En este instar es cilíndrica, con aproximadamente 3 cm de largo y 5 mm de diámetro; cabeza de color castaño; hipognata; patas toráxicas castaño claro; placa protorácica castaño oscuro; casi negros; espínulas poco visibles, un poco más ocuras que el color general del cuerpo.

Las larvas se desarrollan en las ramas nuevas y en los frutos. En las ramas nuevas se alimentan de la médula y de la corteza; en los frutos se alimentan de las semillas o tejidos internos. Cuando se agota el alimento en una rama o fruto, la larva puede migrar hacia otras ramas u otros frutos, hasta completar el desarrollo. Después de esto, las larvas construyen un capullo blanco y suave, por lo general adentro del propio fruto o rama donde se desarrollaron.

Huevo. El huevo tiene forma elíptica y aplastada con córeo arrugado, de color amarillo pálido cuando frescos y rojo después de 24 horas de ser ovipositado. Según Grijpma (34) son de color amarillo pálido a castaño amarillento (2,5Y6/4 – 2,5Y7/4 en la escala de Munsell) y rojo (5R4/8 en la misma escala) respectivamente.

En las plantas jóvenes la oviposición ocurre a lo largo de los tallos, en el pecíolo de las hojas y en las hojuelas, generalmente cerca de las venas. En los árboles adultos la oviposición también ocurre en las ramas jóvenes, pero en la época seca principalmente en los frutos, con mayor énfasis al final del desarrollo de éstos. Se observaron varios huevos en el pedúnculo y en las ramifiaciones de la panícula. Roovers (69) indica que generalmente se observan huevos sobre o alrededor de las cicatrices de las hojas, aunque muchos se encuentran también a lo largo del tallo y sobre las venas en la parte superior de las hojas tiernas. Grijpma* notó que hay una mayor frecuencia de huevos en las cicatrices de las hojas, en el tallo, como también en las lenticelas.

Ciclo de vida. El autor, en observaciones efectuadas en 1970 en Curitiba, sur de Brasil, encontró que el ciclo de vida varía con la temperatura, en larvas criadas en dieta natural de tallos y hojas de *C. fissilis*. En el invierno, junio–setiembre, el ciclo duró de 80 a 95 días y en el verano de 63 a 80 días. En ambos ciclos, los huevos llevaron 11 días para eclosionar.

Grijpma (34) encontró que el ciclo de vida, desde el huevo hasta la emergencia del adulto se completa en 35 días, en promedio. El máximo de empupación ocurrió 25 días después de la oviposición y el máximo de emergencia a los 35 días. El estudio fue realizado bajo condiciones de laboratorio, con temperaturas entre 22 y 32°C (34). Roovers (69) indica que el ciclo completo, de una oviposición hasta otra, se completa en 45 días en promedio y que el período de desarrollo del huevo fue de 96 a 120 horas. Sterringa** indica un período de 92 a 97 horas entre la oviposición y la eclosión. Esta variación se debe probablemente a diferencias de temperatura.

Hospedantes. En Costa Rica se encontró *H. grandella* sobre *C. angustifolia*, *C. odorata*, *C. salvadorensis****. *C. tonduzii*, *S. macrophylla* y *S. mahagoni* (Cuadro 1). Ballou (2) también la encontró sobre *Guarea caoba* C. DC.

Entwistle (23) registra el ataque de *H. grandella* en las siguientes especies en América: *Carapa guianensis*, *C. procera*, *Cedrela odorata* (= mexicana), *C. fissilis*, *C. fissilis* var. *macrocarna* C. DC., *C. lilloi* C. DC., *C. tubiflora* Bertoni, *Guarea trichilioides* L., *Khaya nyasica* Stapf ex Bak. f., *K. senegalensis* A. Juss., *Swietenia macrophylla*, *S. mahogani* y *Trichilia* sp. (Meliaceae); registra también *Erythrina* sp. (Leguminosae) como

* Comunicación personal.
** Información personal.
*** Este dato no está registrado en la tesis del autor (8), pues fue obtenido posteriormente al término de esta.

hospedante. La indicación de *Erythrina* sp. como hospedante de *H. grandella* parece dudosa. Grijpma* obtuvo varias ramas atacadas de una especie de *Erythrina*, cuyos síntomas eran muy parecidos a los de las meliáceas atacadas por *H. grandella.* Todos los adultos que resultaron de las larvas que atacaban estas ramas fueron identificadas como *Terastia meticulosalis* Guenée (Lep., Pyralidae). Además de esto, se intentó criar larvas de *H. grandella* recién emergidas sobre ramas jóvenes de *Erythrina* sp. pero las larvas no aceptaron este alimento y terminaron muriéndose.

El daño principal es ocasionado en las plantaciones jóvenes debido al ataque en el brote terminal. Los daños causados resultan en la muerte del brote terminal, y como consecuencia, la formación de numerosos brotes secundarios que producen deformaciones en el tronco. Los ataques repetidos disminuyen el crecimiento, o incluso pueden causar la muerte de los árboles jóvenes. El daño en los frutos, que en el período observado aparentemente fue más bajo que en otros años, no parece ser un factor significante en la regeneración de la especie en vista de la gran cantidad de semillas producidas.

Hypsipyla ferrealis (Hampson)

Crocidomera ferrealis Hampson (38); *Hypsipyla ferrealis* (Hampson) Heinrich (41).

Adulto. (Fig. 12b). Largo del ala anterior del macho 14,2 ± 1,1 mm (11,3 a 16,4 mm), de la hembra 15,4 ± 1,1 mm (12,8 a 17,7 mm); (n = 40).

Palpos labiales castaño pálido mezclados con escamas gris, más oscuros en el lado dorsal. Fronte y vértex castaño pálido a castaño grisáceo. Antenas castaño pálido; ciliadas en el macho, débilmente ciliadas en la hembra. Tórax castaño pálido mezclado con escamas gris oscuras. Alas anteriores castaño grisáceo pálido con un tenue brillo violáceo; venas R_5 Ms y Cus marcadas con escamas negras; una mancha pálida en el centro del ala desde Rs hasta Cus, cruzando la célula; una banda subterminal pálida, sinuosa, paralela al **termen**; cílios castaño grisáceo con una línea basal oscura. Alas posteriores semihialinas, gris claro en el macho, castaño oscuro en las hembras, con un tenue brillo violáceo; costa iridescente; márgenes externa y anal y cílios castaño grisáceo oscuro; cílios con una línea basal oscura. Patas castaño pálido con escamas negras externamente. Abdomen castaño grisáceo dorsalmente, castaño pálido ventralmente.

Organo genital masculino (Fig. 5a, b), conforme preparaciones microscópicas VOB 132, 137, 139. Uncus estrechado posteriormente a su base amplia, volviéndose a anchar posteriormente; ápice redondeado. **Gnathos** más corto que el uncus; proceso ventral cilíndrico, angosto, con dos dientes terminales. **Transtilla** completa, con dos expansiones ventro–laterales. **Anellus** en forma de U, con una área esclerosada en la parte interna. Vinculum más largo que ancho. Valvas más largas que el uncus, anchas, densamente pilosas en el lado interno; clamps cortos no pronunciados. Edeago cilíndrico, curvado en la base, vesica armada con un diente quitinizado, curvado; un tercio del largo del edeago.

Organo genital femenino (Fig. 5c), conforme preparación microscópica VOB 138. Ostium amplio. Antro en forma de embudo; membranoso. Conducto de la bolsa angosto. Inserción del conducto seminal en la bolsa copulativa cerca de su conexión con el conducto de la bolsa. Bolsa copulativa oblonga, membranosa, estrechada abruptamente del lado posterior. Signum ausente.

Tipo. En el Museo Británico (41).

Localidad del tipo. Río . xaola, Costa Rica (41).

Distribución (Fig. 13). Se recolectó en Turrialba, en Guayacán y cerca de Siquirres. Heinrich (41) indica que también fue recolectada en Cachí, Caín, Juan Viñas, Puerto Limón, Río Sixaola y Tuis, en Costa Rica. Se encuentra también en Colombia, Venezuela, Guyana Francesa, Trinidad (23, 41) y en Brasil (6, 41).

Observaciones. Los adultos de *H. ferrealis* son muy variables en tamaño y color. Los ejemplares recolectados en Costa Rica tuvieron una variación menor que las medidas dadas por Heinrich (41) en su trabajo, y a las observadas en *H. grandella.* No obstante, algunos ejemplares obsequiados por el Dr. F. Bennett, de Trinidad, tenían cerca de 10 mm de largo del ala anterior, aproximándose a las medidas de los menores ejemplares estudiados por Heinrich (41).

La mayoría de los ejemplares de *H. ferrealis* tienen maculación similar a la de *H. grandella,* pero en algunos ejemplares la maculación es menos marcada y otros, casi no presentan máculas y las alas son de color uniforme.

Algunos ejemplares son más oscuros y otros más claros que el normal. Se puede diferenciar fácilmente *H. ferrealis* de *H. grandella* debido a sus alas posteriores oscuras, como también indicó Heinrich (41). La figura de la hembra (Fig. 12b) de *H. ferrealis* presentada en este trabajo fue sacada de un ejemplar más claro que los normales.

La ilustración del órgano genital masculino presentada en este trabajo (Fig. 5a, b) es muy similar a la ilustración dada por Heinrich (41), con la diferencia que la de su trabajo presenta una sola valva. La ilustración del órgano genital femenino (Fig. 5c) presenta más detalles que las presentadas por Heinrich (41) y la bolsa copulativa está más inflada que la de este autor. Las descripciones de estos órganos presentadas por Heinrich (41) son incompletas, principalmente la del órgano genital femenino.

Pupa. Castaño claro en el lado ventral, castaño oscuro en el lado dorsal; fusiforme; superficie con puntuación esparcida con excepción del lado ventral del tórax que es liso. Los machos miden 14,5 ± 0,4 mm de largo y 4,3 ± 0,4 mm de diámetro (n = 50); las hembras miden 14,5 ± 1,5 mm de largo y 4,6 ± 0,5 de diámetro (n = 50). Es prácticamente imposible diferenciar la pupa de esta especie de las de *H. grandella.*

Larva. Después de eclosionar, la larva es de color crema claro; cabeza y placa protorácica negras; placa anal del color del cuerpo. El color crema cambia gradualmente hacia blanco hialino al final del primer

* Información personal.

instar. En el segundo y tercer instar la larva es de color blanco–hialino, volviendo al color crema claro al final del tercer instar. En el segundo instar la cabeza y las placas protorácica y anal son gris oscuro. Los pináculos son minúsculos, gris oscuro, visibles al microscopio. En el tercer instar la cabeza es castaña, manchada de gris; placa protorácica gris oscuro; placa anal y pináculo gris. Al inicio del cuarto período larval son de color crema, algunas levemente pigmentadas con color rosa. Al final de este instar pueden tener una gran variedad de colores. De 21 larvas observadas, tres eran de color crema, siete rosa claro y once verde azulado claro con tonalidad rosácea. Antes de empupar todas son de color azul verduzco. En este instar la cabeza es castaño claro; placa anal con manchas gris. Las que alcanzan el quinto instar son de color azul verduzco con tonalidad rosácea.

Huevo. El huevo de *H. ferrealis* tiene forma elíptica, redondeado en el lado superior y plano en el lado que se queda junto a la superficie en que fue ovipositado; córeo arrugado; de color crema, casi blanco inmediatamente después de la oviposición y rojo acastañado (10R4/10 en la escala de Munsell (63), después de 24 horas de edad). Es parecido al de *H. grandella*, con excepción del color después de 24 horas de la oviposición y de la forma menos aplastada que en ésta. Una hembra recolectada a la luz en Turrialba puso 570 huevos en una caja plástica en el laboratorio.

Hospedantes. En Costa Rica las larvas fueron encontradas solamente en los frutos y semillas de *C. guianensis*. La literatura cita como plantas alimenticias de las larvas de *H. ferrealis* a *C. guianensis* (6, 23, 41), *Spondias mombin* L. y *Rheedia* sp. (23). En dos muestras de frutos de dos árboles de *S. mombin*, recolectadas en el área del IICA–CTEI, no fueron encontradas larvas de *H. ferrealis*. Bennett* informó que no es muy seguro que *S. mombin* y *Rheedia* sp. sean hospedantes de esta especie.

Existe la posibilidad de que hay otro, u otros hospedantes. Una de las razones es que en Turrialba, situada en una región en donde no ocurre naturalmente el cedro macho, se puede recolectar fácilmente los adultos de *H. ferrealis* a la luz, durante todo el año, siendo más frecuente en agosto y setiembre. En el período entre 17 de agosto y el 25 de setiembre de 1971 se recolectaron 238 adultos en una trampa de luz de mercurio y en las ventanas de los laboratorios del CTEI. En una sola noche, el 18 de setiembre, se recolectaron 52 ejemplares de esta especie. Por otra parte, vale mencionar que esta época del año coincide con el período en el cual los frutos del cedro macho caen al suelo. Otra razón es que el 75 por ciento de los adultos de *H. ferrealis* emergen en una época en la cual *C. guianensis* no tiene frutos.

Parásitos. El único parásito encontrado fue *Hypomicrogaster hypsipylae* De Santis. De las cuatro larvas parasitadas emergieron 22 machos y 35 hembras de esta especie.

* Información personal.

Sematoneura atrovenosella Ragonot

Sematoneura atrovenosella Ragonot (65), Heinrich (41).

Adulto (Fig. 12c). Largo del ala anterior del macho 12,1 ± 1,2 mm (10,0 a 15,0 mm; n = 11), de la hembra 14,3 ± 1,4 mm (12,1 a 17,2 mm; n = 18).

Palpos labiales gris claro mezclado con escamas gris oscuro. Antena gris claro; ciliada en el macho, cortamente ciliada en la hembra. Vértex blanquecino a gris claro. Tórax gris claro manchado de oliva ocráceo. Alas anteriores gris claro mezclado con escamas oliva ocráceo principalmente en la parte posterior; venas marcadas con escamas negras; una mancha negra en la base de M_2 y M_3; seis puntos negros a lo largo del termen, entre las venas; cilios gris. Alas posteriores semihialinas, blanco oscuro; costa gris; cilios gris con una línea oscura en la base. Patas blanquecinas mezcladas con escamas negras del lado externo. Abdomen gris; manchado con oliva ocráceo en la parte anterior.

Organo genital masculino (Fig. 6b, c), conforme preparaciones microscópicas VOB 126, 133. Uncus anchamente triangular. Gnathos más corto que el uncus; proceso apical simple y cilíndrico. Transtilla completa, arqueada ventralmente, con proceso ventral en forma de lóbulo aplastado con margen posterior cóncavo. Anellus en forma de U, con pelos en las dos extremidades posteriores. Vinculum más ancho que largo, cóncavo anteriormente. Valvas espatuladas con un diente subterminal en la costa. Edeago cilíndrico, redondeado en la base, agudo en la parte terminal; vesica provista de un diente quitinizado levemente curvado, un tercio del largo del edeago.

Organo genital femenino (Fig. 6d), conforme preparaciones microscópicas VOB 125, 134. Ostium angosto, membranoso. Antro angosto, membranoso. Conducto de la bolsa membranoso. Inserción del conducto seminal en la bolsa, próximo a la unión con el conducto de la bolsa. Bolsa copulativa membranosa con granulación fina; estrechada posteriormente. Signum ausente.

Tipo. En el Museo de Zoología de la Universidad de Berlín (41).

Localidad del tipo. Chanchamayo, Perú (41).

Distribución (Fig. 13). Se encontraron larvas en frutos de *C. tonduzii* cerca de Santa Cruz, de Turrialba, a 1.500 m de altitud. Según Heinrich (41) fue también recolectada en Juan Viñas y Tuís, en Costa Rica. También fue recolectada en México, Colombia, Ecuador, Perú (41).

En Costa Rica esta especie probablemente se distribuye en las regiones superiores a 1.000 m de altitud, aproximadamente. Durante dos años de colectas semanales a la luz no fue posible capturar ejemplares de esta especie, así como en las recolectas esporádicas en las regiones más bajas del país. Mientras que en tres recolectas a la luz en Santa Cruz, cerca del Volcán Turrialba, a 1.500 m de altitud, fue posible recolectar varios ejemplares. Las localidades citadas por Heinrich (41) en su trabajo, para Costa Rica, también se sitúan encima de 1.000 m de altitud.

Observaciones. Heinrich (41) presenta figuras buenas de los órganos genitales de esta especie. En la figura del órgano genital masculino presentada por este autor, el uncus da la impresión de ser bifurcado debido a la superposición del ano. En la misma figura, la **transtilla** es casi imperceptible. En la ilustración que se presenta en este trabajo la **transtilla** fue resaltada por ser una característica que distingue notablemente esta especie de otros Phycitinae. La bolsa copulativa, en el órgano genital femenino, está más inflada que la presentada por Heinrich (41).

Pupa. La pupa es de color castaño claro en el lado ventral; castaño oscuro en el lado dorsal; fusiforme. Con excepción del lado ventral del tórax, la pupa es corrugada, con puntuación más densa que las de *H. ferrealis* y *H. grandella.* El macho mide 12,9 ± 0,8 mm de largo y 3,3 ± 0,2 mm de diámetro (n = 5), la hembra mide 13,9 ± 0,9 mm de largo y 3,5 ± 0,2 mm de diámetro (n = 7).

Es fácil diferenciar la pupa de esta especie de las de *H. grandella* y *H. ferrealis* por el pronotum y mesonotum que son lisos en éstas y arrugado con puntuación medianamente densa en *S. atrovenosella.*

Larva. La larva en el primer instar es de color castaño anaranjado. A partir de este instar va cambiando gradualmente de color en cada instar hacia el color castaño grisáceo en el último instar. En el lado ventral es de color crema. El primero y segundo instar tienen cápsula cefálica, placa protorácica y anal negras; el cuarto y quinto instar tienen cápsula cefálica castaña y placas anal y protorácica castaño claro.

Las larvas viven en el interior del fruto alimentándose de las semillas. Son más lentas en sus movimientos que *H. grandella* y *H. ferrealis.* Parecen ser gregarias, pues fueron encontradas hasta 12 larvas en diferentes edades en un solo fruto. Se empupan en el interior del fruto, en el eje. Antes de empuparse construyen un capullo fusiforme blanco, denso y resistente. Más denso y más resistente que los de *H. grandella* y *H. ferrealis.*

Ciclo de vida. El ciclo de vida de *S. atrovenosella,* desde la eclosión del huevo hasta la emergencia del adulto, tiene una duración de tres meses aproximadamente. Se recolectó una muestra de frutos atacados en Santa Cruz, Turrialba, el 14 de diciembre de 1972, que contenía larvas en varios instar incluyendo algunas recién eclosionadas. Estas larvas fueron criadas en laboratorio y los últimos adultos de esta muestra emergieron el 15 de marzo de 1973, tres meses después de la recolecta.

Hospedantes. La larva de *S. atrovenosella* vive en los frutos de *C. tonduzii.* Según Heinrich (41) no hay información anterior sobre el hospedante de esta especie.

Los frutos atacados presentan uno o más orificios circulares con 2–3 mm de diámetro en la cáscara. Al abrírselos se puede observar las semillas parcial o totalmente destruidas y el espacio interno del fruto ocupado por el excremento del insecto y por restos del fruto, unidos entre sí por hilos de seda blancos.

Parásitos. De una muestra recolectada en Santa Cruz de Turrialba, salieron cuatro adultos de una especie del género *Apanteles* (Hym., Braconidae).

Sematoneura grijpmai Becker

Sematoneura grijpmai Becker (9)

Adulto. (Fig. 12d). Largo del ala anterior del macho 8,9 ± 0,6 mm (8,3 a 9,5 mm; n = 4), de la hembra 10,1 ± 0,6 mm (9,3 a 10,8 mm; n = 6).

Palpos labiales castaño mezclado con escamas blanquecinas. Antena fusco grisácea; ciliada en el macho; levemente ciliada en la hembra. Cabeza fusco grisácea, mezclada con escamas blanquecinas. Tórax fusco grisáceo; tégulas fusco acastañado. Alas anteriores fusco grisáceo; margen posterior con escamas castaño rojizo; venas Ms y Cus marcadas con escamas gris oscuro; dos puntos blancos, uno al final de la célula, sobre Cus, el otro sobre M_3; una banda subterminal pálida, paralela al **termen** desde R_5 hasta el **tornus**; cilios gris, con una línea clara en la base. Alas posteriores gris claro; cilios gris con una línea clara en la base. Patas gris mezcladas con escamas blancas externamente; base de la coxa anterior con escamas castaño rojizo. Abdomen gris. La hembra más castaño rojizo que el macho.

Organo genital masculino (Fig. 7a, b), conforme preparaciones microscópicas VOB 128, 136, 135. Uncus anchamente triangular. **Gnathos** más corto que el uncus; proceso apical redondeado distalmente. **Transtilla** completa, arqueada ventralmente, con procesos irregulares. **Anellus** en forma de U, piloso distalmente. Vinculum redondeado anteriormente. Valvas con los márgenes paralelos con un diente terminal en la costa. Edeago cilíndrico, un poco curvado anteriormente, vesica provista de un diente quitinizado ancho, mitad del largo del edeago.

Organo genital femenino (Fig. 7d), conforme preparación microscópica VOB 130. Ostium amplio, membranoso. Antro amplio. Conducto de la bolsa en forma de embudo, angosto anteriormente. Inserción del conducto seminal en la bolsa, poco posterior al medio de ésta. Bolsa copulativa membranosa con punctuación marcada entre la inserción del conducto seminal y el conducto de la bolsa. **Signum** ausente.

Tipo. Holotipo macho y paratipo hembra en la Universidad de Costa Rica; paratipos macho y hembra en la Colección Nacional de Canadá. En la tesis del autor (8) se indica que el holotipo se encuentra en el Departamento de Zoología de la Universidad Federal do Paraná, Brasil. Como el ejemplar seleccionado fue destruido en el correo al ser enviado de Costa Rica a Brasil, se designa como holotipo al paratipo macho que se encuentra depositado en la Universidad de Costa Rica.

Distribución. (Fig. 13). Se encontraron larvas de esta especie en frutos de *C. odorata* en Chomes, Provincia de Puntarenas (D. Sliwa, col.); Santa Cruz, Provincia de Guanacaste (P. Grijpma y V. O. Becker col.); y en el Parque Nacional de Santa Rosa, Provincia de Guanacaste (V. O. Becker col.).

Observaciones. Esta especie no se ajusta bien a las características del género *Sematoneura,* tampoco a los otros géneros de Phycitinae americanos. Probablemente sea necesario crear un género nuevo para esta especie en el futuro. En *S. grijpmai* las venas M_2 y M_3 están unidas en la base (Fig. 7a) como en *Hypsipyla* (Fig. 4b), mientras que en *S. atrovenosella* son muy próximas pero independientes (Fig. 6a). Se decidió mantenerla en el género *Sematoneura* porque se aproxima más a este género que a cualquier otro de los Phycitinae americanos, y principalmente, debido a su comportamiento y biología que son semejantes a los de *S. atrovenosella*.

Larva. La larva de *S. grijpmai* es de color castaño grisáceo en el lado dorsal y crema en el lado ventral. Vive en los frutos de *C. odorata*, alimentándose de las semillas. Empupa en el eje del fruto de manera similar a *S. atrovenosella*. Antes de empupar construye un capullo fusiforme, blanco y resistente.

La larva de esta especie es muy similar a *S. atrovenosella* tanto en la forma y coloración como al comportamiento. Se puede separarlas por ser alopátricas y por estar asociadas con hospedantes distintos. *S. atrovenosella* fue encontrada en las partes altas del país, en el Bosque Húmedo y Muy Húmedo Montano Bajo, y está asociada a *C. tonduzii*. *S. grijpmai*, asociada con *C. odorata*, fue encontrada solamente en las Provincias de Puntarenas y Guanacaste en el Bosque Húmedo Premontano Tropical y Bosque Seco Tropical, transición a Húmedo, regiones con verano fuerte.

Hospedante. Las larvas de esta especie fueron encontradas en frutos de *C. odorata*. Los frutos, cuando atacados, tienen uno o más orificios circulares en la cáscara. Al abrirlos se pueden observar las semillas parcial o totalmente destruidas y el espacio interno del fruto ocupado por excremento del insecto y restos del fruto, unidos entre sí por hilos de seda blancos. Se observó que los frutos atacados abren antes que los sanos.

Chrysauginae

Humiphila paleolivacea Becker

Humiphila paleolivacea Becker (9).

Adulto. (Fig. 12 e, f). Largo del ala anterior del macho 8,5 ± 0,9 mm (7,0 a 9,7 mm; n = 9), de la hembra 10,1 ± 0,9 mm (8,6 a 12,0 mm; n = 16).

Palpos labiales oliváceos. Frente y vértex oliváceos. Antena amarillo pálido; olivácea en el lado dorsal hasta la mitad. Tórax oliváceo. Alas anteriores oliváceas; bandas antemediales y posmediales sinuosas, blanquecinas, cruzando transversalmente el ala; cilios gris. Alas posteriores fusco blanquecino. Patas blanquecinas internamente, mezclado con escamas de ápice oliváceo y negro en la parte externa; tibias medianas con escamas largas de extremidad negra. Abdomen amarillo pálido por encima, oliváceo por debajo.

Organo genital masculino (Fig. 8c, d), conforme preparaciones microscópicas VOB 142, 143. Uncus corto y ancho, obtusamente triangulado. **Tegumen** ancho. **Gnathos** con el ápice puntiagudo, arqueado dorsalmente, más largo que el uncus. **Transtilla** ausente. **Anellus** en forma de placa aproximadamente circular. Vinculum angosto, más ancho que largo. Valvas simples, anchas en la base, redondeadas en el ápice; sáculo no bien diferenciado. Edeago cilíndrico, curvado en la base; vesica sin modificaciones.

Organo genital femenino (Fig. 8e), conforme preparación miscroscópica VOB 141. Ovipositor setoso. Apófosis corta. Ostium amplio. Antro angosto. Conducto de la bolsa copulativa largo, con rugas transversales en la parte posterior; rugas longitudinales y esclerosado entre el conducto seminal y las rugas transversales; fuertemente curvado próximo a la inserción del conducto seminal; membranoso anteriormente. Bolsa copulativa piriforme, membranosa, con punctuación fina y marcada. **Signum** en forma de dos series opuestas con cinco o siete manchas quitinizadas.

Tipo. Holotipo macho en la Colección Becker, actualmente en el Departamento de Zoología de la Universidad Federal de Paraná, Brasil; dos paratipos hembras en la Colección Nacional de Canadá, dos paratipos, hembra y macho, en el Museo Nacional de los Estados Unidos, y en la Universidad de Costa Rica.

Localidad del tipo. Turrialba, Costa Rica 629 m de altitud.

Distribución (Fig. 13). Encontrado solamente en la localidad del tipo. Se buscaron larvas de esta especie en el mismo hospedante en otras regiones del país y no fue posible encontrarlas.

Observaciones. En la tesis del autor (8) esta especie está registrada como *Saprophila paleolivacea*, pero la Comisión de Nomenclatura Zoológica* informó que este nombre ya fue usado en Zoología como *Saprophilus* Streubel 1839.

Los adultos pierden su color original con mucha facilidad. Los ejemplares que se quedaron por cinco minutos o más en el frasco con éter sulfúrico cambiaron hacia el color amarillo pálido. Lo mismo sucedió con los ejemplares que fueron puestos en la cámara húmeda para suavisarse.

Pupa. La pupa es de color castaño amarillento en el lado dorsal y amarillo pálido en el lado ventral; lisa, con excepción de dos hileras de espinas en el lado dorsal. Las espinas están distribuidas por pares, un par para cada segmento, desde el primer hasta el octavo segmento abdominal. El par del primer segmento es minúsculo, los siguientes pares van alargándose hasta el tamaño máximo del par del sétimo segmento.

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* Comunicación personal.

Las pupas fueron encontradas en el suelo, entre la materia orgánica en descomposición, adentro de capullos de forma elíptica, con color de tierra. Los capullos (Fig. 11d) fueron construidos con partículas de hojas, tierra y excremento, unidos entre sí con hilos de seda.

Larva. La larva (Fig. 11c) de esta especie es alargada, cilíndrica, un poco aplastada dorso–ventralmente; gris, semihialina, un poco más clara en el lado ventral; cabeza y placa protorácica de color castaño; placa anal y pináculos negros. Es muy ágil. Al ser tocada brinca y se mueve rápidamente hacia atrás.

Las larvas fueron recolectadas sobre el tronco y en el suelo junto a éste, entre las hojas muertas y la materia orgánica en descomposición. Probablemente son saprófagas.

Ciclo de vida. Se observó que durante todo el año hay larvas de varios instar. Durante la época de sequía, muy fuerte en los meses de enero–marzo de 1973, la población bajó notablemente, volviendo a aumentar después del inicio de las lluvias. En el mes de junio eran muy abundantes y fue posible recolectar 58 larvas en un período de media hora.

El período entre la empupación y la emergencia fue aproximadamente nueve días. Los adultos emergieron dos meses después de la recolecta de larvas de aproximadamente 1 cm de largo.

Hospedante. Las larvas de esta especie viven en troncos de *C. guianensis,* debajo de la corteza muerta y en el suelo, junto al tronco, entre la materia orgánica en descomposición.

La corteza del tronco presenta excremento de larva saliendo de abajo de las placas muertas de la corteza que se desprende del tronco. Al remover estas placas se puede encontrar una o varias larvas por debajo de cada placa.

Se examinó, también, el tronco y el suelo cerca de otros árboles próximos a los árboles de *C. guianensis* pertenecientes a *S. macrophylla,* y a *Prioria copaifera* Griseb y *Pentaclethra macroloba* (Willd.) Kuntze (Leguminosae). No se encontró larvas en estos árboles.

Stenomidae

De las larvas que atacaban hojas de *C. odorata* en las muestras del Parque Nacional de Santa Rosa, y en Turrialba, se obtuvieron adultos de una especie del género *Antaeotricha.*

Antaeotricha ribbei Zeller

Antaeotricha ribbei Zeller (80), *Stenoma ribbei* (Zeller) Walsingham (74).

Adulto (Fig. 12 g). Largo del ala anterior 10,5 a 11,4 mm en el macho y 12,1 a 14,4 mm en la hembra.

Palpos labiales gris oscuro; segundo segmento pálido por debajo; tercer segmento blanquecino hacia el ápice. Fronte gris oscuro. Vértex gris oscuro anteriormente, blanco posteriormente. Antena gris oscuro; ciliada en el macho; filiforme, blanquecina por debajo en la primera mitad, en la hembra. Alas anteriores blancas; banda gris verduzco oscuro, ancha, en diagonal desde la base de la costa hasta el tercio distal del márgen posterior, con escamas blancas irregularmente distribuidas en la banda; una sombra gris marginada de amarillo ocráceo, exterior a la célula discal; una mancha blanca, pequeña, al final de la célula discal; cilios blancos. Alas posteriores gris; costa blanca; cilios gris. Patas anteriores gris oscuro, con brillo violáceo externamente, blancas internamente. Patas medias gris externamente, blancas internamente. Patas posteriores blancas; tarsos amarillo pálido por debajo. Abdomen blanco; gris oscuro por debajo en el macho.

Organo genital masculino (Fig. 9a, b, c) conforme preparaciones microscópicas VOB 148, 149. Uncus angosto y largo, curvado ventralmente. **Gnathos** corto, con expansión mediana en forma triangular. **Anellus** en forma de dos expansiones laterales espatuladas, con cerdas esparcidas. Vinculum redondeado, levemente cóncavo en la parte anterior. Valvas angostas, largas, setosas; proyección mediana interna gruesa, con numerosas setas gruesas, largas y curvadas, con extremidad bifurcada. Edeago corto, grueso en la extremidad distal; vesica con una placa quitinizada provista de espinas cortas.

Organo genital femenino (Fig. 9d) conforme preparación microscópica VOB 150. Ostium amplio. Antro amplio, esclerosado, transversalmente arrugado. Conducto de la bolsa angosto y membranoso anteriormente, ancho y esclerosado posteriormente; fuertemente curvado en la unión de la parte membranosa con la parte esclerosada. Inserción del conducto seminal en la parte posterior del conducto de la bolsa, cerca del antro. Bolsa copulativa elongada, membranosa; un poco arrugada. Signum en forma de placa circular, quitinizada, con espinas volteadas para el interior de la bolsa.

Tipo. En el Museo Staudinger (74); es probable que se encuentre actualmente en el Museo Británico.

Localidad del tipo. Chiriquí, Panamá (74).

Distribución (Fig. 13). En Costa Rica fue encontrada en Turrialba, Guayacán, y en el Parque Nacional de Santa Rosa, Guanacaste. Según Walsingham (74) también ha sido recolectado en México, Panamá y Bolivia.

Observación. La descripción e ilustración de los órganos genitales de esta especie es dada por primera vez en este trabajo.

Pupa. La pupa mide aproximadamente 10 mm de largo y 5 mm de diámetro; robusta, lisa; un poco aplastada dorso–ventralmente. Los segmentos abdominales son contraídos ventralmente. Es de color verde claro con manchas negras por todo el cuerpo (Fig. 11b).

La empupación ocurre entre dos hojuelas sobrepuestas, en las cuales se desarrolló la larva. Se queda fijada por el cremaster en los hilos de seda distribuidos por la larva en las superficies internas de las hojuelas y protegida por éstas.

Larva. La larva es de color verde con cabeza y placa protorácica castañas, alargada y aplastada dorso–ventralmente.

Viven en las hojas, adentro de una protección formada por dos hojuelas sobrepuestas y unidas entre sí por medio de hilos de seda. Las larvas pequeñas se alimentan del parénquima de las superficies contiguas de las hojuelas. En los últimos instar se alimentan del limbo de las hojuelas, con excepción de la vena principal y base de las venas secundarias. Son muy ágiles. Cuando tocadas, brincan hacia atrás y al suelo adonde es difícil capturarlas.

El comportamiento de esta especie es similar al de las larvas de *A. dissimilis* (5).

Ciclo de vida. Se observó que el período entre empupación y emergencia del adulto fue de ocho días aproximadamente. No fue posible establecer el ciclo completo porque no se pudo conseguir huevos o larvas en los primeros instar.

Es una especie que probablemente tiene generaciones continuas con huevos, larvas, pupas y adultos durante todo el año. Fueron recolectados adultos en enero, febrero, abril, junio, julio, agosto, setiembre, noviembre y diciembre; y larvas en enero, marzo, junio, julio y diciembre.

Hospedantes. Se encontraron larvas de esta especie en las hojas de *C. odorata* en el Parque Nacional de Santa Rosa, Guanacaste, y en Turrialba y en *S. macrophylla* en Turrialba. En Brasil se encontró *A. dissimilis,* especie afín a ésta, en hojas de *C. fissilis.*

Las plantas cuando atacadas presentan las hojuelas sobrepuestas, generalmente a los pares, unidas entre sí con hilos de seda. El ataque de las larvas jóvenes ocasiona lesiones en las superficies contiguas, causando muerte del parénquima foliar en las áreas atacadas. Cuando atacadas por larvas desarrolladas, el limbo de las hojuelas aparece parcialmente comido, con excepción de la vena principal y base de las venas secundarias.

Como la incidencia del ataque es muy bajo, esta especie no ocasiona daño considerable a la planta.

Parásitos. De una de las larvas de la muestra recolectada en el Parque Nacional de Santa Rosa, Guanacaste, salió un parásito del género *Agathis* (Hym., Braconidae).

Gracillariidae

Phyllocnistis meliacella Becker

Phyllocnistis meliacella Becker (9).

Adulto. Largo del ala anterior 2,0 a 2,2 mm en ambos sexos.

Palpos labiales blanco plateados. Fronte blanco plateado. Vértex cubierto con escamas largas, blanco plateado. Antena filiforme, blanco plateado, crema en el medio, con escamas de ápice gris; del largo del ala anterior. Tórax blanco plateado. Alas anteriores blanco plateado; una banda crema, marginada de negro a lo largo del medio del ala; cilios amarillo crema con puntas negras formando bandas. Alas posteriores blanco plateado. Patas blanco plateado; tarsos anteriores y medios con escamas gris oscuro. Abdomen blanco plateado.

Organo genital masculino (Fig. 10a, b) conforme preparaciones microscópicas VOB 145, 146. Uncus ausente. Valvas largas y angostas, con pocas cerdas esparcidas en el ápice. Tegumen corto. Vinculum elongado y redondeado. Edeago cilíndrico, curvado en la base.

Organo genital femenino (Fig. 10c), conforme preparación microscópica VOB 147. Conducto de la bolsa membranoso, angosto. Inserción del conducto de la bolsa en el tercio posterior lateral de la bolsa copulativa. Bolsa copulativa elongada, membranosa, con punctuación esparcida y marcada. Signum en forma de dos placas quitinizadas, una aproximadamente opuesta a la inserción del conducto de la bolsa, el otro en la parte anterior de la bolsa. Inserción del conducto seminal en la extremidad anterior de la bolsa copulativa.

Tipo, Holotipo macho y tres paratipos en el Museo Nacional de los Estados Unidos, Washington, dos paratipos en la Universidad de Costa Rica. En la tesis del autor (8) se indica que el holotipo se encuentra en el Departamento de Zoología de la Universidad Federal de Paraná, Brasil. Como el ejemplar seleccionado fue destruido en el correo al ser enviado de Costa Rica a Brasil, se designa como holotipo un paratipo macho que se encuentra en el Museo Nacional de los Estados Unidos.

Localidad del tipo. Turrialba, Costa Rica. 620 msnm.

Distribución (Fig. 13). Fue encontrado en Liberia, Guanacaste, y en Turrialba, Costa Rica.

Observaciones. Esta especie difiere de las otras especies de este género descritas para la región Neotropical. Bruner, Scaramuzza y Otero, según el Dr. De Santis*, relacionan en su trabajo una especie de *Phyllocnistis* que fue encontrada minando hojuelas de *S. macrophylla* en Cuba. Según la misma información, este material fue remitido al Dr. A. Busck, Smithsonian Institution,

* Comunicación personal.

Washington, D.C., para identificación. El Dr. A. Busck les informó que se trataba de una especie nueva, pero aparentemente no la describió.

Las ilustraciones de los órganos genitales de esta especie presentadas en este trabajo (Fig. 10) son un poco esquemáticas, principalmente la del órgano genital femenino. Esto se debe a la dificultad de dibujar estos órganos debido a sus dimensiones exiguas, y a la falta de equipo adecuado. Se procuró presentar, por lo menos las características más importantes, con la mayor fidelidad posible.

Pupa. La pupa de esta especie mide aproximadamente 3 mm de largo y 0,6 mm de diámetro; crema; parte ventral del tórax casi blanco; tercer y cuarto segmento abdominal con manchas gris oscuro en el lado dorsal. Cabeza con tres espinas quitinizadas en la frente; los dos laterales gruesos en la base, terminando en punta aguda; el central fino y curvado; negro. La extremidad de las alas llega hasta el quinto segmento abdominal; las antenas y el último par de patas llegan hasta el sexto segmento abdominal. Lado dorsal del abdomen con un conjunto de espinas pequeñas en cada segmento, dispuestos en forma de arco invertido; más dos pares de espinas, mayores que las anteriores, en cada segmento, las del par anterior más próximas entre sí y las posteriores más apartadas. Cada segmento con una cerda grande de cada lado. Ultimo segmento abdominal con una proyección de cada lado.

La empupación ocurre en la hojuela en la cual se desarrolló la larva, al final de la galería, por lo general en el borde de la hojuela.

Larva. La larva de esta especie es alargada y aplastada dorso–ventralmente; crema verduzco, hialina; con 5 mm de largo al final de su desarrollo. Es ápoda, sin pelos, con dos apéndices caudales laterales largos. Area ocelar como un pequeño punto negro; parte de las mandíbulas acastañadas.

Las larvas viven debajo de la epidermis del lado ventral de las hojuelas jóvenes, alimentándose de la savia. Al alimentarse construyen una galería irregular que se va alargando y aumentando de ancho a medida que la larva se desarrolla. La galería serpentea sobre todo el limbo foliar (Figs. 11 f, g), a veces cruza la vena principal y las secundarias de las hojuelas, terminando generalmente en el borde de la hojuela. Antes de empuparse la larva construye una cámara pupal doblando ventralmente el borde de la hojuela, uniendo el borde doblado al limbo por medio de una película de seda de color crema.

Generalmente hay sólo una larva por hojuela, raramente dos. En un muestreo sistemático en una parcela de *S. macrophylla* se recolectaron 101 hojuelas atacadas, 89 de ellas tenía una larva y 12 tenía dos.

Ciclo de vida. El ciclo de vida de esta especie tiene una duración de aproximadamente un mes.

En Turrialba se encontraron larvas durante todo el año. Como el ciclo es relativamente corto, comparando esta especie con las demás estudiadas, es probable que *P. meliacella* tenga generaciones continuas durante todo el año.

Hospedantes. Fueron encontradas larvas de esta especie en *C. angustifolia, C. odorata, C. tonduzii, S. macrophylla* y *S. mahagoni* en Turrialba y en *S. macrophylla* en Liberia, Guanacaste.

En Turrialba se encontraron también hojuelas de *C. guianensis* con síntomas similares al de los hospedantes anteriores cuando atacados por larvas de *P. meliacella* (Fig. 11 e).

Fueron marcadas algunas hojas que estaban recién saliendo para determinar la edad en que ocurre el ataque. Las hojas después de haber sido marcadas fueron inspeccionadas dos veces a la semana. A los veinte días se notó en algunas hojuelas marcadas larvas pequeñas que empezaban a construir su galería.

Parásitos. Las larvas de las muestras de *S. macrophylla* recolectadas en Turrialba, estaban parasitadas por una especie del género *Horismenus,* muy cerca de *H. cupreus* (Ashmead) (Hym., Eulophidae). El porcentaje de larvas parasitadas puede llegar hasta 50 por ciento. Una muestra de 53 pupas recolectadas en Turrialba, en hojas de *S. macrophylla* tenía 22 pupas parasitadas. Otra muestra de 8 pupas de la misma localidad tenía 5 pupas parasitadas. Todos los parásitos fueron identificados por De Santis* como *Horismenus* sp. De Santis* indicó que adultos de un parásito de *Phyllocnistis* sp. encontrados sobre *S. macropylla* en Cuba, fueron determinados por Gahan como *Horismenus* sp.

Conclusiones

Con base en el estudio y en la revisión de la literatura se concluye que en Costa Rica existe una especie del género *Carapa: C. guianensis* Aublet, tres del género *Cedrela: C. odorata* Linnaeus, *C. salvadorensis* Standley y *C. tonduzii* C. de Candolle, y una del género *Swietenia: S. macrophylla* King. Aunque la literatura mencione la existencia de *Cedrela angustifolia* Sessé & Moc., no fue posible encontrar árboles de esta especie durante la recolección de las muestras.

En Costa Rica, todas las especies de estos tres géneros, se distribuyen en regiones ecológicas distintas, con excepción de *Cedrela odorata.* La distribución de esta especie coincide con la de las demás, excepto con la de *C. tonduzii.*

De los microlepidópteros asociados con las especies de los géneros *Carapa, Cedrela* y *Swietenia,* en Costa Rica, *Hypsipyla grandella* (Zeller) y *H. ferrealis* (Hampson) causan el mayor daño a sus hospedantes.

H. grandella es la única especie de las incluidas en este estudio que puede ser encontrada en las áreas de distribución de todas las especies de hospedantes estudiadas.

El número de especies de microlepidópteros encontrados es bajo, en comparación con el número de hospedantes estudiados.

MICROLEPIDOPTEROS	HOSPEDANTES *Carapa guianensis*	*Carapa angustifolia*	*Cedrela odorata*	*Cedrela salvadorensis*	*Cedrela tonduzii*	*Swietenia macrophylla*	*Swietenia mahogani*
PYRALIDAE							
Hypsipyla grandella	X	X	X	X	X	X	X
Hypsipyla ferrealis	X						
Sematoneura atrovenosella					X		
Sematoneura grijpmai			X				
Humiphila paleolivacea	X						
STENOMIDAE							
Antaeotricha ribbei			X			X	
GRACILLARIIDAE							
Phyllocnistis meliacella	?	X	X		X	X	X

CUADRO 1. Microlepidópteros asociados con las especies de *Carapa, Cedrela* y *Swietenia* en Costa Rica.

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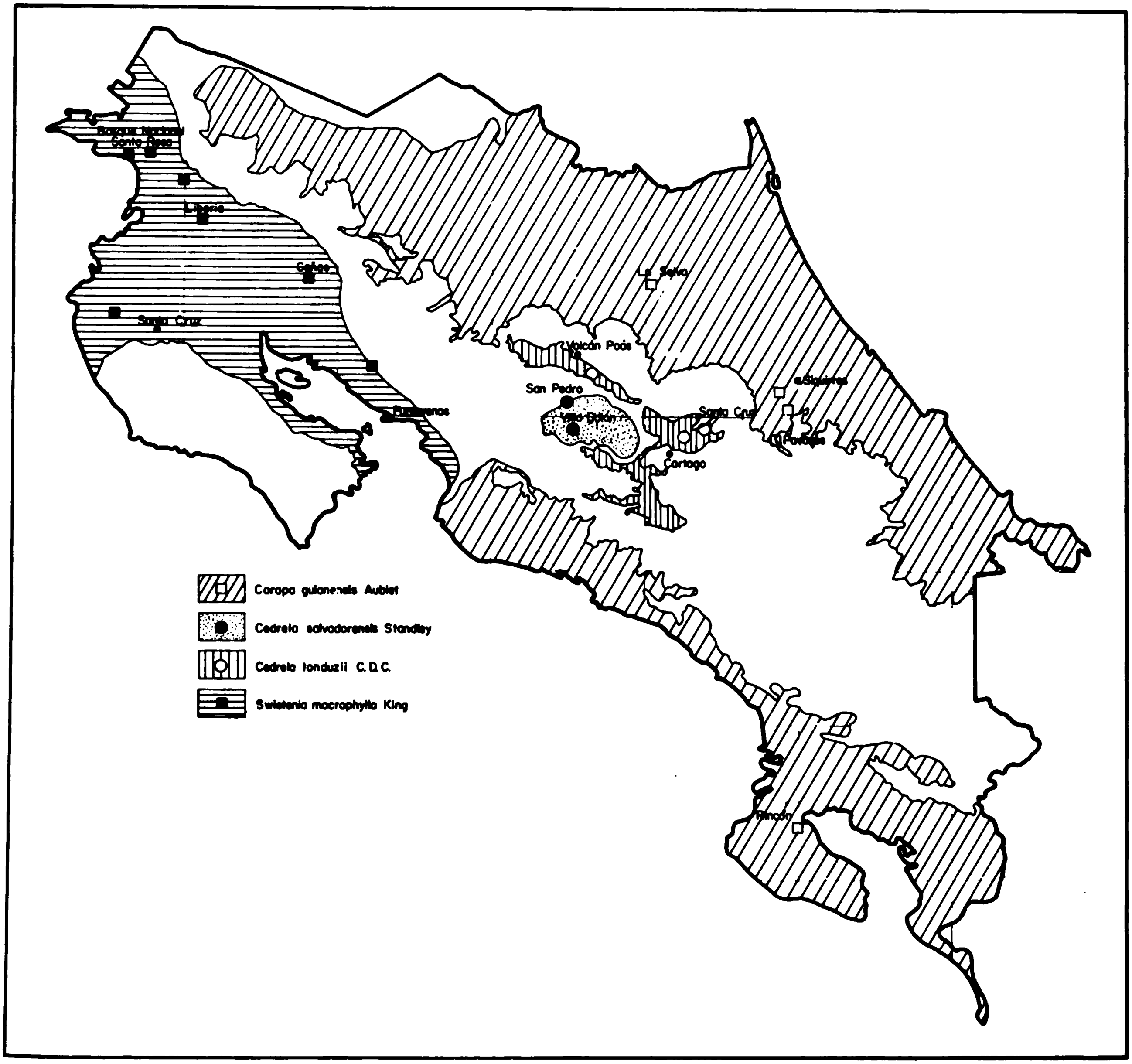

Fig. 1. Distribución geográfica de *Carapa guianensis*, *Cedrela salvadorensis*, *C. tonduzzi* y *Swietenia macrophylla* en Costa Rica.

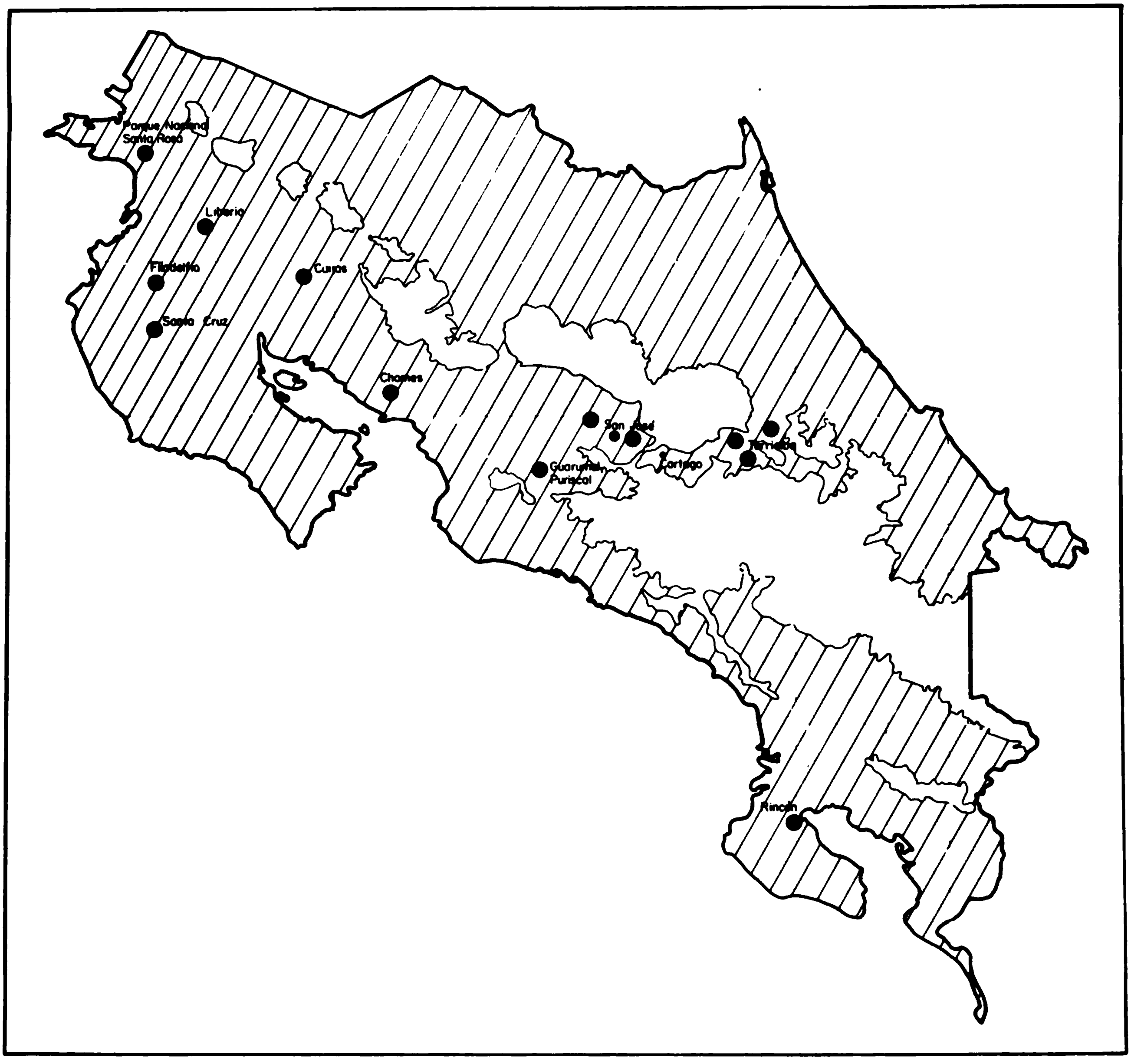

Fig. 2. Distribución geográfica de *Cedrela odorata* en Costa Rica.

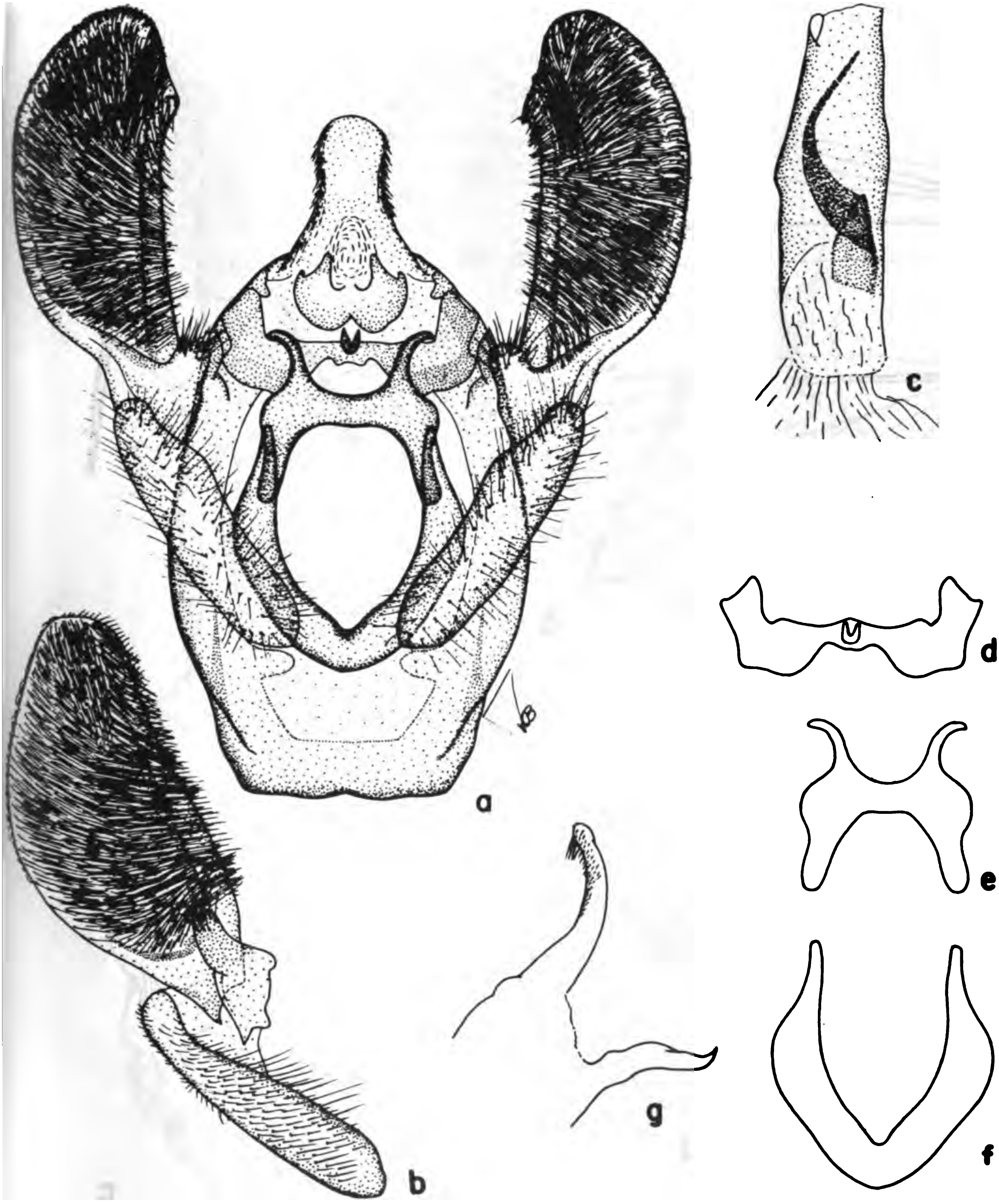

Fig. 3. *Hypsipyla grandella*: a) órgano genital masculino sin edeago; b) valva izquierda; c) edeago; d) gnathos; e) transtilla; f) anellus; g) vista lateral del uncus y gnathos.

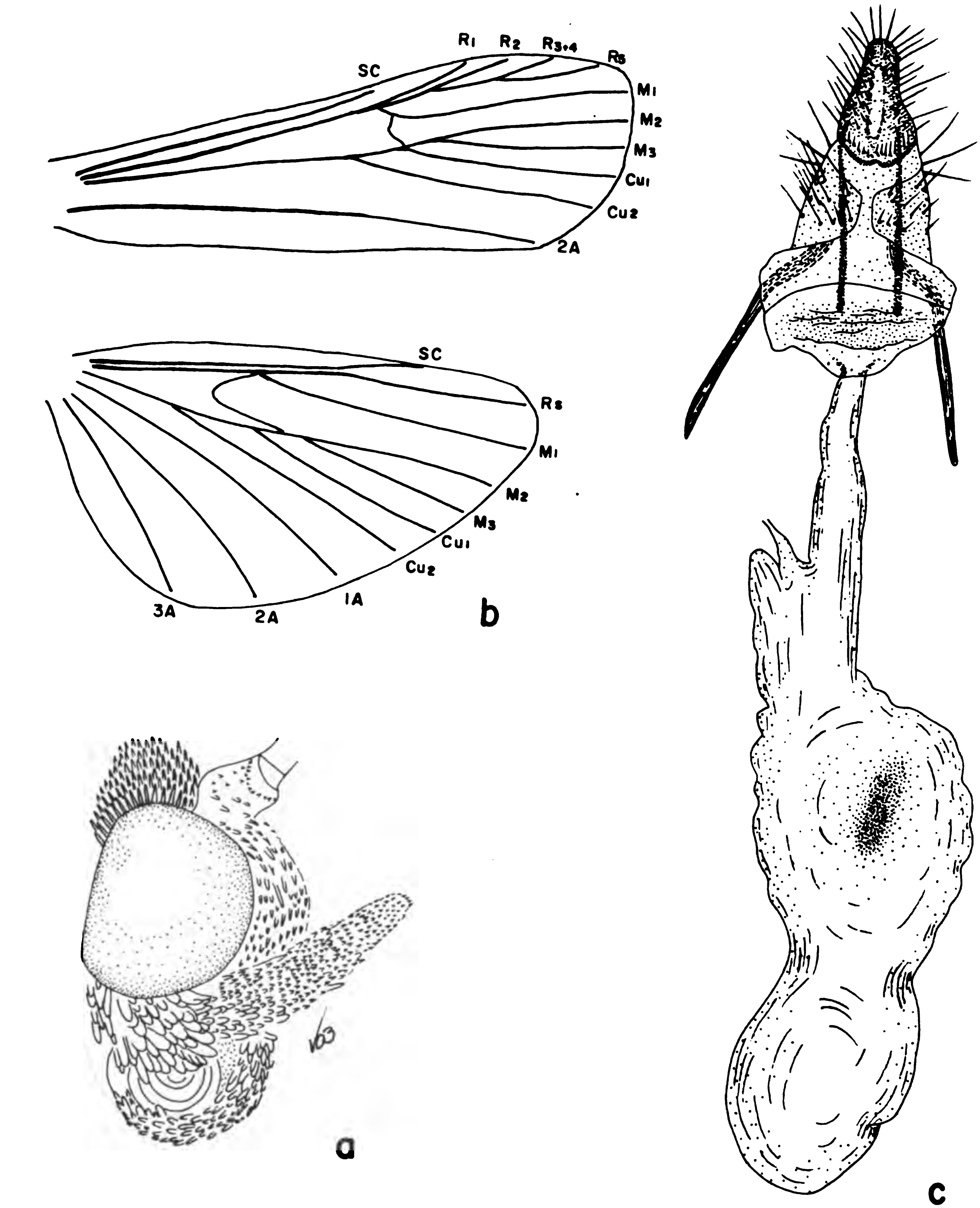

Fig. 4. *Hypsipyla grandella:* a) cabeza; b) venación de las alas; c) órgano genital femenino.

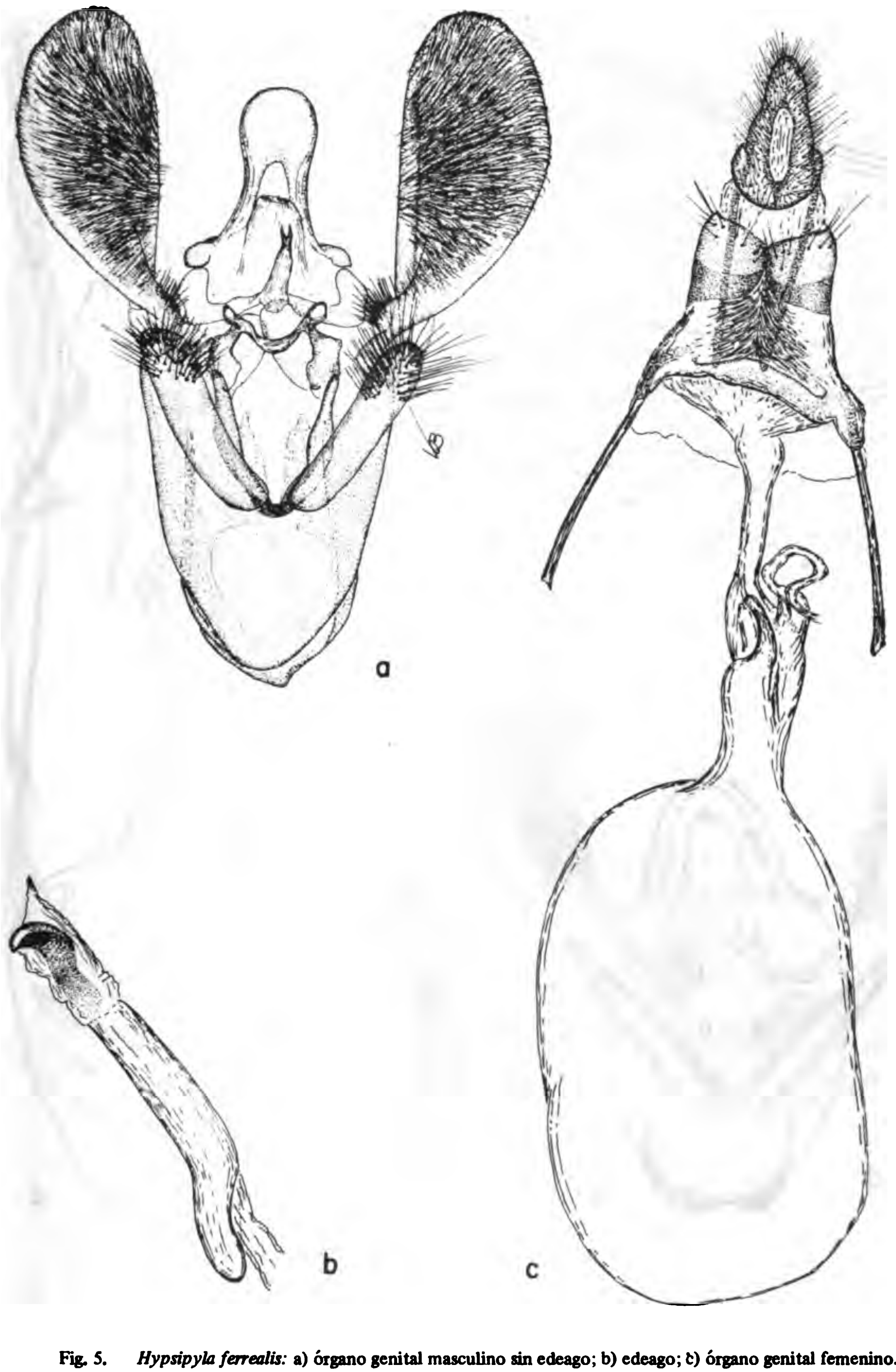

Fig. 5. ***Hypsipyla ferrealis:*** **a) órgano genital masculino sin edeago; b) edeago; c) órgano genital femenino.**

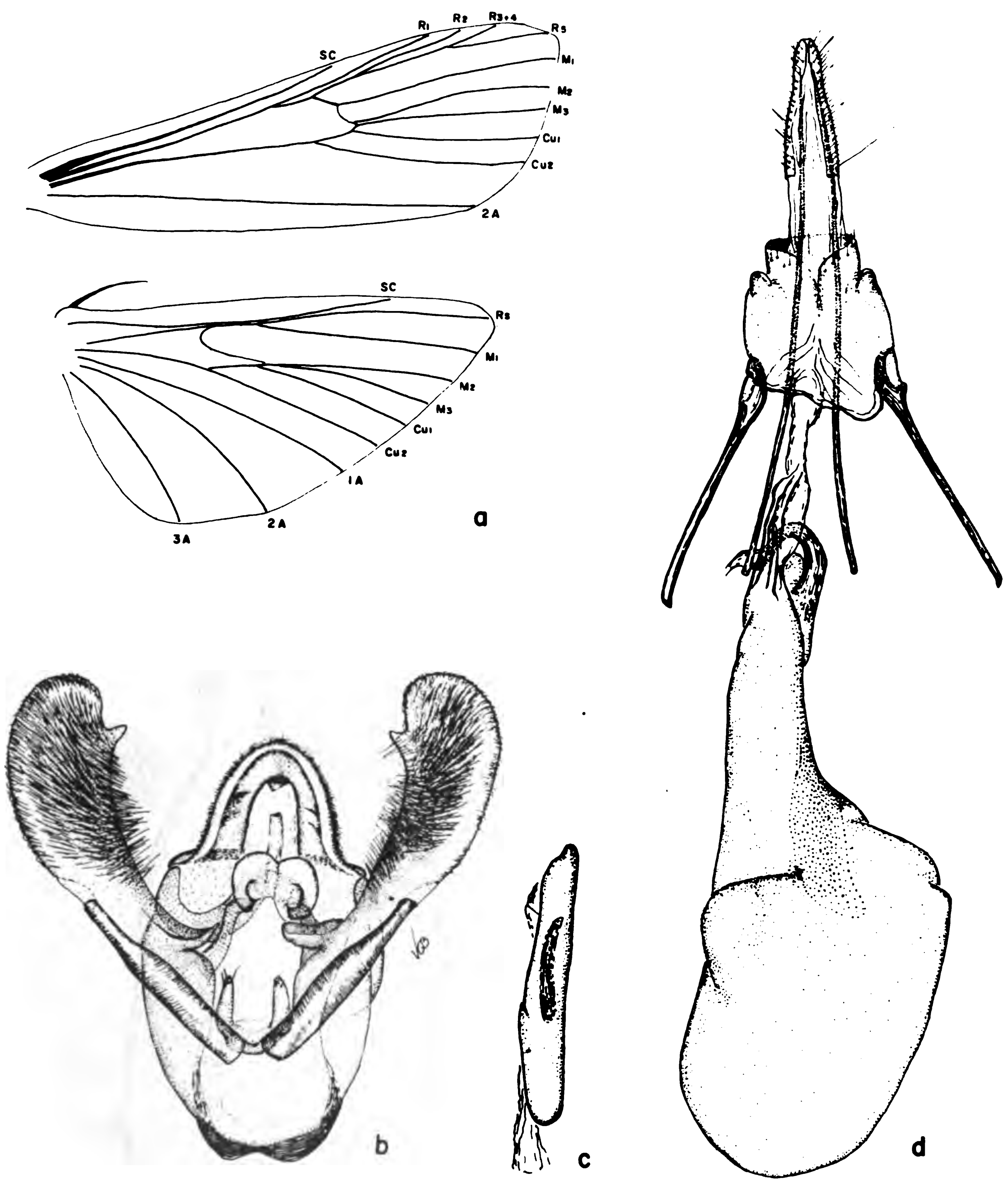

Fig. 6. *Sematoneura atrovenosella:* a) venación de las alas; b) órgano genital masculino sin edeago; c) edeago; d) órgano genital femenino.

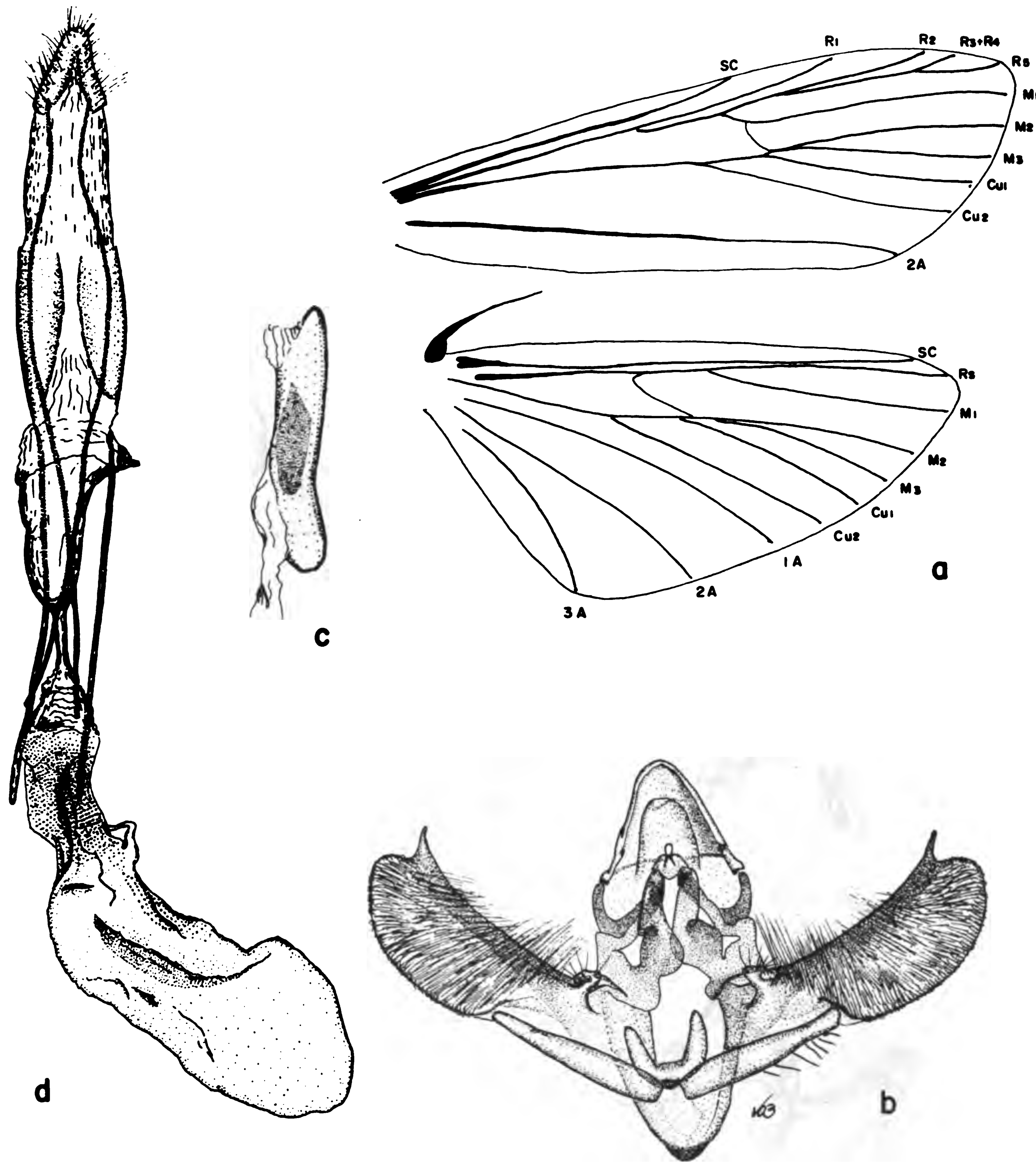

Fig. 7. *Sematoneura grijpmai:* a) venación de las alas; b) órgano genital masculino sin edeago; c) edeago; d) órgano genital femenino.

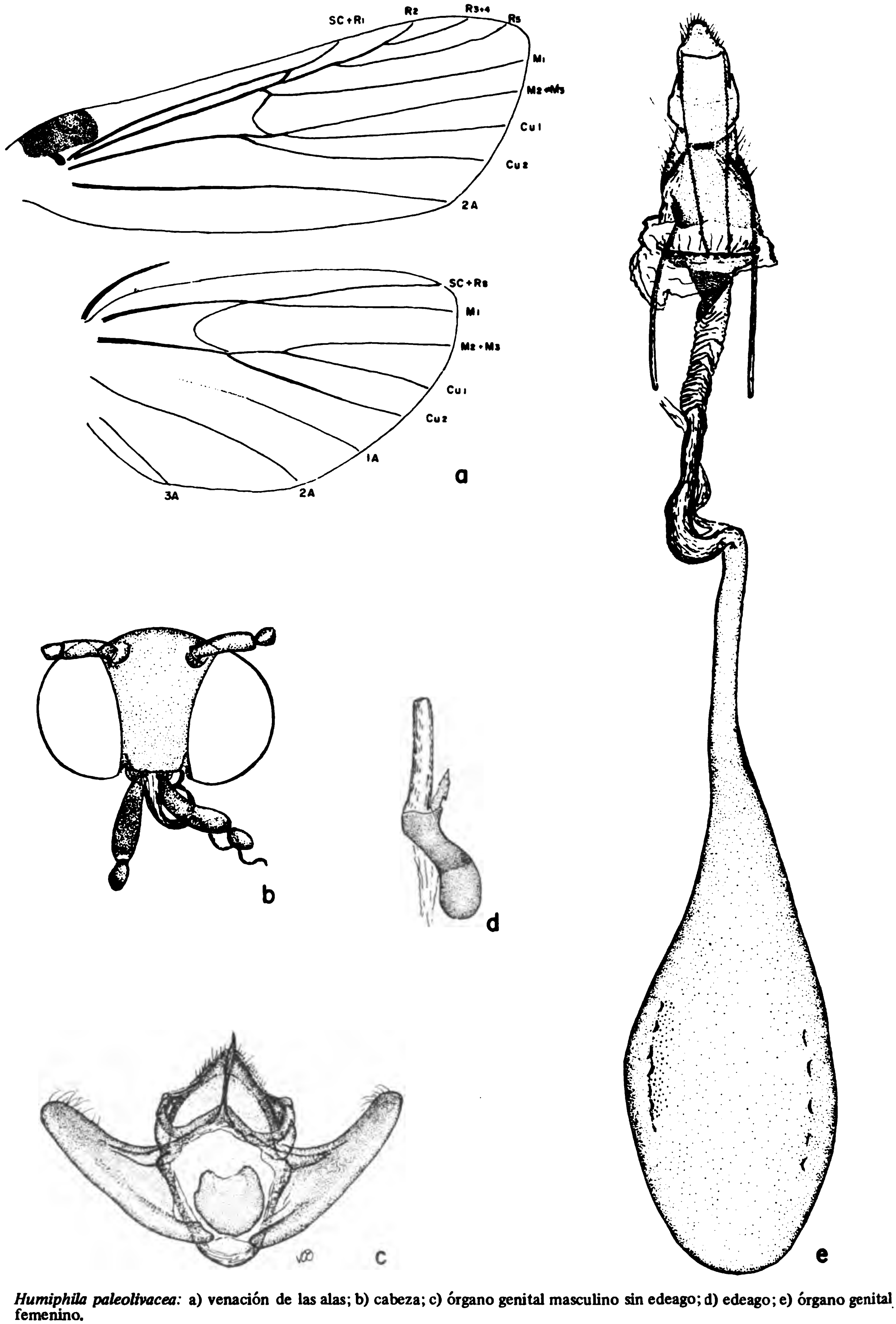

Fig. 8. ***Humiphila paleolivacea:*** **a) venación de las alas; b) cabeza; c) órgano genital masculino sin edeago; d) edeago; e) órgano genital femenino.**

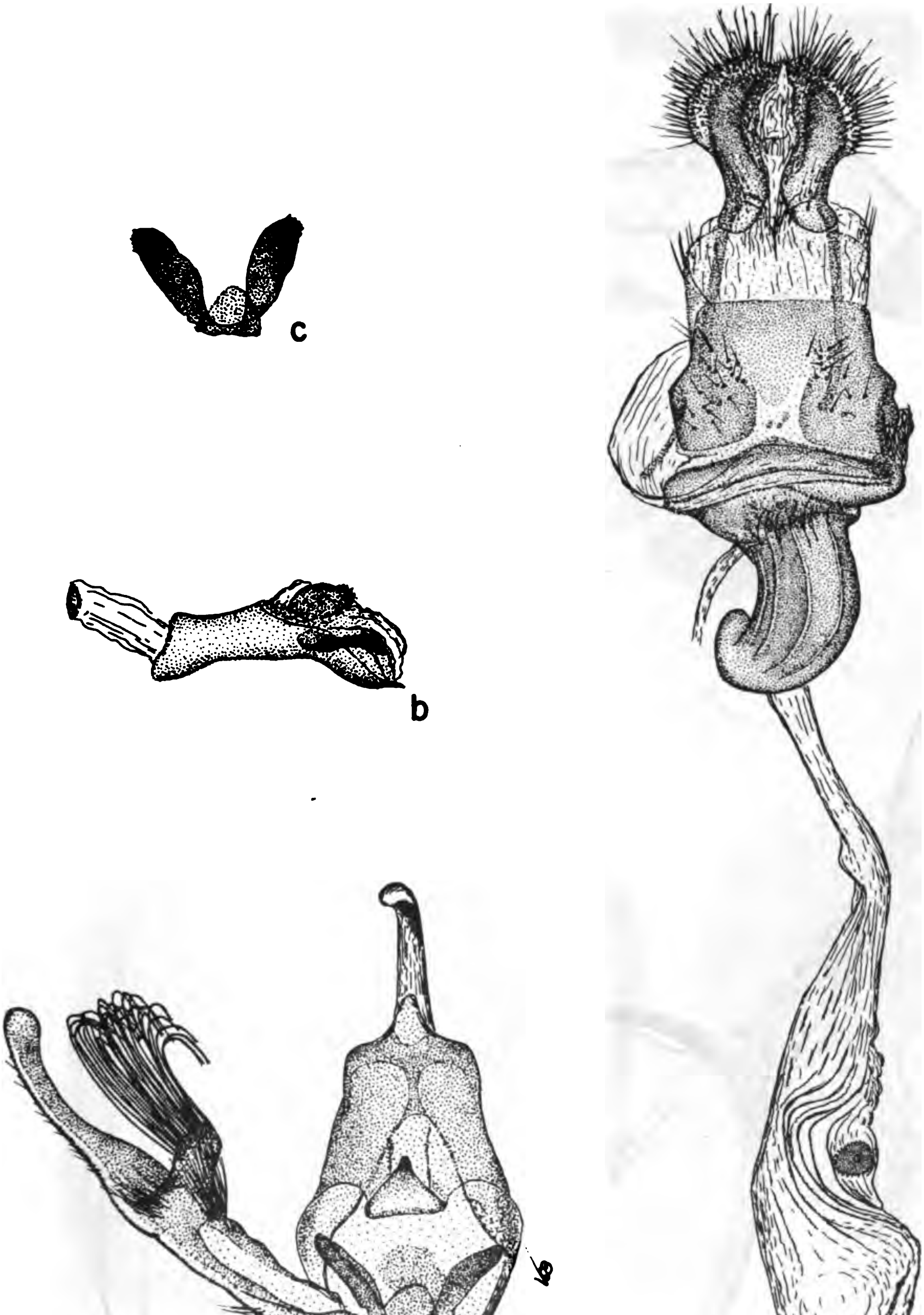

Fig. 9. *Antaeotricha ribbei:* a) órgano genital masculino sin edeago; b) edeago; c) anellus; d) órgano genital femenino.

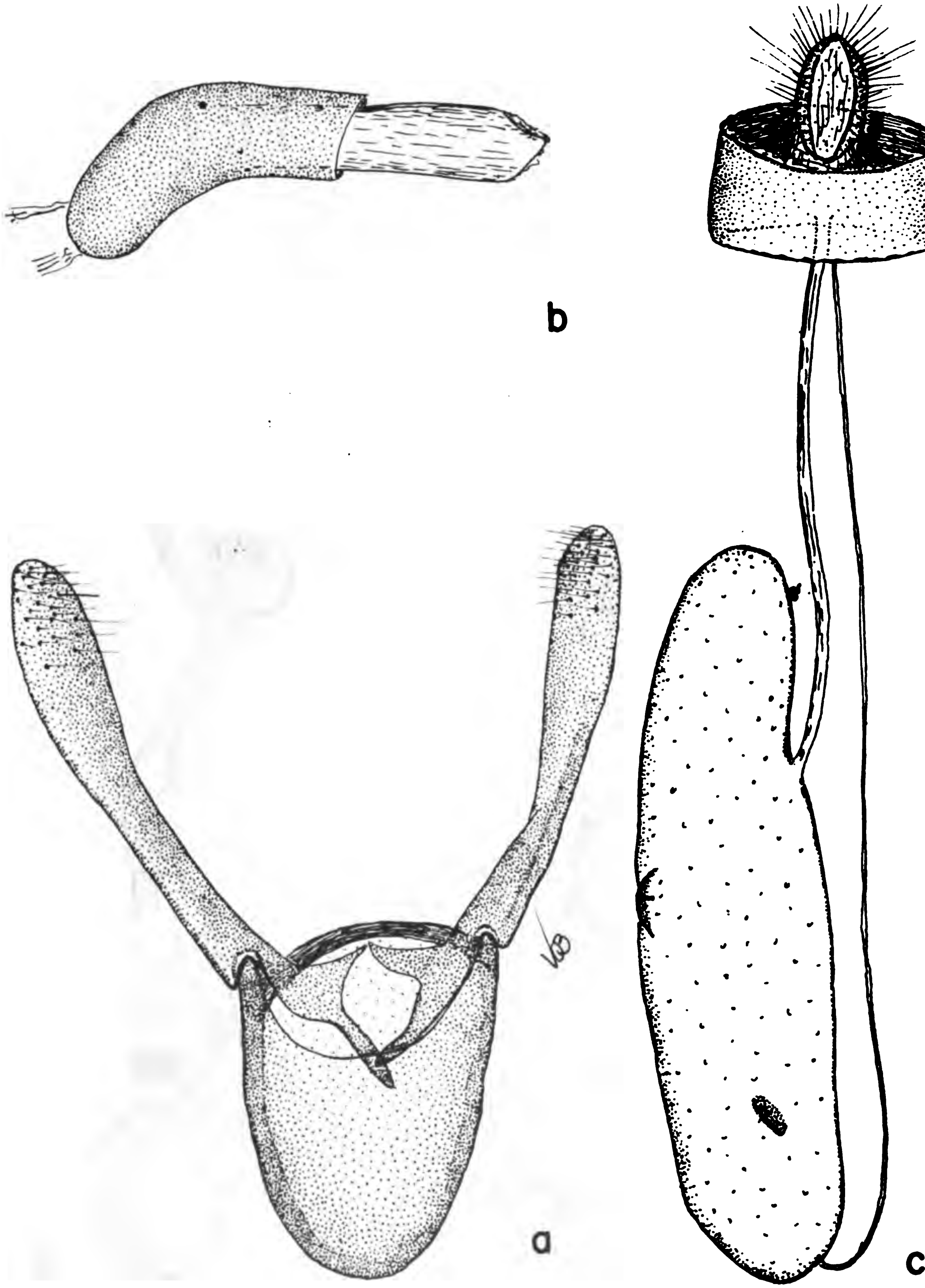

Fig. 10. *Phyllocnistis meliacella:* a) órgano genital masculino sin edeago; b) edeago; c) órgano genital femenino.

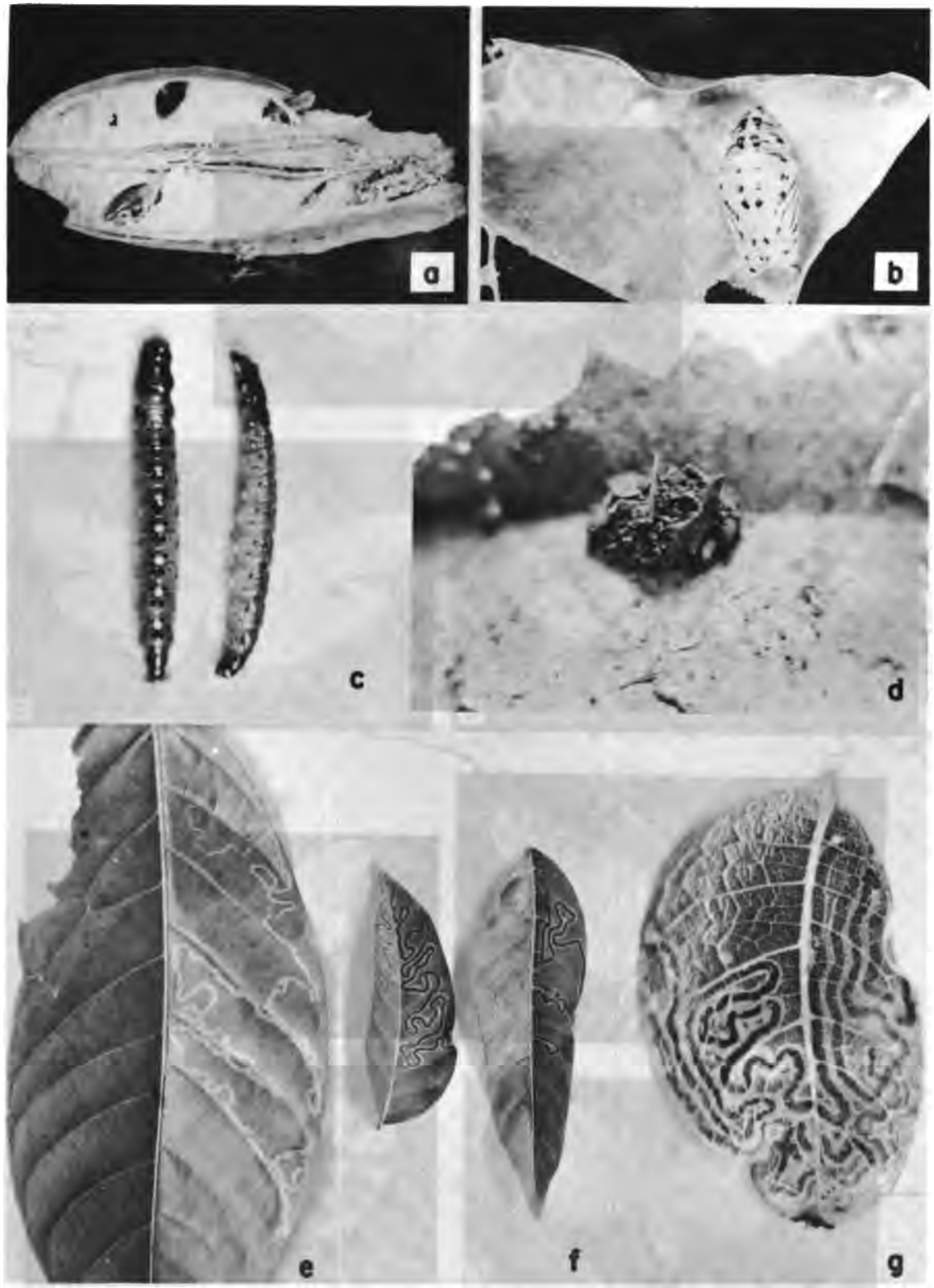

Fig. 11. a) Fruto de *Cedrela odorata* atacado por *Sematoneura grijpmai;* b) pupa de *Antaeotricha ribbei;* c) larvas de *Humiphila paleolivacea;* d) capullo de *H. paleolivacea;* e), f) y g) hojuelas de *Carapa guianensis, Cedrela odorata* y *Swietenia macrophylla* minadas por larvas de *Phyllocnistis meliacella.*

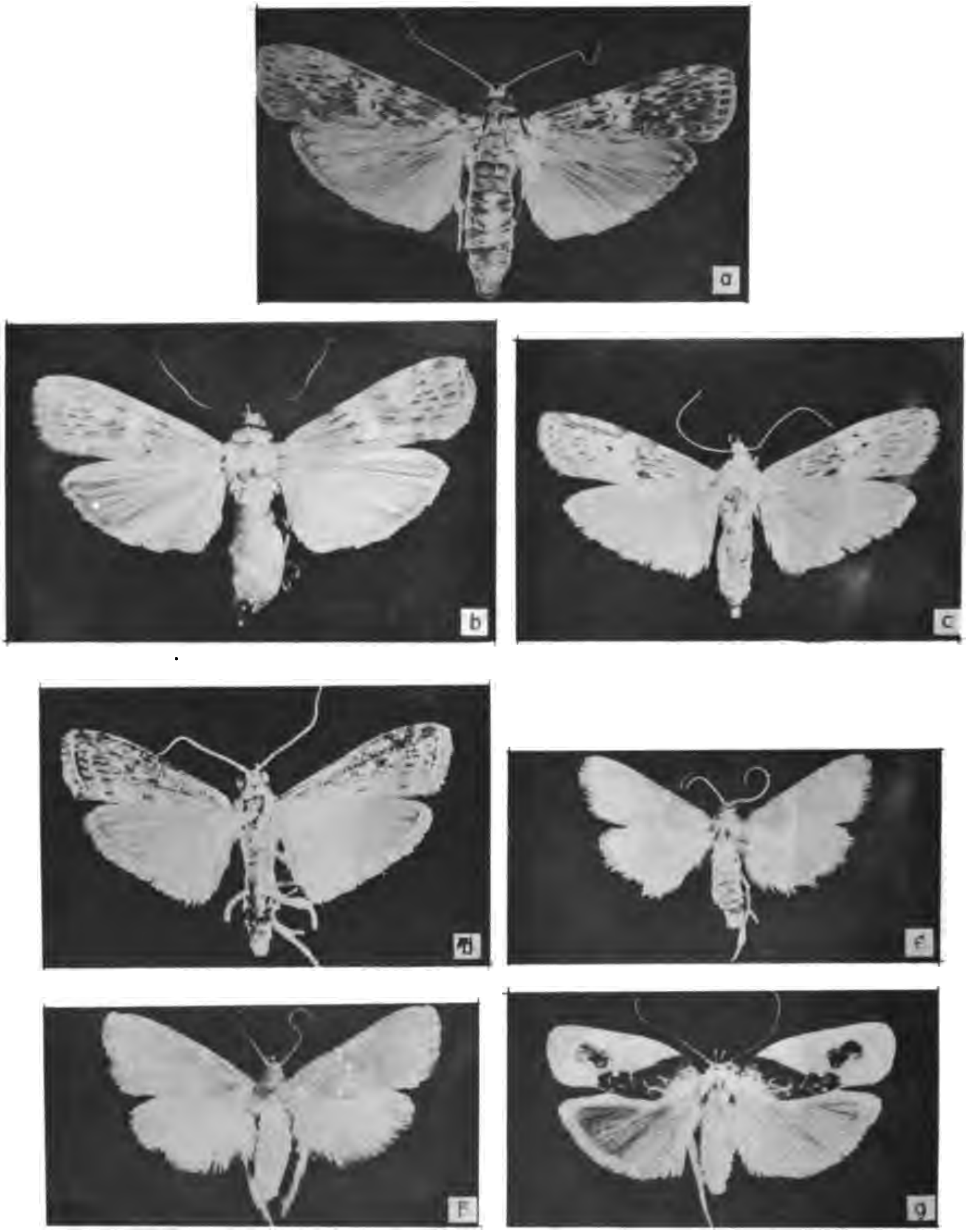

Fig. 12. a) Hembra de *Hypsipyla grandella* (Zeller); b) hembra de *H. ferrealis* (Hampson); c) hembra de *Sematoneura atrovenosella* Ragonot; d) macho de *S. grijpmai* Becker; e) y f) macho y hembra de *Humiphila paleolivacea* Becker; g) hembra de *Antaeotricha ribbei* Zeller.

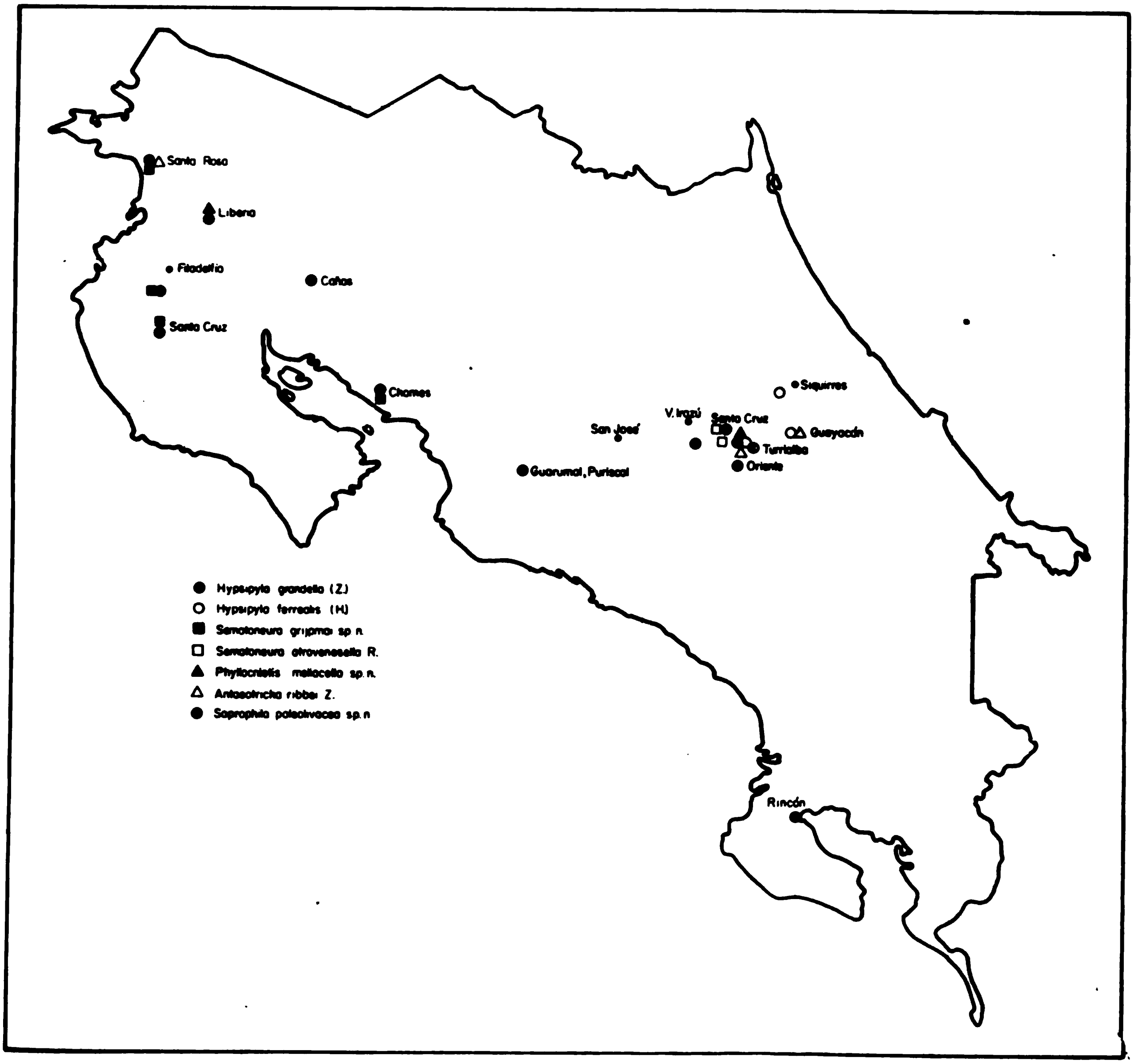

Fig. 13. Distribución de los microlepidópteros asociados con *Carapa, Cedrela* y *Swietenia* en Costa Rica.

BIOLOGICAL AND CHEMICAL SCREENING FOR THE BASIS OF RESISTANCE OF *TOONA CILIATA* M. J. ROEM. VAR *AUSTRALIS**

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COMPENDIO

La presente investigación fue dirigida a estudiar la base química de la inmunidad del cedro australiano (*Toona ciliata* var. *australis*) al barrenador de las Meliáceas, *Hypsipyla grandella*. En plantaciones de campo se constató que el barrenador es atraído y oviposita sobre el cedro australiano pero que las larvas eclosionadas mueren cuando penetran en los tejidos de esta especie. Experimentos con injertos de *Cedrela odorata* L. sobre un patrón de *Toona* resultaron en inmunidad de *Cedrela* al barrenador. En pruebas empleando compuestos de hojas extraídas por una serie de solventes orgánicos mezclados con los ingredientes de dieta artificial para *Hypsipyla* se obtuvo una mortalidad de 50, 53, 42 por ciento de las larvas del barrenador con las extracciones de acetona, agua y hexano respectivamente.

Utilizando la técnica de cromatografía de capa fina, se estableció que existen probablemente dos componentes tóxicos y polares en la extracción acuosa de hojas del cedro australiano, uno de los cuales es altamente polar. En cantidades equivalentes a 50 mg de hojas de *Toona*, estos compuestos cuando inyectados oralmente en larvas del sexto instar de *H. grandella* causaron una mortalidad de 80 por ciento de las larvas. En una dosis equivalente a 150 mg de hojas de *Toona* la fracción purificada del compuesto más polar, resultó en una mortalidad de 100 por ciento de las larvas tratadas.

Los autores

Introduction

The Australian red cedar (*Toona ciliata* var. *australis;* Meliaceae) was introduced in November 1967 in Costa Rica, for inclusion in the tree species trials of the Department of Tropical Forest Sciences of IICA–CTEI. In May 1968, the first field plot was established which soon showed excellent growth and complete absence of attacks of *Hypsipyla grandella* (Zeller). This absence of attacks was particularly striking since neighboring trial plots of *Cedrela odorata* L. were heavily attacked by the shootborer. The undisturbed growth and the fact that the leaves and young shoots of the Australian cedar, in contrast to the native *C. odorata*, did not produce any particular smell led to the hypothesis that *H. grandella* might orient itself towards native hosts by means of chemoreception, but that the shootborer would be unable to detect the possibly different volatile compounds of young leaves and shoots of the exotic Australian cedar (5, 6).

In May 1971 however, eggs of *H. grandella* were found on the Australian cedar during a field survey. This finding resulted in a series of investigations on the resistance of *T. ciliata* var. *australis* against *H. grandella* attacks, which are summarized below under confirmation, grafting and toxicity trials.

Materials and Methods

Resistance confirmation trials

During field inspections in a mixed trial plot consisting of *C. odorata*, *Pinus massoniana* Lamb. and *T. ciliata* var. *australis*, 100 *H. grandella* eggs were found on stems of five, approximately 3 m high *Toona* trees. The eggs were removed from the bark of the trees for laboratory investigations.

Larvae emerging from the eggs were divided into two groups and placed in sealed plastic boxes containing leaves of *C. odorata* and *T. ciliata* var. *australis* respectively. Feeding material was renewed whenever necessary, e.g. from once every five days during the first and

* Received for publication 18 September 1974.

second instars to once every two days in the last instar of the *H. grandella* larvae.

Observations with respect to feeding, mortality, pupation and emergence were made and two male and female adults were dissected for species identification.

Average maximum and minimum temperatures in the laboratory were 30.3°C and 22.0°C respectively; average maximum and minimum relative humidities were 68.9 and 50.5 per cent.

Grafting trials

In order to determine if chemical compounds present in *Toona* could be translocated into *C. odorata* and promote resistance against *H. grandella*, the native *C. odorata* was grafted on stems of *Toona*. The mortality of first instar larvae placed on these grafts was compared with that of larvae placed on seedlings of *Toona* and *Cedrela*.

The experiment was conducted in a greenhouse where the average maximum and minimum temperatures were 31.4°C and 19.0°C; the average maximum and minimum relative humidities were 96.2 and 78.4 per cent, respectively.

Larvae of *H. grandella* reared on *Cedrela* plant material were placed onto the middle of the stem of ten grafts of *C. odorata* on *Toona*, ten *Toona* and ten *Cedrela* plants of approximately 45 cm height. Each plant received two groups of four larvae, the second group being released three days after the first to enhance survival of the individuals.

The plants were checked for small ants and spiders which predate upon first instar *H. grandella* larvae. Stickum–Special* was put around the stems at the height of the grafting to impede any future predation and to prevent larvae from boring into the *Toona* stem.

Survival of the larvae was observed by daily removal of fresh frass from the boring holes. If fresh frass was not produced during two days and no additional boring holes were noted, the larvae were considered dead.

An additional experiment was conducted under laboratory conditions as a control for the greenhouse grafting trial. First instar *H. grandella* larvae were reared on leaflets obtained from potted plants of *C. odorata*, *T. ciliata* var. *australis* and of *C. odorata* grafted on stems of *T. ciliata* var. *australis* respectively. The larvae (100/treatment) were subdivided into groups of ten and placed on the leaflets in small, sealed plastic boxes. Survival of the larvae was checked daily under a microscope.

Toxicity tests

As the previously described tests strongly suggested the existence of chemical toxicity in the plant tissue of the Australian cedar, further experiments were conducted to isolate the responsible fraction(s).

Young shoots and leaves of *T. ciliata* var. *australis* and *C. odorata* (for control) were subjected to solvent extraction and subsequent thin layer chromatography (TLC) in order to produce chemical fractions suitable for bioassay. The solvents employed in the extractions; e.g. hexane, ethyl ether, acetone, benzene, chloroform, methanol and distilled water; were selected to include a wide range of solubility parameters, polarities and hydrogen bonding capabilities (4, 7). All solvents were reagent grade except acetone and water which were distilled prior to use. Hydrophilic solvents such as acetone, benzene and methanol were dried during the extractions with anhydrous sodium sulfate so that their solvent properties would not change as result of hydration from the water in the leaves.

Extracts of *Toona* and *Cedrela* leaves (20 g) were obtained by macerating the foliage suspended in solvent (75 ml) for approximately five minutes in an Osterizer blender. The fibrous mass was soaked overnight and subsequently filtered on the foraminous plate of a Buchner funnel. Frequently further clarification with analytical grade filter aid was required. The resulting clear extracts were refrigerated until use.

In the first series of trials, each separate extract (75 ml) was mixed with the dry components of synthetic diet (64 g) which consisted of commonly used ingredients (1) except for agar and its corresponding water component. These were omitted since they did not add to the nutritional value of the diet and only would dilute any possible toxic compound present in the fractions. The mixtures were placed in an oven (45°C) for two hours to allow the solvents to evaporate. Thereafter the liquid components of the synthetic diet were added and thoroughly mixed with the dry parts which now contained the extractable chemical compounds of *Toona* (or *Cedrela* in case of the controls).

TABLE 1. Characteristics of observable bands of aqueous *Toona* extracts on cellulose TLC–plates.

Sample Code No.	Developing solvent	Rf values	Characteristics of observable bands
T_{33}	Ethyl acetate-Methanol (1:1)	0. 2-0.3	Fluorescent at 254 nm
		0. 8-0.9	Green
		0.95-1.0	Fluorescent at 254 nm
T_{34}	Ethyl acetate-Methanol	0. 2-0.3	Fluorescent at 254 nm
		0. 8-0.9	Green
		0.93-1.0	Fluorescent at 254 nm
T_{34}-TLC-3	Benzene-Ethyl acetate (3:1)	0.92-1.0	Light green

When water extracts of *Toona* or *Cedrela* were mixed with the dry components of the diet, an equal volume of water was omitted from the normal diet composition, as it was virtually impossible to evaporate the water component of these fractions under the humid ambient conditions of Turrialba.

The concentration of the extracted chemicals was equivalent to approximately one gram of *Toona* or *Cedrela* leaves per 8 g of artificial diet. However, the

* Michel and Pelton Co., Oakland, California.

extraction process is not 100 per cent efficient nor is it assured that the active components of *Toona* are chemically stable. Moreover, some chemical interaction (e.g. oxidation or reduction) between the artificial diet components and the *Toona* extract could result in inactivation of the originally toxic *Toona* compound(s). As the chemical constituents of the *Toona* and *Cedrela* fractions might also be influenced by the acidity of the artificial diet ingredients and consequently influence mortality of the second instar larvae employed in these experiments, the pH of samples of these diets was determined and compared with that of the commonly used rearing medium. For this purpose diet samples (2 g) were diluted with distilled water (10 ml) and the acidity of the suspension measured with a Beckman Zeromatic pH meter.

Second instar larvae (10–20/treatment) were placed individually in small rearing bottles containing the diet ingredients mixed with the extractives from one of the methanol, benzene, chloroform, hexane, acetone, water and ethyl ether fractions of *Toona* and *Cedrela*. Development of the larvae, mortality and time needed to pupate and emerge were recorded.

The bioassay of these diets yielded ambiguous results with respect to the toxicity of the *Toona* compounds obtained; nonetheless some indication was provided of which extracts contained possible toxicants.

At this point, additional force–feeding tests (11) were employed: In these experiments sixth instar larvae of *H. grandella* were force–fed with concentrated water and hexane extracts of *Toona leaves* (30 µl/larva). The higher concentration was prepared by extraction of a larger amount of *Toona leaves* (40 g/75 ml solvent). Water and hexane extracts of *Cedrela* obtained in identical procedures, were used as controls in these tests.

In order to avoid any effects of the organic solvent on the larvae an aliquot of the hexane extract (13 ml) was evaporated onto the surface of distilled water (2 ml) containing a few drops of a surfactant Tween–20.* Prior to force–feeding the larvae, the extract film was emulsified in the water, so that it could be readily injected.

An 'Agla' micrometer syringe, Burroughs Wellcome & Co., England and a micrometer, Shardlow Micrometers Ltd., Sheffield, England, in combination with a blunted Yale No. 27 hypodermic needle were used in these trials.

For each extract, ten to fifteen sixth instar larvae were individually anaesthetized prior to treatment by placing them in a CO_2 chamber for approximately 60 seconds. Subsequent force–feeding was facilitated by the use of a stereo microscope to ensure elimination of any larvae that might have been injured during the oral injection. Needle penetration was approximately 3–4 mm into the mouth parts. After treatment, the force–fed larvae were placed in glass jars and mortality during larval and pupal stages was recorded.

Since the aqueous extract of *Toona* proved to be highly toxic, further separation of its components was obtained through thin layer chromatography (TLC) (12).

The TLC plates (Cellulose 300 G with $CaSO_4$ binder, Macherey, Nagel and Co.) were prepared using a Desaga type spreader (12) and dried in an oven at 105°C for 15 minutes. Three plates (20 x 20 cm) were used to separate aliquots of an aqueous extract (T-33) equivalent to 2 g of *Toona* per plate. The developing solvent and the characteristics of the resultant observable bands (by visual or ultraviolet light) are shown in Table 1. Three bands from each of the developed plates were removed. The first contained the starting band and the thin fluorescent band (254 nm) (Rf = 0.0 – 0.3); the second band comprised the middle region with no identifying characteristics (Rf = 0.3 – 0.7) and the third contained the rest of the developed region (Rf = 0.7 – 1.0) including a green band and fluorescent band (254 nm). The isolated TLC samples were extracted with water (10 ml) for 24 hours, concentrated appropriately and the resultant aqueous extracts force–fed to sixth instar *H. grandella* larvae (10–20) as previously described using calculated doses of 50 mg of original foliage per larvae.

In the ensuing bioassay the third band was identified as being active; thus, the separation was repeated on a fresh extract (T–34) with a more polar ethyl acetate–methanol solvent (2:3) in an effort to concentrate the mobile components into the final region (Rf = 0.8 – 1.0). This band was then removed and rechromatographed with a less polar benzene–ethyl acetate (3:1) solvent and separated again into three sections consisting of the starting band, the middle (Rf = 0.2 – 0.8) and the final region (Rf = 0.8 – 1.0). These sections were again removed from the glass plates, extracted with water, concentrated as before and force–fed to larvae in doses equivalent to 150 mg of original foliage per larvae. It was expected that the less polar developing solvent would effect a better separation of the mobile components by removing the non–polar constituents from the sample.

Results and discussion

Resistance confirmation trials

These experiments resulted in a 100 per cent mortality of all first instar *H. grandella* larvae that were placed on a *Toona* leaf diet. Actual boring into the leaflets was observed and excrements were found on the leaflets. Fifty per cent of these larvae died within 24 hours, 42 per cent died within 48 hours and the remainder within 72 hours. The larvae readily accept the offered *Toona* as food and are apparently unable to detect the toxic properties of the plant material prior to feeding. Several dead first instar larvae could be found with their heads still sticking in the boring holes. This prompt death suggested the existence of a toxic compound rather than absence of essential food components.

Seventy two per cent of the first instar larvae placed on *Cedrela* leaflets were reared through to the adult stage; the remainder died due to cannibalism and bacterial infections during the rearing. The dissected specimens were identified as *Hypsipyla grandella* (Zeller).

Since *H. grandella* is attracted to the Australian cedar, oviposits on it and its larvae are stimulated to feed on the plant material notwithstanding its toxicity, the tree acts as a natural trap for the insect. Therefore, it might be conceivable to employ the Australian cedar in

* ICI America Inc., Atlas Chemical Division. Wilmington, Delaware.

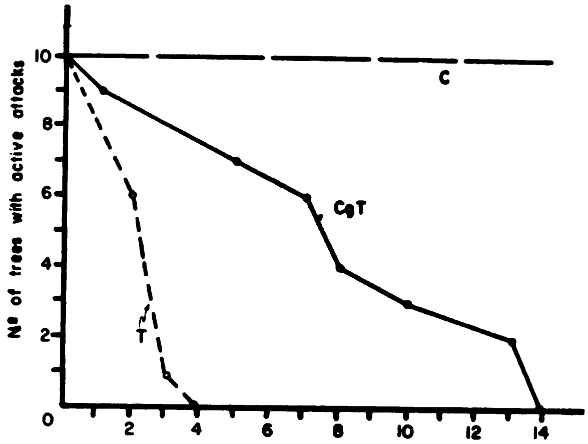

Fig. 1. Time dependence of boring activity of first instar *H. grandella* larvae in plants of *C. odorata* (C), of *T. ciliata* var. *australis* (T) and of *C. odorata* grafted on *T. ciliata* var. *australis* (CgT).

plantations where this exotic is mixed with native Meliaceae, so as to reduce the *Hypsipyla* population and keep its damage below a certain threshold. However, several uncertainties ought to be considered before such planting schemes are put into practice. Firstly, development of resistance of *H. grandella* against *Toona* could well be promoted if larvae which started to feed on this tree species might continue to feed on *Cedrela* or *Swietenia* e.g. by migration from *Toona* trees. Other

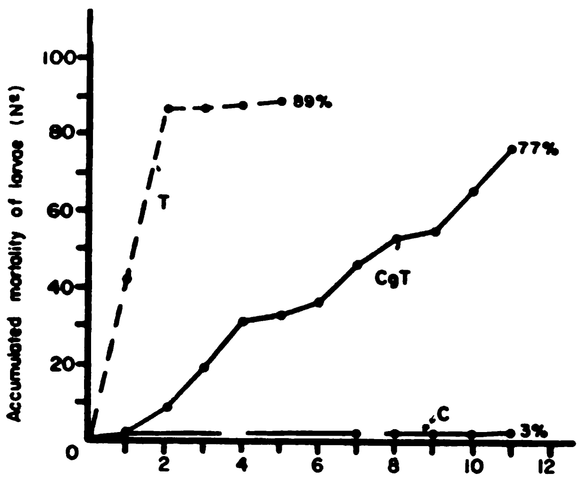

Fig. 2. Time dependence of accumulated mortality of 100 first instar *H. grandella* larvae on leaf diets of *C. odorata* (C), of *T. ciliata* var. *australis* (T) and of *C. odorata* grafted on *T. ciliata* var. *australis* (CgT).

aspects which should still be investigated are, whether the adult has a preference for the different tree species employed in these mixtures and to what degree the shootborer population is effectively influenced by these mixtures. Moreover, a single female can oviposit over 1000 eggs under laboratory conditions, and field observations have shown that over 50 per cent of the trees in a young *Cedrela odorata* plantation contained only 1–3 eggs per tree; consequently deficiencies in the effectiveness of *Toona* as a natural trap for *Hypsipyla* in mixed Meliaceous plantations are expected.

Oviposition by Lepidoptera on introduced plants closely related to native hosts but toxic to their larvae, is also reported by Dethier, Straatman and others (2, 3, 10). Straatman (10) suggests that there has been insufficient time for these insects to adapt to the introduced plants, i.e. the adults are unable to discriminate between the introduced plant and the native host, and the larvae have not yet developed the ability to feed on them.

Painter (8, 9) indicates that the development of host plant preference involves a simultaneous evolution of both the plant resistance mechanisms and of tolerance and host preference of the insect, resulting in the formation of a dynamic equilibrium. In the case of the Australian cedar and *Hypsipyla grandella*, the lack of this simultaneous evolution of resistance mechanisms may have resulted in the present immunity of the tree to this insect species. However, as indicated in earlier publications (5, 6) a number of reports exist which show the successful attack of native *Hypsipyla* spp. on recently introduced exotic Meliaceae. For example, of two Meliaceae introduced from India into Puerto Rico *Chukrasia tabularis* A. Juss. and *Toona ciliata*, the former is successfully attacked by *H. grandella* and the latter not.* Although no explanation has been found yet to account for this difference in susceptibility, it is apparent that resistance does not depend solely on the absence of a simultaneous evolution of the insect and the tree species.

Grafting trials

The results of these trials under both laboratory and greenhouse conditions are shown in Figures 1 and 2. The grafts of *Cedrela* on *Toona* were found to be resistant against *H. grandella* (Fig. 3, a–d) although not to the same extent as the *Toona* plants.

The resistance induced in the grafts of *Cedrela* indicated that the plant constituent(s) responsible for this resistance could be translocated from the *Toona* stock to the *Cedrela* graft. However, it should be pointed out that the grafts were only 4 months of age. Further experimentation with older grafts is indicated to confirm the persistence of the induced resistance. In addition, it would be recommendable to include grafts of *Toona* on *Cedrela* stocks in these trials to determine whether the *Toona* grafts would become susceptible to *H. grandella* attack.

Toxicity trials

The highest mortality of *H. grandella* larvae placed on diets consisting of the commonly used rearing ingredients and the *Toona* leaf extracts was most consistently

* Geary. T. F. Institute of Tropical Forestry. Río Piedras, Puerto Rico. Personal communication. July 1969.

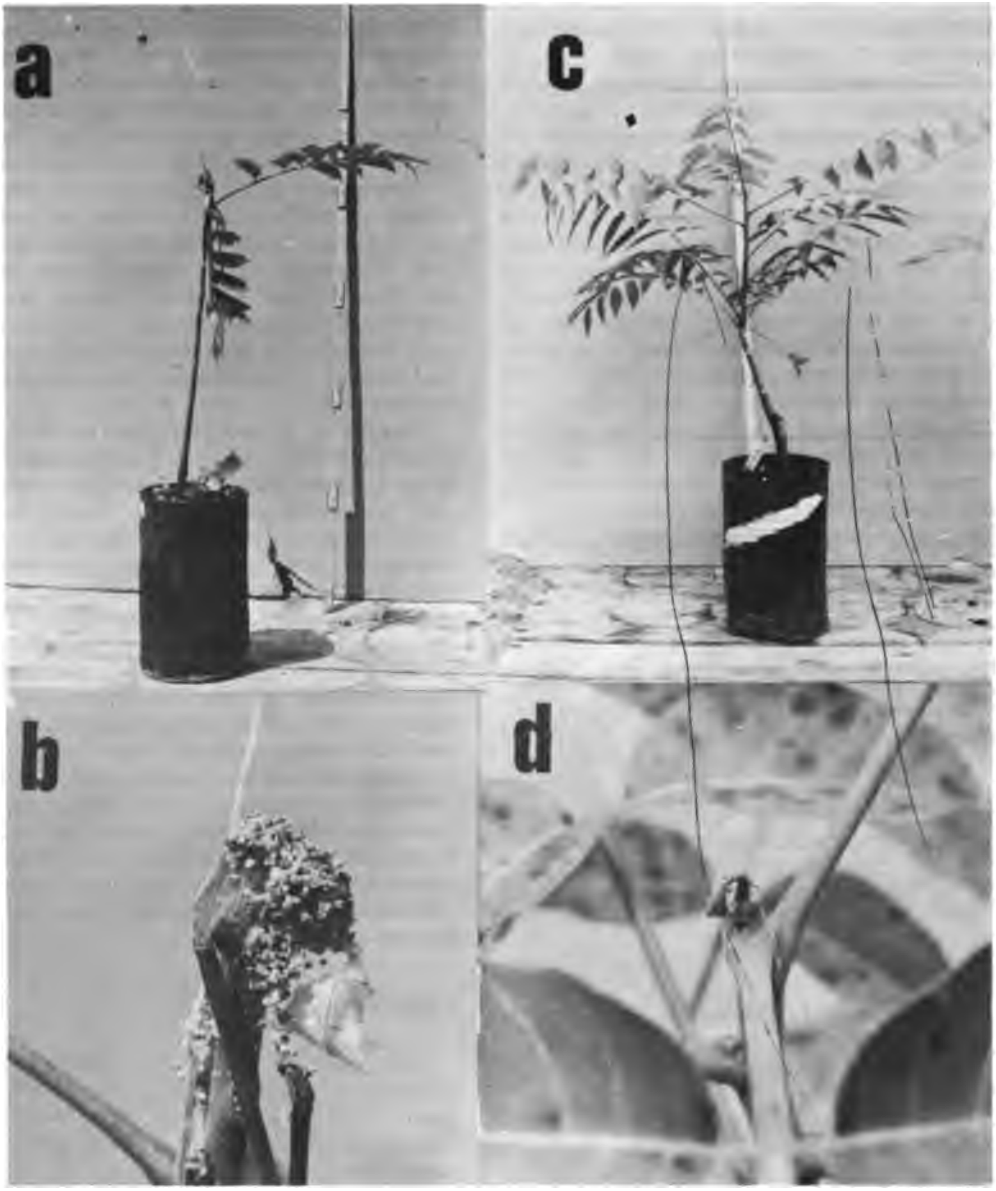

Fig. 3. a–d. Effect of *H. grandella* attack on *C. odorata* seedlings (a, b) and on *C. odorata* grafted on *T. ciliata* var. *australis* (c, d). Pictures taken three weeks after placement of first instar larvae on the plants.

found in the water (T–17) and hexane *Toona* (T–16) extracts (Table 2). Figure 4 demonstrates the anomaly in the development of larvae feeding on diets containing these extracts. The larvae placed on these diets not only developed more slowly but their mortality was higher (25 and 20 per cent respectively) than with other extracts. In particular, initial pupation of the larvae feeding on the mixture of diet ingredients and the aqueous *Toona* extract took more time than pupation of those placed on any of the other diet mixtures. In addition, in this experiment, mortality during the pupal stage was remarkably high for the treatments in which *H. grandella* was placed on mixtures of either the water or hexane *Toona* extracts. In these treatments an additional 25 and 30 per cent respectively of the *H. grandella* died during the pupal stage (Fig. 5). Frequently incomplete wing development was observed in pupae of larvae feeding on these diets.

Pupal weight of *H. grandella* placed on the mixtures of the *Toona* extracts with diet ingredients corresponded to a large extent with the mortality percentages documented above. Average weight of pupae reared on CTL (diet ingredients only), C_7, T_{15}, T_{14}, T_{16}, and T_{17} diet mixtures were respectively 159 mg (range 140–175 mg) 144 mg (range 115–165 mg), 149 mg (range 90–185 mg), 144 mg (range 104–180 mg), 113 mg (range

TABLE 2. Average mortality of *H. grandella* larvae reared on mixtures of artificial diet ingredients and *Toona* (T) or *Cedrela* (C) leaf extracts.

Sample Code No.	Extracting solvent	pH of diet mixtures	No. of replications	No. of replications with dead larvae	Average mortality (%)
Controls					
(CTL)	**Diet ingredients only**	**5.00**	**50**	**7**	**14.0**
Cedrela					
C_3	Methanol	4.95	10	0	0.0
C_2, C_{10}	Benzene	5.08	30	4	13.3
C_5	Chloroform	5.02	10	2	20.0
C_4	Hexane	5.04	10	1	10.0
C_6, C_7, C_{16}	Water	5.04	50	9	18.0
C_{18}	Acetone	5.06	10	0	0.0
C_{17}, C_{19}	Ethyl Ether	5.13	30	3	10.0
Toona					
T_9	Methanol	4.98	10	0	0.0
T_{10}, T_{14}	Benzene	5.06	30	8	26.6
T_{11}, T_{15}, T_{19}	Chloroform	5.00	40	11	27.5
$T_{12}, T_{16}, T_{20}, T_{22}$	Hexane	5.01	50	21	42.0
$T_{13}, T_{17}, T_{21}, T_{25}$	Water	5.01	60	32	53.3
T_{18}	Acetone	4.96	10	5	50.0*
T_{26}	Ethyl Ether	5.12	20	2	10.0

* Contaminated diet; turned sour.

65–170) and 80 mg (range 45–120 mg). In the T_{17} mixtures only 20.0 per cent of the pupae weighed over 100 mg, whereas the corresponding percentages for the T_{16}, T_{15}, T_{14}, C_7 and CTL mixtures (Table 3) were respectively 68.7; 88.9; 100; 100 and 100 per cent.

The acidity of the *Toona* diet mixtures differed little from that of the control and *Cedrela* mixtures (Table 2); thus, any effect of this factor was discounted.

The only other *Toona* fraction that caused high mortality of *H. grandella* larvae, was the acetone extract (T_{18}) (Table 2). However, the mixture of this extract with the diet ingredients resulted in a contaminated (sour) feeding medium. It is unknown whether the contamination *per se*, or a toxic *Toona* compound had caused this mortality. Further investigation of this fraction was not pursued in view of the high mortality encountered in the water and hexane extracts of *Toona*.

The mortality levels observed in the controls which consisted of those larvae reared on either the commonly used rearing ingredients only or the corresponding *Cedrela* fractions mixed with these ingredients, were considered normal.

Since these diet–mixture tests suggested that the active compound(s) of *Toona* might be chemically labile, the hexane (T_{32}) and water (T_{31}) extracts of *Toona* were bioassayed by force–feeding sixth instar larvae. The results of these tests clearly demonstrated the toxicity of the fresh aqueous *Toona* fraction, although a certain degree of toxicity was still present in the hexane fraction (Table 4). Moreover, the reduced mortality

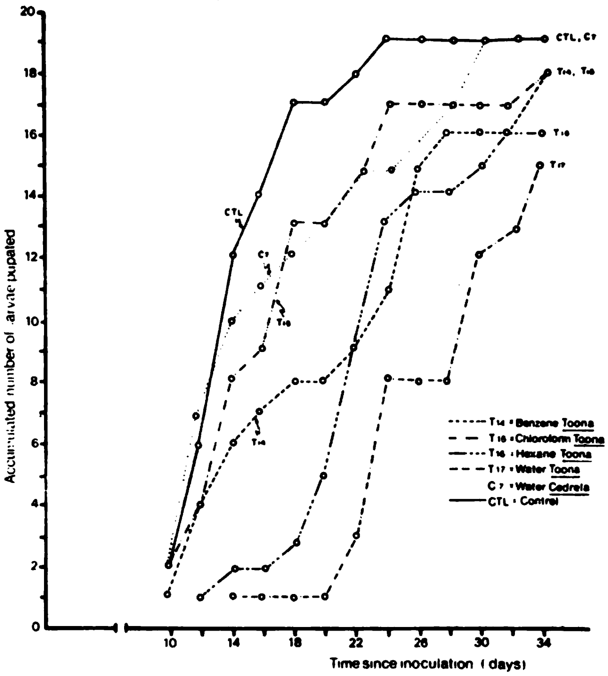

Fig. 4. Time required for 20 *H. grandella* larvae of the second instar to pupate after feeding on artificial diet (CTL) and mixtures of artificial diet ingredients with leaf extracts of *Toona* (T_{14}–T_{17}) and *Cedrela* (C_7). Larvae not accounted for died during the experiment.

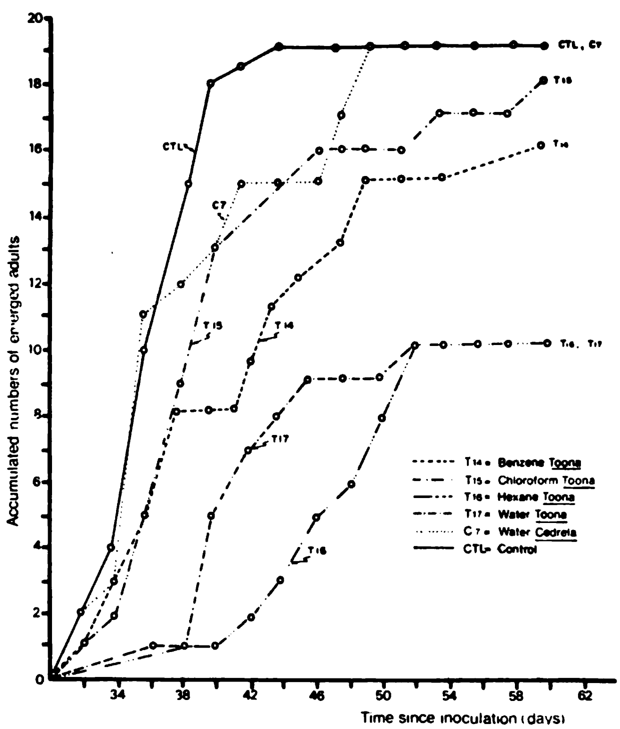

Fig. 5. Time required for 20 *H. grandella* larvae of the second instar to emerge as adult after feeding on artificial diet (CTL) and mixtures of artificial diet ingredients with leaf extracts of *Toona* (T_{14}–T_{17}) and *Cedrela* (C_7). Adults not accounted for died during the larval or pupal stage.

TABLE 3. Pupal weight (mg) of *H. grandella* reared on mixtures of artificial diet ingredients and *Toona* (T) or *Cedrela* (C) leaf extracts.

Pupa No.	Control CTL	Water C_7	Chloroform T_{15}	Benzene T_{14}	Hexane T_{16}	Water T_{17}
1	140	155	157	154	95	92
2	145	160	148	178	115	58
3	161	159	90	172	120	45
4	174	164	155	180	108	46
5	150	152	180	104	170	80
6	149	141	185	128	112	75
7	162	158	163	160	65	105
8	175	128	155	155	140	85
9	173	165	106	161	90	65
10	165	161	166	155	115	100
11	153	143	105	125	95	95
12	162	163	153	135	125	85
13	146	164	171	108	124	70
14	170	156	185	167	116	120
15	171	139	97	136	98	80
16	116	154	155	133	120	–*
17	158	157	168	134	–*	–*
18	149	158	149	107	–*	–*
19	152	149	–*	–*	–*	–*
20	–*	–*	–*	–*	–*	–*
Average weight	159	154	88.9	144	113	80
Percentage > 100 mg	100.0	100.0	149	100.0	68.7	20.0

* Larva died.

TABLE 4. Mortality of sixth instar *H. grandella* larvae force–fed with *Toona* and *Cedrela* hexane and water extracts and TLC–subfractions of *Toona* water extracts.

Extracts and code number	No. of larvae treated	No. of larvae pupated	No. of adults emerged	Mortality %
Toona, water (T_{31})	12	2	2	83.3
Cedrela, water (C_{21})	15	13	11	26.7
Toona, water (T_{31})	12	6	6	50.0
Cedrela water (C_{21})	12	11	11	8.3*
Toona, hexane (T_{32})	17	13	11	35.3
Cedrela, hexane (C_{22})	17	15	15	11.8
Toona, water (T_{33})	15	1	1	93.3
TLC–1	15	3	3	80.0
TLC–2	15	14	14	6.7
TLC–3	15	3	3	80.0
Toona, water (T_{34})				
TLC–3–1	12	1	0	100.0
TLC–3–2	12	10	8	33.3
TLC–3–3	12	12	12	0.0

* Same extracts (T_{31}, C_{21}) as used in previous test, but kept for 7 days in refrigerator at 5°C.

obtained with an aqueous *Toona* extract which had been stored under nitrogen for one week at 5°C seems to confirm the suggested instability of the chemical(s) involved.

Both aqueous subfractions T_{33}–TLC–1 and T_{33}–TLC–3, obtained form the thin layer chromatography plates, caused 80.0 per cent mortality in the treated larvae, whereas the subfraction T_{33}–TLC–2 had practically no effect (6.7 per cent mortality). This finding appears to indicate that two toxic, water–soluble chemical compounds are probably present, one of which is highly polar. Since neither of the subfractions T_{33}–TLC–1 and T_{33}–TLC–3 is as toxic as the original extract T_{33} in spite of more concentrated dosages force–fed (150 mg vs. 50 mg *Toona* leaf equivalents) a synergism is suggested.

Confirmation of the toxicity of the highly polar compound in the chromatographed aqueous extract TLC–3(T_{34}) was obtained when this subfraction was rechromatographed in additional tests (T_{34}–TLC–3–1,2,3). The mortality caused by the subfraction T_{34}–TLC–3–2 is probably due to some overloading of the plates (Table 4).

The solubility of the toxicants in water would be in accordance with the results obtained in the grafting trials, where translocation of the toxicant(s) present in the *Toona* stocks induced resistance against *Hypsipyla* in the *C. odorata* grafts.

Acknowledgements

The authors wish to thank Mrs. Cathi Roberts for her efficient assistance during the experiments. In particular, appreciation is due to Dr. W. Albertin, Head of the Department of Tropical Forest Sciences and Dr. O. Hidalgo–Salvatierra formerly at IICA/NEP in Turrialba, Costa Rica, for facilitating this research. The critical review of the manuscript by Dr. G. G. Allan, Dr. L. M. Schoonhoven and Dr. J. de Wilde is gratefully acknowledged. The support of this study was provided by the Netherlands Ministry of Development Co–operation.

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THE CONCEPT OF CONTROLLED RELEASE INSECTICIDES AND THE PROBLEM OF SHOOTBORERS OF THE MELIACEAE

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COMPENDIO

Se discuten las dificultades para controlar *Hypsipyla grandella* (Zeller) con insecticidas convencionales en plantaciones de Meliaceae y se les contrasta con las características de los insecticidas sistémicos organofosforados y de carbamatos. Se define termodinámicamente el concepto de liberación controlada y se esbozan las ventajas biológicas y económicas, con particular referencia a la ciencia forestal tropical. Se revisan consideraciones para diseños específicos de combinaciones para la protección de plántulas de cedro y caoba en términos de la estructura química de los insecticidas eficaces y de los polímeros asociados.

With the expanding global demand for wood and paper (14) increasing attention is being turned to the tropics where tree growth can be remarkably rapid (13). However, a typical acre of tropical forest contains perhaps 150 tree species of which only one or two are commercially useful (10). Planting efforts to establish a monoculture of these marketable trees have often been unsuccessful because of the damaging effects of some insect pests and particularly lepidopteran shootborers. The frequent destruction of juvenile native meliaceous plantations in the Americas, Africa and Asia/Australasia by *Hypsipyla grandella* (Zeller) and *Hypsipyla robusta* (Moore) respectively rank as classic examples of the problems of tropical reforestation (11). The larvae of these moths tunnel into the stem of seedlings during much of the year causing crippling distortion or death.

Protection of the plant by exterior sprays is rather ineffective because after penetration into the plant the often overlapping generations of larvae become essentially physically inaccessible to the insecticides. The deployment of the newer systemic insecticides which are translocated from the soil into the sap can overcome this insect accessibility factor. However, the effective insecticidal systemic chemicals are usually either carbamates or organophosphates and both of these classes of compounds are readily biodegradable especially in the vigorous biological regime of the tropical environment. Their use is therefore economically crushing because even the period of effectiveness of the most durable is only a few days and this may be further reduced by the frequent and heavy rains which tend to leach the insecticide out of the root zone and into the subsoil. Of course, these difficulties also occur to a lesser extent in the temperate regions and are overcome to some extent by the application of large quantities of insecticide. This is recognized as wasteful and ecologically undesirable but the magnitude of the waste illustrated in Fig. 1 is not always fully appreciated. Thus, the duration of effectiveness of three levels of application of a typical nonpersistant pesticide with a half–life of 15 days is graphically represented by curve A. If the loss of pesticide were a result of an unimolecular reaction then obviously treatment at a level just above minimum necessary (say 1 mg), for insect control would afford protection for only a few hours. To attain a practical period of control for many agricultural crops, of say 50 days, the level of application would have to be increased tenfold. Under these conditions most of the insecticide is not used for actual control of the pest but functions as a primitive reservoir to maintain the critical control level. An even more wasteful spewing of biocide into the environment follows if the longevity of pesticidal effectiveness is to be doubled or tripled. The levels of treatment must then be increased one hundred –or one

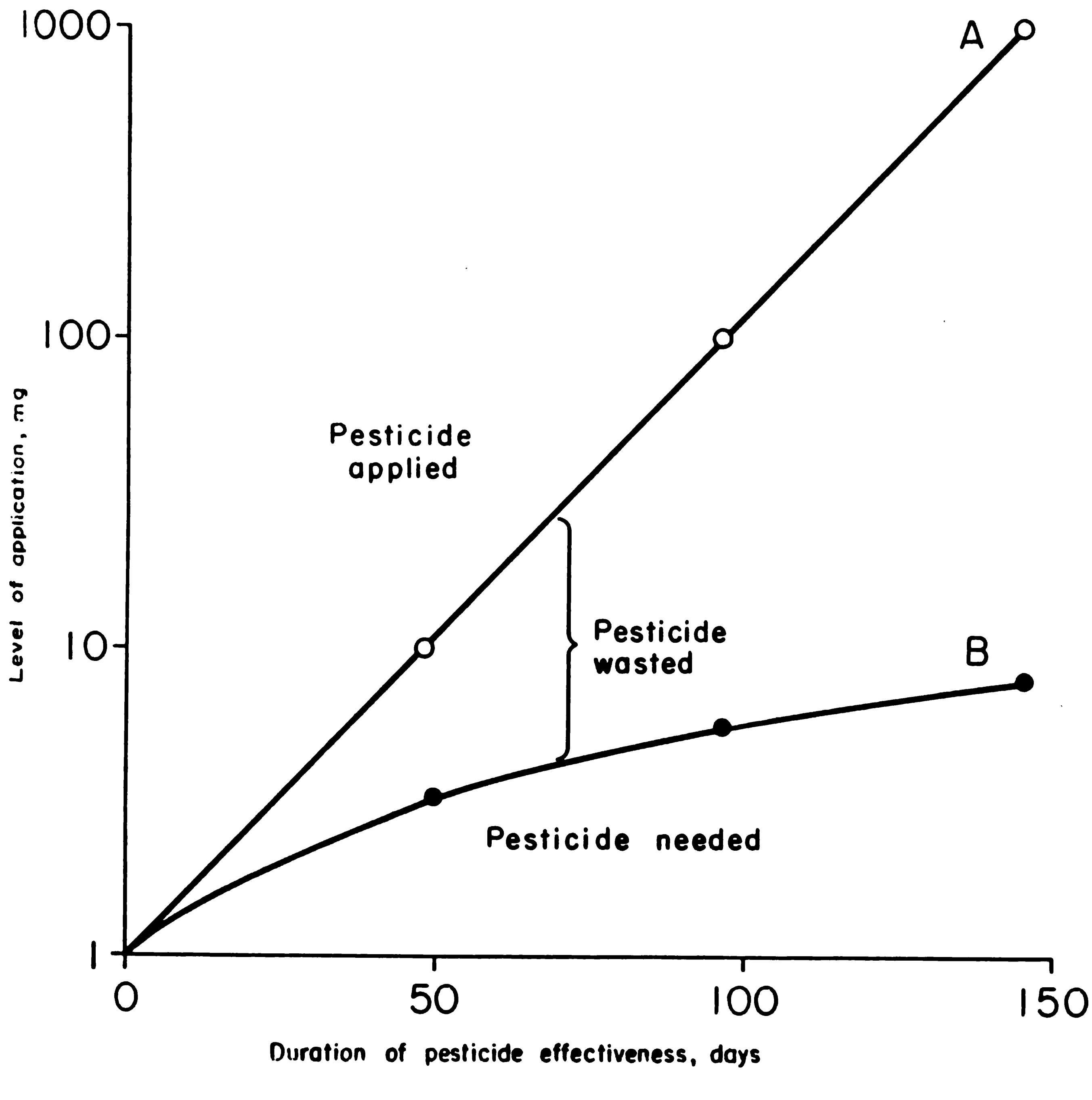


Fig. 1. Comparative duration of pesticide effectiveness for practical and ideal application levels.

thousand– fold respectively. However, the level theoretically required to achieve these periods of protection is only that needed to replace the amount dissipated i.e. to restore the insecticide level in the plant to the minimum necessary for effective pest control. It is therefore apparent that, for optimum performance, pesticides should be supplied from some efficient reservoir at a rate exactly equivalent to the loss which takes place following initial application. Curve B in Fig. 1 then indicates the amount necessary to maintain this minimum effective level. The logarithmic area between curves A and B represents the excessive amount of pesticide wasted in conventional treatments and delineates the opportunity for improvement of application technology. For example, if the biocide level were to be continuously restored by some mechanism the total amount needed for 50, 100 and 150 days of pest control would be about 3, 6 and 8 mg respectively instead of the treatment levels of 10, 100 and 1000 mg which would normally be applied.

In practice, of course, this gross extent of wastage is sometimes diminished by efforts to stabilize the pesticide applied on adsorbents such as corn grits, clays and the like. The movement of pesticide from these carriers into the soil does constitute a mechanism for restoring the amount of material biodegraded in the soil biosphere. This restorative effect is however shortlived, even for agricultural or horticultural needs, and is hopelessly inadequate for forestry purposes when longterm protection is mandatory. Nonetheless, some extension of the period of pesticidal effectiveness has clearly been realized by an apparently irrational miscellany of different formulations and inventions. This jumble can nonetheless be rationalized using thermodynamics. Thus, if a free pesticide Ⓟ is placed in the soil it is depleted immediately at some rate determined by its structure and the environment. That is Ⓟ $\xrightarrow{K_p}$ consumption, and the rate of disappearance of Ⓟ is given by the product, K_p (pesticide). Since K_p is a constant, the rate of depletion can only be modified by manipulation of the pesticide concentration in the soil. In the simplest pesticide formulations this is done by adsorption on an inert substrate denoted by Ⓟ. Although this is often described as a physical phenomenon it is preferable fundamentally to regard the adsorption as a low energy chemical process. This chemi–absorption therefore must afford a quasi–chemical structure, Ⓢ–Ⓟ, which axiomatically is biologically inactive because the pesticide is chemically bound within a micro–environment and insulated from the pest and the biodegrading action of the surrounding macro–environment. Naturally, if the formulation is to be of value, then in use the following sequence must transpire;

$$\textcircled{S}\text{–}\textcircled{P} \xrightarrow{K_r} \textcircled{S} + \textcircled{P} \xrightarrow{K_p} \text{consumption}$$

For maximum efficiency of the pesticide the level of Ⓟ should be the minimum necessary to control the pest and this is usually rather small, and much smaller than the levels invariably applied by conventional techniques. Of course, the separation of the components in the formulation Ⓢ–Ⓟ does require an energy input and for simple adsorption systems the interactions to be overcome are low energy van der Waal's forces. The rate constant, K_r of this quasi–chemical bond cleavage is theoretically calculable from the theory of absolute reaction rates (9) where $K_r = (RT/N_0h)e^{-\Delta F/RT}$ and the symbols have the usual physico–chemical meanings. In practical terms, the inverse logarithmic dependence of K_r on the free energy of formation of Ⓢ–Ⓟ is worthy of comment since the more stable the linkage between Ⓢ and Ⓟ the smaller will be the value of K_r. This in turn implies that, for a specified quantity of Ⓟ in the formulation, the higher the energy of the linkage the longer will be the period of release. Thus all forms of pesticide–substrate formulations can be regarded as a spectrum of controlled release bonded pesticides –some bonded with more, and some with less energy. Since simple adsorption is the low end of the spectrum, then high energy covalent bonding must be the other extremity. Of course, the energy of bonding must be selected such that K_r has a reasonable value. Otherwise Ⓟ cannot be maintained at the necessary levels without the initial employment of huge quantities of the pesticide–polymer combination. Obviously then, from fundamental considerations, a longer term controlled release and a significant reduction in the amount of pesticide used cannot be expected from simple adsorption of insecticides on materials like activated carbon, walnut shells, simple clays or silica gel. For the protection of cedar and mahogany seedlings from *H. grandella* the period of insecticidal activity must last until the trees are sufficiently tall to cope successfully with larvae attack. While the length of this period has not yet been established a period of 3–5 years seems a reasonable working assumption since unattacked trees reach a height of about 6 meters in 3–4 years (12). Initial efforts to attain commensurate longevity of larvicidal action attention should therefore logically to focussed on systemic chemicals firmly attached to a substrate by a high energy bond. Release of the pesticide then must depend on the cleavage of a definite identifiable chemical bond such as an ester or amide. The attachment can take any one of several forms (3). The simplest of these has the pesticide attached as a pendent substituent to a natural or synthetic, water–soluble or insoluble polymer having a replaceable hydrogen as formulated in equation (I).

$$\text{Polymer–X–H+RCOOH} \underset{\text{environment}}{\overset{\text{synthesis}}{\rightleftharpoons}} \text{Polymer–X–COR} \qquad \text{(I)}$$
$$(X = N, O \text{ or } S)$$

Ideally, the pesticide should contain a structural moiety suitable for use as a link to the macromolecule and the carboxyl group is only one of the many alternatives possible. Pesticide–polymer combinations of this type can readily be synthesized by conventional organic chemical procedures. If the pesticide cannot be directly attached to the substrate to form a bond of suitable stability, a bridging entity may be interposed. An example of this would be the linkage of pesticide alcohol to a polysaccharide substrate by means of a phosphate or a diurethane bridge. Alternatively, the pesticide may be initially converted to a polymerizable derivative, e.g., vinyl 2,4–dichlorophenoxyacetate, which is then homo– or co–polymerized to give a wholly synthetic pesticide–polymer combination.

For all of these variations, side chain degradation occurs in the biological environment so that the chemical bonds holding the pesticide within its polymeric prison are sequentially broken to provide a sustained liberation of the pesticide over an extended period of time. The rate of release will clearly be determined by the nature of the pesticide–polymer bond, the hydrophilicity of the neighboring groups, the chemical characteristics of the pesticide and polymer, and the dimensions and structure of the resultant macromolecular combination.

Where the polymeric backbone is water–soluble, the rate of hydrolytic degradation (R_h) for n spherical particles (radius r and density ρ) of the pesticide–polymer combination in a water–saturated heterogeneous surface reaction is given by

$$R_h = r\rho 4\pi r^2 \; dr/dt \qquad \text{(II)}$$

and by

$$R_h = nK_h 4\,\pi r^2 C_0 \qquad \text{(III)}$$

where C_0 is the initial concentration of pesticide–polymer linkages on the surface of a particle of radius r_0 and K_h is the hydrolysis rate constant. Since each pesticide molecule released by hydrolysis exposes another pesticide–polymer linkage beneath, C_0 is a constant and the duration of pesticide release (D_{pr}) by combination of equations (II) and (III) can be predicted from the relationship

$$D_{pr} = \rho r_0/K_hC_0 - (R_h\rho^2/n\,\pi K_h^3 C_0^3) \qquad \text{(IV)}$$

or

$$D_{pr} = A - BW^{-1/2} \qquad \text{(V)}$$

where A and B are constants and W is the amount of pesticide–polymer combination employed. The validity of this equation was demonstrated by measuring the persistence of herbicidal activity exhibited by a polymer synthesized by the partial acylation (56.1 %) of poly-vinyl alcohol with 2–methyl–4–chlorophenocyacetyl chloride (4). The minimum amount of pesticide–polymer combination ($W_{min} = B^2/A^2$ when $D_{pr} = 0$) which must be applied for herbicidal activity is defined by equation V.

On the other hand, the polymeric backbone selected may be water–insoluble and cellulose and lignin, the macromolecules of wood and bark, are particularly good choices because of their biodegradability and ecological acceptability and because of their abundance as clean, low–cost solid wastes (5). The particular species of wood waste used is not as important to the rate of release as the degree of substitution achieved. Marine and proteinaceous solid wastes (2) can also be used as the polymeric backbone. Acylation of these natural polymers (6) with a herbicide acid affords combinations which degrade by a heterogeneous surface reaction to provide a sustained release of pesticide. The rate of degradation (R_d) can be expressed by

$$R_d = W\, dC/dt \qquad \text{(VI)}$$

or by

$$R_d = K_d\, WC \qquad \text{(VII)}$$

where W is again the amount of pesticide–polymer combination used, C is the concentration of pesticide per unit weight contained therein at time t and K_d is the degradation rate constant.

The duration of effectiveness (D_{pr}) of this type of controlled release herbicide polymer combination, obtained from equations (VI) and (VII) is therefore given by

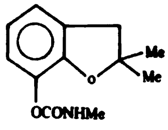
$$D_{pr} = (1/K_d)(\ln W - \ln R_d/K_d) \qquad \text{(VIII)}$$

or

$$D_{pr} = M\log_{10} W - N \qquad \text{(IX)}$$

where M and N are constants. In this case, the minimum amount of herbicide–polymer combination necessary for pesticide activity is $10^{N/M}$ ($D_{pr} = 0$). Experimental confirmation of the usefulness of equations (VIII) and (IX) in the design of controlled release pesticide–polymer combinations was provided by measurement of the release of herbicide acids from esters of cellulose and lignin (4). On the other hand, the biocide need not be a pendent substituent on the macromolecule. If it is polyfunctional, the pesticide can be part of the polymer backbone (3). While the number of pesticides having two or more groups suitable for condensation polymerization is not large, extremely long term control may be attainable since the rate of release is clearly inversely proportional to the degree of polymerization and this can be very large. Tordon (4–amino–3,5,6–trichloropicolinic acid) Endothall (7–oxabicyclo[2.2.1] –heptane–2,3,–dicarboxylic acid) and Amiben (3–amino–2,5–dichlorobenzoic acid) are examples of herbicides which can be polymerized to this type of controlled release material. However, all of these covalent alternatives can be closed off if the insecticide cannot readily be chemically attached to the substrate. This is frequently the case with both established organophosphorus and carbamate insecticides where these materials have been developed for control using conventional application technology. Of course, it is possible to synthesize and test analogs containing suitable functional groups until an equally active material is found; but this could be very laborious and time–consuming. A more practical alternative is provided by solid solutions of pesticides in suitable plastics. The basic thermodynamic considerations again apply but the low energy of specific pesticide–matrix interactions are supplemented by the additional constraints of diffusion through appreciable masses of plastic. Apart from the necessity that the pesticide and polymer are mutually soluble no specific structural moiety within the pesticide molecule is necessary. Furthermore a broad range of polymer matrices are operative. The method therefore has general applicability to systemic insecticides. The rate of release of a particular insecticide from such combinations depends on the concentration of insecticide and the molecular weight of the polymer and is directly proportional to the square root of elapsed time. The basic factors influencing the design of a controlled release insecticide system of this type are therefore the chemical structures of the pesticide and the polymer matrix. The former is determined by host–insect relationships and the finite number of insecticides commercially available. The latter, for reasons of convenience and economy, will also usually be a commercially available plastic. For example, substantially linear polyamides and polyurethanes are excellent choices because in both the molten and solid state these materials are solvents for many of the systemic biocides now used (1).

Now, with all this controlled release technology how can an effective program of *Hypsipyla* control be developed? Obviously the preferred insecticide must first be selected because its structure and properties may exclude certain controlled release alternatives. The evaluation of most of the insecticides commercially available was carried out in Turrialba (7) and it was found that the most effective materials included carbofuran (i), methomyl (ii), isolan (iii), phosphamidon (iv) and monocrotophos (v).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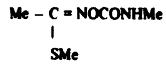
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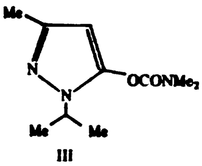
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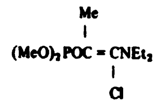
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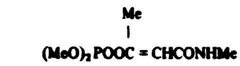
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It is apparent from the chemical structures (i) to (v) that while both carbofuran, methomyl and monocrotophos contain replaceable hydrogen atoms attached to sites which could be used as one end of a covalent bridge to a substrate, isolan and phosphamidon do not. In spite of the reactive sites in carbofuran, methomyl and monocrotophos it was decided to focus initially on controlled release combinations which could be speedily made in Turrialba and to later prepare covalently–linked insecticides at the University of Washington in Seattle.

Accordingly, the insecticides were dissolved in a polyamide derived from the dimer of linoleic acid and ethylenediamine. The molten solutions were cast into a block. By immersing blocks in water the amount of insecticide escaping can be measured.

From a knowledge of this rate and the amount of insecticide needed the size and shape of the controlled release pesticide–polymer combination can be predicted (8). Field tests of larger quantities of the designed combination were undertaken and these are described in the paper by Wilkins.* In these tests, as will be apparent, the attacks of *H. grandella* on *Cedrela odorata* were prevented for more than one year.

Thus, it is clear that the concept of controlled release is applicable to the problem of protecting meliaceous seedlings against *H. grandella* and systemic insecticides in sustained release forms may become major components of international integrated programs for the effective suppression of the attacks of this shootborer scourge of cedar and mahogany.

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COMPORTAMIENTO EN VUELO Y SELECCION DE HOSPEDERO DEL BARRENADOR DE LAS MELIACEAS, *HYPSIPYLA GRANDELLA* ZELLER (LEPID., PHYCITIDAE)*

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ABSTRACT

Field and laboratory studies were established in Puerto Rico to investigate the flight and host selection behavior of *Hypsipyla grandella.* Barrier traps, placed in *Cedrela odorata* stands were used to elucidate patterns of host selection; laboratory feeding studies were used to determine the effects of native hosts and an introduced Meliaceae *(Toona ciliata)* on larval survival. The studies showed that moths fly between midnight and 0500 h, with a peak flight at 0300 h. Flight takes place at temperatures between 15–24°C. Infestations concentrate on *C. odorata* that evidence new growth, and the tallest trees are most subject to attack; open grown trees are more readily infested than shaded stems. The trapping studies also indicated the presence of a female sex pheromone. Acetone, methanol, and hexane extracts of new or mature foliage were ineffective in attracting moths. However, moths were attracted to acetone extracts of new leaves in association with *Cedrela* plants having only mature leaves. Feeding studies showed that *T. ciliata* acetone extracts were toxic to *H. grandella* larvae; hexane and methanol extracts were nontoxic. Results from the field and laboratory studies suggested a chemical affinity between primary *Cedrela* attractants and toxic *Toona* extracts; a hypothesis explaining these relationships is proposed.

Introducción

El barrenador de las Meliaceae, *Hypsipyla grandella* Zeller (Lepid., Phycitidae), es el insecto–peste más severo de las plantaciones jóvenes de Meliaceae. En muchas áreas de América Tropical, plantaciones de cedro (*Cedrela odorata* L.) y caoba (*Swietenia* spp.) han sido abandonadas después de repetidas infecciones de *H. grandella* (Fors 1941; Holdridge 1943). El daño ocurre debido a que la larva de las palomillas perforan los fustes y brotes terminales de las plantas jóvenes, resultando en una interrupción en el crecimiento de altura y las plantas en consecuencia ramifican profusamente (Fig. 1). Con el tiempo, después de un ataque continuo, los árboles o mueren o son tan severamente deformados que sus probabilidades de crecimiento para alcanzar un tamaño de árboles maderables son mínimos.

La biología y distribución de *H. grandella* ya han sido descritas (Ramírez Sánchez 1964; Tillmanns 1964). Otros estudios han demostrado diferencias en la susceptibilidad o aceptabilidad del hospedero entre diferentes géneros de Meliaceae (Burgos 1954; Carter 1945; Gray 1972; Grijpma 1970; Holdridge 1943; Lamb 1969). Por ejemplo, cuando *Toona ciliata* M. Roem var. *australis* (una Meliaceae australiana) es introducida en América Latina, las palomillas de *H. grandella* no atacan a los árboles, pero cuando esta especie es cultivada en su habitat nativo, es fuertemente atacada por *Hypsipyla robusta* (Moore). Las larvas de *H. grandella*, en efecto, son matadas cuando son alimentadas con material de este hospedero introducido; las larvas también mueren cuando son expuestas a las emanaciones volátiles de hojas trituradas de *T. ciliata* (Grijpma y Gara 1970 b). En general, Meliaceae introducidas en áreas nuevas, como especies exóticas, no son atacadas por *Hypsipyla.* Sin embargo, hay indicaciones que el *Hypsipyla* nativo sí ataca ciertas Melicaeae introducidas (Gray 1972).

Estudios en Costa Rica indican que las palomillas de *H. grandella* prefieren hospederos con hojas producidas recientemente, y hay evidencia que las poblaciones en vuelo son atraídas a las hojas nuevas en respuesta a la estimulación olfatoria (Grijpma y Gara 1970 a). La

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influencia de los factores ambientales sobre el vuelo de la palomilla es pobremente entendido. Es conocido que las palomillas son voladores nocturnos y que la actividad voladora es mucho mayor entre 17–21°C (Grijpma y Gara 1970 a); las palomillas parece que tienen sus vuelos mucho más densos poco después de períodos de lluvia (Tillmanns 1964).

El propósito de este artículo es describir algunos de los eventos por los cuales los barrenadores localizan su material hospedante cuando son condicionados por factores del hospedero y estados ambientales.

Fig. 1. Planta de *Cedrela odorata* de 2 años de edad, la cual tiene el brote terminal destruido debido al ataque de *H. grandella.*

Materiales y Métodos

Durante el período de agosto 15 a octubre 2, 1971, estudios sobre el comportamiento en la selección del hospedero de *H. grandella,* fueron establecidos en plantaciones de *Cedrela* localizadas en campos pertenecientes a la Estación Experimental de la Universidad de Puerto Rico. Una investigación estuvo situada en la Subestación de Gurabo, la otra en la Subestación de Corozal.

La plantación de Gurabo tenía siete años de edad y durante años, la plantación ha sido severamente atacada por *H. grandella.* Al momento de estos estudios, solamente cerca de tres por ciento de la plantación estuvo atacada debido a que la mayoría de la plantación estaba cubierta con malas hierbas y gramíneas altas. La plantación de Corozal tenía menos de dos años de edad, periódicamente era deshierbada y los árboles tenían un crecimiento anual mayor de 0,6 m. Alrededor del 75 por ciento de los árboles fueron plantados bajo sombra proporcionada por *Inga* spp. La *Inga* fue previamente establecida como sombra para café. Las *Cedrelas* que estaban creciendo en las áreas sin sombra estuvieron siendo fuertemente atacadas por el barrenador.

Los patrones de vuelo de *H. grandella* fueron estudiados en ambas áreas por medio de barreras de vuelo. Básicamente, los aparatos fueron paneles de 0,75 x 3 m construidos con tela metálica con redecilla de 1,7 cm de diámetro. Las barreras de vuelo fueron instaladas como un tablero a una altura de 1,3 m; subsecuentemente, la superficie de las barreras fue cubierta con un material pegajoso. La altura de 1,3 m fue escogida debido a como lo indicó un experimento previo sobre estandarización en que los barrenadores vuelan a una altura promedia de 1 a 2 m (Grijpma y Gara, a).

Dos grupos de sistemas de trampas fueron colocadas en Corozal; una barrera de 25 trampas fueron colocadas en un campo adyacente a la plantación, otra barrera de 25 trampas se colocó dentro de la plantación. Solamente los árboles que crecían en el abierto fueron escogidos para el estudio. El sistema fue diseñado para que hubiera una trampa cerca de cada árbol. En otras palabras, 25 *Cedrelas* que crecían en el abierto, tenían barreras de vuelo colocadas en medio de ellos.

Se colocaron también trampas en la plantación de Gurabo. Al principio fue establecido un sistema de 50 barreras en el centro de la plantación. Más tarde, la mitad de la plantación fue deshierbada; en ese momento, 25 de las barreras fueron removidas de la mitad sin limpiar y colocadas en la porción aclarada de la plantación. En todos los casos, las barreras de vuelo fueron colocadas cerca de los árboles de *Cedrela.*

Seis instrumentos, llamados olfactómetros de campo, también fueron colocados en la plantación de Gurabo. Los olfactómetros eran cilindros de tela metálica de 45,75 cm de diámetro por 76,25 cm de largo. Estos cilindros fueron colocados a 61 cm arriba del suelo sobre estacas de madera y subsecuentemente cubiertas con un material pegajoso. Los olfactómetros de campo fueron diseñados para evaluar la atractividad de varios señuelos suspendidos en el centro de cada cilindro. En detalle, los materiales colocados dentro de los olfactómetros fueron como sigue: follaje nuevo recogido dos días después de su crecimiento; follaje maduro y extractos de acetona, hexano, metanol de follaje nuevo procedente de árboles bajo sombra y/o de árboles al abierto y extractos de acetona, hexano y metanol de follaje maduro procedente de árboles creciendo bajo sombra o al abierto.

Los extractos foliares fueron subsecuentemente puestos en frascos de polietileno. Las muestras foliares fueron suspendidas y sumergidas en recipientes llenos de agua para conservar la frescura del material.

Para relacionar factores de la fenología del hospedero con el comportamiento del ataque y vuelo de las palomillas, fue anotada la frecuencia del ataque en cada sitio de prueba junto con la relación de hojas nuevas y total de hojas de cada planta atacada.

Lluvia, temperatura ambiental y humedad relativa en cada sitio de prueba fue monitorizado por medio de pluviómetros regulares e higrotermógrafos Foxboro T.M. Los higrotermógrafos fueron colocados en cajas de madera como se usa en las estaciones meteorológicas.

Los ensayos preliminares indicaron que *H. grandella* puede ser criada exitosamente sobre una modificación de la dieta de McMorran–Grisdale (vea Fatzinger 1970). Según el caso, la dieta de McMorran–Grisdale fue usada como una base para una serie de pruebas de alimentación donde extractos foliares de *Cedrela* y *Toona* fueron incorporados dentro de la dieta. En la práctica fueron puestas muestras del segundo instar de *H. grandella* sobre cuatro réplicas incorporándosele a cada una de las dietas extractos de hexano, metanol y acetona de *Toona;* y extractos de hexano, metanol y acetona de *Cedrela;* y cuatro réplicas a las que se incorporó celulosa molida.

Resultados y Discusión

Vuelo

El vuelo de las palomillas del barrenador ocurrió entre la media noche y cerca de las 0500 h., con vuelos mucho más densos a las 0300 h. (Fig. 2). Anotaciones horarias

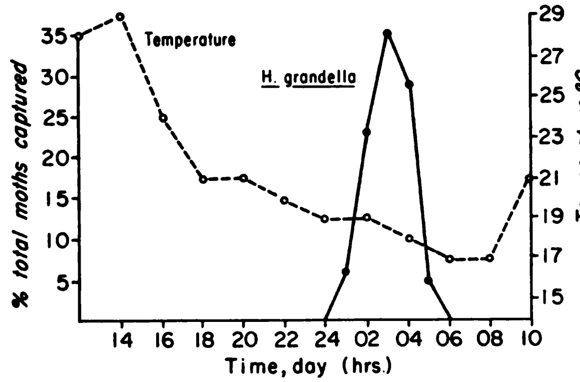

Fig. 2. Patrón de vuelo nocturno de *H. grandella* con relación a la temperatura media en los lotes de prueba en Corozal y Gurabo (setiembre 5–18, 1971).

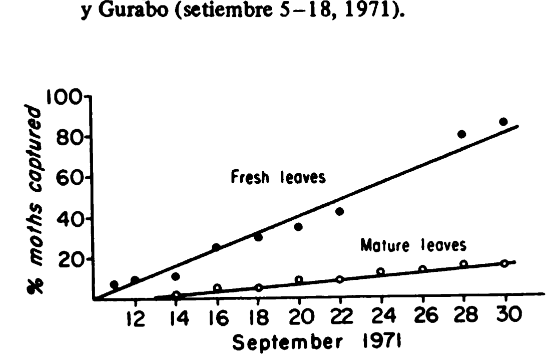

Fig. 3. Porcentaje acumulado de capturas de *H. grandella* en trampas de barrera cerca de *C. odorata* con hojas frescas comparado con capturas cerca de *Cedrela* con solamente hojas maduras. Lotes de prueba en Corozal y Gurabo (setiembre 5–18, 1971).

del vuelo fueron tomadas para 12 noches y en cada caso el máximo de vuelo fue evidente a las 0300 h. Los insectos volaron a temperaturas entre 15–24°C; no se registraron vuelos cuando durante la noche, las temperaturas cayeron abajo de 15°C. Durante el curso del estudio los vuelos continuaron con lluvias entre 0,4–2,0 mm; lluvias más fuertes no ocurrieron, y por lo tanto la intensidad de lluvia necesaria para terminar los vuelos es desconocida. Estudios previos llevados a cabo en Costa Rica indicaron que las trampas de malla colocadas en rodales adyacentes de árboles no hospederos, no capturaron barrenadores; todos los insectos capturados fueron aquellos activos dentro de plantaciones severamente atacadas (Grijpma y Gara 1970 a). En el estudio de Corozal, cerca del 2 por ciento de la población atrapada fue cogido en las trampas de malla colocadas en un campo vecino. El hecho de que fueran capturadas palomillas fuera de la plantación sustenta la hipótesis de que las palomillas, quienes han estado dispersas al azar, llegan a ser orientadas hacia las plantaciones jóvenes por la detección de material hospedero disponible. Se cree que una vez la infección está fuertemente establecida, la población de palomillas dejará de dispersarse y ataca en sincronía con la disponibilidad de material hospedero. Eventualmente la plantación será destruida y el material hospedante adecuado llegará a ser limitado. Entonces la población de palomillas una vez más se dispersará al azar.

Selección del Hospedero

La mayoría de las palomillas capturadas fueron atrapadas en barreras cerca de árboles con hojas nuevas (Fig. 3). Más aún, árboles ya atacados fueron los que tenían nuevos brotes de crecimiento. Esta relación se puede notar en la Fig. 4, donde la mayor frecuencia de ataque ocurrió en árboles con una gran cantidad de hojas nuevas en relación al total de hojas. La respuesta inmediata de *H. grandella* al crecimiento de brotes nuevos fue remarcable. En el estudio de Gurabo, la mitad aclarada (deshierbada) de la plantación estuvo bajo ataque severo cerca de seis días después de que los árboles fueron liberados. De nuevo, es visto que las infecciones estuvieron concentradas en los árboles que evidenciaron renovación de crecimiento (Fig. 5). Después de dos semanas la porción aclarada de la plantación fue 12 por ciento atacada. Es interesante notar que los ataques en la mitad no aclarada del lote aumentó hasta 5,8 por ciento de los árboles que ya estaban bajo ataque. Aparentemente, atrayentes primarios emanan del incremento del crecimiento nuevo en la sección aclarada donde se hace efectiva la población de *Hypsipyla* a través de toda el área. La respuesta de los barrenadores a los árboles de mayor crecimiento también fue evidente en el estudio de Gurabo. En la porción aclarada de la parcela, la altura media para los 224 árboles fue de 1,8 m, mientras que la altura media para los 29 árboles atacados fue de 2,3 m. Similarmente, de los 192 árboles en la porción no aclarada, 11 fueron recién infectados y la altura media de éstos fue 1,9 m; los árboles restantes no atacados tuvieron una altura media de 1,2 m. Estas relaciones indican que los árboles de más rápido crecimiento producen más hojas frescas y por consiguiente, estuvieron más sujetos al ataque.

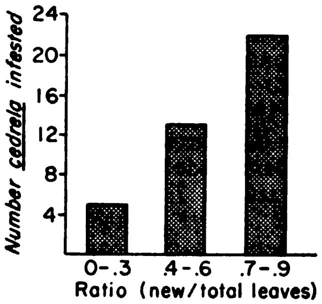

Fig. 4. Relación entre la frecuencia del ataque de *H. grandella* y proporción de hojas nuevas/total de *C. odorata* infectada (planta con todas las hojas nuevas = 1,0); parcela de prueba en Gurabo (setiembre 11–30. 1971).

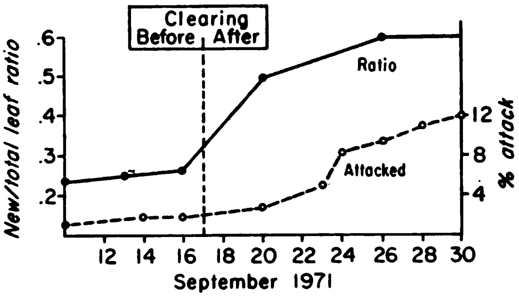

Fig. 5. Aumento de ataques de *H. grandella* relacionado a la proporción de hojas nuevas/total de *C. odorata* antes y después de que la parcela de prueba de Gurabo fuera aclarada de malas hierbas y gramíneas.

Atrayentes

Cuando las muestras foliares fueron suspendidas en el centro de la serie de olfactómetros, las palomillas respondieron en mayor número al follaje fresco procedente de árboles creciendo al abierto; no hubo respuesta a las trampas con señuelos de follaje maduro. Desde que los árboles al abierto fueron los de más rápido crecimiento, de nuevo se ve que las palomillas responden a los fustes de mayor actividad de elongación. El hecho de que los árboles sombreados son más lentos en crecimiento y por lo tanto menos expuestos al ataque, puede explicar los resultados encontrados en estudios silviculturales, los cuales recomiendan cultivar Meliaceae bajo cobertura de árboles para prevenir ataques de *Hypsipyla* (Gray 1972). Los extractos de acetona, hexano y metanol, todos fueron inefectivos en atrapar *H. grandella* cuando los frascos de polietileno sólo contenían extractos cuando fueron suspendidos en los olfactómetros. Sin embargo, las palomillas fueron atraídas cuando los frascos contenían extractos de acetona de crecimiento nuevo, colectado de árboles creciendo al abierto y fueron colocados en las trampas con señuelos junto con *Cedrela* en potes. Los árboles envasados en estos estudios contenían solamente hojas maduras. Evidentemente, parte del complemento volátil necesario para atraer a *H. grandella* estuvo ausente en el extracto de acetona, pero en asociación con hojas maduras de *Cedrela*, la combinación fue parcialmente efectiva en atraer barrenadores (Tabla 1).

Durante los estudios de vuelo, se notó que los primeros insectos capturados fueron principalmente hembras, luego fueron capturados machos (Tabla 2. Entre la media noche y las 0300 h., el 75 por ciento de las hembras fueron capturadas; durante el mismo período solamente el 36 por ciento de los machos fueron

TABLA 1. Respuesta de las palomillas de *H. grandella* a los olfactómetros de campo con señuelos con: follaje nuevo y maduro; extractos de acetona de follaje nuevo y maduro de árboles al abierto y bajo sombra (A.A. y B.S.); extractos de acetona de A.A. y B.S. hojas nuevas y maduras en presencia de *Cedrela* en potes con sólo hojas maduras. Extractos de hexano y metanol de A.A. y B.S. con follaje nuevo y maduro también fueron probados; no hubo respuesta de los insectos bajo ninguna condición.

Material del señuelo	No. de insectos capturados (Días) 1	2	3	4	5	6	7	Total de capturas
Follaje nuevo	4	3	1	0	0	0	1	9
Follaje maduro	0	1	0	0	0	0	0	1
Extractos de acetona								
A.A. follaje fresco	0	0	0	0	0	0	0	0
B.S. follaje fresco	0	0	0	0	0	0	0	0
A.A. follaje maduro	0	0	0	0	0	0	0	0
B.S. follaje maduro	0	0	1	0	0	0	0	1
Extractos de acetona más *Cedrela* en potes								
A.A. follaje fresco	0	1	1	0	0	0	0	2
B.S. follaje fresco	3	2	0	0	0	0	0	5
A.A. follaje maduro	0	0	0	0	0	0	0	0
B.S. follaje maduro	0	0	0	0	0	0	0	0

TABLA 2. Patrones de vuelo nocturno de ♀♀ y ♂♂ de palomillas de *H. grandella* muestreadas por medio de barreras de vuelo localizadas en Gurabo y Corozal.

Tiempo (horas)	RESPUESTA ♀♀	♂♂
2400	1	0
2430	2	0
0100	2	0
0130	3	1
0200	6	3
0230	4	2
0300	10	7
0330	5	9
0400	4	5
0430	0	2
0500	0	1
0530	0	1
Total	37	31

capturados. Estos resultados sugieren que las hembras responden más a los primeros atrayentes y son las primeras en detectar hospederos susceptibles. Las sexo–feromonas entonces explicarían las llegadas posteriores (p.e. atracción) de los insectos machos.

Estudios de Alimentación

Los resultados del estudio de alimentación demostraron que los extractos de *Toona ciliata* son distintamente tóxicos para las larvas de *Hypsipyla* (Tabla 3). Los extractos de metanol y hexano fueron completamente no tóxicos. Además de los tres correspondientes extractos del follaje de *Cedrela*, solamente la fracción de acetona mostró propiedades atrayentes. Una afinidad estrecha entre el principal atrayente de *Cedrela* y el componente tóxico de *Toona* es por lo tanto sugerido. Se están llevando a cabo investigaciones para clarificar esta relación. Estos experimentos, junto con los datos de campo, dan crédito a una hipótesis de que *T. ciliata* y *H. robusta* asiático han co–evolucionado. De acuerdo con las ideas de Fraenkel (1953, 1959), los mismos volátiles pueden ser usados por *H. robusta* como un estímulo en la selección del hospedero desde que las palomillas de *H. robusta* fácilmente atacan a *Toona*. Similarmente, es interesante que *Cedrela odorata* plantado en Africa y las Filipinas, no sean atractivos a *H. robusta* (Gray 1972); de nuevo, un caso donde los mecanismos de defensa de la *Cedrela* parecen adecuados en contra de las palomillas todavía no adaptadas al hospedero.

Los varios fragmentos de evidencia apuntan a las siguientes generalizaciones: las Meliaceae han desarrollado la producción de sustancias venenosas para *Hypsipyla*. El barrenador, el cual ha co–evolucionado con cada especie particular de árbol, se ha adaptado a estas sustancias secundarias y de hecho usa los materiales en cuestión como un atrayente. Debido a las sutiles diferencias químicas entre las sustancias producidas por las diferentes especies de Meliaceae, los árboles que han sido introducidos geográficamente dentro de nuevos ambientes serán, por un tiempo desconocido, inmunes al ataque del barrenador.

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TABLA 3. Tasa de mortalidad del 2° instar de *H. grandella* colocadas en la dieta McMorran–Grisdale suplementada con los siguientes aditivos: Extractos de hexano, metanol y acetona de *Toona ciliata;* extractos de hexano, metanol y acetona de *Cedrela odorata;* y celulosa molida.

Material del señuelo (Dieta + Aditivos)	% Mortalidad (acumulada) Días de prueba 1	2	3	4	5	6	7	8
Toona –Extracto de hexano	0	0	5	5	5	6	10	10
Toona –Extracto de metanol	0	0	0	0	0	0	0	0
Toona –Extracto de acetona	5	25	60	85	90	100	100	100
Cedrela –Extracto de acetona	0	0	0	0	0	5	6	6
Cedrela –Extracto de metanol	0	0	0	0	0	5	5	6
Cedrela –Extracto de hexano	0	0	0	0	0	0	0	0
Celulosa	0	0	0	0	0	0	2	2
Dieta sola (testigo)	0	0	0	0	0	0	0	0

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ESTUDIO PRELIMINAR DE EXTRACTIVOS DE LAS MELIACEAS QUE ATRAEN A *HYPSIPYLA GRANDELLA* ZELLER

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COMPENDIO

Hypsipyla grandella is the limiting factor on the production of the plantations of *Swietenia* sp., *Cedrela* spp. and other species of the same family. The extractive could be the answer for their economical control. The essential oils of the terminal buds and fresh leaves of *Cedrela odorata, Cedrela angustifolia, Swietenia macrophylla* and *Guarea guara* have been extracted with steam distillation and then separated according to their polarity. In comparing the non polar extractives, the common substances have been determined. One of them was present in all these species, in quantities which resulted equal to the preference of *H. grandella* for these four hosts. The infrared and spectrometer study of the masses revealed a sesquiterpene with 2 or 3 double bonds and a molecular weight of 204.

Introducción

El problema de ataque de *Hypsipyla* a las especies *Cedrela odorata, Cedrela angustifolia* y *Swietenia macrophylla,* en Venezuela se ha intensificado tanto en los últimos años que aun en los mismos viveros de Barinitas y Ticoporo el ataque del barrenador ha sido alarmante. Por tal motivo, y debido a que los controles hasta ahora ensayados en el país no han resultado económicos, ha surgido la duda de si es recomendable o no continuar la regeneración de estas especies mientras la plaga no se tenga bajo control.

En Venezuela se han experimentado los siguientes métodos para controlar el ataque de *Hypsipyla:*

a. Finol* del Instituto de Silvicultura, ULA, ha ensayado un cultivo mixto de *Swietenia macrophylla* y *Cassia siamea* a campo abierto, en la estación Sabana El Irel, con los siguientes resultados:
   1. Parcela mixta: 20 por ciento de ataque al material plantado al cabo de cinco años.
   2. Parcela testigo: totalmente atacada y destruida a los dos años.
b. Mundarain** también del Instituto de Silvicultura, ULA, recientemente ha ensayado los insecticidas Metasystox y Cebicid 85, mediante aplicación directa sobre la planta, en parcelas experimentales de *Swietenia macrophylla* y *Cedrela odorata.* Los resultados indicaron una disminución del ataque en las parcelas de *Swietenia* tratadas con los dos insecticidas. El Cebicid 85 también dio buen resultado en las parcelas de *Cedrela odorata,* sin embargo, en las parcelas de esta especie tratadas con Metasystox el ataque fue aún más intenso que en las parcelas no tratadas. Mundarain argumenta que, tal vez, el Metasystox activa la producción de las sustáncias que atraen a *Hypsipyla.*
c. Ramírez Sánchez (1966) del Instituto Forestal Latino Americano, en el año 1966, ensayó siete insecticidas para conocer su eficacia y la duración de sus efectos en una plantación de *Cedrela odorata* de hasta 3 m de altura en Barinitas, Venezuela. Llegó a la conclusión de que no sería mediante la aspersión de los insecticidas probados como se podría controlar el ataque de *Hypsipyla.*
d. Tillmanns (1964) en sus apuntes bibliográficos sobre control y combate de *Hypsipyla grandella* Zeller concluye que ninguno de los procedimientos ensayados ha resultado satisfactoriamente económico para controlar la plaga. Parece ser que la literatura no contiene información sobre el estudio de compuestos que actúen como atrayentes de insectos en las Meliáceas. Trabajos de esta índole ya se han realizado satisfactoriamente con especies de otras familias botánicas. A continuación se citan tres ejemplos:
   1. Se ha comprobado que en América Tropical la composición química de la fragancia de algunas especies de la familia Orquidiaceae (Dodson 1969) determina el tipo de abejas que atraen. Combinaciones apropiadas de compuestos con las mismas proporciones encontradas en las fragancias de las orquídeas, atraen las mismas abejas que son atraídas por las flores.
   2. Del aceite de las semillas de *Angelica archangelica* (Fornasiero, *et al.,* 1969) se han aislado dos

* FINOL, H. Cultivos a campo abierto de *Swietenia macrophylla* con especies nodrizas. Mérida, Instituto de Silvicultura, ULA, 1972. (Comunicación personal).

** MUNDARAIN, P. Control de *Hypsipyla* con insecticidas Metasystox y Cebicid 85 en parcelas experimentales de *Swietenia macrophylla* y *Cedrela odorata.* Mérida, Instituto de Silvicultura, ULA, 1972. (Comunicación personal).

compuestos: el copaene y el isómero a–ylangene, los cuales han sido considerados responsables de atraer la mosca de frutas *Ceratitis capitata*.

3. Cadahia (1965) por cromatografía de capa fina ha observado la presencia de un compuesto químico en los extractos acuosos y alcohólicos del género *Populus*, el cual estimula la oviposición del *Crytorrhynchus lapathi* L.

Sobre las sustancias atrayentes de *Hypsipyla* en las Meliáceas, Seelkopf* en 1964, usando el método de arrastre de vapor, obtuvo los aceites volátiles de ramas jóvenes de *Cedrela odorata*. Según versión de Seelkopf estos aceites colocados sobre ramitas secas, papel, trapos y piedras, en una jaula con mariposas de *Hypsipyla*, estimularon, en esos lugares, la oviposición de los insectos.

Con base en las informaciones de Seelkopf y Finol, Usubillaga, del Instituto de Investigaciones Químicas de la ULA y el autor, del Laboratorio Nacional de Productos Forestales, decidieron en setiembre de 1972 hacer un estudio intensivo de los aceites volátiles de las especies de Meliáceas: *Cedrela odorata*, *Cedrela angustifolia*, *Swietenia macrophylla* y *Guarea guara*, las cuales son atacadas en el mismo orden con intensidad decreciente. El autor considera que de existir un compuesto atrayente del insecto debe encontrarse en mayor concentración en *C. odorata*, la cual presenta mayor ataque en las plantaciones, en menor proporción en *C. angustifolia* y *Swietenia macrophylla*, las cuales son menos atacadas, y en muy poca concentración en *Guarea guara*, la cual casi no presenta ningún ataque en las mismas condiciones. Como complemento indispensable de esta investigación, G. Raets, del Instituto Forestal Latinoamericano de Investigación y Capacitación (IFLAIC) y el autor, comenzaron la crianza de mariposas en el laboratorio, siguiendo muy de cerca la técnica descrita por P. Grijpma (1971).

Experimentos y Métodos

Extracción

Para realizar la extracción del aceite se utilizaron 2 kg de material fresco de *Cedrela odorata* obtenido desde la yema terminal 30 cm hacia abajo, traído desde Barinitas, Venezuela (Lat. N. 8° 20'; alt. 500 m). Este material fue triturado con un molino de martillo y extraído con vapor de agua en un destilador de acero inoxidable durante 135 minutos, hasta obtener 8 litros de condensado. El condensado se dividió en fracciones de 2 litros, los cuales fueron saturados con NaCl y extraídos con 3 porciones de éter etílico, con volúmenes de 300, 200 y 100 cc. El material extraído fue secado con $CaCl_2$ y el éter evaporado en un evaporador rotatorio. En esta forma se obtuvieron 4 gramos de un aceite amarillo claro con olor fuerte a cedro.

Utilizando el mismo procedimiento se obtuvieron 2,9 gr de aceite de *C. angustifolia*, 4 gr de aceite de *S. macrophylla* y 4,5 gr de aceite de *G. guara*.

* SEELKOPF, C. Sustancias atrayentes de *Hypsipyla* en las Meliáceas. Mérida. Instituto de Investigaciones Químicas, ULA, 1972. (Comunicación personal).

Cromatografía de gas

Los compuestos de las muestras de aceites obtenidos fueron separados analíticamente en un cromatógrafo de gas Perkin–Elmer, modelo 880, utilizando una columna capilar Golay de silicone 500 DC con un arrastre de helio de 2 ml/min, temperatura de inyección 180°C, temperatura en la columna 134°C y temperatura en el detector 170°C. En esta forma se detectó un mínimo de 35 compuestos para *C. odorata*, 33 para *C. angustifolia*, 25 para *S. macrophylla* y 35 para *G. guara*.

Fraccionamiento del aceite de acuerdo a su polaridad

Un gramo de aceite de *C. odorata* fue disuelto en n–pentano y colocado sobre una columna de 1½ cm de diámetro con 30 gramos de sílica gel activada. Luego fue eluida sucesivamente con 200 cc de n–pentano, 200 cc de $CHCl_3$ y 200 cc de metanol, con objeto de dividir las sustancias en no polares, poco polares y más polares. Cada uno de los solventes fue recogido en fracciones de 20 cc.

Obtención de espectrogramas de uno de los compuestos

Tres de los volúmenes de 20 cc eluidos con n–pentano resultaron contener un compuesto casi puro (según lo reveló la cromatografía a gas); con uno de ellos se obtuvo un espectro infrarrojo en un espectrofotómetro Perkin–Elmer modelo 237 B y un espectro de resonancia magnética nuclear en un espectrómetro Varian 60 D. Luego se obtuvo su peso molecular utilizando un espectrógrafo de masa Varian Mat 111 diseñado para trabajar con un cromatógrafo de gas.

Ensayos nocturnos en el campo

Pequeñas porciones de las tres fracciones con diferentes grados de polaridad, así como de la muestra original, fueron colocadas por la noche sobre papel engomado en la plantación de las Meliáceas en estudio, para determinar el grado de atracción que tiene *Hypsipyla* por dichas sustancias. También se trituraron hojas terminales frescas de *Cedrela odorata* y se colocaron a campo abierto en un plato de petri dentro de una jaula.

Ensayos nocturnos en el laboratorio

En una jaula con 20 mariposas, en un cuarto acondicionado a una temperatura de 25°C, y humedad relativa de 65 por ciento, se colocaron durante la noche, sobre papel de servilleta y sobre pequeñas plantas de *Cordia alliodora*, las diferentes fracciones obtenidas, con objeto de determinar cuál de ellas estimulaba la oviposición del insecto. En otra oportunidad se colocaron en la jaula dos pequeñas plantas de *C. odorata*.

Resultados y Discusión

Cromatografía de gas

La separación de los compuestos del aceite en el cromatógrafo de gas con la columna Golay de silicone 500 DC resultó imperfecta, ya que aparecen picos superpuestos de compuestos oxigenados y no oxigenados. Sin embargo, la columna es más adecuada una vez que los compuestos han sido fraccionados de acuerdo a su polaridad.

Espectrogramas de un compuesto

Sobre sílica gel resultó fácil separar los aceites volátiles en no polares, poco polares y polares. Sólo hubo tiempo para iniciar el estudio de los no polares. Los volúmenes 4° 5° y 6° eluidos con n–pentano, cada uno resultó tener un compuesto en mayor abundancia, lo cual se demostró, en el cromatógrafo de gas, por tener diferentes tiempos de retención. El espectrógrafo de masa reveló un peso molecular de 204 para los compuestos principales de los tres volúmenes y el infrarrojo, un hidrocarburo con dobles enlaces.

Parece que se trata de un sesquiterpeno, es decir, $C_{15}H_{24}$. En la colección Saddler no se encontró ningún espectro de $C_{15}H_{24}$, similar al obtenido en este caso: actualmente se está trabajando en una mayor purificación de la sustancia, para obtener un espectro de la resonancia magnética nuclear, ya que los picos del espectro obtenido resultaron redondeados.

Resultados en el campo

En el campo, ni los paneles engomados sobre los cuales se colocaron las diferentes fracciones, ni la jaula con las hojas terminales trituradas, revelaron la visita del insecto. La plantación fue revisada y tan sólo se encontraron 8 huevos frescos esa mañana, distribuidos en la siguiente forma: 4 sobre dos *Cedrela odorata*, 2 sobre una *C. angustifolia* y 2 sobre una *S. macrophylla*, todos cerca del sitio sobre el cual se había colocado una de las muestras de los compuestos no polares. Este tipo de investigación fue suspendido hasta los meses de lluvia (abril, mayo y junio) en los cuales, según Roovers (1971), la población de la mariposa alcanza su pico máximo. Según Roovers y experiencia del autor, en los meses de noviembre y diciembre el ataque de *Hypsipyla* en la plantación de Barinitas es insignificante.

Resultados en el laboratorio

En el experimento con las mariposas en el laboratorio, trabajo realizado junto con G. Raets del IFLAIC, las mariposas que nacieron en el cuarto acondicionado, a partir de larvas y pupas recolectadas de plantas atacadas, no mostraron interés en aparearse, ni tampoco ovipositaron en forma regular en los sitios marcados. Los pocos huevos encontrados resultaron infértiles. En uno de los experimentos se notó mayor oviposición (huevos infértiles) sobre sitios sin ningún atrayente. Cuando se colocaron las pequeñas plantas de *C. odorata* no se notó ningún cambio en la conducta de apareamiento y oviposición.

Conclusiones y Recomendaciones

a. Tan pronto como se compruebe el experimento de Seelkopf o resulten positivas algunas de las extracciones con solventes orgánicos realizadas en el Laboratorio Nacional de Productos Forestales, quedaría demostrado que hay, por lo menos, un compuesto que atrae a *Hypsipyla;* esto daría una gran ventaja en el control del insecto, ya que se podría sintetizar el compuesto, de no encontrarse en el mercado. Después se podría:
   1. Poner un sebo para atraer y envenenar la mariposa, o, por lo menos, para que oviposite en sitios en los cuales el huevo no pueda germinar, o de hacerlo que la larva no encuentre las condiciones apropiadas para su desarrollo.
   2. Los genetistas podrían obtener una nueva variedad botánica que no posea el compuesto atrayente.

b. Sin la presencia de *Hypsipyla* es imposible hacer un trabajo estadístico con objeto de determinar qué compuesto la estimula a ovipositar. Si sólo se piensa trabajar con las mariposas de las plantaciones la investigación deberá limitarse a los meses de abril, mayo y junio, en los cuales la población es máxima; por lo tanto, la crianza de este insecto, a disponibilidad del investigador químico, es indispensable para no interrumpir el trabajo.

c. Una vez que se haya identificado el compuesto a los compuestos que atraen a *Hypsipyla* y su proporción en las cuatro especies estudiadas, sería conveniente investigar la posible presencia de un compuesto repelente a la mariposa en *Guarea guara.*
   El uso de un repelente natural con un atrayente natural sería instrumento suficiente para ejercer pleno control sobre el insecto.

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WHITE PEACH SCALE ATTACK ON TOON IN PUERTO RICO*

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COMPENDIO

Toona ciliata var. *australis* crece sobre una variedad de sitios, y resiste el ataque de *Hypsipyla grandella.* Por estas razones se cree que servirá en el lugar de *Cedrela odorata* para plantaciones neotropicales. Pero en Puerto Rico se ha notado que es atacada por el *Pseudaulacaspis pentagona,* frecuentemente, con efectos indeseables. Cualquier plan de reforestar con *Toona ciliata* var. *australis* en gran escala, debe incluir un método para controlar este insecto.

The white peach scale, *Pseudaulacaspis pentagona* (Targioni–Tozzeti) Homoptera–Diaspididae, has been reported by Martorell[1] to attack several tree species in Puerto Rico. These include *Erythrina glauca* Willd., *E. poeppigiana* (Walp.) O. F. Cook, *Hibiscus tiliaceus* L., *Mammea americana* L., *Mangifera indica* L., *Montezuma speciosissima* Sessé & Moc., *Trema lamarckiana* (Roem. & Schult.) Blume, *T. micrantha* (L.) Blume, and others. When abundant it can defoliate and cause dieback, deformation and eventually death of the tree (Martorell[2], Anon.[3]).

Toona ciliata M. Roem. var. *australis* (F.v.M.) C. DC. (toon) is promising as a tree to replace *Cedrela odorata* L. (cedar), possibly the most commercially important timber tree native to the neotropics. It is an Australian member of the mahogany family (Meliaceae), to which cedar also belongs. Plantations of cedar are hindered in the Western Hemisphere by two factors: 1) specific site requirements (which are as yet poorly understood); and 2) susceptibility to attack by the mahogany shootborer (*Hypsipyla grandella* Zeller). Toon has shown itself unhindered by both of these factors in Puerto Rico and in Costa Rica (Grijpma[4]; Grijpma and Ramalho[5]). The woods of the two species are said to be nearly identical (Grijpma and Ramalho[5]), although further tests are needed, especially on plantation–grown material.

Seeds of toon were received early in 1971 from the Interamerican Institute of Agricultural Sciences at Turrialba, Costa Rica and sown in the Institute of Tropical Forestry nursery at Río Piedras, Puerto Rico. In December of 1971, fifty of the resulting seedlings were planted at the Puerto Rico Commonwealth Forest at Vega Alta (50 m altitude), and another fifty at the Corozal Station of the University of Puerto Rico Agricultural Experiment Station (200 m altitude). Other sites around the island were also sown with fifty trees. Those at Corozal were planted among 1 year old seedlings of cedar under the shade of *Inga laurina* (Sw.) Willd. Those at Vega Alta were planted in the open sun of a grassy meadow between limestone hills. Both of these sites have red clay soils.

After one year in the field the toon at Corozal was outgrowing the 2 year old cedars. At Vega Alta it grew even more rapidly than at Corozal, the best individuals being 3.5 m in height. It was not attacked by *Hypsipyla grandella* and its form was excellent with the following exceptions: a) branches, while not frequent, were present occasionally and threatened to ruin its form if not pruned, and b) at Corozal, some trees were hampered by overhead *Inga* shade and grew in non–vertical directions toward the light. *Hypsipyla grandella* was present at Corozal, at least, since the adjacent cedar seedlings were attacked.

The white peach scale was nofed in great numbers on several of the toon seedlings at both sites during the first year measurement (December, 1972)[6]. In some cases, a 1.5 m section of the stem was covered with the scale, giving it a white aspect, much as if painted white. At Corozal none were found on the adjacent cedars. In most cases, toon appeared quite vigorous. Indeed, the insect attacked trees of all sizes, regardless of vigor or growth rate (Figure 1). At Corozal, one seedling was

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Fig. 1. Heavy incrustation of the white peach scale on a vigorous 1 year old seeding of toon at Vega Alta. The white aspect of a few months earlier is now dark, as shown above.

seen to be both lacking in vigor and covered with the white peach scale. Three months later (March, 1973), this seedling's main stem was dead and the only leaves present were from basal sprouts. In May 1973, toon plantations at Yabucoa and at Río Abajo, of the same age as those at Corozal and Vega Alta, were also being attacked by the white peach scale. Also in May 1973 toon which were attacked earlier at Vega Alta were sprouting adventitious buds, and resinous bleeding along the stem was common.

Toon has many features to recommend it for large-scale planting in Puerto Rico and elsewhere. However, such a planting program would need to provide for control of the white peach scale, because loss of vigor and form of trees may prove of economic consequence.

FLIGHT OF THE MAHOGANY SHOOTBORER, *HYPSIPYLA GRANDELLA* *

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ABSTRACT

Field studies were undertaken in Costa Rica to elucidate flight preparation and behavior of released *Hypsipyla grandella*. In a cleared area, 83% of the released moths flew toward and landed in the foliage of larger trees; the rest landed on the lower, grassy vegetation. Flight was correlated with ambient air temperature but not with relative humidity. Wind speed was important in determining the direction in which moths flew, and no difference was observed in flight behavior between males and females.

The mahogany shootborer, *Hypsipyla grandella* Zeller, is a serious problem in the establishment of Spanish cedar (*Cedrela* spp.), mahogany (*Swietenia* spp.), and other Meliaceae plantations in the American tropics. This lepidopteran borer lays eggs on shoots and leaves of young plants and the larvae bore into terminals and stems of their host plants. Height growth is greatly reduced and the trees are completely stunted due to repeated attacks. Ramírez Sánchez (1964) has described the biology of *H. grandella*.

H. grandella is believed to select its host at night as adult insects are most active during the evening hours (Ramírez Sánchez 1964, Gara *et al.* 1973). It has been suggested (Grijpma and Gara 1970) that *H. grandella* remains inactive during daylight hours and hides in grass cover associated with Meliaceae plantations. Flight occurs when nightly temperatures are above 17°C; apparently rainfall above 11 mm inhibits flight (Gara *et al.* 1973). However, detailed studies on *H. grandella* flight behavior and nocturnal movements are lacking. Accordingly, the objective of this study was to observe and describe flight preparation and behavior of released insects.

Materials and Methods

An initial study was undertaken in a small, 20 x 20 m *Cedrela odorata* L. plantation located in Florencia Sur, Turrialba, Costa Rica. The 2 x 2 m spaced trees were ca. 5 years old and averaged 1.5 m in height. The grass cover of the plot had been cut previously. Insects utilized in the study were reared on artificial diet (Hidalgo–Salvatierra 1971).

On 2 consecutive days and hourly, from 0800 to 1800 and from 2300 to 0300 h, 6 *H. grandella* adults (3 ♂ and 3 ♀), 12–72 h old, were relased from a 1.5 x 0.5 m piece of canvas, stretched on a frame and supported 15 cm above the ground. Later, a 2nd study was carried out in an adjacent mixed Meliaceae plantation with an abundant grass cover. Under these conditions 2 releases of 10 ♂ and 10 ♀ were made at 1000 h on 2 different dates. This grassy area was later swept with an insect net to determine the number of released *H. grandella* adults present.

Ambient temperature and relative humidity (RH) were recorded by a hygrothermograph and wind speed and precipitation were determined by a Hastings anemometer® and a standard rain gauge, respectively. Flight distance and elevation, as well as flight termination, were watched whenever light conditions permitted.

Results

Half the insects tested during dayling hours flew. Of those that flew, 83% flew toward and landed in the foliage of larger trees (8–10 m). The rest landed on the ground and remained motionless on the grassy vegetation. In several instances, the moths, having flown to the ground, quickly climbed the grass and flew again, finally coming to rest in a 10 m high tree. Flight preparation consisted of rapid wing flutterings for 5–30 s while the moths orientated into the wind. In all cases the antennae were held in a forward position throughout flight preparation. Of the 40 adults released in the grassy, mixed Meliaceae plantation, 32 flew out of grass and upwards, finally coming to rest in the crowns of 7 m tall trees. The remaining 8 flew into the grass and remained there. Three days later, the grassy area was thoroughly swept, but no *H. grandella* adults were retrieved. At the time of the study, this plantation was under heavy shootborer attack.

Between 2300 and 0200 h, all insects readily flew. Later, between 0200 and 0300 h, none flew, even when tossed in the air. On the average, ambient temperatures varied from 19.5°C at 2300 to 17°C at 0300. Even during precipitation of 2.5 mm/h, ½ the insects flew (an 1800 h test).

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* Lepidoptera: Phycitidae.
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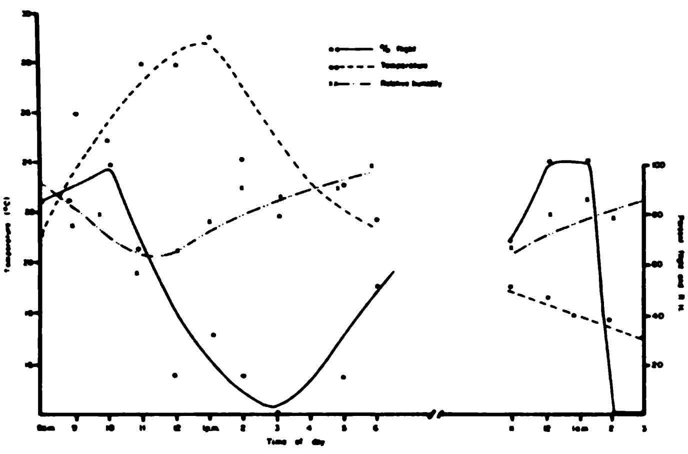

Fig. 1. *H. grandella* flight activity as related to time of day, temperature, and relative humidity.

When wind speed was less than 0.5 m/s, 63% of the moths began and continued to fly into the wind. With wind speeds above 0.5 m/s, 75% of the moths which flew began to fly into the wind but quickly circled and flew with the wind. There was no difference in flight behavior between males and females.

Discussion

Only a few of the released *H. grandella* flew to ground cover; whereas 83% came to rest in the crowns of larger trees. Apparently, tree crowns are highly attractive to *H. grandella* as resting spots. The study undertaken in the grassy area confirmed the fact that *H. grandella* most likely spend daylight hours in surrounding trees.

Fig. 1 indicates that *H. grandella* readily flew when temperatures ranged from 22–25°C. A temperature of ca. 25.5°C was associated with a decrease in per cent flight. Also, between 0200 and 0300 h, when temperatures were 17°C or lower, no flight was observed, confirming earlier findings of Gara *et al.* (1973). RH appeared to have little or no effect on the percentage of insects that flew.

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BIBLIOGRAFIA SOBRE *HYPSIPYLA*

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Instrucciones para el usuario. Las referencias están en orden alfabético según autor. Cada referencia está clasificada, por las siguientes materias, en un índice localizado al final de la bibliografía.

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IV. **Silvicultural control**, including resistant species introduction and breeding, site selection, etc.
V. **Mass rearing techniques**, including diet, captive behavior, etc.
VI. **General**, including information on the host species, entomological study techniques, etc.

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FECHA DE DEVOLUCION

ESTA OBRA SE TERMINO DE IMPRIMIR EL 27 DE FEBRERO DE MIL NOVECIENTOS SETENTA Y SEIS EN ARTES GRAFICAS DE CENTROAMERICA S.A.

SE HIZO UN TIRAJE DE 800 EJEMPLARES.

Autor	STUDIES ON THE SHOOTBORE[R] HYPSIPYLA GRANDELLA ...
Título	

Fecha Devolución	Nombre del solicitante

Impreso en Costa Rica
por Artes Gráficas de Centroamérica S.A.