# STUDIES ON THE SHOOTBORER *Hypsipyla grandella* (Zeller) Lep. Pyralidae

## Volume I



P. GRIJPMA
editor

IICA
MISCELLANEOUS PUBLICATION No. 101

**Cover:** Pupae of *Hypsipyla grandella* (Zeller) reared on artificial diet.

**Portada:** Pupas de *Hypsipyla grandella* (Zeller) criadas en dieta sintética.

## SONATINA

*Rubén Darío*
(nicaragüense)

La princesa está triste . . . ¿qué tendrá la princesa?
Los suspiros se escapan de su boca de fresa,
que ha perdido la risa, que ha perdido el color.
La princesa está pálida en su silla de oro.
Está mudo el teclado de su clave sonoro,
y en un vaso, olvidada, se desmaya una flor.

El jardín puebla el triunfo de los pavos reales.
Parlanchina, la dueña dice cosas banales,
y, vestido de rojo, piruetea un bufón.
La princesa no ríe, la princesa no siente;
la princesa persigue por el cielo de Oriente
la libélula vaga de una vaga ilusión.

¿Piensa acaso en el príncipe de Golconda o de China,
o en el que ha detenido su carroza argentina
para ver de sus ojos la dulzura de luz?
¿O en el rey de las islas de las rosas fragantes,
o en el que es soberano de los claros diamantes,
o en el dueño orgulloso de las perlas de Ormuz?

¡Ay! , la pobre princesa de la boca de rosa
quiere ser golondrina, quiere ser mariposa,
tener alas ligeras, bajo el cielo volar,
ir al sol por la escala luminosa de un rayo,
saludar a los lirios con los versos de mayo
o perderse en el viento sobre el trueno del mar.

Ya no quiere el palacio, ni la rueca de plata,
ni el halcón encantado, ni el bufón escarlata,
ni los cisnes unánimes en el lago de azur.
Y están tristes las flores por la flor de la corte;
los jazmines de Oriente, los nelumbos del Norte,
de Occidente las dalias y las rosas del Sur.

¡Pobrecita princesa de los ojos azules!
Está presa en sus oros, está presa en sus tules,
en la jaula de mármol del palacio real;
el palacio soberbio que vigilan los guardas,
que custodian cien negros con sus cien alabardas,
un lebrel que no duerme y un dragón colosal.

¡Oh, quién fuera *hipsipila* que dejó la crisálida!
(La princesa está triste, la princesa está pálida).
¡Oh visión adorada de oro, rosa y marfil!
¡Quién volara a la tierra donde un príncipe existe
(la princesa está pálida. La princesa está triste),
más brillante que el alba, más hermoso que abril!

—Calla, calla, princesa —dice el hada madrina—;
en caballo con alas, hacia aquí se encamina,
en el cinto la espada y en la mano el azor,
el feliz caballero que te adora sin verte,
y que llega de lejos, vencedor de la Muerte,
a encenderte los labios con su beso de amor.

To establish a rotary fund for continuation of this series, this publication is being sold at US$4.00.

A fin de establecer un fondo rotatorio para la continuación de esta serie, esta publicación se vende al precio de US$4.00.

# STUDIES ON THE SHOOTBORER *Hypsipyla grandella* (Zeller) Lep. Pyralidae

## Volume I



P. GRIJPMA
editor

00000248 ~~001148~~

## TABLE OF CONTENTS

## *PREFACE*

*This publication is a collection of articles and communications resulting from investigations carried out by members of the Inter–American Working Group on the shootborer* Hypsipyla grandella *(Zeller). The papers, which are arranged chronologically, were published earlier in "Turrialba", the journal of the Inter–American Institute of Agricultural Sciences at Turrialba, Costa Rica.*

*The contents of this booklet reflect the growth of the research by the members of the Working Group 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.*

*New information, which eventually may result in control of this borer was obtained and is being developed continuously. The participants in the Inter–American Working Group, which was established at Turrialba in September 1970, grew in two years from 5 to 72 members, representing 26 countries. This vivid interest justified the establishment of an international working party on integrated control of* Hypsipyla *spp. under the auspices of IUFRO, at Gainesville, Florida in March 1971.*

*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, in the research projects deserves special mention.*

*Funds for this publication were made available by the Netherlands Bureau of International Technical Assistance which, together with the Department of Tropical Forest Sciences of IICA–CTEI, and the Nuclear Energy Program at Turrialba, also supported the investigations.*

*Pieter Grijpma*
*Inter–American Working Group on* Hypsipyla

# *TOONA* SPP., POSIBLES ALTERNATIVAS PARA EL PROBLEMA DEL BARRENADOR *HYPSIPYLA GRANDELLA* DE LAS *MELIACEAE* EN AMERICA LATINA*

*P. Grijpma***, R. Ramalho****

Silvicultor, Departamento de Ciencias Forestales
Instituto Interamericano de Ciencias Agrícolas de la OEA, Turrialba, Costa Rica

Profesor de Dendrología de la Escola Superior de Florestas
de la Universidade Federal de Viçosa, Minas Gerais, Brasil (respectivamente)

## ABSTRACT

*Toona ciliata* and *T. ciliata,* var. *australis* are fast growing valuable forest trees, which, when planted in forest plantations in their native countries, suffer heavily from attacks of *Hypsipyla robusta,* a shoot borer of *Meliaceae*. Trials with *T. ciliata* and its variety *australis* in Latin America reveal that these exotic *Meliaceae,* as well as some others, are not attacked by *Hypsipyla grandella,* present in this continent. Parallel to this experience are findings that *Cedrela odorata* introduced in other continents is not or is less attacked by *Hypsipyla robusta* than native *Meliaceae.* Apparently some preference exists, and selection is made by the native *Hypsipyla* moth. Grijpma supposes that this selection is based on the attraction of the *Hypsipyla* moth by a specific odour of the host tree, which would be different for various *Meliaceae.* It was noted that when leaves and young shoots of *Cedrela odorata* were crushed, a strong garlic–like smell was produced, which was not noticed when leaves of *T. ciliata* var. *australis* were treated in the same way. Of the plots of both species, established in the Puente Cajón Species Trials in Turrialba, Costa Rica, *Cedrela odorata* is heavily attacked by *Hypsipyla grandella,* while *Toona ciliata* var. *australis* growing in the same area is not attacked at all. It is thought that the hypothesis of the olfactory orientation of the moth of *Hypsipyla* spp. might open new ways toward the solution of the *Hypsipyla* problem: e.g. by means of (gas) chromatography, the main odorific components of the essential oils in the leaves and the shoots of the *Meliaceae* could be analized and at a later stage perhaps be used as attractants against *Hypsipyla.*

If the hypothesis of olfactory orientation of the *Hypsipyla* moth is correct, it would also be possible to select and breed *Meliaceae,* which do not possess or have a low content of the attractant component in the leaves and young shoots.

Crossing of the attack–free species (e.g. *Toona* with *Cedrela*) could offer other interesting aspects. The fact that *Toona* and *Cedrela* can be propagated vegetatively, is an additional advantage.

This paper further reviews the available botanical, ecological, silvicultural and technological data on *T. ciliata* and its variety *australis.* It also includes a short summary of information on *Toona sureni* and *Toona calantas,* other species of this genus which may be of very much interest to Latin America.

The authors

* Recibido para la publicación el 15 de octubre de 1969.

** En un convenio bilateral del IICA con el Programa de Asistencia Técnica de Holanda.

*** Actualmente estudiante graduado del IICA.

## Introducción

La caoba y el cedro son dos especies forestales latinoamericanas cuyas maderas tienen un prestigio mundial y están bien cotizadas en el mercado internacional. Ambas, especies de los géneros *Swietenia* y *Cedrela,* pertenecen a la familia de las *Meliaceae* y tienen su distribución natural limitada exclusivamente a América Latina y las Islas del Caribe.

No obstante el alto valor económico que tienen estas especies para el continente, ha sido virtualmente imposible, hasta el momento, cultivarlas económicamente en plantaciones en América Latina, debido a los frecuentes ataques de la larva de *Hypsipyla grandella* Zeller, un barrenador que ataca varias partes del árbol pero principalmente los brotes. Los daños causados son generalmente la muerte del brote terminal, y como consecuencia la formación de numerosos brotes secundarios que producen deformaciones del tronco. Los repetidos ataques disminuyen también el crecimiento, o incluso pueden causar la muerte de árboles jóvenes. Este problema existe en otros continentes, donde otras especies forestales pertenecientes a la familia de *Meliaceae* son atacadas por otras especies *Hypsipyla* (24). En Asia y Oceanía por ejemplo, los ataques de *Hypsipyla robusta* impiden el desarrollo de plantaciones de *Toona* spp., mientras en Africa las larvas de *Hypsipyla albipartalis* e *Hypsipyla robusta,* causan grandes daños en *Meliaceae* nativas (2, 3, 15, 22, 38, 50, 53).

El control de la *Hypsipyla* con insecticidas es difícil; en primer lugar porque las larvas cuando emergen de los huevos (depositados generalmente en los tallos, el haz de los folíolos en las axilas de las hojas) penetran rápidamente en los tejidos de la planta. Es probable que el tiempo en que serían susceptibles a insecticidas no pasaría de algunas horas (50). Según Ramírez (45) la eclosión es más frecuente durante las primeras horas de la noche, lo que también dificultaría el combate de este barrenador. Otro problema consiste en la alta frecuencia de los ataques debido al relativamente corto ciclo que tiene la *Hypsipyla.* El ciclo completo de *H. grandella* dura 35 ± 5 días (45), pudiéndose repetir este ciclo durante todo el año, lo que hace que el control con insecticidas sea muy costoso.

Para reducir los ataques de *Hypsipyla* sobre las *Meliaceae* existen varias recomendaciones, las cuales generalmente se refieren a la mezcla en plantaciones de las *Meliaceae* con otras especies forestales, manejo de sombra, tipo de suelo y tratamientos que limitan la propagación del insecto. También se está investigando el uso de insecticidas sistémicos y el control biológico para combatir la *Hypsipyla;* en Puerto Rico, un parásito *Calliephialtes* spp., ha sido mencionado y en la India han sido encontrados 11 parásitos de los cuales *Trichogramma minutum* es probablemente el más importante porque parasita los huevos de *Hypsipyla robusta* (46, 50).

Aunque los métodos mencionados anteriormente podrían constituir los puntos claves para el control sistemático de la *Hypsipyla,* y como consecuencia podrían dar la solución para el establecimiento de plantaciones económicas de *Meliaceae* en América Latina, éstos no serán discutidos aquí.

En esta contribución los autores quieren llamar la atención al hecho que **hay indicaciones de que existe cierta preferencia de parte de la(s) especie(s) de *Hypsipyla* nativa(s) por ciertos representantes de la familia de la *Meliaceae.***

Analizando la literatura sobre la introducción de *Meliaceae* exóticas en los países tropicales de los diferentes continentes es notable que **algunas** de estas especies tiendan a ser menos atacadas por la(s) *Hypsipyla(s)* nativa(s).

En Queensland, Australia, la exótica *Cedrela odorata* proveniente de América Latina es considerada como una especie forestal de gran promesa, en vista de su rápido crecimiento y el hecho de que no es atacada por la *Hypsipyla robusta* el barrenador que destruye *Toona ciliata* var. *australis* que crece a la par (53).

Paralelamente, se ha observado el mismo fenómeno en Turrialba, Costa Rica, en las pruebas de especies forestales del Instituto Interamericano de Ciencias Agrícolas, donde *Toona ciliata* var. *australis* introducida de Australia está libre de ataques de *Hypsipyla grandella,* mientras las especies nativas, *Cedrela odorata, Swietenia macrophylla* y *Swietenia humilis,* en parcelas colindantes a las de *Toona,* son frecuentemente atacadas.

En Puerto Rico, Geary (27) informa que las especies *Toona ciliata* y *Toona sureni* tampoco están atacadas, mientras *Cedrela odorata* y *Swietenia macrophylla* en el mismo arboreto de Ciénaga Alto son atacadas fuertemente.

*Cedrela odorata* introducida en Africa no parece susceptible al barrenador (44). En Java, Indonesia esta especie sí es atacada por *Hypsipyla robusta,* pero los daños no se consideran serios y la especie es usada para reemplazar a *Toona sureni* que es fuertemente atacada (3, 56).

En Filipinas las plantaciones de *Swietenia macrophylla* están libres de ataques, y la especie se está usando para reforestaciones (50).

Chable (16) observó en Honduras que los ataques de *Hypsipyla grandella* son mínimos en las especies: *Khaya nyassica, Khaya ivorensis, Toona ciliata* y *Entandrophragma rederi,* todas miembros de las *Meliaceae,* procedentes de otros continentes. Según sus datos *Entandrophragma* nunca ha sido atacada.

En Turrialba, *Khaya ivorensis* plantado a la par de *Swietenia macrophylla* no es atacada tampoco, mientras la parcela de la caoba es un verdadero fracaso debido al barrenador.

No obstante estos datos positivos hay que enfatizar que no se trata de una estricta especialización de la(s) *Hypsipyla(s)* nativa(s) sobre las *Meliaceae* nativas. Existen varios informes en los cuales se mencionan serios ataques de la *Hypsipyla* nativa en *Meliaceae* exóticas. En Ceilán e India, *Hypsipyla robusta* es considerada como una seria plaga para la introducida *Swietenia macrophylla* (53); en Australia la introducción de *Swietenia mahogani* fracasó debido a los ataques del barrenador nativo (53) y en las Antillas francesas se observó que *Khaya senegalensis,* proveniente de Africa sí es atacada por el barrenador *Hypsipyla grandella* (50).

En vista de la aparente inmunidad de *Toona ciliata, T. ciliata* var. *australis, T. sureni, Khaya ivorensis* y *Entandophragma* spp., a los ataques del barrenador *Hypsipyla grandella,* sumado a la buena calidad de su madera, estas especies merecen recibir una alta prioridad en las pruebas experimentales con especies forestales en América Latina.

Ya que *Toona ciliata* y *ciliata* var. *australis* figuran entre las más prometedoras *Meliaceae* exóticas para

América Latina, se da a continuación una revisión amplia de la literatura existente sobre esta especie y su variedad, y un resumen de datos para algunas otras especies de *Toona*, de posible interés.

## General

### Nombres botánicos:

a) *Toona ciliata* M. Roem.
b) *Toona ciliata* M. Roem. var. *australis* (F.v.M.) C.D.C.

### Sinónimos:

a) *Toona ciliata: Cedrela toona* Roxb. ex Rotll.
b) *Toona ciliata* var. *australis: Toona australis* Harms; *Cedrela australis* F.v.M.

Ha existido mucho desacuerdo entre los botánicos sistemáticos sobre *Toona;* las especies de este género asiático se parecen tanto a las de *Cedrela* de América Latina, que de Candolle (11) las agrupó todas en *Cedrela.* En 1846 Roemer (49) separó los cedros asiáticos en el género *Toona,* pero de Candolle las mantuvo en *Cedrela.* Harms (18, 29) distinguió en 1896 nuevamente el género *Toona* de las *Cedrela,* pero de Candolle en 1908 los reunió otra vez (12). Recientemente Smith (52), en su revisión del género *Cedrela,* comprueba que la separación hecha por Roemer fue correcta y distingue por las siguientes razones los dos géneros: *Toona* en Asia y *Cedrela* en América Latina:

a. Entre las más importantes diferencias morfológicas figura la columna que forma el ginóforo en *Cedrela* y su ausencia en *Toona.*
b. En *Cedrela* los filamentos están adnatos a la superficie del ginóforo, mientras en *Toona* los filamentos expandidos forman un tipo de almohadilla en el cual el ovario está involucrado parcialmente (Figura 1).
c. Los pétalos de *Cedrela* están adnatos al ginóforo por medio de una carina en la superficie interior. Los pétalos de la flor de *Toona* están conectados por su base misma al ápice del pedicelo, muy abajo de la masa de filamentos expandidos.

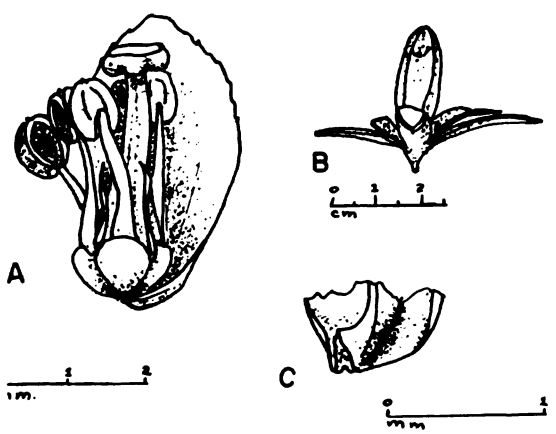



Fig. 1. Morfología de una flor de *Toona.* A– Flor disecada de *Toona serrata* (Royle) Roem., mostrando un pétalo insertado abajo de la masa de filamentos expandidos y los estaminodios alternando con los estambres. La base del ovario está rodeada por el tejido en forma de almohadilla, anteriormente descrito como disco. B– Fruta de *Toona sinensis* A. Juss. C– Base de un pétalo mostrando la pequeña área de conexión. Tomado de Smith Jr., E. A.: (38).

d. En *Toona* el cáliz está formado por cinco lóbulos bien diferenciados, soldados sólo brevemente en la base, consecuentemente el cáliz se abre en forma plana o refleja al antesis. En vista de que los pétalos están soldados solamente al ápice del pedicelo, éstos también se abren ampliamente. En *Cedrela,* los segmentos del cáliz están soldados formando una copa, la adnación de los pétalos al ginóforo permite su abertura solamente encima del punto de conexión.

Hace falta una revisión del género *Toona,* para establecer con claridad los nombres actualmente usados para las especies de este género. No está bien establecido por ejemplo, si el *Toona* de los trópicos de Australia es una especie distinta *(T. australis)* o solamente una variedad *(T. ciliata* var. *australis);* también se desconoce si *T. ciliata* del Pacífico Oeste (33) es el mismo que el que se encuentra en la India. Según algunos autores (42) existen 20 variedades de la especie *Toona ciliata.*

### Nombres comunes:

1. *Toona ciliata:* Toon tún, túni, maha nim (Hindi); Túni, tún, túna, lúd (Bengalí); Maha limbu (Uriya); Mahlun (Satpuras); Drawi (Pb.); Túni, bobich (Nepalés); Simal (Lepcha); Somso (Bhutia); Poma, Henduri Poma (Assamés); Goria ním (Melghat); Grawa (Khond); Mahalimo (Saora); Kujya (Tippera); Katangat (Kól); Madagiri vembu (Madura) Santhana vembu, thevatharam (Tamalés); Mathagiri vembu (Mal); Vedi vembu (Trav. Hills); Súli, máli (Salem); Kal kilingi (Nilgiris); Sandani vembu (Tinnevelly); Tundú, kempú gandagheri (Kan); Nogé, chikado, tseetkado (Maghalés); Shurúzbed (Chakma); Thitkado, tawtama, ni, kashitka (Burmés); Moulmein cedar, Indian mahogany, Singapore cedar, Sandal neem, Happy Tree, White toon, Yomhom, Burma cedar. En Nueva Guinea la especie se encuentra bajo los siguientes nombres vernaculares: Red cedar mafus. (Lower Markham Valley), Epi (Suku), Kapere (Vailala), Mufus (Yulu). (5, 7, 31, 33, 47).

2. *Toona ciliata* var. *australis:* Red cedar, Australian (red) Cedar, Australian Toon (13, 14, 26, 31, 59).

### Distribución natural:

*Toona ciliata* y sus variedades tienen una distribución muy amplia; la especie se encuentra en la India, al este de Pakistán, Birmania, Tailandia, el sur de China, Nueva Guinea y Malaya, en el Archipiélago de Bismarck, Celebes, Moluccas y Filipinas, y en los valles del Himalaya hasta 1.300 m. Se encuentra generalmente en los bordes de los ríos o en precipicios sombríos desde Assam, Bengalia, hasta el oeste de los Ghats. Muchas veces se encuentra también en pantanos al pie del Himalaya. En el sur de la India ocurre principalmente en los bosques tropicales húmedos (5, 30, 33, 34). Según Kraemer (33) la especie se encuentra en toda la región del Pacífico Oeste, pero en ningún lugar es abundante; se extiende desde las zonas de bajura de la costa hasta 1.700 m en las laderas de las montañas. *Toona ciliata* var. *australis* tiene una distribución natural en el este de Australia, desde Ulladulla, al sur de Sydney, en el Estado New South Wales hasta Atherton en el norte de Queensland (26, 30).

## Descripción

### General:

*Toona ciliata* y su variedad *australis* son árboles deciduos, grandes, que pueden alcanzar 50 m de altura, con diámetros de 1,50 m (15, 18, 33, 38). El tronco es generalmente recto y por 75 por ciento libre de ramas (15, 33); las gambas son frecuentes, por lo menos en áreas tropicales y subtropicales (26, 33; Figura 2).

### La corteza:

La corteza de árboles maduros tiene un color gris, café o rojizo, con un grosor de aproximadamente 6–15 mm. La caída de la corteza es por escamas grandes (Figura 2). Cortando la corteza se pueden diferenciar dos capas, la capa exterior que tiene un color rojizo, mientras que la capa interior es de color blanco; generalmente posee un líquido de color oscuro con un olor agradable y un sabor amargo (5, 15, 26, 33, 60).

Fig. 2. *Toona ciliata* M. Roem. var. *australis* (F. v. M.) C.D.C. en el bosque tropical pluvial australiano. La foto muestra el desarrollo de las gambas y aspectos de la corteza. Tomado de Francis (26).

### Las hojas:

Las hojas son deciduas, alternas, pendientes, compuestas, frecuentemente paripinadas, de 30–50 cm de largo, 6–12 pares de folíolos, con frecuencia 7. Benthall (5) indica que las hojas de *Toona ciliata* pueden tener hasta 100 cm de largo. Los folíolos son opuestos o casi opuestos, glabros, oval–lanceolados, obtusos en la base, ápice acuminado, 8–13 cm de largo y 7–8 cm de ancho, los márgenes a veces ondulados, los lados desiguales y curvados de un color verde brillante en las hojas maduras y rojizo en las hojas jóvenes. Los pecíolos de los folíolos miden aproximadamente 6 mm (5, 15, 18, 54, 58).

La variedad *australis* tiene hojas alternas compuestas, pinadas, con 3–8 pares de folíolos, cada una con apariencia de una hoja ordinaria, lados desiguales, con pecíolos de menos de 1 cm de largo (26, Figura 3).


Fig. 3. Inflorescencia, hojas y frutos de *Toona ciliata* M. Roem var. *australis* (F.v.M.) C.D.C. Tomado de Francis, (26).

### Flores:

La inflorescencia es una panícula terminal pendiente, que lleva, flores blancas, hermafroditas, olorosas, cáliz en

cupela, puberulento en el exterior, con 5 sépalos cilioides; 5 pétalos oblongos, cilioides; disco amarillo, pubescente, más corto que el ovario, ovario pubescente, estilo glabro, 5 estambres con filamentos glabros o pubescentes (5, 15, 18, 58, Figura 3).

Francis (26) da la siguiente descripción de la variedad *australis:* flores olorosas, en panícula grande, terminal; las flores individuales miden aproximadamente 4 mm de largo, el cáliz formado de 5 lóbulos y de 1–2 mm de largo. En el interior del cáliz están los 5 pétalos ovalados que tienen aproximadamente 4 mm de largo. Más adentro y más corto que los pétalos están los estambres variando en número de 4 a 6. El ovario en el centro de la flor tiene un estilo culminado en un estigma redondo y plano.

Smith (52) cree que el androceo de *Toona* se deriva de un tubo estaminal por la razón de que los estaminodios están alternados con sus estambres y por la existencia de la masa de tejido, alrededor del ovario (Figura 1).

En Calcuta, India, *Toona ciliata* florece en febrero–marzo (5). La variedad *australis* florece en setiembre–octubre (26). En general la floración y fructificación ocurre todos los años (5, 39).

### Frutos y semillas:

Cápsulas pediceladas, oblongas, leñosas, 2–2,5 cm de largo y 0,75–1,0 cm de diámetro, abertura apical en 5 valvas; contiene semillas con alas membranosas a ambos lados insertadas en 5 cavidades de la columna central; 4–5 semillas en cada cavidad (26, Figura 3). Smith (52) indica que los frutos de *Toona* y *Cedrela* son básicamente similares; siendo la diferencia entre los frutos de los dos géneros, principalmente en el grado de desarrollo. Los frutos de *Toona* son más pequeños, y la columna en el fruto es solamente angulada, mientras en *Cedrela* es alada. En *Toona* no se encuentra un área apical estéril como en *Cedrela.*

## Aspectos silviculturales

### Clima:

En su distribución natural *T. ciliata* se presenta en regiones con una precipitación que varía desde 1.125–4.000 mm al año (15, 17, 53); la estación seca es generalmente de 3 a 4 meses. La especie prefiere sitios húmedos pero crece también en lugares más secos. Streets (53) indica que la especie puede crecer bajo condiciones climatológicas relativamente secas si se la riega en su juventud (como se hace en la India). Chevalier (18) observa que la especie crece también en la zona de los monzones con una estación seca prolongada, y a la vez se encuentra en zonas templadas de China. *Toona ciliata* también se adapta en condiciones bastante secas (800 mm por año) si está plantado en un suelo bueno, con un nivel freático accesible en la época seca (15).

La especie pierde sus hojas al comienzo de la estación seca (15). En Turrialba, Costa Rica, donde la precipitación en el mes más seco es todavía 50 mm, no se ha notado que la especie pierda sus hojas. Benthall (5) informa que en Calcuta, India, la especie pierde sus hojas al comienzo de la época fría.

La especie y su variedad *australis* son de árboles de rápido crecimiento y exigentes de luz, aunque en su juventud son tolerantes a la sombra.

La variedad *australis* se encuentra en los bosques tropicales pluviales de la costa de Nueva Gales del Sur y Queensland, Australia, donde la precipitación generalmente equivale o excede 1.500 mm (26). La distribución de la lluvia varía desde uniforme en Nueva Gales del Sur, hasta concentrada en el verano en Queensland.

La temperatura en el área de distribución natural de *T. ciliata* varía de 43°C (máximo absoluto) a −1°C (mínimo absoluto). Streets (53) indica que esta especie tolera ligeras heladas. En la meseta de Atherton, Queensland, la temperatura mínima absoluta es −2°C mientras el máximo es de 28°C.

### Suelos:

La especie y su variedad *australis* se desarrollan preferentemente en la parte inferior de las pendientes con un suelo rico y bien drenado; también se encuentra con frecuencia en los bordes de los ríos. No soportan suelos compactos arcillosos, ni suelos arenosos pobres. *T. ciliata* tiene aparentemente cierta preferencia para suelos calcáreos (15). El sistema radical es superficial, necesitando un buen abastecimiento de agua y elementos minerales en los horizontes superiores del suelo. En comparación con *Cedrela odorata, Toona ciliata* parece ser un poco menos exigente en cuanto al drenaje del suelo (15). Kraemer (33) indica que en la región del Pacífico Oeste, la especie prefiere los suelos aluviales profundos, bien drenados y crece también en inclinaciones de las montañas bajas hasta 1.700 m de altura.

## Semillas y manejo de las plantas

### Recolección:

Los árboles maduros de *T. ciliata* y su variedad *australis* fructifican prácticamente todos los años (15, 29); se recomienda colectar las cápsulas de los árboles poco antes de que estén maduras. Luego las cápsulas son colocadas en el sol para abrirse y las semillas separadas por mano o con ayuda de una zaranda (15). La recolección de las cápsulas de *T. ciliata* se practica en mayo (provincias centrales y norte de la India), octubre–noviembre (Birmania) agosto–octubre (Travancore) (18–39). Las semillas de la variedad *australis* son recolectadas en agosto–setiembre en Hawaii donde la especie está introducida.

### Semillas y almacenamiento:

Las semillas son livianas; hay 280–425 semillas de *T. ciliata* por gramo (39). En la variedad *australis* encontramos 306 semillas por gramo. Las semillas pueden ser almacenadas por un año si se les envasa en seco en latas herméticamente cerradas (39). Según Letourneux (38) se pueden almacenar las semillas en sacos de yute bajo techo por año, sin perder más que el 20 por ciento de su poder germinativo. Otra fuente (15) indica que la viabilidad natural de las semillas de *T. ciliata* no dura más que 1–3 meses, pero que se puede mantener la viabilidad por un año en latas herméticamente cerradas y mantenidas a una temperatura de 4 a 5°C.

## Germinación:

La germinación de semillas frescas de *Toona ciliata* es buena. Letourneux (38) indica un promedio de 90 por ciento, después de 8–12 días. Magini y Tulstrup (39) mencionan que la germinación comienza 9 días después de la siembra y que se puede obtener 60–80 por ciento. En Turrialba, semillas frescas de la variedad *australis* germinaron en un 84 por ciento, en condiciones de laboratorio; aparentemente existe una correlación entre el peso de las semillas y el porcentaje de germinación (Figura 4). La especie puede también ser propagada vegetativamente con estacas (53).

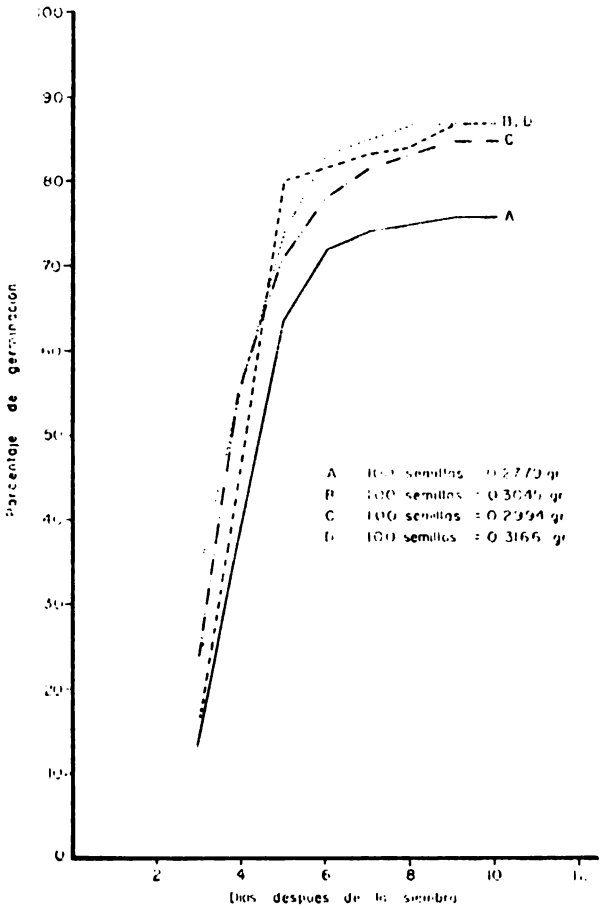



**Fig. 4.** Diagrama que indica la germinación de semillas frescas de *Toona ciliata* M. Roem. var. *australis* (F.v.M.) C.D.C. en condiciones de laboratorio.

En Turrialba, Costa Rica se efectúa la germinación en pequeñas cajas de madera o zinc de un tamaño de 50 x 30 x 10 cm, de las cuales se sumerge la parte inferior en agua, mojando así el medio de germinación por capilaridad. Como medio se usa un suelo fértil, fino y se tapan las semillas con una capa delgada de aproximadamente 2 a 3 mm de grosor. En vista de que las semillas son pequeñas y livianas, es necesario poner las cajas de germinación bajo techo transparente para proteger las plantitas recién germinadas contra la lluvia.

En Africa tropical (15) se cubren las semillas con una capa de tierra fina de 2 cm de espesor, mientras se las protege bajo un techo de paja contra las lluvias fuertes y el sol. El techo es levantado cuando comienza la germinación, generalmente en el octavo día después de la siembra, y reemplazado por sombra parcial en las horas más calientes. El repique* se efectúa generalmente cuando las plantitas tienen un mes de edad y miden entonces alrededor de 10 cm. En Turrialba se repica después de 2 semanas cuando las plantitas tienen sus primeras hojas y miden aproximadamente 5 cm.

La siembra directa en el campo es un método poco utilizado, pero a veces practicado cuando las semillas son abundantes. Esta práctica no es recomendable en vista de las lluvias que destruyen las plantas jóvenes frágiles (15).

## Plantación:

La plantación en el campo se efectúa con tocones, plantones deshojados o plantas del vivero. En Africa tropical se usan plantas de un año de edad, que tienen alrededor de 1,20 m de altura. Estas son plantadas con éxito ya sea en la época seca, cuando las plantas han perdido sus hojas, o al comienzo de la época lluviosa; en el último caso se usa tocones que tienen un tronco de 7 cm de largo y alrededor de 25 cm de raíces (15).

En Asia tropical también se hacen las plantaciones con tocones (36, 38). En India se probó que tocones plantados inmediatamente tienen mejores resultados; no obstante se puede almacenarlos por 6 semanas si son mantenidos húmedos; en estado seco permanecen bien sólo por 3 ó 4 días (36). En Rhodesia del Sur (53) se usa en la plantación plantas de 8–15 cm. En Turrialba, las plantas tienen generalmente 30 cm cuando son transplantadas en el campo.

## Espaciamiento:

El enraizamiento superficial y las grandes necesidades de la especie en cuanto a agua y elementos nutritivos exigen que el espaciamiento sea amplio; en Africa tropical (15) un distanciamiento de 4 x 4 m es sugerido como mínimo y 6 x 6 m como un buen promedio. No obstante, se ha constatado en Puerto Rico que la especie tiene una forma más pobre que *Cedrela odorata* en campo abierto, y se recomienda un espaciamiento más estrecho (55). Letourneux (38) indica un espaciamiento de 2 x 2 m. La experiencia con la variedad *australis* en Turrialba, indica que las plantaciones de 2 x 2 m, se cierran en un año, por lo cual se podría recomendar un un espacio más amplio, de 2,5 x 2,5 m por ejemplo.

## Tratamientos culturales:

Las plantas jóvenes de esta especie y su variedad *australis* son sensibles a la competencia de las malas hierbas, por lo cual se debe mantener limpias las plantaciones durante los primeros años (15, 53). Generalmente la poda no es practicada (15).

En Hawaii, hasta 21 por ciento de los árboles, plantados a 3 x 3 m, en las parcelas de prueba de la variedad *australis* necesitaba podas (Cuadro 1). De otros

* Repique: pasaje de plántulas de un medio de germinación a bolsas, recipiente o camas con tierra, donde se desarrollan para ser luego transplantadas al terreno definitivo.

CUADRO 1. *Toona ciliata* var. *australis*. Resumen de datos de algunas parcelas experimentales en Turrialba, Costa Rica y Honaunau Forest, Hawaii (14).

| Especificaciones | Turrialba, Costa Rica | | | | | Honaunau Forest, Hawaii | | |
|---|---|---|---|---|---|---|---|---|
| | Atirro | Bajo Reventazón | Puente Cajón | Florencia Sur | Florencia Sur | SP No. 14 | SP No. 10 | SP No. 8 |
| Tamaño de la parcela (ha) | 0,04 | 0,04 | 0,04 | 0,012 | 0,012 | 0,4 | 0,4 | 0,4 |
| Tipo de suelo | arcilloso | arenoso | franco arcilloso arenoso | arcillo arenoso | arcillo arenoso | A[1] | P[1] | A&P[1] |
| Precipitación (mm) | 2.600 | 2.600 | 2.600 | 2.600 | 2.600 | 2.500 | 2.500 | 1.250 |
| Edad después de la plantación (meses) | 15 | 12 | 17 | 13 | 13 | 12 | 36 | 48 |
| No. de árboles | 100 | 100 | 100 | 20 | 20 | 411 | 302 | 307 |
| Supervivencia (%) | 95 | 91 | 94 | 100 | 95 | 86 | _[2] | _[2] |
| Altura promedio (m) | 4,1 | 4,4 | 5,7 | 3,0 | 3,4 | 1,1 | 4,0 | 5,0 |
| Arbol más grande (m) | 6,8 | 6,1 | 7,1 | 4,6 | 5,0 | 3,3 | 14,0 | 13,0 |
| Arbol más pequeño (m) | 1,1 | 0,8 | 1,9 | 1,4 | 1,6 | 0,3 | 0,3 | 0,3 |
| Diámetro promedio (DAP, cm) | 3,8 | 4,1 | 5,7 | 3,2 | 3,8 | _[2] | _[2] | _[2] |
| Diámetro más grande (DAP, cm) | 7,4 | 6,5 | 8,4 | 4,7 | 3,0 | _[2] | _[2] | _[2] |
| Diámetro más pequeño (DAP, cm) | 1,0 | 1,0 | 1,5 | 1,0 | _[2] | _[2] | _[2] | _[2] |
| Arboles podados (%)[3] | 10 | 13 | 29 | 25 | 30 | 6 | 12 | 21 |
| Arboles enfermos (%) | 0 | 0 | 0 | 0 | 0 | 0 | | 0 |
| Arboles muertos por enfermedades (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Daños a las yemas (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Arboles inclinados (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Arboles bifurcados (%) | 6 | 11 | 14 | 5 | 5 | _[2] | 1 | 2 |
| Fertilización[4] | si | no | si | no | si | no | _[2] | _[2] |
| Rebrotes al pie del tronco (%) | 3 | 5 | 5 | 15 | 15 | _[2] | no | no |
| | | | | | | | _[2] | _[2] |

1. A: roca con suelo orgánico y mineral. P: roca de tipo Pahoehoe, con capa delgada de suelo.
2. No indicado.
3. En las parcelas de Hawaii, todas las ramas fueron podadas hasta 4 m de altura. En las parcelas de Turrialba (Atirro, Bajo Reventazón y Puente Cajón) se podó hasta 2 metros de altura.
   Para las parcelas de Florencia Sur, solamente está indicado el porcentaje de árboles que necesitaría una poda.
4. La fertilización en Atirro era un abonamiento inicial, lo cual se efectuó dos meses después de la siembra en el campo, con 50 g de 20–20–0 por árbol. La fertilización en Puente Cajón fue trimestral con 50 g de 20–20–0 por árbol. La fertilización en Florencia Sur fue trimestral con 62 g de 20–20–0 por árbol.

países de Africa tropical, también se informa sobre la mala forma de los árboles de *T. ciliata* en parcelas de prueba (53).

## Crecimiento y rotación:

Son pocos todavía los datos que existen sobre el crecimiento en plantaciones de esta especie y la variedad *australis;* no se encuentra en la literatura tablas volumétricas. Indudablemente esto se debe a la seriedad de los ataques de *Hypsipyla robusta* en los países donde la especie y su variedad se encuentran naturalmente. En Africa tropical (15) se calcula que las plantaciones en buenos suelos pueden obtener un diámetro de 50 cm en unos 20 años (Cuadro 2).

Letourneux (38) indica para Asia tropical un crecimiento inicial mucho más lento: 30 cm al primer año, pero observa que el desarrollo subsecuente es rápido, y que *T. ciliata* a los 22 años tiene una altura de 19 m y una circunferencia de 55 cm. En Hawaii, la variedad *australis* es considerada como la especie más prometedora de las especies introducidas. Una plantación de 22 años tenía una altura que variaba de 30–36 m y un diámetro de 25–55 cm. En esta isla se

ejecuta la plantación a raíz desnuda, con plantas de 30–60 cm. Aparentemente esta variedad se adapta mejor en zonas de bajura (13, 14).

CUADRO 2. Estimación del crecimiento de *T. ciliata* en Africa Tropical en buenas condiciones (15).

| Edad en años a partir de semillas | Diámetro (a 1,30 m en cm) | Altura m |
|---|---|---|
| 1 | – | 1,20 |
| 2 | 4 | 3 |
| 3 | 7 | 5 |
| 6 | 17 | 10 |
| 9 | 25 | 14 |
| 12 | 33 | 18 |
| 15 | 40 | 22 |
| 20 | 50 | 25 |
| 30 | 60 | 30 |
| 40* | 70 | 35 |

* Después de 40 años el crecimiento disminuiría mucho.

Geary (27) da la siguiente información sobre 2 parcelas de *T. ciliata* en Arboreto de Ciénaga Alta en Puerto Rico (elevación 650 m, precipitación anual 2.500 mm):

**Parcela 34 A**: edad 5,5 años; 9 árboles; altura promedio 3,3 m (rango de 1,70 a 6,70 m); diámetro promedio al pecho 5,3 cm (rango de menos de 2,5 a 9,5 cm), sin ataques de *Hypsipyla grandella*, buena forma; mejor que las especies de *Cedrela*.

**Parcela 34 B**: edad 5,5 años; 6 árboles altura promedio 3,6 m (rango de 1,70 a 6,70 m); diámetro promedio al pecho 4,0 cm (rango de menos de 2,5 cm a 11,0 cm), sin ataques del barrenador *Hypsipyla grandella*, buena forma, vigoroso.

En Turrialba, Costa Rica, *Toona ciliata* var. *australis* procedente de Hawaii, está plantado en 12 localidades y no ha sido atacado en ningún lugar por *Hypsipyla grandella*. El desarrollo de *T. ciliata* var. *australis* aquí es sumamente rápido y prometedor (Cuadro 1, Figuras 5, 6 y 7).

La inmunidad de *T. ciliata* var. *australis* al ataque de *Hypsipyla grandella* es lo más convincente en las pruebas de parcelas de árboles individuales, donde está plantado a 3 m de distancia de *Cedrela odorata, Swietenia humilis, Swietenia macrophylla* y *Khaya ivorensis*. En estas parcelas, establecidas en 3 localidades con 4 replicaciones cada una, ningún árbol de *T. ciliata* var. *australis* está atacado, mientras *Cedrela odorata, Swietenia humilis* y *Swietenia macrophylla*, que crecen colindantes a esta variedad, sí son atacadas (Figuras 5, 7).

En cuanto a la rotación, no se dispone de suficientes datos para determinar el turno óptimo. Tomando en cuenta las experiencias existentes y el uso más rentable de la madera (para ebanistería y la fabricación de chapas), se puede estimar que la rotación debería ser en el orden de 40 a 50 años (15). En Turrialba, Costa Rica, se observó que el crecimiento de *T. ciliata* var. *australis* responde altamente a la fertilización (Cuadro 1) con lo cual se podría acortar el turno.

Una vez establecida la plantación, la segunda rotación es facilitada por la abundante regeneración natural, que se establece generalmente. Muchos países de Africa tropical como Rhodesia del Sur, Nyasaland y Uganda indican que la regeneración natural es profusa. La especie y su variedad son tolerantes a la sombra en su juventud (38, 53).

### Daños entomológicos:

En Africa, Asia y Asutralia, los principales enemigos, particularmente en la juventud, son los barrenadores *Hypsipyla robusta* y *Zeuzera coffea* (8, 15, 17, 18, 22, 24, 26, 38, 44, 53). Para *Toona ciliata* y *T. ciliata* var. *australis* introducidas en América Latina, no se han notado hasta el momento ataques de la larva de *Hypsipyla grandella* en las plantaciones experimentales establecidas en Costa Rica, Honduras y Puerto Rico (16, 27).

En Australia, larvas de *Pingasa* sp. *(Geometridae)* comen las hojas de la variedad *australis* y pueden causar daños considerables (9). En Turrialba, Costa Rica se ha observado que las hormigas cortadoras, en particular del género *Atta*, pueden causar daños considerables en plantaciones jóvenes. También se notó un ataque de *Planococcus* sp. (Cochinilla harinosa) en las hojas de *T. ciliata* var. *australis* durante una época extraordinariamente seca, pero el ataque duró corto tiempo y no provocó muchos daños.

### Daños fitopatológicos:

Letourneux (38) indica que plantaciones de *Toona ciliata* pueden ser atacadas fuertemente por el hongo *Fomes lucidus*.

### Daños mecánicos:

Las hojas de *T. ciliata* son bien apreciadas por herbívoros, que pueden causar daños en plantaciones jóvenes (5, 8, 15, 38). Debido a su delgada corteza la especie y su variedad no resisten el fuego (53).

## Características y usos de la madera

### General:

Record y Hess (34) indican que la madera de *Toona ciliata* es indistinguible de *Cedrela odorata*. La madera de la especie y su variedad *australis* es idéntica a la madera del cedro, tiene las mismas excelentes calidades y es usada para los mismos fines. Tiene un color rojizo atractivo, brillante, con grano recto y una bonita figura. Es fácil de sazonar y trabajar; moderadamente resistente a las termites (5, 18, 20, 25, 26, 53). La madera es moderadamente durable; experimentos en Hawaii indican que la durabilidad natural de postes sin tratamientos sería alrededor de 4 años (51). El peso específico varía entre 0,46 – 0,64 (20, 31, 33). Chevalier menciona un promedio de 0,57 g/cm$^3$ (18). La albura de *T. ciliata* es de color rosado hasta café claro (15), la de la variedad *australis* tiene color blanco amarillento (18).

**Fig. 5.** Pruebas de parcelas de árboles individuales. Puente Cajón, Turrialba, Costa Rica. *Toona ciliata* M. Rowm, var. *australis* (F.v.M.) C.D.C. a 3 metros de distancia de *Cedrela odorata* L. El *Toona* es libre de ataques de *Hypsipyla grandella* Zell., mientras el *Cedrela* es fuertemente atacado (flechas). La *Toona* es atacado por las hormigas cortadoras del género *Atta*. Ambas especies tienen 13 meses de plantadas; la regla tiene 3 metros.

Fig. 6. Parcela de *Toona ciliata* M. Roem. var. *australis* (F.v.M.) C.D.C., de 12 meses de edad en Bajo Reventazón, Turrialba, Costa Rica. La regla tiene 4 metros.


**Fig. 7.** Pruebas de parcelas de árboles individuales, Bajo San Lucas, Turrialba, Costa Rica. *Cedrela odorata* L., plantado a 3 metros de distancia de *Toona ciliata* M. Roem. var. *australis* (F.v.M.) C.D.C. es fuertemente atacado, por *Hypsipyla grandella* mientras la *Toona* es libre de ataques. Ambas especies tienen 13 meses de plantadas; la regla tiene 3 metros.

## Anatomía:

Francis (26) da la siguiente descripción de la variedad *australis:* los poros son solitarios y en cadenas radiales; 2–5 poros por cadena; frecuentemente se encuentran anillos concéntricos de poros grandes, desconectados al principio de cada zona de crecimiento (¿anillo anual? ); el parénquima está ubicado en la confluencia de las zonas de crecimiento en forma de líneas concéntricas que muchas veces son interrumpidas por las cadenas concéntricas de poros largos. La figura de la madera es causada por estas cadenas concéntricas de poros, y solamente es prominente cuando están bien desarrolladas. Para *T. ciliata* se da la siguiente descripción (19, 20): la madera es porosa en forma de anillos o semiporosa en anillos (Figura 8). El número de poros varía de 144–368 por $cm^2$ (33). Los anillos de crecimiento están presentes y visibles en el corte transversal y longitudinal; hay alrededor de 2–15 por 2,5 cm. Los poros de la madera formada en primavera, son ovalados y grandes, sin tílides pero conteniendo frecuentemente una sustancia gomosa de color café oscuro. La transición a la madera de verano es gradual. Los poros de la madera de verano son más pequeños en diámetro; solitarios o en grupos radiales de 2–3 poros.

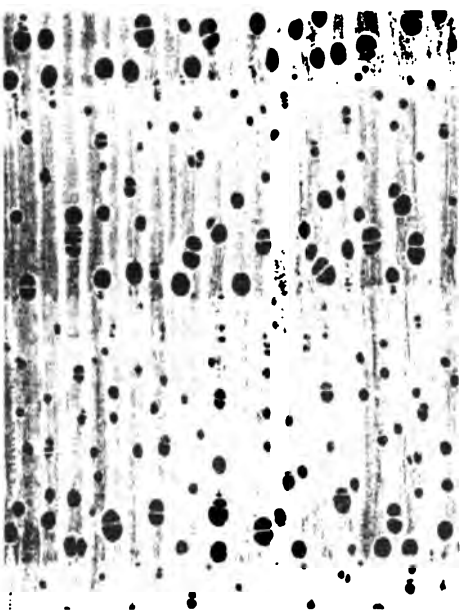


Fig. 8. Corte transversal de la madera de *Toona ciliata* M. Roem., que muestra la diferencia en el tamaño de los poros de la madera de primavera y de verano. Tomado de Chowdbury (20).

Los conductos gomosos están generalmente ausentes, pero a veces encontrados en grupos concéntricos conteniendo sustancias oscuras. Parénquima visible con un lente alrededor de los poros de la madera de primavera. Los rayos son moderadamente anchos y visibles al ojo en el corte transversal, aproximadamente 40 por centímetro (33). El largo de las fibras cambia dentro de un anillo de crecimiento. En *T. ciliata* var. *australis* las fibras de la madera de primavera tienen un largo promedio de 0,75 mm, mientras las de la madera de verano tienen un promedio de 1,37 mm (6).

## Usos de la madera:

La madera es considerada como una de las mejores en la India y Australia. Inglaterra importó anteriormente grandes cantidades de madera de *T. ciliata* desde Birmania. Sus usos principales son para enchapados, muebles, ebanistería, carpintería, cajas de puros y de té, construcciones generales, barcos, fósforos y además leña (1, 3, 4, 5, 6, 15, 18, 22, 23, 25, 31, 33, 47, 53, 60).

## Otros usos:

En algunos lugares se usan las flores como colorante rojo y amarillo y son empleadas para teñir algodón. En India las hojas son usadas como forraje para el ganado. La corteza es mencionada como un astringente en el tratamiento de la disentería y también es considerada como remedio contra la fiebre (5). Los árboles mismos son ornamentales y frecuentemente plantados en parques, jardines y avenidas (5, 18, 21, 26, 54).

## Otras especies de *Toona* de interés forestal

Casi todas las especies del género *Toona* producen una madera de buena calidad. Chevalier (18) y Begemann (4) mencionan en adición a *T. ciliata* y la variedad *australis,* las siguientes especies: *T. calantas, T. fargesii, T. microcarpa, T. mollis, T. multijuga, T. paucijuga, T. serrata, T. serrulata, T. sinensis* y *T. sureni,* de los cuales *T. calantas* y *T. sureni* probablemente son las de mayor interés para América Latina. A continuación se da alguna breve información sobre estas dos especies. De las otras especies mencionadas llama la atención que *T. sinensis* se ha adaptado en París, Francia (18) y podría ser una especie interesante para la introducción desde las zonas subtropicales hasta las templadas de América Latina.

## 1. *Toona sureni* (Bl.) Merill

## Sinónimos:

*Swietenia sureni* Bl., *Cedrela febrifuga* Bl., *Toona febrifuga* (Bl.) Roem, *Surenus febrifuga* O. Kze, *Cedrela toona* mult. auct. (2, 18).

## Nombres comunes:

Soerén, laoet, redani (Java); Soren (Madura); Horeni, Linoe (Sumba); Suntang putch, incoe, soeren (Malaya); Xúong mót, zúeng mót, xúong moc, hong dao (Annam); Chham Chhar, chamcha (Cambodia); Ka xua, sa tam, so banne (dialecto Moi) (2, 18, 34).

## Distribución natural:

Vietnam: Bienhoa, Phanrang, Provincia de Baria. Cambodia: Phnon Changor, Royaung. Annam: Blao. Annam: Blao. Indonesia: Java, Sumatra, Ambón, islas Sunda, Sumba, Madura. Península de Malaya, Birmania (2, 3, 18, 34).

En Java, *T. sureni* se encuentra en la zona de los monzones, en áreas de bajura así como en las montañas (3). En Annam ha sido encontrada también en la

cordillera cerca de Djiring, a una altura de 1.020 m pero en general es una especie del denso bosque ecuatorial pluvial (18).

## General

*T. Sureni* es una especie de rápido crecimiento, un árbol grande que puede tener de 20–40 m de altura con diámetros de 0,60–2,00 m (3). Ramas fuertes, pubescentes en su juventud pero luego glabras. Hojas grandes de 60–80 cm, compuestas, generalmente paripinadas, 5–12 pares de folíolos, frecuentemente 8; los folíolos miden de 12–16 cm de largo y 4–5 de ancho, acuminados, glabros en ambos lados, a veces pubescentes en las nervaduras, con 12–16 nervaduras secundarias; peciolulos delgados, 6–15 mm de largo. Pecíolo de 6–10 cm. Flores en panículas pendientes, del mismo tamaño o más corto que las hojas, raquis pubescente; pedicelo pubescente; flores blancas,olorosas, 4 mm de largo; sépalos redondeados, adnatos en la base; pétalos oblongos, pubescentes en el exterior, ciliados; disco pubescente en la parte engrosada; ovario igualando al disco, pubescente en la base; estilo y filamentos de los estambres glabros. Cápsula leñosa, con lenticelas, 2 cm de largo (3, 18, 34).

La madera es de buena calidad, bien cotizada, atractiva, de color café–rojizo, liviana con un peso específico de 0,39–0,45, olorosa, fácil de sazonar y trabajar, usado como madera para aserrar, cajas de cigarros y de té, muebles y carpintería interior (3, 18, 34).

En Asia las plantaciones de esta especie son fuertemente atacadas por *Hypsipyla robusta* (3).

De Puerto Rico, Geary (27) indica que la parcela de *T. sureni* en el Arboreto de Ciénaga Alta, no es atacada por *Hypsipyla grandella,* aunque *Cedrela odorata* y *Swietenia macrophylla* son fuertemente atacadas en el mismo Arboreto. No existen muchos datos sobre el crecimiento de esta especie en América Latina. Los siguientes provienen del Arboreto Ciénaga Alta, (27) Puerto Rico (elevación 650 m, precipitación 2.500 mm).

## Parcela 35 A: *Toona sureni:*

Edad 5,5, años; 14 árboles; altura promedia 4,3 m (rango de 2,30 a 8,30 m); diámetro promedio 7,0 cm (rango de menos de 2,5 a 13,5 cm); sin indicaciones de ataques de *Hypsipyla grandella;* algunos árboles de mala forma, posiblemente debido a bejucos; la mayoría de los árboles con troncos rectos.

## 2. *Toona calantas* Merrill et Rolfe.

## Sinónimos:

*Cedrela odorata* Blanco non L., *Cedrela toona* F. Vill. non Roxb.

## Nombres comunes:

Kalantas (Pangasinan, Sambai, Tagalog); Aláñgi, alánki, anteng, bakóog, porak (Iloko); Antáng (Ibanag); Kantiñgen (Iloko, Sambali); lanigda, lanigpa (Bikol); lanigpa (Samar–Leyte Bisaya, Cebú Besaya) (10, 18).

## Distribución natural:

La especie es encontrada con frecuencia en las provincias de Mindanao, Palawan, Luzón, Isla de Batán, Visayas y Mindoro de Filipinas. En su distribución natural se encuentra generalmente cerca de los ríos y en los planos de bajura sujetos a inundaciones.

El árbol puede tener una altura de 40–50 m y diámetros hasta 1,50 m, pero frecuentemente el diámetro es menor. El tronco es recto y cilíndrico, alrededor de 50 por ciento está libre de ramas. Corteza de 5–18 mm de grosor de un color café–rojizo al interior. Hojas grandes, compuestas, alternas, agrupadas en manojos al final de las ramas, 7–11 pares de folíolos, pubescentes cuando joven, luego glabros, 5–13 cm de largo y 3,5–6 cm de ancho (figura 9). Inflorescencia y flores como en *T. sureni,* las cápsulas un poco más grandes, de 3–5 cm, frecuentemente 4 cm (10, 18).

La madera de *T. calantas* es bien cotizada en Filipinas y entre otros, usada para la fabricación de cajas de puros, instrumentos musicales, muebles y carpintería (10, 18, 34). Contiene un aceite esencial oloroso, muy parecido al de *Cedrela odorata.* Entre los aceites esenciales, Brown (10) citando a Brooke, indica que *cadinene* es uno de los principales. La madera es suave, fácil de trabajar y tiene

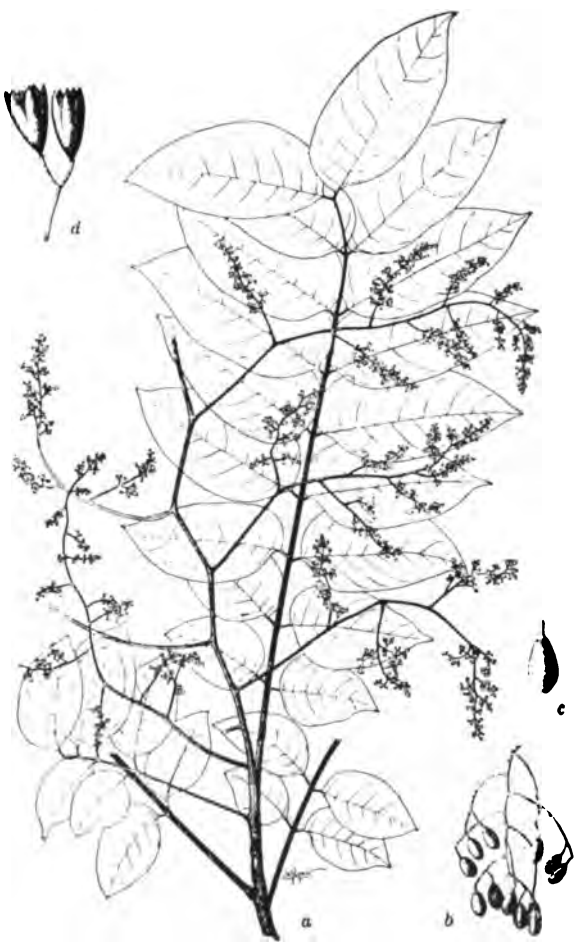



Fig. 9. *Toona calantas* Merrill y Rolfe. a– Inflorescencia y hojas. b y c– Frutos. d– Cápsulas maduras. Tomado de Brown (10).

un color rojo claro hasta rojo oscuro. El peso específico varía de 0,41–0,44 g/cm$^3$. Un estudio para enraizar estacas de *T. calantas* bajo condiciones naturales en camas de vivero falló en Filipinas (32).

## Discusión

El hecho de que las actuales plantaciones experimentales de *T. ciliata* y la variedad *australis*, así como varias otras *Meliaceae* exóticas, no son atacadas por *Hypsipyla grandella*, o solamente en forma mínima en América Latina, no sólo ofrece buenas perspectivas para el establecimiento de plantaciones de estas valiosas especies de rápido crecimiento, sino da también lugar al desarrollo de varias hipótesis y especulaciones que posiblemente podrían indicar otro camino para la solución del problema de la *Hypsipyla*.

De los datos disponibles se puede deducir que la mariposa (imago) de *Hypsipyla grandella* obviamente selecciona el árbol huésped para ovipositar. ¿Cómo lo hace? ¿Sería posible que sea atraído por el olor típico de *Cedrela odorata?* Frotando las hojas y los brotes de esta especie suelta un olor fuerte a ajo o cebollas, lo que no es el caso cuando se frotan las hojas de *T. ciliata* var. *australis*. ¿Sería posible aislar este constituyente (probablemente un aceite esencial) con cromatografía de gases, producirlo sintéticamente y usarlo en el combate de la *Hypsipyla?* ¿Sería posible cruzar las *Toonas* que no son atacadas, con *Cedrelas* y obtener un cruce que tampoco es atacado por la *Hypsipyla?* ¿Es posible seleccionar razas de *Cedrela* que estarán libres de ataques? En Australia, la investigación al respecto del problema de *Hypsipyla robusta* en *Toona ciliata* var. *australis* indica que se puede obtener una raza resistente a los ataques de *H. robusta* (50). No resultó claro en esta información en qué sentido *T. ciliata* var. *australis* sería resistente.

**Nota:**

Existe el peligro grave en la experimentación con *Meliaceae* exóticas, que con la importación de las semillas se tiene la posibilidad de que se importe también la *Hypsipyla robusta*, por la cual siempre se deben tomar las más estrictas medidas fitosanitarias.

## Literatura citada


1. ALI, S. F. y WALLIN, W. B. Suitability of indigenous wood species for furniture manufacture. Pakistan Forest Research Laboratory, Bulletin No. 1, 1963. 3 p.
2. BACKER, C. A. y BRINK, R. C. B. van der. Flora of Java. Netherlands, Noordhoff–Groningen, 1965. pp. 641–643.
3. BECKING, J. H. *et al.* Agrarische Winkler Prins. Amsterdam, Elsevier, 1954. v. 3. pp. 545–546.
4. BEGEMANN, H. F. Lexikon del Nützhölzer. Augsburg, Verlag Emmi Kittel, 1963. p. 526.
5. BENTHALL, A. P. The trees of Calcutta and its neighbourhood. Calcutta, Thacker Spink, 1946. pp. 110–111.
6. BISSET, I. J. W., DADSWELL, H. E. y AMOS, G. L. Changes in fibre–length within one growth ring of certain angiosperms. Nature 165(4192):348–349. 1950.
7. BOONE, R. S. Paintability of two Hawaii grown woods... Pacific Southwest Forest and Range Experiment Station. Research Note PSW No. 116. 1966. 6 p.
8. MOR, N. L. Manual of Indian forest botany. Oxford University Press, 1953. pp. 257–258.
9. BRIMBLECOMBE, A. R. Control of the Cedar looper Queensland Journal of Agricultural Science 15(3):159–160. 1958.
10. BROWN, W. H. Useful plants of the Philippines. Manila, Department of Agriculture and Natural Resources, Technical Bulletin No. 10, 1954. v. 2. pp. 273–275.
11. CANDOLLE, C. de. *Meliaceae* DC. *In* Monographiae Phanerogamarum. Paris, 1878. v. 1, pp. 399–752. (Original no consultado, citado por Smith, Jr., C. E. A revision of *Cedrela (Meliaceae)* Fieldiana: Botany 29(5):298. 1960).
12. _____ . A revision of the Indo–Malayan species of *Cedrela*. Records of the Botanical Survey of India. 3(4):368. 1908. (Original no consultado, citado por Smith, Jr., C. E. A revision of *Cedrela (Meliaceae)*. Fieldiana: Botany 29(5):298. 1960).
13. CARLSON, N. K. Honaunau forest. American Forests 66(4):16–19. 53, 55–58. 1960.
14. _____ and BRYAN, L. W. The Honaunau forest: an appraisal after seven years of planting. Journal of Forestry 61(9):643–647. 1963.
15. *CEDRELA ODORATA* Linné et *Toona ciliata* M. Roem, Caracteres sylvicoles et méthodes de plantation. Bois et Forêts des Tropiques No. 81:29–34. 1962.
16. CHABLE, A. C. Reforestation in the Republic of Honduras, Central America. Ceiba 13(2):1–56. 1967.
17. CHAMPION, H. J. y BRASNETT, N. V. Choice of tree species. FAO Forestry Development Paper No. 13. 1958. pp. 176–177. 180.
18. CHEVALIER, A. Les Toona's on Cèdres bâtards, arbres de reboisement. Revue de Botanique Appliquée et d'Agriculture Tropicale No. 272–274;152–165. 1949.
19. CHOWDHURY, K. A. The formation of growth rings in Indian trees. Part II. Indian Forest Records 2(2):41–57. 1939.
20. _____ . The identification of Burma commercial timbers. Indian Forest Records (new series), (Utilization) 3(6):14, plate III. 1945.
21. CORNER, E. J. H. Wayside trees of Malaya. 2nd ed. Singapore, Government Printing Office, 1952. v. 1, 772 p.
22. DABRAL, S. N. Silviculture of the little known timbers of India and their uses. Proceedings of the Symposium on Timber, New Delhi, 1959. s.l., 1961. pp. 48–60.
23. DAS, E. S. Building timbers of the Punjab. Proceedings of the Symposium on Timber, New Delhi, 1959. sl., 1961. pp. 35–41.
24. ENTWISTLE, P. F. The current situation on shoot, fruit and collar borers of the *Meliaceae*. Oxford, Commonwealth Forestry Institute, 1968. 15 p. Paper of the 9th Commonwealth Forestry Conference. New Delhi, 1968. 15 p. (Original no consultado, compendiado en Forestry Abstracts).

25. FORS, A. J. Maderas cubanas. 4a ed. Habana, Impresora Mundial, 1957. 162 p.

26. FRANCIS, W. D. Australian rain-forest trees. Forestry and Timber Bureau. Sydney, Commonwealth of Australia. 1951. pp. 202-203, 206.

27. GEARY, T. F. Instituto Forestal Tropical, Río Piedras, Puerto Rico. Comunicación personal a P. Grijpma, 1969.

28. HADEN-GUEST, S., WRIGHT, J. K. y TECLAFF, E. M. A world geography of forest resources. New York, Ronald Press, 1965. 736 p.

29. HARMS, H. *Meliaceae. In* Die natürlichen Pflanzenfamilien. 1896. v. 3, pp. 258-308. (Original no consultado, citado por Smith, Jr., C. E. A revision of *Cedrela (Meliaceae)* Fieldiana Botany 29(5):298. 1960.

30. HARMS, H. y MATTFELD, J. *Meliaceae, In* Die natürlichen Pflanzenfamilien. Leipzig, Verlag von Wilhelm Engelmann, 1940. v. 1961. pp. 44-45.

31. HOWARD, A. L. A manual of the timbers of the world. 3rd. ed. London, MacMillan 1951. p. 363.

32. JACALNE, D. V. y GALINATO, P. F. A study on the propagation of *Cananga odorata* Lamark, *Phaeanthus ebracteolatus* Merril, *Intsia bijuga* (Colebrooke) O. Kuntze, *Cassia javanica* Linnaeus, and *Toona calantas* Merril and Rolfe by cuttings. Philippine Journal of Forestry 14(1-4):97-110. 1958(1961).

33. KRAEMER, H. H. Native Woods for construction purposes in the Western Pacific Region. Washington, D.C. Bureau of Yards and Docks, Dept. of the Navy, 1944. pp. 140-141.

34. _____ . Native woods for construction purposes in the South China Sea Region. Washington, D.C. Bureau of Yards and Docks, Navy Department 1945. pp. 120-121.

35. KRIBS, D. A. Comparative anatomy of the woods of the *Meliaceae.* American Journal of Botany 17(8):724-738. 1930.

36. KRISHNASWAMY, V. S. y DABRAL, S. N. The life of stumps of some forest species under moist and dry conditions. Indian Forester 81(7):408-410. 1955.

37. _____ y MATHAUDA, G. S. Phenological behaviour of a few forest species at New Forest. Dehra Dun. Indian Forester 80(3):124-153. 1954.

38. LETOURNEUX, C. Les méthodes de plantations forestieres en Asie Tropicale. FAO: Cahier No. 11. 1957. 178 p.

39. MAGINI, E. y TULSTRUP, N. P. Notas sobre semillas forestales. FAO: Cuaderno de Fomento Forestal No. 5. 1956. pp. 121-122.

40. MARRERO, J. Forest planting in the Caribbean National Forest, past experience as a guide for the future. Caribbean Forester 9(1):85-148. 1948.

41. METCALFE, C. R. y CHALK, L. Anatomy of the Dicotyledons. Oxford Clarendon Press, 1950. v. 1. 724 p.

42. NEAL, M. C. In gardens of Hawaii. Lancaster, Bishop Museum Press, 1965. 924 p.

43. NELSON, R. E. y HONDA, N. Plantation timber on the island of Hawaii 1965. Pacific Southwest Forest and Range Experiment Station. Resource Bulletin PSW-3 1966. 52 p.

44. PARRY, M. S. Métodos de plantación de bosques en el Africa Tropical. FAO. Cuaderno de Fomento Forestal No. 8. 1957. p. 320.

45. RAMIREZ SANCHEZ, J. Investigación preliminar sobre biología, ecología y control de *Hypsipyla grandella* Zeller. Boletín del Instituto Forestal Latino Americano de Investigación y Capacitación No. 16:54-77. 1964.

46. RAO, V. P. y BENNET, F. D. Possibilities of biological control of the Meliaceous shoot borer *Hypsipyla* spp. *(Lepidoptera: Phycitidae).* Trinidad, Commonwealth Institute of Biological Control, 1968. 14 p. Paper of the 9th Commonwealth Forestry Conference, New Delhi, 1968. 14 p. (Original no consultado, compendiado en Forestry Abstracts).

47. RECORD, S. J. y HESS, R. W. Timbers of the new world. Yale University Press, 1947. p. 363.

48. RICHARDS, P. W. The tropical rain-forest, an ecological study. Cambridge University Press, 1964. pp. 193-349.

49. ROEMER, M. J. *Familiarum naturalium regni vegetabilis synopsis monographicae, I. Wimariae, Hesper.* 137, 1846 (Original no consultado, citado en Smith, Jr. C. E. A revision of *Cedrela (Meliaceae)* Fieldiana: Botany 29(5):298. 1960.

50. SHOOTBORERS of the *MELIACEAE* Unasylva 12(1):30-31. 1958.

51. SKOLMEN, R. G. Natural durability of some woods used in Hawaii... preliminary findings. U. S. Forest Service. Research Note PSW-167. 7 p.

52. SMITH, Jr., C. E. A revision of *Cedrela (Meliaceae).* Fieldiana: Botany 29(5):295-341. 1960.

53. STREETS, R. J. Exotic trees in the British Commonwealth. Oxford, Clarendon Press, 1962. pp. 219, 223-224.

54. STURROCK, D. y MENNINGER, E. A. Shade and ornamental trees for South Florida and Cuba. Stuart, Florida. Stuart Daily News Inc., 1946. 172 p.

55. TROPICAL FOREST EXPERIMENT STATION. Puerto Rico. Ninth annual report and program. Caribbean Forester 10(1):81-119. 1949.

56. VERHOEF, L. Cedrela mexicana M. Roem. Sebagai pohon kajoe jang dipenoesahakan. Korte Mededelingen. Bosbouwproefstation, Buitenzorg. No. 80; 11 p. 1942.

57. WADSWORTH, F. H. Five years of forest research on the North Coast of Puerto Rico, Caribbean Forester 9(4):373-380. 1948.

58. WHITE, F. Forest Flora of Northern Rhodesia. Oxford University Press, 1962. 455 p.

59. WONG, Jr., W. H. C., HONDA, N. y NELSON, R. E. Plantation timber on the island of Lanai, 1966. Pacific Southwest Forest and Range Experiment Station Resource Bulletin PSW-7. 1967. 18 p.

60. WORTHINGTON, T. B. Ceylon trees. Colombo, Ceylon, The Colombo Apothecaries' Co. 1959. 429 p.

# IMMUNITY OF *TOONA CILIATA* M. ROEM. VAR. *AUSTRALIS* (F.v.M.) C.D.C. AND *KHAYA IVORENSIS* A. CHEV. TO ATTACKS OF *HYPSIPYLA GRANDELLA* (ZELLER) IN TURRIALBA, COSTA RICA*

*Pieter Grijpma*
Silviculturist, Department of Forest Sciences
Inter-American Institute of Agricultural Sciences (IICA), Turrialba, Costa Rica,
attached to IICA through the Dutch Technical Assistance Program

## COMPENDIO

En las parcelas experimentales del Instituto Interamericano de Ciencias Agrícolas, Turrialba, Costa Rica, *Toona ciliata* var *australis* (Cedro australiano) y *Khaya ivorensis* (Caoba de Nigeria) no son atacadas por el barrenador de las *Meliaceae (Hypsipyla grandella),* mientras que las *Meliaceae* nativas como el Cedro (*Cedrela* spp.) y la Caoba (*Swietenia* spp.) son fuertemente atacadas. En muchos países tropicales de otros continentes estas *Meliaceae* latinoamericanas no son atacadas o son menos atacadas por las *Hypsipylas* nativas, aunque algunos países informan sobre serios ataques.

El autor supone que aceites esenciales volátiles en los brotes y las hojas, los cuales serían diferentes para varias pero probablemente no para todas las *Meliaceae,* atraen la mariposa de *Hypsipyla* spp., a los árboles huéspedes. Una especialización de la mariposa de *Hypsipyla* sobre ciertos aceites esenciales de las *Meliaceae* nativas conduciría a la inmunidad de las *Meliaceae* exóticas que no tendrían estos aceites esenciales como componentes principales. Se proponen algunos proyectos de investigación relacionados con la hipótesis de la orientación por el olfato de la mariposa de *Hypsipyla* a su árbol huésped. En vista de su rápido crecimiento, su valiosa madera y la ausencia de ataques de *Hypsipyla grandella,* la especie *Toona ciliata* var. *australis* parece ser muy prometedora para plantaciones forestales en América Latina.

El autor

## Introduction

The literature about tree species trials in various tropical countries, indicates that several exotic *Meliaceae* are not, or are less attacked by the native shootborer of *Meliaceae (Hypsipyla* spp.*).*

*Cedrela odorata* from Latin America, introduced in Queensland, Australia, is considered a very promising plantation tree because it is not attacked by *Hypsipyla robusta,* which destroys plantations of *Toona ciliata* var *australis,*** growing next to it (21). Lamb (18) and Parry (19) indicate that in Africa, *Cedrela odorata* is unattractive to *Hypsipyla robusta.* In the Philippines, *Swietenia macrophylla* is reported to be free of attack and is used for reforestation (17). Damage to this mahogany species is also insignificant in Malaya (20).

In Latin America however, it is impossible at present to establish commercial plantations of these native *Meliaceae* because of the heavy attacks of *Hypsipyla grandella,* present in this continent.

It should be emphasized however, that apparently the native *Hypsipyla* does not attack the native *Meliaceae* exclusively; there are reports of native *Hypsipyla* attacks on introduced *Meliaceae.* For example, in Java, Indonesia *Cedrela odorata* is attacked by *Hypsipyla robusta,* but the damage is not considered serious, and the species is used to replace the native *Toona sureni* in plantations, a species heavily attacked by the borer (1, 15). In India and Ceylon, *Swietenia macrophylla* is reported to be heavily attacked by *Hypsipyla robusta* even though the tree is an exotic (21). Moreover, *Hypsipyla grandella* attacks the introduced *Khaya senegalensis* in Martinique, French Antilles (20).

Although strict specialization of native *Hypsipyla* spp. on native *Meliaceae* is not evident, the native shootborers seem to prefer certain meliaceous species. The available information on trials with exotic and native *Meliaceae* in Latin America seems to support this theory.

Chable (6) observed in Honduras that the attacks of *Hypsipyla grandella* on the exotics: *Khaya nyassica, Khaya ivorensis, Toona ciliata* and *Entandrophragma rederi* are minimal, whereas the native *Cedrela odorata* and *Swietenia macrophylla* are heavily attacked.

* Received for publication January 20, 1970.
** Synonyms: *Cedrela toona* var. *australis; Toona australis; Cedrela australis.*

According to his information *Entandrophragma* was never attacked. In Puerto Rico the exotics *Toona ciliata* and *Toona sureni* are not attacked either by *Hypsipyla grandella* (13).

In the species trials of the Department of Forest Sciences of the Inter–American Institute of Agricultural Sciences in Turrialba, Costa Rica, a similar experience is being observed (14); here *Toona ciliata* var *australis* and *Khaya ivorensis* are free of any attack of *Hypsipyla grandella,* while plots of *Cedrela odorata, Swietenia humilis, Swietenia macrophylla* and *Swietenia macrophylla* x *mahogani* are seriously attacked.

Although the species trials in Turrialba are of a relatively recent date, a very prominent difference in attack of the shootborer on the various *Meliaceae* included in the trials exist, so that a report on these observations together with growth data of the plots is justified.

## Species Trials with *Meliaceae* in Turrialba, Costa Rica

Presently two types of trials are established: Single Tree Plot Trials and Block Trials. The Block Trials vary in size, number of trees per plot and treatment (fertilized and non–fertilized), while the Single Tree Plot Trials only vary in treatment (fertilized and non–fertilized).

## Single Tree Plot Trials

These are preliminary selection trials in which usually many species are planted together in a randomized design. The data of each tree are considered as coming from one plot, the "single tree plot". This system of preliminary selection trials was devised by C. B. Briscoe of the Institute of Tropical Forestry in Puerto Rico (5). H. Barres, who worked with Briscoe in Puerto Rico, introduced this design in Turrialba, Costa Rica. The Single Tree Plots have the advantage of offering a better statistical base for comparisons of growth and adaptation of trees to new environments. This experimental design is also less costly to install as less trees of each species are needed and a smaller area is utilized. The Single Tree Plot Trials also have a number of disadvantages, which are discussed in the draft of "A Guide to Tree Species Trials in Tropical America" (12) and will not be elaborated on here.

The Single Tree Plot Trials at the Inter–American Institute of Agricultural Sciences in Turrialba, Costa Rica contain 77 tree–species, varieties and provenances, planted in 3 locations (Puente Cajón, Bajo San Lucas and Florencia Sur) with 4 replications in each location of which 2 replications are fertilized quarterly with 62 grams of 20–20–0 (250 grams per year), and 2 are not fertilized. Planting distance is 3 x 3 meters. Among the 77 species, varieties and provenances the following *Meliaceae* have been planted: *Toona ciliata* var *australis* (Provenance: Hawaii), *Khaya ivorensis* (provenance: Ivory Coast), *Cedrela odorata* (provenance: Costa Rica), *Cedrela odorata* (provenance: Ghana), *Swietenia humilis* (provenance: Costa Rica), *Swietenia macrophyla* (provenance: British Honduras) and *Swietenia macrophylla* x *mahogani* (provenance: Virgin Islands). These *Meliaceae* were planted in a group, but randomized within the group; the group itself was located at random in each replication. Each replication contained one tree of each species, variety or provenance (Single Tree Plot).

In October 1969, when all trees were 13 months in the field, an evaluation of *Hypsipyla* attack on these *Meliaceae* was made; this information, together with growth data on the trees, is presented in Table 1.

## Block Trials

A number of Block Trials containing the following *Meliaceae: Cedrela odorata, Swietenia macrophylla,*

TABLE 1 Growth data and incidence of *Hypsipyla grandella* attacks on native and exotic *Meliaceae* in Single Tree Plot Trials in Turrialba, Costa Rica. All trees included in these trials were 13 months in the field.

| Species | *Cedrela odorata* | | | | *Khaya ivorensis* | | *Swietenia humilis* | | *Swietenia macrophylla* | | *Swietenia macrophylla* x *mahogani* | | *Toona ciliata* var *australis* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Provenance | Ghana | | Costa Rica | | Ivory Coast | | Costa Rica | | British Honduras | | Virgin Islands | | Hawaii | |
| Number of replications (= trees) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Trees attacked by *Hypsipyla grandella* (%) | 100 | 83 | 60 | 80 | 0 | 0 | 20 | 17 | 33 | 33 | 33 | 50 | 0 | 0 |
| Fertilization | yes* | no | yes* | no | yes* | no | yes* | no | yes* | no | yes* | no | yes* | no |
| Number of surviving trees (out of 6) | 5 | 6 | 5 | 5 | 6 | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Average height (m) | 1.30 | 1.30 | 1.30 | 1.10 | 1.60 | 1.50 | 0.90 | 1.10 | 1.20 | 1.20 | 1.20 | 1.40 | 3.10 | 3.30 |
| Tallest tree (m) | 1.80 | 1.90 | 2.20 | 2.00 | 2.70 | 2.30 | 0.95 | 2.00 | 1.75 | 2.15 | 1.40 | 0.95 | 5.00 | 5.70 |
| Smallest tree (m) | 0.70 | 0.50 | 0.75 | 0.55 | 1.00 | 0.90 | 0.85 | 0.90 | 0.80 | 0.80 | 0.95 | 1.95 | 0.80 | 1.20 |
| Average diameter (B.H. cm) | 1.3 | 1.3 | 1.5 | 1.3 | 1.3 | 1.1 | 1.0 | 1.0 | 1.0 | 1.1 | 1.0 | 1.1 | 3.4 | 3.3 |
| Biggest diameter (B.H. cm) | 2.0 | 2.0 | 3.7 | 2.5 | 2.5 | 2.0 | 1.0 | 1.0 | 1.0 | 1.5 | 1.0 | 1.3 | 6.5 | 4.8 |
| Smallest diameter (B.H. cm) | 1,0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

* Fertilization consisted of a quarterly application of 62 grams of 20–20–0 per tree (250 grams per year).

*Swietenia humilis, Toona ciliata* var *australis* and *Khaya ivorensis,* has been established in various locations in the Turrialba area. The size of these plots varies from 0.012–0.040 ha and the number of trees per plot from 20–100. Fertilized treatment was from non–fertilized to 62 grams of 20–20–0 per tree, applied quarterly. In view of the fact that all plots of the native Latin American *Meliaceae* are so heavily attacked by *Hypsipyla grandella* that growth data are of little value, only three representative plots are included in Table 2.

## Conclusions

From the data presented in Tables 1 and 2 the following conclusions may be drawn:

1– All the Latin American *Meliaceae* included in the trials were attacked by *Hypsipyla grandella,* but the exotics *Toona ciliata* var *australis* and *Khaya ivorensis* were not attacked, although the latter two species were planted at a distance of only 3 meters from the attacked Latin American *Meliaceae,* in the Single Tree Plot Trials (Figs. 1, 2 and 3). In the Block Trials the distance between the plots of the native *Cedrela odorata* and *Swietenia humilis* (both heavily attacked) and the exotic *Toona ciliata* var *australis* (not attacked) was 55 meters (Figs. 4 and 5).

2– Although only 7 out of 77 trees in the replications of the Single Tree Plots were *Meliaceae,* attacks of *Hypsipyla grandella* occurred. At least for the Turrialba area, this indicates that mixtures of *Meliaceae* with other forest species do not guarantee that *Hypsipyla* attacks will not occur.

3– Of the Latin American *Meliaceae* in the Single Tree Plot Trials, *Cedrela odorata* is attacked most frequently and *Swietenia humilis* the least, although the Block Trials indicate that *Swietenia humilis* (Fig. 4) is also highly susceptible to attacks of the shootborer. There is apparently no difference in susceptibility to attack between the two provenances of *Cedrela odorata* (Costa Rica and Ghana).

4– Of the two exotic *Meliaceae* that are not attacked by *Hypsipyla grandella, Toona ciliata* var.

TABLE 2. Growth data and incidence of *Hypsipyla grandella* attacks on native and exotic *Meliaceae* in Block Trials in Turrialba, Costa Rica.

| Plot location | *Toona ciliata* var *australis* Provenance: Hawaii | | | | | *Cedrela odorata* Prov. Costa Rica | *Swietenia humilis* Prov. Costa Rica | *Khaya ivorensis* Provenance: Ivory Coast | | *Swietenia macrophylla* Prov. Venezuela |
|---|---|---|---|---|---|---|---|---|---|---|
| Plot location | Atirro | Bajo Reventazón | Puente Cajón | Florencia Sur | | Puente Cajón | Puente Cajón | Florencia Sur | | Puente Cajón |
| Plot Size (ha) | 0.04 | 0.04 | 0.04 | 0.012 | 0.012 | 0.04 | 0.04 | 0.012 | 0.012 | 0.04 |
| Soil type | river bank clay | sandy river soil | sandy clay loam | sandy clay loam | sandy clay loam | sandy clay loam | sandy clay loam | sandy clay loam | sandy clay loam | sandy clay loam |
| Drainage | imperfect | good | imperfect | good | good | imperfect | imperfect | good | good | imperfect |
| Age after field planting (months) | 15 | 12 | 17 | 13 | 13 | 12 | 24 | 13 | 13 | 48 |
| Number of trees planted | 100 | 100 | 100 | 20 | 20 | 100 | 100 | 20 | 20 | 49 |
| Planting distance (m) | 2x2 | 2x2 | 2x2 | 2.5x2.5 | 2.5x2.5 | 2x2 | 2x2 | 2.5x2.5 | 2.5x2.5 | 3x3 |
| Survival (%) | 95 | 91 | 94 | 100 | 95 | 88 | 81 | 100 | 100 | 70 |
| Trees attacked by *Hypsipyla grandella* (%) | 0 | 0 | 0 | 0 | 0 | 100 | 88 | 0 | 0 | 100 |
| Fertilization* | yes | no | yes | no | yes | yes | yes | yes | no | yes |
| Average height (m) | 4.10 | 4.40 | 5.70 | 3.00 | 3.40 | 0.57 | 0.67 | 1.43 | 1.47 | 1.45 |
| Tallest tree (m) | 6.80 | 6.10 | 7.10 | 4.60 | 5.00 | 1.20 | 1.40 | 2.25 | 2.30 | 3.30 |
| Smallest tree (m) | 1.10 | 0.80 | 1.90 | 1.40 | 1.60 | 0.10 | 0.10 | 1.00 | 1.10 | 0.60 |
| Average diameter (B.H. cm) | 3.8 | 4.1 | 5.7 | 3.2 | 3.8 | ** | ** | 1.2 | 1.3 | 3.3 |
| Biggest diameter (B.H. cm) | 7.4 | 6.5 | 8.4 | 4.7 | 3.0 | ** | ** | 2.5 | 2.5 | 5.6 |
| Smallest diameter (B.H. cm) | 1.0 | 1.0 | 1.5 | 1.0 | 1.0 | ** | ** | 1.0 | 1.0 | 1.0 |

* Fertilizer applications:
Atirro: one initial application of 50 grams of 20 20 0 per tree, applied two months after field planting.
Puente Cajón: quarterly applications of 50 grams of 20- 20- 0 per tree. (200 grams/year).
Florencia Sur: quarterly applications of 62 grams of 20–20–0 per tree. (250 grams/year).

** Trees are too small to be measured for diameters, due to repeated attacks by *Hypsipyla grandella,* and consequent ramification.

*australis* is far more promising than *Khaya ivorensis* in view of its faster growth and better form (Figs. 6 and 7); its valuable wood equals the qualities of *Cedrela odorata* and other Latin American *Cedrela* species.

## Discussion

The present available data from the Single Tree Plot and Block Trials clearly indicate a preference of *Hypsipyla grandella* for the native *Meliaceae* included in the trials, and in particular for *Cedrela odorata*. It is also apparent that *Hypsipyla grandella* is able to detect and select these specific hosts from other trees. This host specificity may be due to the moth's ability to seek out and attack trees which emit specific volatile materials. It is interesting to note, that when young shoots and leaves of *Cedrela odorata* were crushed, a strong penetrating, garlic–like smell was produced, which was absent when leaves and shoots of *Toona ciliata* var *australis* were treated in the same way.

It is known that for oviposition some insects select hosts that produce specific essential oils. Ehrlich and Raven (11) discussing the selection of host plants by butterflies, indicate that analysis made it clear that their choices have a chemical basis.

These authors mention that Dethier had noted already several years ago that the apparently unrelated citrus and parsley families, which are both attractive to a group of swallowtail butterflies, had in common certain essential oils **(methyl chavicol, anethole** and **anisic aldehyde)** which presumably acccounted for the attractiveness to the group of swallowtails. Dethier had also found that caterpillars of the black swallowtail butterfly would even try to feed on filterpaper soaked in these essential oils.

It seems probable that host selection of *Hypsipyla* on the *Meliaceae* may have a similar chemical basis.

Many investigations on essential oils of the *Meliaceae*, of which only a few are cited here, have already taken place (2, 3, 4, 7, 8, 16), but as far as the available literature could be reviewed, they have not been related to the attacks of *Hypsipyla* spp.

Much research on the essential oils of *Meliaceae* has, for instance, been done in Nigeria by Bevan and collaborators (2, 3, 4). These authors (4) indicate that **anthotecol**, an extractive from *Khaya anthoteca* is related chemically and probably biologically to substances obtained from other *Meliaceae*. This could be the common chemical basis and reason why in all continents *Meliaceae* are attacked by *Hypsipyla* spp.

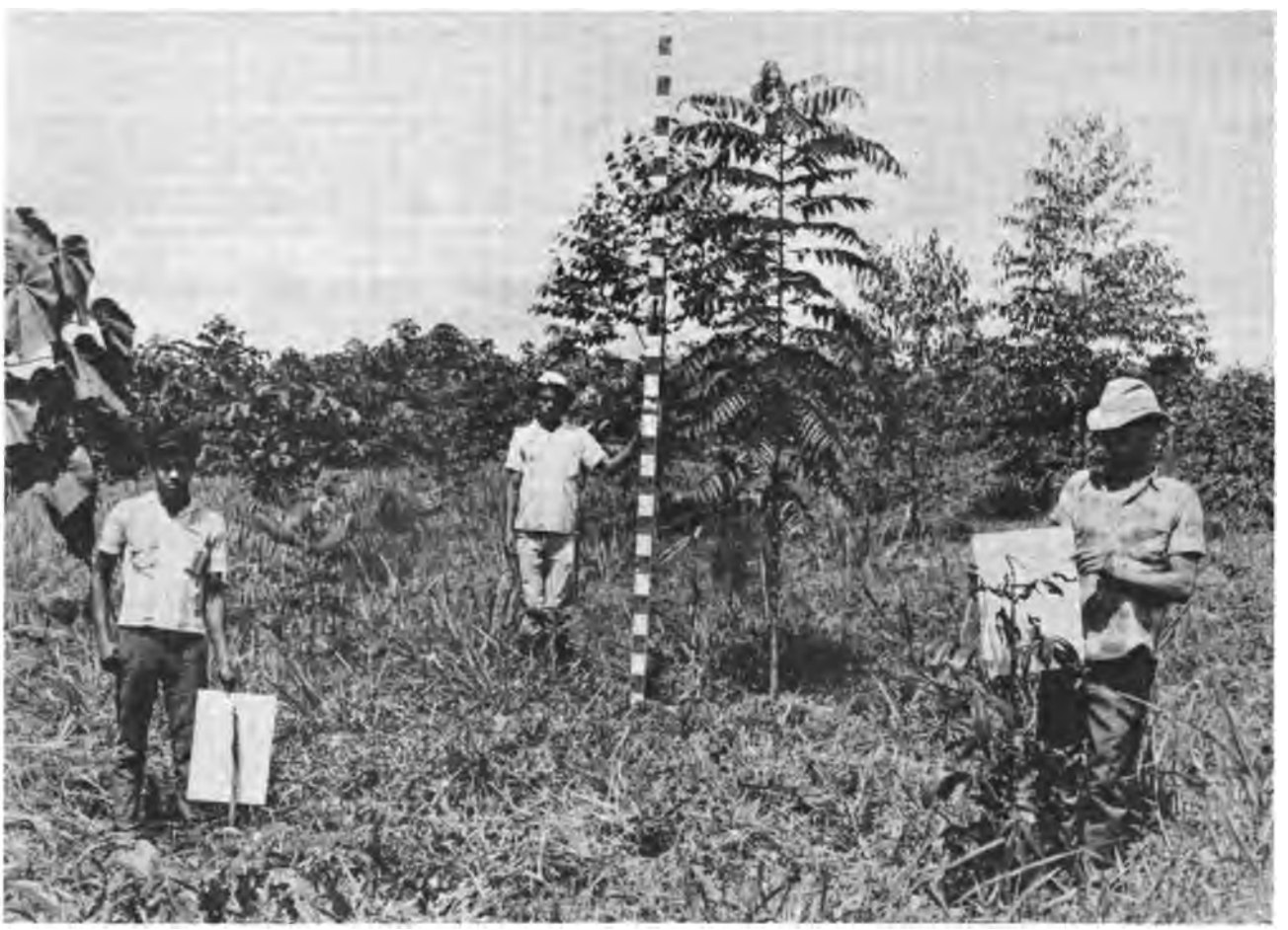


Fig. 1. Single Tree Plot Trials, Puente Cajón, Turrialba, Costa Rica. *Cedrela odorata* (left) and *Swietenia macrophylla* (right) are heavily attacked by *Hypsipyla grandella*, while *Toona ciliata* var *australis* is not attacked. Planting distance: 3 m; all trees 13 months in the field. Height of *Toona*: 3,25 m.

Fig. 2. Single Tree Plot Trials, Puente Cajón, Turrialba, Costa Rica. *Cedrela odorata* (foreground) is heavily attacked by *Hypsipyla grandella. Toona ciliata* var *australis* (left) and *Khaya ivorensis* (right, arrow) are unattacked. Planting distance: 3 m; all trees 13 months in the field. Height of *Toona:* 2.75 m.

Fig. 3. Single Tree Plot Trials, Bajo San Lucas, Turrialba, Costa Rica. *Cedrela odorata* (foreground) is attacked by *Hypsipyla grandella,* while *Toona ciliata* var *australis* (arrow, above) and *Khaya ivorensis* (arrow, left) are unattacked Planting distance: 3 m; all trees 13 months in the field. Height of *Toona:* 5.00 m.

Fig. 4. Block trial of *Swietenia humilis,* 24 months after planting, heavily attacked by *Hypsipyla grandella.* In the background at a distance of 55 m, a plot of *Toona ciliata* var *australis* (arrow), free of attacks. Puente Cajón, Turrialba, Costa Rica.

Fig. 5. Block Trial of *Cedrela odorata*, 18 months after planting, heavily attacked by *Hypsipyla grandella*. In the background is the plot of *Toona ciliata* var. *australis*, free of attacks. Puente Cajón, Turrialba, Costa Rica.

Specialization of the *Hypsipyla* spp. on certain essential oils, might then result in immunity of exotic *Meliaceae* to borer attack if the trees lack or hardly possess these specific chemicals on which the native *Hypsipyla* have specialized.

With respect to the serious attacks of *Hypsipyla robusta* on *Swietenia macrophylla*, introduced into India and Ceylon (21), a comparative study on the essential oils of the shoots and leaves of local and exotic *Meliaceae*, may clarify these and similar cases, e.g. attack

Fig. 6. Plot of *Toona ciliata* var. *australis*, 18 months after planting, average height: 6.20 m; fertilized quarterly with 50 grams of 20–20–0; unattacked by *Hypsipyla grandella*, Puente Cajón, Turrialba, Costa Rica.

Fig. 7. Appearance of the trees of *Toona ciliata* var. *australis* (unpruned) in the Block Trials in Puente Cajón.

of *Hypsipyla robusta* on *Swietenia mahogani* in Australia (21). On the other hand it is also possible that the native *Hypsipyla* spp. may adapt to the new smell or taste of similar essential oils found in exotic *Meliaceae*, which after all, are probably related chemically to those of the native *Meliaceae*. A last possibility which is not very probable, but should not be completely discarded, is that an exotic *Hypsipyla* spp. may have been imported together with the exotic *Meliaceae*.

If the hypothesis of the olfactory orientation of the *Hypsipyla* moth is correct it might open new ways towards the solution of the *Hypsipyla* problem. A number of interesting research projects could then be initiated which ultimately would make it possible to establish, on a commercial basis, plantations of the fast growing valuable *Meliaceae* in the tropics.

The following research projects in relation to the *Hypsipyla* problem might be considered.

1– The olfactory orientation of *Hypsipyla* to its hosts. A preliminary investigation by the author is presently underway in Turrialba.

2– Investigation on other reasons why some exotic *Meliaceae* are not, or are less, attacked by the native *Hypsipyla* spp. If the moth of *Hypsipyla grandella* would be given no other choice but to oviposit on seedlings of *Toona* or *Khaya*, would the larvae then enter the shoots? Or are the shoots and bark chemically unpalatable to the larvae? This is also being investigated presently in Turrialba.

3) Analysis of the volatile essential oil(s) in the leaves and shoot of economically important *Meliaceae*, by means of gas chromatography and N.M.R. and the subsequent determination of the host selection attractant(s) of *Hypsipyla* spp.; such chemicals might be used conceivably in the combat of *Hypsipyla* spp.

4– Selection and breeding of *Meliaceae* which do not have, or only have a low content of the attractant component in the leaves and shoots.

5– Crossing of the attack–free, or little attacked *Meliaceae* with species that are attacked by *Hypsipyla* (e.g. *Toona* with *Cedrela*) might also offer interesting aspects. The fact that *Toona* as well as *Cedrela* spp. can be propagated vegetatively is an additional advantage.

Finally the chemical composition of the essential oils of the *Meliaceae* may also be of interest to botanists and entomologists, who study the evolution and distribution of the *Meliaceae* and the genus *Hypsipyla* in the world.

## Summary

*Toona ciliata* var. *australis* (Australian Red Cedar) and *Khaya ivorensis* (Nigerian Mahogany) included in Single Tree Plot and Block Trials in Turrialba, Costa Rica, are not attacked by *Hypsipyla grandella*, whereas native *Meliaceae* such as *Cedrela odorata* and *Swietenia* spp. are heavily attacked. In many countries of other continents these Latin American *Meliaceae* are not, or are less attacked by native *Hypsipyla* spp., although some countries report serious attacks.

The author supposes that volatile essential oils in the shoots and leaves, which would be different for various but probably not all *Meliaceae*, attract the moth of *Hypsipyla* spp. to its host trees. Specialization of the *Hypsipyla* moth on certain essential oils of native *Meliaceae* would lead to immunity of exotic *Meliaceae* which do not possess these essential oils as main elements. Some research projects related to the hypothesis of olfactory orientation of the *Hypsipyla*–moth to its host trees are suggested. In view of its fast growth valuable timber and absence of attacks of *Hypsipyla grandella*, *Toona ciliata* var. *australis* seems a most promising plantation tree for Latin America.

## Acknowledgements

The author wishes to thank Dr. Robert I. Gara, of the College of Forest Resources, University of Washington, for the critical review of the manuscript and the helpful suggestions. Thanks are also due to Dr. Herster Barres, Department of Forest Sciences, Inter–American Institute of Agricultural Sciences, Turrialba, Costa Rica, for having made available the growth data of the Single Tree Plot Trials.

## Literature cited

1. BECKING, J. H., *et al.* Agrarische Winkler Prins. Amsterdam, Elsevier 1954, v. 3. pp. 545–546.

2. BEVAN, C. W. L., EKONG, D. E. U. and TAYLOR, D. A. H. Extractives from West African members of the family *Meliaceae*. Nature No. 4991:1323–1325, 1965.

3. _____, POWELL, J. W. and TAYLOR, D. A. H. West African Timbers. Part VI. Petroleum extracts (of heartwood) from species of the genera *Khaya, Guarea, Carapa* and *Cedrela*. Journal of the Chemical Society. Feb. 1963:980–982.

4. _____, REES, A. H. and TAYLOR, D. A. H. West African Timbers. Part VII. *Anthotecol*, an extractive from *Khaya* anthoteca. Journal of the Chemical Society. Feb. 1963:983–989.

5. BRISCOE, C. B. Statistically valid planting trials. Proceedings of the 2nd. World Eucalyptus Conference. Sao Paulo, 1961. pp. 525–531.

6. CHABLE, A. C. Reforestation in the Republic of Honduras, Central America. Ceiba 13(2):29. 1967.

7. CHAN, W. R. *et al.* Extractive of *Cedrela odorata* L. Part II. The structures of the *Cedrela* tetracyclic triterpene, odoratol, iso–odoratol and odoratonen. Journal of the Chemical Society. 20 C:2485–2489, 1968.

8. _____, MAGNUS, K. E. and MOOTOO, B. S. Extractives from *Cedrela odorata* L.: the structure of methyl–angolensate. Journal of the Chemical Society (2C):171–177. 1967.

9. CHINTE, F. O. Trial planting of Large–leaf Mahogany (*Swietenia macrophylla* King). The Philippines Journal of Forestry 6(4):322. 1949.

10. DUTT, S. Indian oleoresins and their essential oils. Indian Oil Soap 27(1):3–10. 1961. Abstract in Chemical Abstracts 56(4):3577. 1962.

11. EHRLICH, P. R. and RAVEN, P. H. Butterflies and plants. Scientific American 216(6):105–113. 1967.

12. FOOD AND AGRICULTURAL ORGANIZATION OF THE UNITED NATIONS, Forestry and Forest Industries Division, Rome. A Guide to tree species trials in tropical America (Draft), 1968. pp. 33–34.

13. GEARY, T. F. Tropical Forest Research Center, Rio Piedras, Puerto Rico. Personal communication to P. Grijpma, July 1969.

14. GRIJPMA, P. y RAMALHO, R. *Toona* spp., posibles alternativas para el problema del barrenador *Hypsipyla grandella* de las *Meliaceae* en América Latina. Turrialba 19(4):531–547. 1969.

15. HELLINGA, G. Houtsoorten voor aanplant op bedrijfsgrootte. Tectona XL. (3/4):200. 1950.

16. HODGES, R., McGEACHIN, S. G. and RAPHAEL, R. A. The chemistry of cedrelone. (Extracted from the heartwood of *Toona ciliata)*. Journal of the Chemical Society, April 1963. pp. 2515–2526.

17. JACALNE, D. V., MEIMBAN, Jr., J. R. and RADLE, J. F. A study of the stump planting of Mahogany (*Swietenia macrophylla* King). The Philippine Journal of Forestry, 13(½):64. 1957.

18. LAMB, A. F. A. Artificial regeneration within the humid lowland tropical forest. Commonwealth Forestry Review 48(1):49. 1969.

19. PARRY, M. S. Tree planting practices in tropical Africa. FAO Forestry Development Paper No. 8, p. 168. 1956.

20. SHOOTBORERS of the *Meliaceae*. Unasylva 12(1):30–31. 1958.

21. STREETS, R. J. Exotic trees in the British Commonwealth. Oxford, Clarendon Press, 1962. 765 p.

# STUDIES ON THE SHOOT BORER *HYPSIPYLA GRANDELLA* (ZELLER).

# I. HOST SELECTION BEHAVIOR*

*P. Grijpma*** and R. I. Gara****

Silviculturist, Forestry Program, Inter–American Institute of Agricultural Sciences,
Turrialba, Costa Rica; attached to IICA through the Dutch Technical Assistance Program
and
Associate Professor of Forest Entomology, College of Forest Resources
University of Washington, Seattle

## COMPENDIO

Este trabajo presenta los resultados de una serie de experimentos relacionados con la selección de los árboles hospederos por el adulto de *Hypsipyla grandella* (Zeller). Los objetivos fueron: 1) investigar el comportamiento del adulto en cuanto al vuelo; 2) estudiar las condiciones microclimáticas asociadas con el vuelo; y 3) determinar modelos de los ataques del barrenador en relación a la fenología del árbol hospedante y posibles atrayentes. Los datos obtenidos indicaron que, en una parcela de *Cedrela odorata* L. fuertemente atacada por el barrenador, los adultos volaron a alturas más altas que los arbolitos, los cuales tenían una altura promedio de 0,6 m. También se observó otro modelo de vuelo que consistía en un vuelo corto de los árboles hospederos a la vegetación baja que cubre el suelo y viceversa. En cuanto a la relación entre las condiciones microclimáticas y el vuelo, la duración del estudio fue demasiado corta para llegar a conclusiones definitivas. No obstante se observó que los adultos no volaron cuando la temperatura fue inferior a 17°C. Precipitaciones menores que 11 mm no impidieron el vuelo. La actividad mayor durante el período de estudio se registró después de una fuerte lluvia.

Hay indicaciones bastante definidas que el adulto de *H. grandella* se orienta a su hospedero por medio del olfato y que existe una alta correlación entre el número de ataques por árbol y la proporción de hojas frescas.

Los autores

## Introduction

The shoot borer of *Meliaceae, Hypsipyla grandella* Zeller, is the greatest detriment to the establishment of *Cedrela* and *Swietenia* plantations in the American tropics. The main damage occurs when larvae bore into the growing tips of young plants, thus destroying height growth for a season or more. The biology and distribution of *H. grandella* has been described by Ramirez Sanchez (9).

It is believed that *H. grandella* selects new hosts at night as adults are active during this period. Ramirez Sanchez (9) observed that greatest flight activity occurred affter dark and egg eclosion took place from 18:20 h. and on through the night.

Since trees over 6 meters in height are not as heavily attacked as the shorter stems, it is assumed that host selection flights occur mainly at lower levels, i.e., the heights of seedlings and younger stems. Field experience shows that once a plot is invaded by the shoot borer the incidence of infestation augments rapidly and eventually all of the plantation is infested. In time, trees degenerate to such an extent that heavy mortality ensues. Apparently, once a plantation is infested, the moths tend to concentrate within the outbreak.

Unfortunately, little is known on microclimatic and other environmental conditions that influence *H. grandella* flights. The only reports available indicate that heavier attacks occur at the beginning of the rainy season, with only minor infestations occuring at the initiation of the dry season (1, 2, 8, 9, 11). In addition, attacks primarily occur in open grown plantations, indicating that temperature among other physical factors may be important in guiding host selection flights.

* Received for publication April 7th, 1970.
** Study to be used by P. Grijpma for Ph.D. dissertation.
*** Study supported by Ford Foundation grant No. 57–74 and conducted under OTS project No. 68–38.

Recent studies (7, 10) indicate that *Hypsipyla* spp. exhibit definite host preferences for certain meliaceous species. In Costa Rica, Grijpma (7) conclusively showed that *H. grandella* preferred *C. odorata* over *Swietenia macrophylla* King, *S. humilis* Zucc. and a hybrid cross of *S. mahogany* (L.) Jacq. x *S. macrophylla*. However, the insects did not attack *Toona ciliata* M. Roem. var *australis* (F.v.M.) C.D.C. and *Khaya ivorensis* A. Chev. He suggested that the preference of *H. grandella* for *Cedrela* was due to the olfactory response of shoot borers to volatile materials emanating from the plant.

It is believed that a more complete knowledge on the host selection behavior of the shoot borer would provide insights into more imaginative and applicable control methods. In particular, verification of primary attractants could lead to isolation and identification of such compounds; synthesis of these materials could be used in the protection of valuable stands. Identification of these compounds also could lead to a tree breeding program in which less susceptible trees would be developed. Accordingly, the main objectives of the studies described here were to gain insights into the host selection mechanisms of *H. grandella*. Specific objectives were as follows: 1) to investigate the flight behavior of shoot borer populations within a *Cedrela* plantation; 2) to outline some of the micrometeorological conditions associated with flight; and 3) to investigate patterns of shoot borer attack in relation to host phenology and possible attractants.

## Materials and methods

**Flight behavior.** Preliminary tests, designed to measure the height of *H. grandella* flights, were established in a plot of *C. odorata* in the pure block species trials in the Puente Cajón area, Turrialba, Costa Rica. The plot contained 100 trees, planted at distances of 2 x 2 m. The stand averaged 0.6 m in height and was about 18 months old. The plantation, over the last 12 months, was completely infested and 18 per cent of the trees were dead.

Heights of flight was determined by means of 0.75 x 3.00 m wooden frames covered with polyethylene sheeting stretched over the frames. Twelve of these frames were set up in diagonals across the plantation, each 3 m from its neighbor. The frames were then coated with Stickem–Special,* a sticky compound that remains viscous for a long time. For a period of a week the frames were checked daily and about 90 per cent of the shootborers caught were recovered from a height of 1–2 m.

Taking into account results of this preliminary test, the main set of trapping devices was established at a mean height of 1.30 m. These devices, termed trapping barriers (6), were constructed from 0.5 x 1 m polyethylene sheets and covered with Stickem–Special.

* Michel and Pelton Co., Oakland, California.

Fig. 1. Two types of sampling barriers used to determine flight patterns of *H. grandella*. The taller barriers sampled height of flight, the others sampled flight in relation to host selection.

Eighty barriers were hung from wires in such a manner that most of the surviving trees had a barrier within a meter distance (Fig. 1). A similar set of barriers was established in a non–host stand of *Fraxinus uhdei* and in a stand of *S. macrophylla* that was grown under shade of *Gmelina arborea* Roxb. The barriers were checked nightly and in the morning; shoot borers trapped on the barriers were collected and their position in regards to the individual *Cedrela* trees was noted. Temperature and rainfall were noted and the results correlated with the shoot borer collections.

**Temperature relations.** Selected *Meliaceae* were also enclosed within a 90 x 90 x 90 cm screened cage. The enclosed trees were infested by shoot borer larvae. As the larval mines extended into the stem the temperature inside the stem was measured by inserting a small thermistor into the larval mine; temperature adjacent to the stems were measured by placing another thermistor next to the stem; resultant temperatures were recorded from a Yellow Springs, model 4256, meter. In practice, one enclosed and infested tree under shade, and another similarly infested tree in open sunlight, were instrumented.

**Adult movement.** The movements of adults within an infestation also were investigated by means of ground barriers. In this case, 70 x 35 cm polyethylene panels were coated with Stickem–Special and located as follows: 1) two were positioned directly under selected trees, and 2) others in three concentric circles around the trees. The radii of the circles were 0.5, 1.25 and 2 m respectively (Fig. 2). In the experiment two types of trees were used, one type was flushed with fresh leaves, the other type only had older, mature leaves. Additional barriers (tree barriers) were placed vertically in the form of a cross around one tree with new leaves and another with mature ones.

To further check adult movement, male and female adults were dusted with fluorescent powder. The insects were then released on selected trees and on the ground. Their subsequent activity was followed by observing their fluorescent trails on foliage and on foliage and on the ground by means of an U.V. light.

Fig. 2. Ground barriers around a *Cedrela* tree.

**Host phenology.** In order to relate possible synchrony of fresh leaf production with incidence of attack, periodical surveys were made of the *Cedrela* plantation. Attack frequency was recorded together with the number of fresh and mature leaves.

**Attraction.** The possibility of primary host attractants was investigated in a series of experiments. Two small tables were constructed, and located adjacent to the *Cedrela* infestation. Four wooden frames, 0.5 x 0.5 x 1 m were placed on the tables, each occupying an equal area. Strips of sticky polyethylene were strung around the periphery of each frame in effect forming a cage (Fig. 3) for convenience called an olfactory cage.

Various potted meliaceous tree species were placed in frames according to the test in progress.

## Results

**Flight activity.** Flight of *H. grandella* mainly occurred when nightly temperatures were over 17°C (Figure 4). There was not a strict correlation between flight and temperature; however, flight activity took place between 17 and 24°C. Temperature differences between the shaded *Swietenia* and the open grown *Cedrela* only varied between 2° – 3.5°C in the larval gallery and next to the stem. The open grown trees demonstrated these higher temperatures between the hours of 0900–1500. Ambient temperatures for 24 hour periods virtually were the same under shaded and isolated conditions. Even though ambient temperatures under the shade were equal to the non–shade areas, fewer attacks occurred in the *Swietenia* than in the *Cedrela* plots. Rainfall between 0.5 and 8.0 mm did not hinder flight, but heavier rains, over 11 mm prevented flight.

The emergence of *H. grandella* from pupae placed in outdoor cages correlated with flight activity of the field population as the greater barrier catches occurred within 1–2 days after emergence (Fig. 4). During periods of moth emergence only 4 insects were caught in the non–host stand.

Data from Table 1 indicate that moths are active on the ground near the tree during early night, but invade their host later. The possibility that moths may inhabit ground vegetation during daylight hours is also seen in a cage test where moths were observed resting during daylight (Fig. 5). When moths were dusted with fluorescent powder and liberated on trees and ground, fluorescent trails left by moths indicated that they moved from the ground to the trees and vice versa.

**Host selection.** During the study it was noted that most of the barrier catches occurred near trees with new leaves (Fig. 6). Periodic surveys of the infestation supported observations that moths preferred to oviposit on trees bearing new leaves. Data from Fig. 7 indicate

Fig. 3. Olfactory cages containing potted *Toona* (left table) and *Cedrela* (right table).

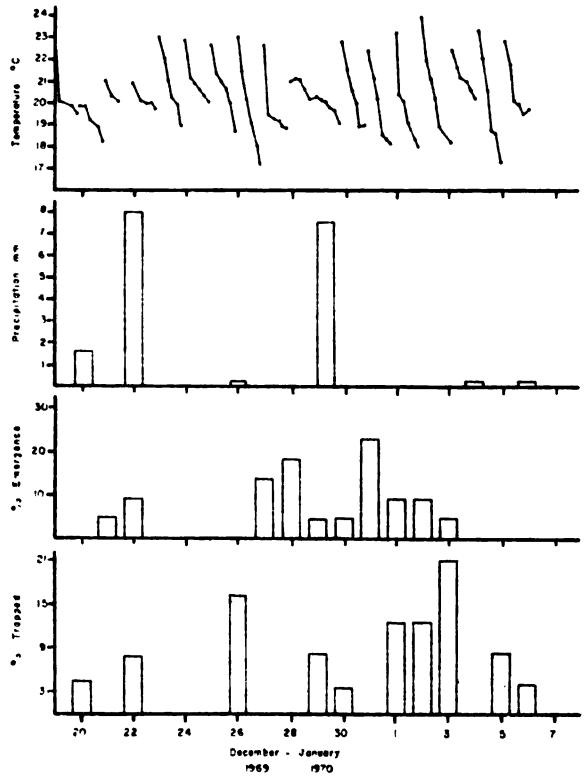



Fig. 4. Relationships between ambient temperatures, precipitation and emergence as well as catches of *H. grandella* on flight barriers.

that the trees most heavily attacked had a predominance of fresh leaves, although trees sustaining a few new leaves among the mature also were attacked. At least during the later part of the rainy season, just one tree with only mature leaves was attacked.

In tests where *Cedrela* and *Toona* trees were used as potted material in the olfactory cages only the *Cedrela* trees were selected by moths. Similarly only *Cedrela* trees with new leaves were chosen by *H. grandella* when new–leaved *Cedrela* trees were compared to cedar trees with only mature leaves (Table 2).

## Discussion

Although night temperatures between 17 and 24°C did not seem to influence *H. grandella* flight, increased flight activity was noted within 4–5 days after rain. It is possible that the additional moisture may stimulate plant growth which in turn may attract more moths. It is also conceivable that *H. grandella* emergence may be induced by periods of higher relative humidity. Tillmanns (11) suggests that after the dry season new attacks by the borer occur soon after the first rains.

The fact that few insects were caught outside of their host area indicated that *H. grandella* do not disperse readily from an active infestation. Within an outbreak, a short distance relationship between emergence and subsequent host selection seems likely. Apparently during the day, adults rest in ground cover at a short distance from their hosts. It also is interesting that the moths' wing and body markings blend in well with herbaceous material.

TABLE 1. Accumulated numbers of male and female *H. grandella* caught on ground and tree barriers, near *Cedrela odorata* trees with fresh and mature leaves on the nights of 5/6 January and 5/6 March, 1970.

| Time | Barrier Catches | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Fresh Leaves | | | | Mature Leaves | | | |
| | | Ground Barriers | | | | Ground Barriers | | |
| | Tree Barriers | Inner ring | 1.25 m | 2.00 m | Tree Barriers | Inner ring | 1.25 m | 2.00 m |
| 19.10 | 0 | 5♀ | 2♀ | 1♂ | | | | |
| 19.15 | 0 | 1♀ | 0 | 0 | | | | |
| 19.22 | 0 | 1♀ | 1♂ | 0 | | | | |
| 19.50 | 0 | 1♀ | 1♀ | 0 | | | | |
| 20.00 | 1♀ | 2♀ | 1♂ | 1♂ | | | | |
| 20.12 | 0 | 1♀, 1♂ | 0 | 0 | | | | |
| 20.20 | 2♀, 1♂ | 0 | 0 | 0 | | | | |
| 20.50 | 1♀ | 0 | 0 | 0 | – No insects caught – | | | |
| 21.17 | 1♂ | 4♀ | 1♂ | 0 | | | | |
| 21.35 | 0 | 0 | 1♂ | 1♂ | | | | |
| 22.15 | 1♀ | 0 | 0 | 0 | | | | |
| 22.30 | 1♀ | 0 | 0 | 0 | | | | |
| 23.50 | 2♀ | 0 | 0 | 0 | | | | |
| 23.30 | 1♀ | 0 | 0 | 1♂ | | | | |
| Totals | 9♀, 2♂ | 11♀, 1♂ | 3♀, 4♂ | 4♂ | 0 | 0 | 0 | 0 |

**Fig. 5.** A *Hypsipyla grandella* moth resting on vegetation.

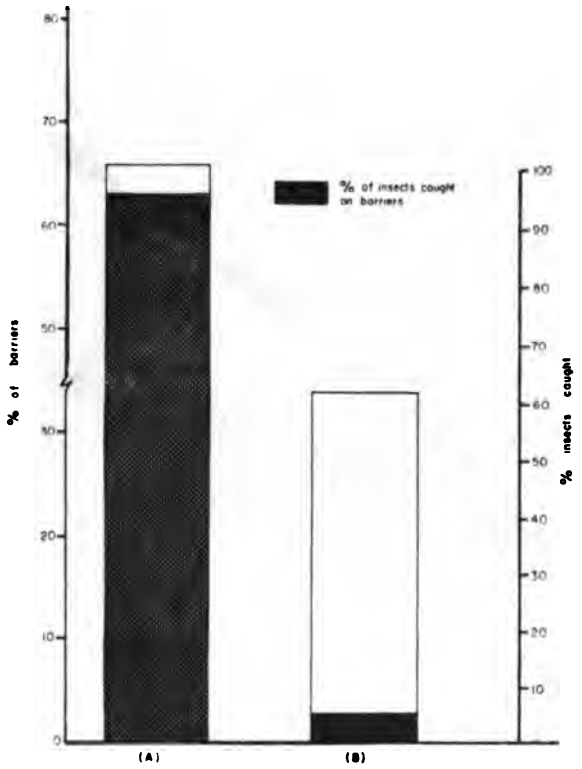



Fig. 6. Percentage of barriers adjacent to *Cedrela odorata* with fresh leaves (A) and without fresh leaves (B), and the respective catches of *H. grandella*. December 20, 1969 – January 7, 1970.

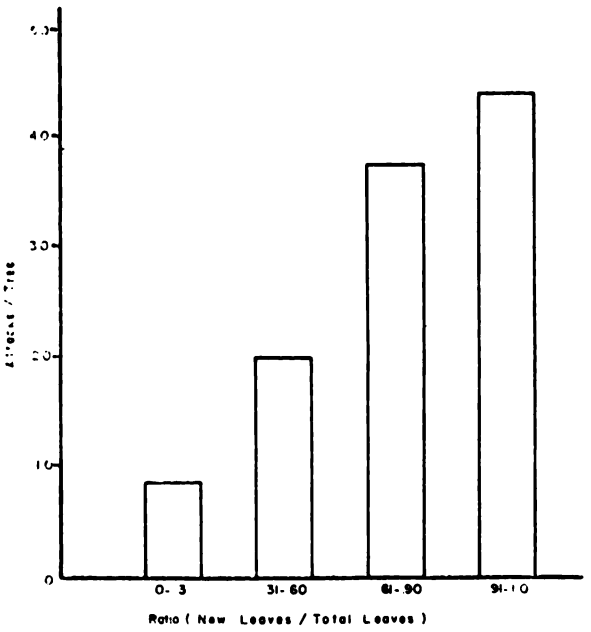



Fig. 7. Relationships between *H. grandella* attack and amount of young foliage available on its hosts tree, *Cedrela odorata*.

TABLE 2. Catches of *H. grandella* on olfactory–cages with different types of potted plant material.

| Data Feb.–Mar 1970 | Host Material | |
|---|---|---|
| | *Cedrela* fresh leaves | *Toona* fresh leaves |
| Feb. 23 | 3 | 0 |
| Feb. 24 | 1 | 0 |
| Feb. 25 | 1 | 0 |
| Feb. 26 | 0 | 0 |
| Feb. 27 | 2 | 0 |
| | | *Cedrela* Mature leaves |
| Mar. 5 | 2 | 0 |
| Mar. 6 | 1 | 0 |
| Mar. 7 | 2 | 0 |

The results of the experiments strongly indicate that *H. grandella* select hosts by olfactory response to volatiles emanating from fresh leaves. Evidence for this olfactory orientation was supplied by the high percentages of catches on barriers located near fresh–leaved trees and by the response of adults to fresh–leaved *Cedrela* placed in olfactory cages. Further evidence for response to host volatiles was the fact that *H. grandella* detected its host plant outside of the infestation. All these data, however, were taken during the rainy season and most of the trees had fresh leaves. Oviposition on trees without leaves also was observed during the dry season, hence, the presence of new growth cannot explain host selection during this season. As it was noted that leafless trees mostly had thin, papery bark, it could be that volatiles responsible for host orientation emanate through lenticels and the bark *per se*. It also is possible that insects may select host in response to volatiles emanating from old borings and frass produced by larvae from previous atracks (Fig. 8).

It has been observed that *Swietenia* and *Cedrela* plots often prosper for a short time, then gradually deteriorate as *H. grandella* attacks augment. When one plantation is destroyed, neighboring meliaceous plantations come under ever increasing attack. These observations indicate that flourishing infestations tend to localize *H. grandella* populations within the outbreak areas. It is likely that as long as the emergence of moths is synchronized with the abundance of new growth, the population remains within the infestation. On the other hand, when plantations finally collapse, the moths disperse and become established in new focal centers. Accordingly, the present study shows that insects emerging in the outbreak area did not disperse but rather attacked in synchrony with the production of fresh leaves. It is interesting that during the dry season, when the *Cedrela* plot was leafless, new attacks began in the shaded, neighboring *Swietenia* plot that was fully foliated and unattacked.

It is thought that attacks on *Meliaceae* are less severe when trees are planted under shade (1, 2, 3, 4, 5, 8, 11). An explanation, in part for this phenomenon may be the lack of suitable ground cover. Another factor may be the

Fig. 8. Egg of *Hypsipyla grandella* oviposited on boring frass of previous atrack.

differences in temperatures developed under open or shaded stands; possibly larvae boring in the cooler shaded stems emerge when their new hosts are unsuitable for attack. Moreover, present observations indicate that shaded *Swietenia* trees often successfully prevent attacks by exudation of gum.

Although olfactory orientation is indicated, it is not clear whether both sexes are equally responsive to host volatiles. Data from ground and tree barriers show that most catches in immediate vicinity of the trees are females. Perhaps females are more responsive to volatile host material than are the males. Female produced sex attractants could then explain the catches of male *H. grandella* on barriers near fresh leaved trees.

## Literature cited


1. BEARD, J. S. Summary of silvicultural experience with cedar, *Cedrela mexicana* Roem., in Trinidad. Caribbean Forester 3:91–102. 1942.
2. BURGOS, L. Un estudio de la silvicultura de algunas especies forestales en Tingo María, Perú. Caribbean Forester 15:14–53. 1954.
3. CATER, J. C. The silviculture of *Cedrela mexicana* Caribbean Forester 6:89–113. 1945.
4. DE LEON, D. Some observations on forest entomology in Puerto Rico. Caribbean Forester 2:160–163. 1941.
5. FORS, A. J. Informe sobre plantaciones forestales en Cuba. Caribbean Forester 2:138–140. 1941.
6. GARA, R. I., VITE, J. P. and CRAMER, H. H. Manipulations of *Dendroctonus frontalis* by use of a population aggregating pheromone. Contributions from the Boyce Thompson Institute 23:55–66. 1965.
7. GRIJPMA, P. Immunity of *Toona ciliata* M. Roem. var. *australis* (F.v.M.) C.D.C. and *Khaya ivorensis* A. Chev. to attacks of *Hypsipyla grandella* Zeller, in Turrialba, Costa Rica. Turrialba 20(1):85–93. 1970.
8. HOLDRIDGE, L. R. Comments on the silviculture of *Cedrela*. Caribbean Forester 4:77–80. 1943.
9. RAMIREZ SANCHEZ, J. Investigación preliminar sobre biología, ecología y control del *Hypsipyla grandella* Zeller. Boletín del Instituto Forestal Latino Americano de Investigación y Capacitación No. 16:54–77. 1964.
10. ROBERTS, H. A survey of the important shoot, wood, flower and fruit loving insects of the *Meliaceae* in Nigeria. Nigerian Information Bulletin No. 15:1–25. 1965.
11. TILLMANNS, H. J. Apuntes bibliográficos sobre *Hypsipyla grandella* Zeller. Boletín del Instituto Forestal Latino Americano de Investigación y Capacitación No. 14:82–92. 1964.

# STUDIES OF THE SHOOTBORER *HYPSIPYLA GRANDELLA* (ZELLER). II. HOST PREFERENCE OF THE LARVA*

*P. Grijpma*** and R. I. Gara****

Silviculturist, Forestry Program, Inter-American Institute of Agricultural Sciences
Turrialba, Costa Rica. Attached to IICA through the Dutch Technical Assistance Program
and
Associate Professor of Forest Entomology
College of Forest Resources, University of Washington, Seattle

## COMPENDIO

En una investigación previa (Turrialba 20(2):85–93) se informó que el adulto de *Hypsipyla grandella* (Zeller), tenía preferencia por las *Meliaceae* americanas. Sin embargo, muy poco se sabe acerca de los estímulos que inducen a las larvas de *H. grandella* a alimentarse y desarrollarse en forma adecuada.

La presente investigación fue dirigida a estudiar el comportamiento nutricional de larvas de *H. grandella*. Se empleó como fuente de alimento, material vegetal de cuatro especies de Meliaceas, *Cedrela odorata* L., *Khaya ivorensis* A. Chev., *Swietenia macrophylla* King., y *Toona ciliata* M. Roem. var *australis* (F.v.M.) C.D.C., y una especie no Meliacea, *Aucoumea klaineana* Pierre. Se constató que las larvas de *H. grandella* también tienen preferencia por las Meliaceas americanas usadas en la investigación.

Durante el curso de la investigación, se observó una alta mortalidad en larvas que se alimentaron con material de *T. ciliata* var *australis*, por lo cual se iniciaron una serie de experimentos complementarios para determinar si el material vegetal de *T. ciliata* var. *australis* es tóxico para las larvas. También hubo evidencia de que dicha sustancia tóxica tiene características volátiles con lo cual se podría explicar la no oviposición de *H. grandella* sobre *T. ciliata* var *australis*. Se piensa que esta característica podría ser empleada en el futuro como base para la selección genética de Meliáceas resistentes a *Hypsipyla* spp.

Los autores

## Introduction

Studies indicate that *Hypsipyla grandella* Zeller display a definite species preference for the various native *Meliaceae* (2). Recent observations reveal that *Cedrela odorata* L. trees with a predominance of fresh leaves are attacked more frequently than those with mature leaves (3). It is believed that the adult *H. grandella* find these preferred *C. odorata* through olfaction (2, 3, 4). Accordingly, the females must have a well developed ability of select a host wherein subsequent larvae will prosper. Little is known, however, on the chain of stimuli and responses that induce larvae to feed and develop properly.

The fact that other *Meliaceae*, such as *Toona ciliata* M. Roem var. *australis* (F.v.M.) C.D.C., are not attacked in Turrialba, Costa Rica, may be attributed to: a) adults are not attracted to *Toona*, or b) adults are attracted and oviposit on *Toona*, but the larvae after eclosion soon die. However, recent studies indicate that *H. grandella* are not attracted to *T. ciliata* var *australis* (3). Investigations in Australia indicate that *T. ciliata* var *australis* is more resistant to attacks of *Hypsipyla robusta* Moore than are the other *Meliaceae* (7). No information exists, however, on whether larvae of *H. grandella* would be able to complete their life cycle in *Toona*.

The present study was undertaken to determine the behavior of *H. grandella* larvae with respect to food selection and feeding activity.

* Received for publication April 7th, 1970
** Study to be used by P. Grijpma for Ph.D. disertation.
*** Study supported by Ford Foundation grant No. 57–74 and conducted under OTS project No. 68–38.

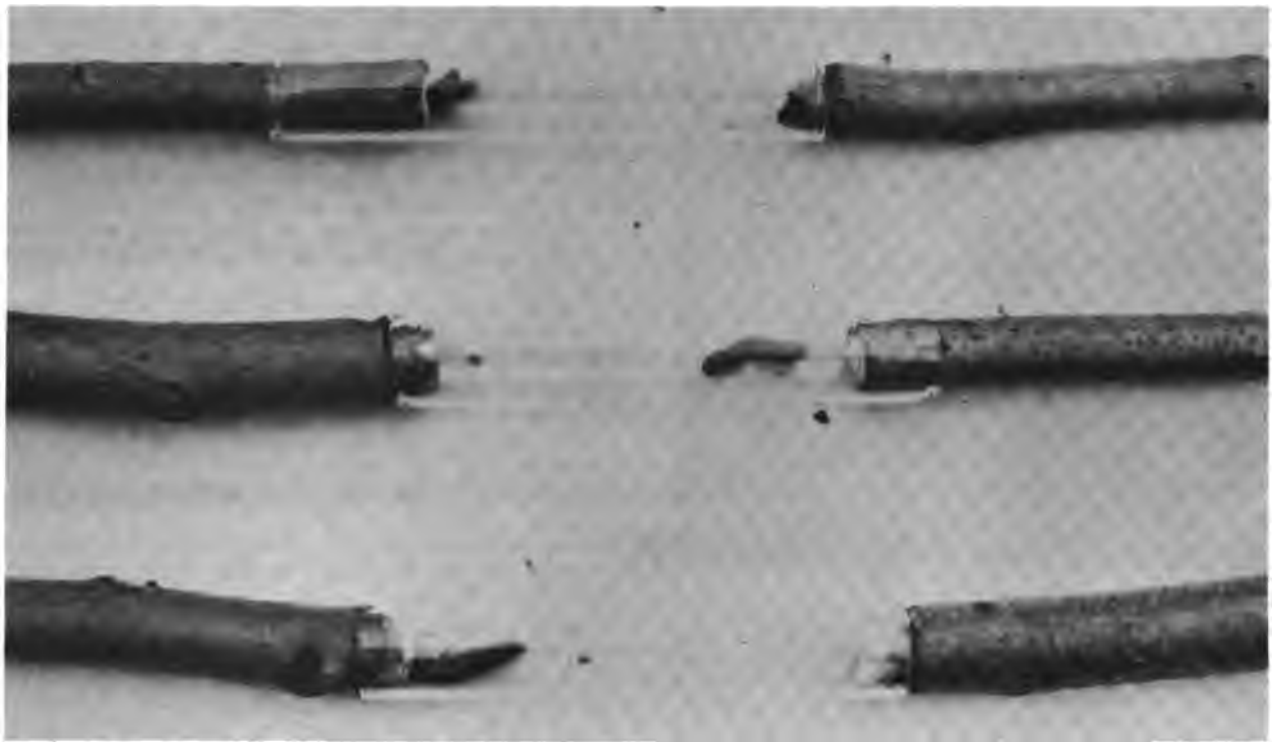


Fig. 1. Host preference test for *H. grandella* larvae, using glass tubes with stems of 4 meliaceous species and a non–host.

## Materials and methods

**Feeding preference.** A number of techniques was developed to evaluate the existence of food preference among *H. grandella* larvae. Although it would have been ideal to use instars of equal ages, the tests were performed with larvae of 3rd–5th instars; most were of the 4th instar. Instars were determined by measuring head capsules and were classified in accordance with a table provided by Ramirez Sanchez (6).

To test food preference between four meliaceous species and a non–host, *H. grandella* larvae were placed in the center of glass tubes of about 1 cm in diameter and 8 cm long (Fig. 1). Each tube contained 1 larva and was given the choice of two tree stems which were inserted into the ends of the tube. Normally, the larva selected one of the species as a boring or feeding medium. The experiment was carried out in a cross treatment design (5). In this design all possible combinations of the 5 species were tested; hence, there were 15 treatments in total, of which 10 were combinations of distinct species, while 5 were of the same species. The species used were *T. ciliata* var. *australis, C. odorata, Swietenia macrophylla* King, *Khaya ivorensis* A. Chev. and *Aucoumea klaineana* Pierre, as a non–host. After larvae were introduced into the glass cylinders, their boring was checked 24 hours later. The length of each larval mine was recorded and the amount of boring was assumed to be a measure of host acceptance or preference. About 20 per cent of the larvae soon died within the tubes as a result of a nematode (Mermithidae, probably *Hexamermis* sp.*) infestation. These dead larva were replaced immediately with healthy ones and their boring measured 24 hours later.

In another experiment, larvae were allowed to select a variety or homogenized host materials. In this case 3 plates, 24 cm in diamter, were divided into five chambers by means of plastic separators. (Figure 2). Each chamber contained macerated leaves and shoots of the same meliaceous species and the non–host as described in the previous experiment. However, in one test series only *C. odorata* and *S. macrophylla* were used as choices and larvae introduced into tests were separated as either reared in *Swietenia* or *Cedrela*. On the average, 4 larvae were placed in the center of each plate. Subsequently, the three plates were placed in a darkened growth chamber. The location of each larva was recorded within one hour and 24 hours later.

The possibility that larvae exhibit food preference through olfaction as well as gustation was tested by means of an olfactometer. Basically, the device was made by fastening strips of wood in such a manner as to form 5 distinct canals with a common entrance chamber (Figure 3). By means of a vacuum pump, air was pulled

* Personal communication from Dr. Calvin L. Massey, Rocky Mountain Forest and Range Experimental Station, Albuquerque, New Mexico. U.S.A.

Fig. 2. Host preference test for *H. grandella* larvae using plates divided into 5 chambers containing various host materials.

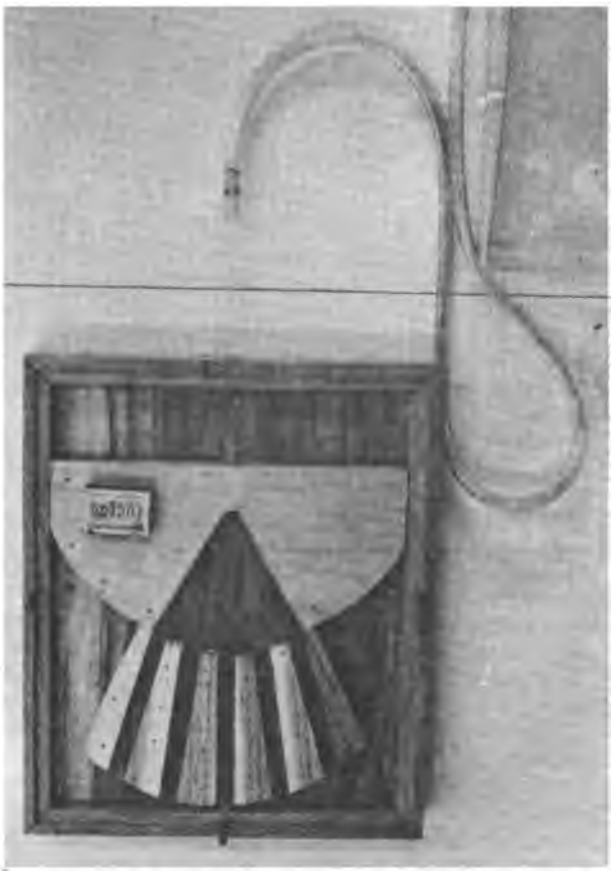


Fig. 3. Laboratory olfactometer used in establishing hosts preferences of *H. grandella* larvae. Food choises were placed in the 5 canals, air drawn through glass tube, presented larvae placed in common entrance chamber, with 5 airstreams containing hosts volatiles.

through the 5 canals which, in turn, contained the 5 different host materials used in the previous tests. The air stream passing around the various homogenized host materials would thus contain the different volatiles and transfer them to the entrance chamber. Here, larvae would be able to select the canal of their choice through olfaction. In designing the device, smoke was introduced into the air intakes and demonstrated, in theory, that the technique was usable as five distinct air streams flowed within each canal and then united at the entrance chamber.

**Host toxicity.** In the host preference tests, as above, it was noted that larvae selecting *Toona*, frequently died. In view of this, another series of tests were designed to determine the posssible toxic effects of *Toona* on *Hypsipyla* larvae. In one experimental series, slivers of bark containing eggs of *H. grandella*, collected from a *C. odorata* field plot, were attached to stems of potted *Cedrela* and *Toona* seedlings. In total 10 *Toona* and 5 *Cedrela* seedlings were used; each tree received two bark slivers (with one egg each). All but 4 of the eggs hatched, two of the eggs positioned on one of the cedar plants died, while the other 2 were on 2 different *Toona* plants. During a period of 10 days, observations were made of the first instars' boring activity and associated behavior.

An additional experiment was designed to study the possible toxic effects of *Toona* on larvae. A standard food medium was created by macerating 16 gr of *Swietenia* and *Toona* plant material; the control merely contained 16 gr of homogenized *Swietenia*. All of the controls and treatments, in addition, contained 34 gr of water, 1.5 gr of agar, and 0.5 gr of sodium benzoate. Apart from the controls, the remaining 4 treatments contained macerated *Swietenia* and *Toona* in the following proportions respectively; 75–25, 50–50, 25–75 and 0–100 per cent. Thus, the last treatment contained solely *Toona*. The macerated food media were placed into petri dishes; each treatment and the control was replicated 5 times. One larva was introduced into each petri dish and checked for survival and boring activity twice daily during a period of 6 days.

Another experiment was designed to determine if larval mortality induced by *Toona* was a result of toxic

Fig. 4. Containers used to determine toxic effect of volatiles emanating from *Toona*. Larvae prevented from physical contact with macerated leaf material by means of Saran screening.

volatile compounds or by ingestion of an unsuitable diet. In this case macerated *Toona* material was placed in the botom of each of 6 containers. Saran™ screening was placed on top of this material so that the larvae would not be in direct contact with the macerated material. A similar series of 6 containers was established, but with *Swietenia* as the host media, and, as a control, 6 additional containers contained water soaked cotton (Fig. 4).

## Results and discussion

In the feeding preference tests where *H. grandella* larvae were placed in glass tubes with different host materials as choices, the following represents total boring (cm) into selected choices: *Toona*, 0.00; *Aucoumea*, 1.24; *Khaya*, 9.68; *Swietenia*, 19.26; and *Cedrela* 19.68. An analysis of variance (Table 2) shows that these differences are significant at the 95 per cent level of confidence. A non parametric ranking test confirmed the sequence of these preference. It is clear (Table 1) that the larvae avoided boring into *Toona*, while they readily chose *Cedrela* or *Swietenia*.

The selection of host material by larvae placed in plates with 5 food choices is seen in Fig. 5. It is seen that, within one hour, most of the larvae chose to enter compartments with the non–host material. During the one hour period, the remaining larvae showed approximate equal preference for *Cedrela*, *Swietenia* and *Khaya;* there was a somewhat lower response for *Toona*. After 12 hours a large proportion of the larval population left the non–host and went into *Swietenia* and *Cedrela;* less were found in *Toona* and *Khaya*. Moreover, many of the larvae remaining in the exotic *meliaceae* and in the non–host died. The unusually high number or larvae found in the non–host, may have been due to an arresting stimuli (1, 8). The non–host, *Aucoumea* seems to have a high level of strong volatile materials.

In another test, larvae obtained from either *Cedrela* or *Swietenia* plantations could choose as host material cedar, mahogany, or the non–host. From Table 3 it is noted that, within an hour, most of the larvae went into the *Aucoumea*. However, after 12 hours, the larvae moved from this non–host to either *Swietenia* or *Cedrela*. In fact, the larvae exhibited no differences in their subsequent choice of these two meliaceous host materials. The olfactometer test (Fig. 6) showed that 85 per cent of the total number of larvae entered the canals with non–host material *Aucoumea* (45 per cent) and the native *Meliaceae*, *Swietenia* and *Cedrela* (40 per cent). Again the arresting stimulus of *Aucoumea* was observed. Fifteen per cent of the larvae entered the canals with *Toona*, of which 33 per cent died subsequently.

Conclusions from these tests seem to indicate that *H. grandella* larvae prefer their native hosts. It is seen that, although the larvae may be initially stimulated to select an exotic host species, they later leave these hosts and

TABLE 1. Results of feeding–preference test where larvae were placed inside tubes with stems of various hosts inserted at both ends of the tubes; data expressed as distances (cm) larvae bored into different host material: *Toona ciliata* var *australis*, *Cedrela odorata*, *Swietenia macrophylla*, *Khaya ivorensis* and *Aucoumea klaineana* as the non–host control.

| | *Toona* | *Cedrela* | *Swietenia* | *Khaya* | *Aucoumea* |
|---|---|---|---|---|---|
| *Toona* | 0.00<br>0.00 | 2.35<br>0.00 | 1.40<br>0.00 | 3.20<br>0.00 | 0.00<br>0.00 |
| *Cedrela* | 0.00<br>0.72 | 0.00<br>2.57 | 0.00<br>1.05 | 0.00<br>2.98 | 0.00<br>8.40 |
| *Swietenia* | 0.00<br>1.65 | 0.00<br>1.51 | 0.00<br>6.28 | 0.00<br>1.15 | 0.00<br>1.92 |
| *Khaya* | 0.00<br>0.41 | 0.60<br>1.05 | 1.19<br>0.00 | 2.61<br>0.90 | 0.00<br>1.23 |
| *Aucoumea* | 0.00<br>1.24 | 1.01<br>0.00 | 4.16<br>0.00 | 0.18<br>0.00 | 0.00<br>0.00 |

TABLE 2. Analysis of variance of the influence of host on the boring activity of larvae placed in glass tubes with stems of various hosts inserted in the ends of the tubes.

| Source of Variation | D.F. | S.S. | M.S. |
|---|---|---|---|
| Specific vs. General | 1 | .7328 | .7328 |
| Specific Preference | 4 | 13.9047 | 3.47617 |
| General Preference | 19 | 56.9716 | 2.99851 |
| *Toona* vs. others | 1 | 7.6452 | 7.6452* |
| *Cedrela* vs. others | 1 | 10.9634 | 10.9634* |
| *Swietenia* vs. others | 1 | 8.8620 | 8.8620* |
| *Khaya* vs. *Aucoumea* | 1 | .4970 | .4970N.S. |
| Remaining components | 15 | 29.0040 | 1.9336N.S. |
| Error | 25 | 66.1917 | 1.6477 |
| TOTAL | 49 | 137.8008 | |

* Significant at 95%
N.S. Not significant

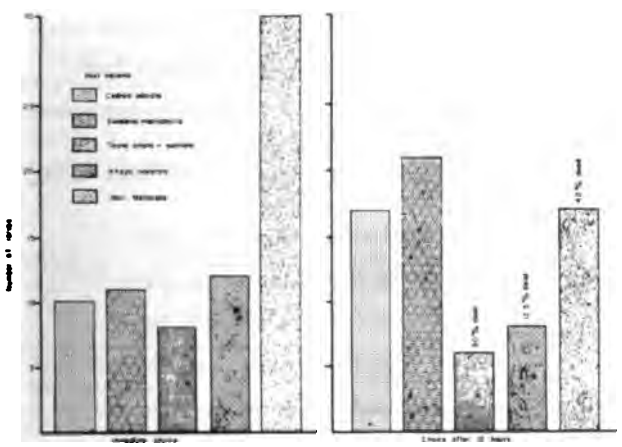


Fig. 5. Selection of macerated host material by *H. grandella* larvae, placed in the centre of plates; subsequent choice expressed by the number of larvae found in chambers containing either *Swietenia*, *Cedrela*, *Khaya*, *Toona* or a non–meliaceous species.

TABLE 3. Relationship between *H. grandella* larvae reared in either *Swietenia* or *Cedrela* and their subsequent choice of either macerated *Swietenia, Cedrela* or non–host plant material.

| Plate | Larval Origin | HOST MATERIAL Choices of the larvae | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Swietenia* | | *Cedrela* | | Non–Host | |
| | | Immediate | After 12 hrs | Immediate | After 12 hrs | Immediate | After 12 hrs |
| 1 | *Swietenia* | 0 | 1 | 0 | 1 | 6 | 0 |
| 2 | *Swietenia* | 1 | 1 | 1 | 1 | 1 | 2 |
| 3 | *Cedrela* | 1 | 1 | 2 | 2 | 0 | 0 |
| 4 | *Swietenia* | 1 | 1 | 1 | 2 | 1 | 1 |
| 5 | *Cedrela* | 1 | 2 | 0 | 0 | 2 | 1 |
| 6 | *Swietenia* | 0 | 0 | 0 | 0 | 1 | 1 |
| TOTALS* | | 4 | 6 | 4 | 6 | 11 | 5 |

* Two larvae raised in *Swietenia* made no choice and remained in middle of the plate.

are recovered in native *Meliaceae*. Frequently, when *H. grandella* larvae were found for protracted periods in non–hosts, they were moribund and died. It is likely that the larvae left the exotic host material because adequate gustatory stimulation was lacking.

The food preference tests showed that a high percentage of larvae that chose *Toona* as a host of *H. grandella* were attached to potted *Toona* and *Cedrela* plants (Table 4), indicate that all first instars hatched on *Toona* died within 24 hours after eclosion; larvae hatched on *Cedrela* trees could be reared through their complete cycle. It was interesting to note that 20 per cent of the eggs on both tree species failed to hatch. Accordingly, *Toona* did not adversely affect eclosion. In many cases, the recently hatched larvae, were likely killed by heavy gum exudation of the *Toona*. This, however, may not be the major cause of larval mortality in *Toona*, as several first instars were found dead with only their heads penetrating the plant's epidermis.

The patterns of larval mortality in the macerated mixture of *Toona* and *Swietenia* material is seen in Table 5. The mortality rate of introduced larvae increased markedly when the proportion of *Toona* increased from 75 to 100 per cent. For example, the response of *H. grandella* to the toxic effects of *Toona* can be noted on

TABLE 4. Activity of 1st. instar *H. grandella* larvae on potted plants of *Toona ciliata* var *australis* and *Cedrela odorata*. Larvae hatched from eggs attached to the seedlings.

| Tree No. | Species | No. eggs Hatched | Larval Activity | | | Remarks |
|---|---|---|---|---|---|---|
| | | | Boring | Dead | Active | |
| 1 | *Toona ciliata* v. *australis* | 2 | x | x | | Gum exudations; larvae died in 24 hrs. |
| 2 | *Toona ciliata* v. *australis* | 2 | x | x | | Gum exudations; larvae died in 24 hrs. |
| 3 | *Cedrela odorata* | 2 | x | | x | No gum produced. Larvae active. |
| 4 | *Toona ciliata* v. *australis* | 2 | x | x | | Gum exudations; larvae died in 24 hrs. |
| 5 | *Toona ciliata* v. *australis* | 2 | x | x | | No gum exudation; larvae died in 24 hrs. |
| 6 | *Cedrela odorata* | 0 | 0 | 0 | 0 | Both eggs did not hatch, attacked by fungi. |
| 7 | *Toona ciliata* v. *australis* | 1 | x | x | | 1 egg did not hatch, attacked by fungi. 1 larva died in 24 hrs. |
| 8 | *Toona ciliata* v. *australis* | 2 | x | x | | No gum exudation, 1 larva died while boring, other dead in 24 hrs. |
| 9 | *Cedrela odorata* | 2 | x | | x | No gum produced, larvae very active |
| 10 | *Toona ciliata* v. *australis* | 2 | x | x | | Gum exudations; larvae died in 24 hrs. |
| 11 | *Toona ciliata* v. *australis* | 1 | x | x | | 1 egg did not hatch, attacked by fungi. |
| 12 | *Cedrela odorata* | 2 | x | | x | No gum exudation, larvae very active. |
| 13 | *Cedrela odorata* | 2 | x | | x | No gum exudation, larvae very active. |
| 14 | *Toona ciliata* v. *australis* | 1 | x | x | | 1 egg did not hatch, attacked by fungi; no gum exudation. Larva died. |
| 15 | *Toona ciliata* v. *australis* | 1 | x | x | | 1 egg did not hatch, attacked by fungi; gum exudation. Larva died in 24 hrs. |

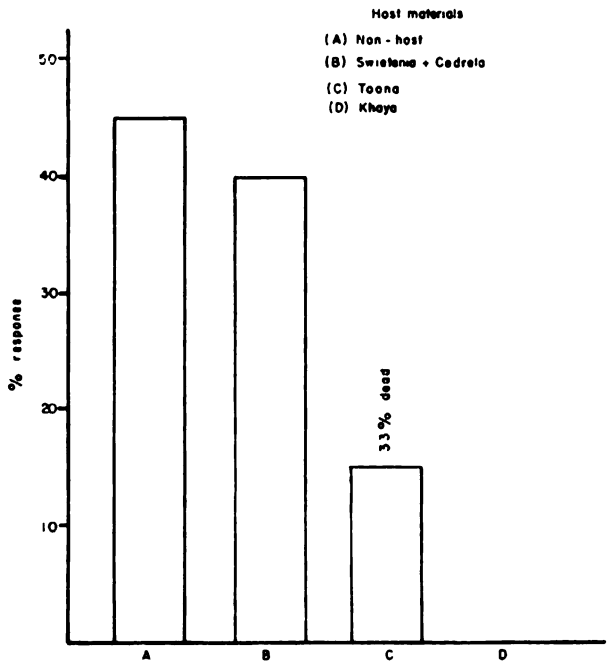



Fig. 6. Choices made by *H. grandella* larvae in olfactometric tests. Larvae were placed within a laboratorial olfactometer containing macerated host material of 4 meliaceous species and a non–host.

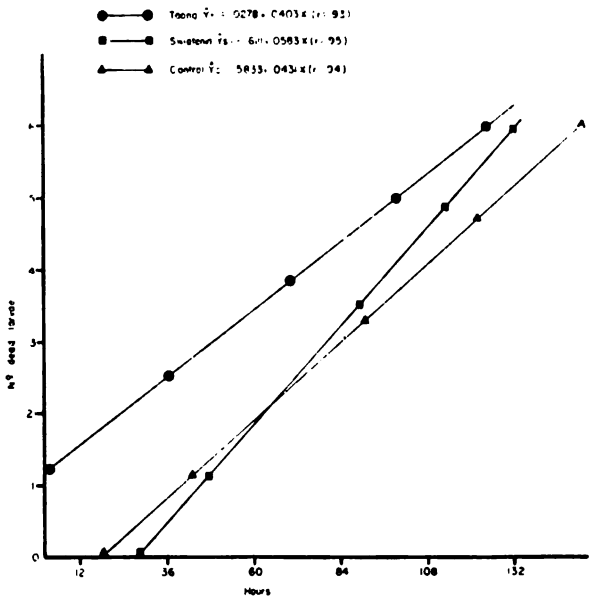



Fig. 7. Regression equations of the death rate of *H. grandella* larvae exposed to volatiles emanating from macerated *Toona* and *Swietenia* leaf material; larvae placed over water soaked cotton were used as a control.

the third day of the test when an increasing percentage of *Toona* in the diet resulted in increased mortality rates, ranging from 0 in the control to 80 per cent in the 100 per cent *Toona* medium.

In the final experiment there was evidence that toxic volatiles emanating from *Toona* were responsible for an accelerated mortality rate among the larvae (Fig. 7). The results of the regression analysis revealed a significant difference at the 95 per cent confidence level between *Toona* versus *Swietenia* and control. The difference between *Swietenia* and control was not significant.

From the foregoing results it may be concluded that *T. ciliata* var *australis* is toxic to *H. grandella* larvae. It is even possible that volatile, toxic compounds from *Toona* may deter *H. grandella* moths from selecting *Toona* for oviposition. These observations may offer prospects for the breeding of resistant *Cedrela* species.

TABLE 5. Mortality of *H. grandella* larvae placed in petri dishes containing macerated *Toona* and *Swietenia* plant material.

| Percentage of plant material | | Accumulated mortality (%) days of test | | | | | |
|---|---|---|---|---|---|---|---|
| *Toona* | *Swietenia* | 1 | 2 | 3 | 4 | 5 | 6 |
| 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 75 | 0 | 0 | 20 | 20 | 40 | 80 |
| 50 | 50 | 0 | 0 | 20 | 40 | 60 | 80 |
| 75 | 25 | 20 | 40 | 40 | 80 | 80 | 100 |
| 100 | 0 | 60 | 60 | 80 | 80 | 80 | 80 |

## Acknowledgement

The authors wish to thank Dr. Gilberto Páez, IICA, Turrialba, Costa Rica, for his assistance in the statistical evaluation of the experiments.

## Literature cited

1. DETHIER, V. G. Mode of action of sugar baited fly traps. Journal of Economic Entomology 48(3):235–239. 1955.

2. GRIJPMA, P. Immunity of *Toona ciliata* M. Roem. var *australis* (F.v.M.) D.D.C. and *Khaya ivoresnsis* A. Chev. to attacks of *Hypsipyla grandella* (Zeller) in Turrialba, Costa Rica. Turrialba 20(1):85–93. 1970.

3. _____ and GARA, R. I. Studies on the shootborer *Hypsipyla grandella* (Zeller). I. Host selection behavior. Turrialba 20(2):233–240. 1970.

4. _____ and RAMALHO. R. *Toona* spp. posibles alternativas para el problema del barrenador *Hypsipyla grandella* de las *Meliaceae* en América Latina. Turrialba 19(4):531–547. 1969.

5. KEMPTHORNE, O. An introduction to genetic statistics. New York, John Wiley, 1957. 545 p.

6. RAMIREZ SANCHEZ, J. Investigación preliminar sobre biología, ecología y control de *Hypsipyla grandella* Zeller. Boletín del Instituto Forestal Latinoamericano No. 16:54–77. 1960.

7. SHOOTBORERS of the *Meliaceae*. Unasyla 12(1):30–31. 1958.

8. THORSTEINSON, A. J. Host selection in phytophagus insects. Annual Review of Entomology 5:193–218. 1960.

# THE EVALUATION OF SOME SYSTEMIC INSECTICIDES FOR THE CONTROL OF LARVAE IN *CEDRELA ODORATA* L.*

*G. G. Allan, R. I. Gara and R. M. Wilkins*

Associate Professor of Fiber and Polymer Science,
College of Forest Resources, University of Washington, Seattle.

Associate Professor of Forest Entomology,
College of Forest Resources, University of Washington, Seattle.

Research Assistant, College of Forest Resources, University of Washington, Seattle

## COMPENDIO

Continuando la serie de estudios sobre *Hypsipyla grandella* (Zeller) (Cf. Turrialba 20(1):233–247. 1970), la principal plaga que obstaculiza el establecimiento de Meliáceas nativas en el Trópico americano, se hizo una investigación para evaluar un grupo de veintiocho insecticidas sistémicos en el control de larvas de *H. grandella* que atacaban plantas de *Cedrela odorata*. Los experimentos se llevaron a cabo en un invernadero del Instituto Interamericano de Ciencias Agrícolas, Turrialba, Costa Rica, en dos etapas sucesivas: en primer lugar se determinaron las curvas características de traslado de cada insecticida en la planta por medio de muestreo de hojas colectadas en diferentes tiempos después de la aplicación del insecticida al suelo de las plantas. Los niveles tóxicos relativos en las hojas de las plantas fueron determinados luego por medio de técnicas estandarizadas de bio–ensayo.

En la etapa siguiente, plantas de *C. odorata* tratadas con dosis iguales de estos sistémicos (75 mg) fueron expuestas al ataque de larvas (primer instar) de *H. grandella* en el momento en que la acumulación del tóxico estaba al máximo. La exposición de las plantas al ataque fue efectuada por medio de la fijación en las hojas de huevos de *H. grandella* que estaban por eclosionar. El efecto de los sistémicos fue evaluado 7 días después de la fijación de los huevos en las plantas, usando un "factor de daño" basado en la condición de la planta y la mortalidad de las larvas.

De los 28 sistémicos probados, cinco: carbofuran, methomyl, phosphamidon, monocrotophos e Isolan, dieron protección completa. Plantas tratadas con Monitor trichlorofon, dimethoate y dicrotophos fueron dañadas, pero ninguna larva sobrevivió después de 7 días. Los otros sistémicos no dieron resultados completamente satisfactorios.

Methomyl y carbofuran fueron los únicos sistémicos que 23 días después de ser aplicados, dieron todavía protección completa contra los ataques.

Los autores

## Introduction

The members of the family *Meliaceae*, especially those of the genera *Cedrela* and *Swietenia*, are fast growing trees yielding high quality, commercially valuable wood. The most important insect pests of the *Meliaceae* are the shootborers, *Hypsipyla* spp. (Lepidoptera: Phycitidae). In tropical America repeated attacks of the shootborer, *Hypsipyla grandella* (Zeller), cripple host trees and effectively prohibit establishment of successful plantations (2, 6, 10, 14). In the old–world tropics, *Meliaceae* are attacked by other *Hypsipyla* species (3, 8, 9).

Control of *H. grandella* by insecticides has been suggested (7), but chemical treatments are considered too costly. The high cost is due to the short life cycle and overlapping generations of the pest, which in turn, calls, for repeated applications of insecticides to reduce damage to economically tolerable levels. Because of the short residual life of the more effective insecticides, especially in tropical climates with high rainfall, and because of the relatively long period during which *Meliaceae* trees must be protected, many investigators feel that biological or silvicultural remedies are indicated (2, 8, 9).

* Received for publication June 12, 1970.

However, new methods for the sustained delivery of pesticides are being developed in which the pesticide is chemically or physically combined with a polymer (1). The polymer may be synthetic or natural (15) and the lignocellulosic solid wastes of the forest product industries are particularly suitable for the chemical release method. Over a prolonged period of time, the pesticide gradually escapes from its protective polymeric prison by, hydrolysis or diffusion to provide a continuous supply of the active material to the environment of the plant. The characteristics of *H. grandella* suggest that this sustained release pesticide concept may be a practical solution to the problem of establishing plantations of *Meliaceae* susceptible to attack by this insect.

To better understand the applicability of this concept, the biology of *H. grandella* is reviewed. Eggs of the shootborer generally are laid singly on the bark of their hosts, close to young leaves. After about 4 days (6) each larva emerges through a small hole in the corium and begins to bore into the leaf axis. The larva, making a short tunnel, becomes too large for the axis which it then leaves to bore into the main stem, generally just above a leaf axil. Entrance holes of the larvae are covered by mixtures of frass and silk which provide protection from pedators, rain and insecticide sprays. The remainder of the larval life (in all, six instars) is spent in boring the main stem or side branches. Larval activity not only severely defoliates the tree but also kills the leader. As a consequence of leader mortality, trees send out side branches and growth is reduced; frequently, trees die from repeated *H. grandella* attacks. All larvae do not survive to continue to damage the plant since many are entombed within the resin produced by the tree. This host defense is more effective in *Swietenia* than in *Cedrela* species (2). Furthermore, it has been observed (4) that approximately 20 per cent of the larvae are killed by a parasitic nematode, *Hexamermis* sp.

Meliaceae plants can be most effectively protected by use of systemic insecticides when the recently hatched larvae bore into the leaf rachis or stem of the plants. Presumably vascular tissue would always contain higher concentrations of the toxicant than pith; the tissue on which later and more resistant instars feed. Thus, if a toxic concentration of an insecticide can be maintained in the young plant for a prolonged period by a controlled release insecticide–polymer combination, then protection from attacks by the shootborer may be secured.

As a preliminary to developing such long–acting controls this paper describes the screening of systemic insecticides for an appraisal of their use in protecting *Meliaceae* from *H. grandella* attacks. The insecticides were evaluated by means of laboratory and greenhouse trials at the Inter–American Institute of Agricultural Sciences of Turrialba, Costa Rica. A report concerning field trials in Turrialba will be published later.

## Materials and methods

Two phases were used in the evaluation of the 28 technical grade, systemic insecticides listed in the appendix. The first phase was the determination of the relative rate of translocation and accumulation of each insecticide in *Cedrela odorata* L. (also known as *Cedrela mexicana*) (12). This "systemicity" was ascertained by first extracting leaf samples at different periods after application of the insecticide to the soil. The leaf extracts then were bioassayed to determine their relative insecticidal contents by standard methods using *Drosophila melanogaster* Meig (13). These measurements revealed the time when the insecticide concentration in the leaves would be at a maximum.

The second phase was the exposure of plants to attack by *H. grandella* at the period when insecticide concentrations within the plant tissues were at their maximum. In this way an estimate of the protection offered by each insecticide was obtained. Exposure of the plant to attack, either too early or too late in relation to the maximum protection offered by an insecticide, could give an underestimate of the potential effectiveness of a particular toxicant.

### Preparation of the leaf extracts of *C. odorata*

Individual potted trees of *C. odorata* (8 months old and about 0.5 m in height) were treated with an insecticide (75 mg active ingredient); the material was applied in a small volume of acetone to the soil surface. The acetone was allowed to evaporate and the insecticide then was watered into the soil. This treatment was applied to 3 trees for each of the 28 insecticides studied. The use of acetone alone had no apparent adverse effect upon the plants as evidenced by appropriate controls.

The trees were watered daily and grown in a green–house at temperatures between 18° – 30°C (Fig. 1). The potting soil had a high organic content and a pH of 6.35. At six intervals, regularly spread over a period of 21 days, a 9 g leaf sample was taken from one of the three plants treated with each insecticide in order to obtain (for each insecticide) six samples. Due to the small size of the plants, the order of taking samples was timed so as to minimize changes in the translocation

Fig. 1. General aspect of young *C. odorata* trees in greenhouse. In the foreground are trees attacked by *H. grandella* larvae (note considerable loss of leaves). Behind are trees not exposed to attack.

rates of the trees. The first two samples were taken successively from one plant, the third and fourth from the second plant and the fifth and sixth from the third plant.

Leaf samples were blended immediately at high speed for one minute with dried commercial acetone (70 ml) and anhydrous sodium sulfate (20 g). The mixture was left in the blending jar for 2o hr. The slurry then was filtered through a pad of diatomaceous earth and washed with more acetone (100 ml). The dark green filtrate was concentrated under reduced pressure at 60°C. The concentrate (15 ml) was transferred to a volumetric flask and made up to 25 ml with dry acetone.

The efficiency of the extraction method was close to 100 per cent, as determined by comparing bioassays of samples to which known amounts of insecticides were added before and after extraction.

### Biossay of insecticidal activity of leaf extracts

The bioassay technique used was based on standard methods (5, 11, 13). An aliquot (2.0 ml) of the leaf–extract solution was pipetted into a test–tube (15 x 2 cm). Subsequently, the tube was mounted at right angles to a disk which was rotated slowly in an almost vertical position. The evaporation rate of the solvent was increased by a fan blowing air over the mouth of the test–tube. This procedure left an 8 cm long film of leaf–extract on the inside of the tube. The film was allowed to stand overnight to reduce its tackiness. About 25 unsexed, non–anaesthetized, 1–day old fruit flies (*D. melanogaster*, wild strain, Turrialba), were added and the free volume of the tube was reduced to 25 ml with a cotton plug. Food and moisture for the flies was provided by dropping into the bottom of the tube a small ball (0.5 ml) of cotton, soaked with a solution (0.5 ml) of sugar (5 per cent) and sodium benzoate (1 per cent) solution. The mortality of the flies was observed periodically for 3 days. Each sample had 2–3 replicates. A dosage–mortality curve was plotted for each tube with hours instead of dose as the logarithmic unit. Control films were prepared by extracting samples from untreated plants by the same procedure as before.

### Bioassay of insecticidal activity in *C. odorata*

Young potted *C. odorata* trees were treated with the systemic insecticides (75 mg active ingredient) in a manner similar to the translocation studies. Eggs of *H. grandella* were collected from *Swietenia* or *Cedrela* trees in IICA plantations around Turrialba. The eggs were contained on small bark slivers removed from the host trees. About 12 eggs then were taped to each of the test plants, generally to leaflets midway on each leaf. This attempt to create an even distribution of emergent larvae was transient, due to the high mobility of the first instars. The attachment of the eggs to the plant was so timed, that when larvae emerged, the insecticide concentration in the foliage was at a maximum. This time of maximum insecticide concentration was determined by the bioassay with *Drosophila* for each insecticide under similar conditions, as described above. The time when the eggs were attached to the plant is indicated for some of the insecticides by the position of the arrows in Figs. 2–4; the average time for eclosion was two days after egg placement. Each tree was isolated by its placement and by a ring of a sticky substance spread around the base of the main stem.

In some cases it was necessary to postpone the time of eclosion of collected eggs by placing them in a 8–9°C cold box. This treatment, used for a maximum of six days, reduced the average rate of hatching from 80 per cent for fresh eggs, to 70 per cent, when desiccation was prevented.

Two days after attachment, the eggs were removed from the plants. The originally bright–red eggs changed to a white, transparent shell with a small hole if the larva emerged; otherwise the egg was black, brown, or simply collapsed. In this way the number of larvae on the plant was determined. This deductive approach was necessary as the minute size of these first instars precluded extensive handling. A week after egg attachment, the plants were assessed for larval damage and compared with infested control plants containing no insecticides.

Damage was estimated for each tree by counting the number of dead leaves, the number of boreholes in the main stem, and by observing whether the leading shoot had been killed. The number of larvae still living was determined by brushing away all frass of the previous day and later watching for production of fresh frass; the accuracy of this assessment was confirmed by dissecting some of the infested stems.

### Persistence of insecticidal activity

The persistence of those insecticides which gave good protection was studied at 18 to 23 days after application of the insecticide to the soil. Those plants, which were previously undamaged, were reinfested with fresh eggs in the same way as before. Similarly, damage and larval survival were determined seven days after attachment of the eggs on the reinfested plants.

## Results and discussion

### Bioassay of insecticidal activity of leaf extracts

Translocation and accumulation of 28 systemic insecticides was followed by extraction and bioassay of leaf samples; examples of the characteristic curves for a number of insecticides are given in Figs. 2, 3 and 4. Points in the curves were obtained by first plotting a log dose probit mortality curve for each tube assayed. This information then gave the number of hours to obtain 50 per cent mortality, which was averaged over the several tubes used for each leaf sample, to give the points in Figs. 2, 3 and 4. Thus, the vertical axis represents $LD_{50}$ values, in terms of hours of the standard treatment. The $LD_{50}$ of 100 hr is represented as zero toxicity even though the controls (similar plant extracts containing no toxicants) had $LD_{50}$ values of 60 to 100 hr. Dead *D. melanogaster* were not counted for more than 70 hr.

In most cases, the insecticides reached their maximum concentration in leaves by about the third day after application to the soil. Thereafter, the concentration in both the leaves and the soil was reduced by degradation. This method of bioassay is not specific for any insecticide but measures total toxicity in terms of mortality, which includes toxic metabolites, if

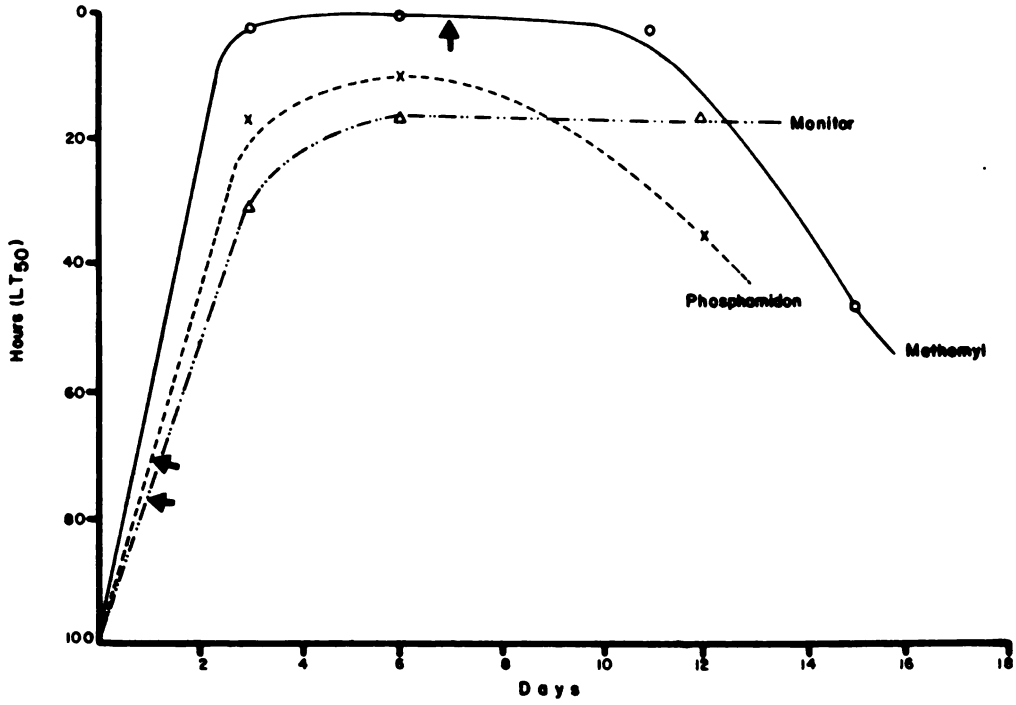



Fig. 2. Characteristic curves of "systemicity" for phosphamidon, Monitor and methomyl in *C. odorata*. The arrows indicate the time of attachment of *H. grandella* eggs to the young trees. Attack occurred generally about 2 days later.

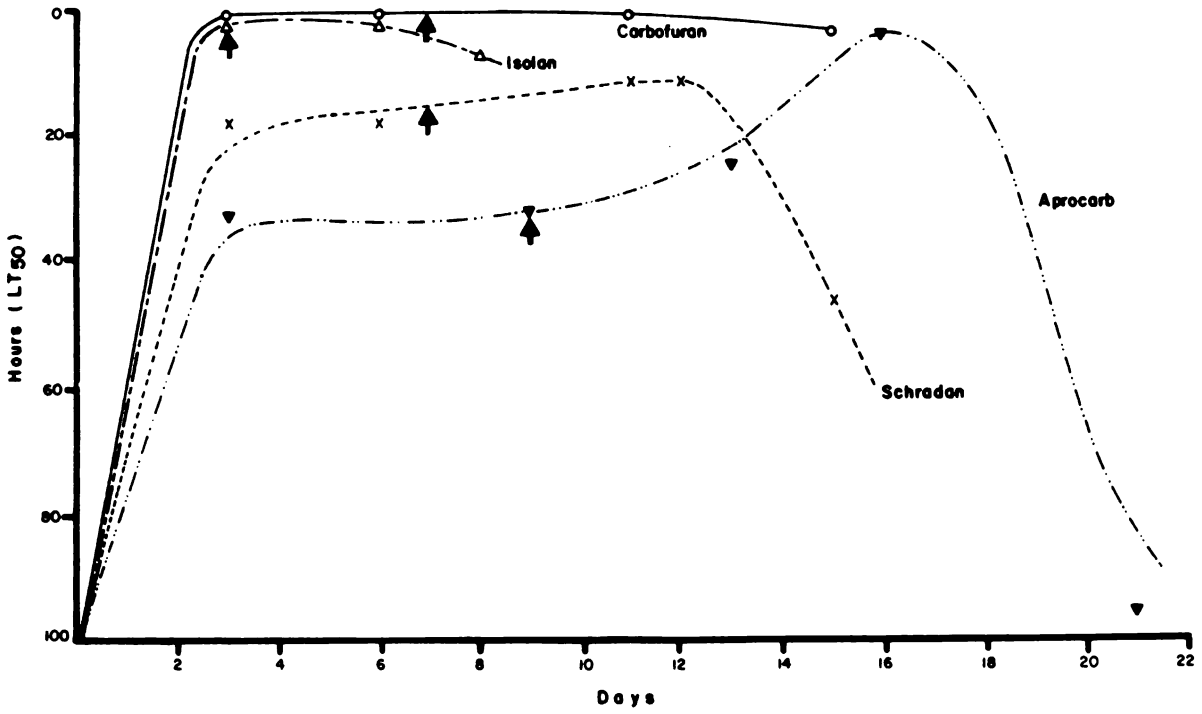



Fig. 3. Characteristics curves of "systemicity" for carbofuran, arprocarb, Isolan and schradan in *C. odorata*.

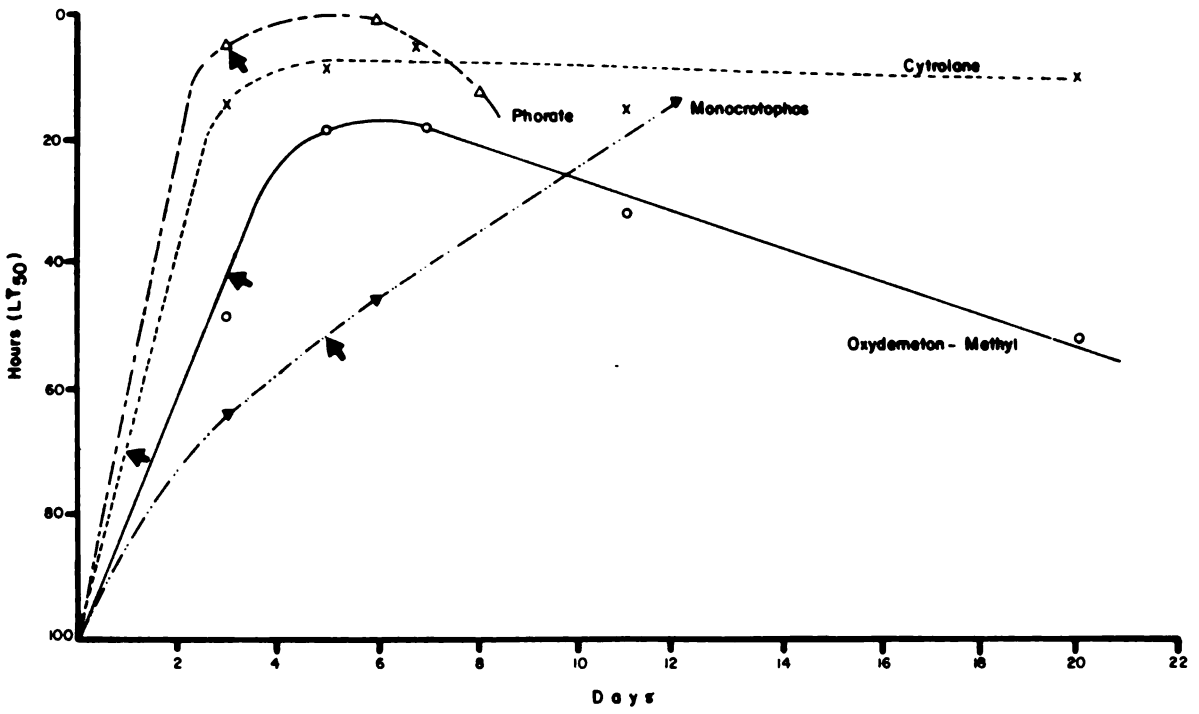



Fig. 4. Characteristic curves of "systemicity" of phorate, cytrolane, monocrotophos and oxydemeton–methyl in *C. odorata*.

any were present. However, a specific method of analysis may miss active metabolites which could be important in controlling *H. grandella*. In view of this, no attempt was made to establish insecticide concentration in the leaf samples by calibrating each of the bioassays against films containing known amounts of a toxicant. However the sensitivity of the method was estimated for a few of the insecticides and was of the order of 1 p.p.m.

## Bioassay of insecticidal activity in *C. odorata*

Toxicity trials with the insecticides (75 mg per pot) using *H. grandella* larvae were conducted, and the time of attachment of the eggs was scheduled so that upon eclosion the larvae would attempt to bore into the plant when each insecticide was present at its respective maximum concentration. This time of egg placement is represented in Figs. 2, 3 and 4 by the position of the arrows for a number of the insecticides. The conditions achieved in the infestation resembled the situation that occurs in natural attack.

The value of *Cedrela* is found in the straight, fast–growing trunk, thus any damage to the young tree, and particularly to the leading shoot, is more serious than attacks on the branches. In evaluating each insecticide the shoot borer damage was categorized and combined into a number by means of a formula. The formula expressed the effectiveness of the insecticide in relation to the damage; the damage factor for each tree was defined as:

$$D_f = \frac{(H + F)\,N + 1}{E} \cdot L$$

where H is the number of boreholes in the mainstem, F the number of leaves severed by the action of *H. grandella*, N the number of larvae still alive, E the original number of larvae and L had the value of 10 if the leader was killed or the value of 1 if the leader was unattacked. This information was determined for each tree, 7 days after eggs had been attached to the leaves. The damage factor formula was useful in expressing the quality of protection each insecticide gave to the tree, especially as related to the desirability of maintaining a straight trunk. Details of the types of damage, the number of larvae surviving and the resultant damage factors ($D_f$) for all pesticides tested are listed in Table 1.

All insecticides were assessed on the basis of their damage factors; the lower the $D_f$ value the greater the protection provided. Untreated plants had $D_f$ values ranging from 18 to 101, with an average of 59. Complete protection gave values around 0.1, or the reciprocal of the number of larvae originally placed on the plant. The damage factors also include larval survival which was generally less than 100 per cent even for the controls.

The insecticidal protection was classified as excellent ($D_f < 1.0$), good ($D_f > 1$ but $< 10$) or poor ($D_f) > 10.0$). Trees treated with fenchlorphos, Pirimor, arprocarb, schradan, disulfoton and phorate fared no better than untreated plants, even though the insecticides were detectable by the bioassay. In these cases, translocation apparently did not occur at a sufficiently high rate to reach a level toxic to *H. grandella* larvae. Degradation of these insecticides in the soil also may have reduced their availability to the plant.

Eight insecticides gave a damage factor of less than 1.0 which was considered excellent. These were;

TABLE 1. Damage and damage factors for young *C. odorata* trees treated with insecticides and untreated.

| Insecticides | Interval between applic. & attack (days) | Original No. of larvae (E) | No. of larvae 5 days after attack (N) | No. of holes in main stem (H) | No. of leaves severed (F) | Condition of leader* (L) | $D_f$, |
|---|---|---|---|---|---|---|---|
| methomyl | 9 | 11 | 0 | 0 | 0 | A | 0.09 |
| carbofuran | 9 | 10 | 0 | 0 | 0 | A | 0.10 |
| monocrotophos | 7 | 10 | 0 | 0 | 0 | A | 0.10 |
| Isolan | 4 | 9 | 0 | 0 | 0 | A | 0.11 |
| dimethoate | 4 | 9 | 0 | 0 | 0 | A | 0.11 |
| phosphamidon | 3 | 8 | 0 | 0 | 0 | A | 0.13 |
| Cytrolane | 3 | 7 | 1 | 0 | 0 | A | 0.14 |
| trichlorfon | 5 | 11 | 0 | 1 | 3 | D | 0.91 |
| C-2307 | 8 | 6 | 1 | 2 | 3 | A | 1.0 |
| Cyolane | 5 | 3 | 2 | 1 | 0 | A | 1.0 |
| Monitor | 2 | 9 | 0 | 0 | 1 | D | 1.1 |
| Bay 68138 | 8 | 4 | 2 | 1 | 1 | A | 1.3 |
| demeton | 3 | 9 | 3 | 4 | 0 | A | 1.4 |
| dicrotophos | 7 | 6 | 0 | 1 | 2 | D | 1.7 |
| menazon | 5 | 8 | 3 | 3 | 2 | A | 2.0 |
| dimetilan | 3 | 8 | 3 | 2 | 4 | A | 2,4 |
| I-19 | 9 | 7 | 2 | 0 | 1 | D | 4,3 |
| aminocarb | 11 | 6 | 1 | 3 | 0 | D | 6.7 |
| I-12 | 2 | 9 | 2 | 1 | 2 | D | 7.8 |
| fensulfothion | 11 | 5 | 1 | 2 | 1 | D | 8.0 |
| oxydemeton-methyl | 5 | 7 | 2 | 2 | 1 | D | 10.0 |
| fenthion | 5 | 8 | 2 | 3 | 1 | D | 10.1 |
| fenchlorphos | 4 | 10 | 5 | 1 | 3 | D | 21.0 |
| arprocarb | 11 | 6 | 3 | 3 | 1 | D | 21.7 |
| disulfoton | 3 | 6 | 4 | 1 | 2 | D | 21.7 |
| schradan | 9 | 6 | 6 | 1 | 1 | D | 21.7 |
| phorate | 4 | 9 | 5 | 3 | 1 | D | 23.3 |
| Pirimor | 5 | 9 | 4 | 5 | 4 | D | 41.1 |
| Control 1 | – | 6 | 2 | 3 | 2 | D | 18.4** |
| Control 2 | – | 10 | 4 | 5 | 3 | D | 33.0 |
| Control 3 | – | 8 | 5 | 3 | 3 | D | 38.8 |
| Control 4 | – | 11 | 6 | 4 | 3 | D | 39.1 |
| Control 5 | – | 9 | 5 | 6 | 4 | D | 56.7 |
| Control 6 | – | 6 | 4 | 6 | 3 | D | 61.7 |
| Control 7 | – | 9 | 9 | 3 | 3 | D | 101.1 |

* A: alive
D: dead

** Average value for all control plants: 49.8

methomyl, carbofuran, monocrotophos, Isolan, dimethoate, phosphamidon, Cytrolane and trichlorfon. Trees treated with dimethoate, Cytrolane and trichlorfon were damaged by the larvae but the others showed no signs of injury. The only traces of attack were small bite–marks on the underside of leaf–stems from which exuded a drop of liquid.

In another test the high toxicity of these insecticides was demonstrated when a leaflet taken from a plant treated with methomyl was enclosed in a small container with seven, 1–day old *Hypsipyla* larvae. Within 4 hr all the larvae were dead with only one bite–mark on the leaflet. A similar leaflet from an untreated tree gave zero mortality in a comparable experiment.

The effectiveness of the insecticide treatment is exemplified in Figs. 5, 6 and 7 which show the results of attacks on treated trees compared to unprotected trees. These photographs were taken 21 days after each tree was initially attacked.

Fourteen insecticides gave limited protection at the rate of 75 mg per plant. In order of increasing activity these are as follows: fenthion, oxydemeton–methyl, fensulfothion, I–12, aminocarb, I–19, dimetilan, menazon, dicrotophos, demeton, Bay 68138, Monitor, Cyolane and C–2307.

In these treatments some larvae were still alive by the seventh day with the exception of dicrotophos where the larvae died earlier. Thereafter, as the insecticides degrade to inactive products, the surviving larvae bore in the stem uncontrolled; only one larva is needed to badly damage a young *Cedrela* tree.

## Persistence of insecticidal protection

The period of protection given to the trees by the seven most promising pesticides was studied. About 20 days after applications of the toxicants the pest plants were reinfested and the attack measured as described above: resultant damage factors and larval survivals are given in Table 2. The protection given by methomyl and carbofuran was sustained and complete, except for slight damage to one leaf of the tree treated with methomyl. The plants treated with dimethoate and Isolan suffered a little damage but those treated with phosphamidon, monocrotophos and Cytrolane were attacked severely.

Fig. 5. Effect of the attack of *H. grandella* larvae, under standardized conditions, on a tree not treated with insecticide at 21 days after the attack occurred. (The $D_f$ at 5 days was 33.0).

Fig. 6. Example of the effect of attack, at 21 days after attack, on a tree treated with an insecticide (1–12) which gave only partial protection. The larvae which survived the insecticide treatment have attacked the plant severely. (The $D_f$ at 5 days was 7.8).

TABLE 2. Persistence of some insecticides determined from damage to young *C. odorata* trees.

| Insecticides | Days after applic. to soil | Orig. No. of larvae (E) | No. of larvae 5 days after attack (N) | No. of holes in main stem (H) | No. of leaves severed (F) | Condition of leader (L)* | $D_f$ | $D_f$ at max. insecticide conc.** |
|---|---|---|---|---|---|---|---|---|
| Phosphomidon | 20 | 13 | 6 | 5 | 2 | D | 33 | 0.13 |
| monocrotophos | 20 | 9 | 1 | 2 | 2 | D | 5.5 | 0.10 |
| Cytrolane | 20 | 9 | 0 | 1 | 3 | D | 1.1 | 0.14 |
| Carbofuran | 23 | 12 | 0 | 0 | 0 | A | 0.08 | 0.10 |
| methomyl | 23 | 12 | 0 | 0 | 1 | A | 0.08 | 0.09 |
| Isolan | 18 | 7 | 1 | 0 | 1 | A | 0.29 | 0.11 |
| dimethoate | 18 | 15 | 0 | 0 | 1 | D | 0.67 | 0.11 |

* A: alive
D: dead

** Values of D as given in Table 1.

## Summary

The evaluation of twenty –eight systemic insecticides for the control of *H. grandella* larvae in young *C. odorata* trees by soil application is described.

Testing was carried out in greenhouse experiments, but under conditions that simulated natural attack.

The eight most promising insecticides were carbofuran, methomyl, monocrotophos, phosphamidon, Isolan, dimethoate, Cytrolane and trichlorfon. With the exception of the last three, these insecticides gave complete immunity at the level of 75 mg active ingredient per 1 gallon pot. Most of the other insecticides tested gave partial protection but permitted some larval survival at seven days after their application to the soil at the same level.

Fig. 7. Example of the protection at 21 days after attack, given to a tree treated with one of the insecticides (methomyl) whose damage factor ($D_f$) was less than 1.0. There is no sign of damage on this plant.

## Acknowledgements

We wish to thank P. Grijpma for his help and interest in this study. Appreciation also is due to the Departments of Forestry and Plants Sciences of IICA for the use of laboratory and greenhouse facilities. In particular, we thank T. McKenzie and H. Barres of the Department of Forestry and H. Fassbender, and G. Paez of the Department of Plant Sciences. The support for this study was administered by the Institute of Forest Products, College of Forest Resources, University of Washington, Seattle, and provided by Ford Foundation Grant 57–74, O.T.S. Project 68–38 and by U.S. Public Health Research Grant 1 RO 1 EC 00319–01 of the Environmental Control Administration, Consumer Protection and Environmental Health Service of the Department of Health, Education and Welfare.

## Literature cited

1. ALLAN, G. G. Pesticide à liberation retardée, son procédé d'obtention. Belgian Patent 706,909. Nov. 14, 1966.

2. DOUROJEANNI, R. M. El barreno de los brotes *(Hypsipyla grandella)* en Cedro y Caoba. Agronomía (Perú) 30(1):35–43. 1963.

3. ENTWISTLE, P. F. The current situation on shoot, fruit and collar borers of the *Meliaceae*. Oxford, Commonwealth Forestry Institute 1968, 15 p. Paper of the 9th Commonwealth Forestry Conference, New Delhi, 1968.

4. GRIJPMA, P. and GARA, R. I. Studies on the shootborer *Hypsipyla grandella* (Zeller). II. Host preference of the larva. Turrialba 20(2):242–247. 1970.

5. PHILLIPS, W. F. *In* Zweig G., Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives, Vol. 1. 1964. pp. 471–490.

6. RAMIREZ, S. J. Apuntes sobre control de *Hypsipyla grandella* Zeller con insecticidas. Boletín del Instituto Forestal Latinoamericano de Investigación y Capacitación No. 22:33–37. 1966.

7. _____ . Investigación preliminar sobre biología, ecología y control de *Hypsipyla grandella* Zeller con insecticidas. Boletín del Instituto Forestal Latinoamericano de Investigación y Capacitación No. 16:54–77. 1964.

8. RAO, V. P. and BENNET, F. D. Possibilities of biological control of the Meliaceous shootborer *Hypsipyla* spp. Lepidoptera: (Phycitidae). Trinidad, Commonwealth Institute of Biological Control, 1968. 14 p. Paper of the 9th Commonwealth Forestry Conference, New Delhi. 1968.

9. ROBERTS, H. An outline of the biology of *Hypsipyla robusta* Moore, the shootborer of the *Meliaceae* (Mahoganies) of Nigeria, with brief comments on two stem borers and one other lepidopteran fruit borer also found in Nigerian *Meliaceae*. Commonwealth Forestry Review 47(3):225–232. 1968.

10. SHOOTBORERS OF THE *MELIACEAE*. Unasylva 12(1):30–31. 1958.

11. SINGH, P., TAYLOR, B. and RODRIGUEZ, J. G. Bioassay of insecticides for control of *Drosophila melanogaster*. Journal of Economic Entomology 61:336–337. 1968.

12. SMITH, C. E. Jr. A revision of *Cedrela (Meliaceae)*. Fieldiana Botany 29(5):314. 1960.

13. SUN, Y. and PANKASKIE, J. E. *Drosophila*, a sensitive insect, for the microbioassay of insecticide residues. Journal of Economic Entomology. 47:180–181. 1954.

14. TILLMANS, H. J. Apuntes bibliográficos sobre *Hypsipyla grandella* Zeller. Boletín del Instituto Forestal Latinoamericano de Investigación y Capacitación No. 16:82–92. 1964.

15. WILKINS, R. M. The design of polymers for the sustained release of selected herbicides. M.S. Thesis in Forestry, Univ. of Washington, 1969.

## APPENDIX – Insecticides Evaluated

| Common Names | Chemical Names |
|---|---|
| aminocarb | 3-methyl-4-dimethylaminophenyl N-methylcarbamate |
| arprocarb | 2-*iso*propoxyphenyl N-methylcarbamate |
| Bay 68138 | ethyl 3-methyl-4-methylthiophenyl *iso*propyl phosphoramidate |
| carbofuran | 2,3-dihydro-2,2-dimethylbenzofuran-7-yl N-methylcarbamate |
| Cyolane | 2-(diethoxyphosphinylimino)-1,3-dithiolane |
| Cytrolane | 2-(diethoxyphosphinylimino)-4-methyl-1,3-dithiolane |
| C-2307 | 3-(dimethylphosphinyloxy)-B-methoxy-N-methyl*cis*crotonamide |
| demeton | 2:1 mixture of 0,0-diethyl 0- and S-2-(ethylthio)ethyl phosphorodithioate |
| dicrotophos | 3-(dimethylphosphinyloxy)-N,N-dimethyl*cis*crotonamide |
| dimethoate | 0,0-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate |
| dimetilan | 2-dimethylcarbamyl-3-methyl-5-pyrazolyl dimethylcarbamate |
| disulfoton | 0,0-diethyl S-2-(ethylthio)ethyl phosphorodithioate |
| fenchlorphos | 0,0-dimethyl 0-(2,4,5-trichlorophenyl) phosphorothioate |
| fensulfothion | 0,0-diethyl 0-4-methylsulfinylphenyl phosphorothioate |
| fenthion | 0,0-dimethyl 0-3-methyl-4-methylthiophenyl phosphorothioate |
| I-19 | hexamethylditin |
| Isolan | l-*iso*propyl-3-methyl-5-pyrazolyl dimethylcarbamate |
| menazon | 0,0-dimethyl S-(4,6-diamino-1,3,5-triazin-2-ylmethyl) phosphorodithioate |
| methomyl | S-methyl N-[(methylca-bamoyl)oxy] thioacetimidate |
| I-12 | S-(N-methoxycarbonyl-N-methylcarbamoylmethyl)dimethyl phosphorothiolthionate |
| Monitor | 0,S-dimethyl phosphoramidothioate |
| monocrotophos | 3-(dimethoxyphosphinyloxy)-N-methyl*cis*crotonamide |
| oxydemeton-methyl | 0,0-dimethyl S-2-(ethylsulfinyl)ethyl phosphorothioate |
| phorate | 0,0-diethyl S-ethylthiomethyl phosphorodithioate |
| phosphamidon | 2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate |
| Pirimor | 5,6-dimethyl-2-dimethylamino-4-pyrimidinyl dimethylcarbamate |
| schradan | octamethylpyrophosphoramide |
| trichlorofon | dimethyl 2,2,2-trichloro-l-hydroxyethyl phosphonate |

# *TRICHOGRAMMA* SP., AN EGG PARASITE*

*Oscar Hidalgo–Salvatierra and Luis R. Madrigal Sánchez*

Instituto Interamericano de Ciencias Agrícolas de la OEA, Turrialba, Costa Rica

## SUMARIO

En investigaciones ejecutadas recientemente en Turrialba, Costa Rica, se descubrió que *Trichogramma* sp. parasita los huevos de *Hypsipyla grandella*. Aunque *Trichogramma* spp. han sido descritos como parásitos de los huevos de *H. robusta* en Asia y Australia, se desconocía la existencia de *Trichogramma* como parásito de huevos de *H. grandella* en América Latina. Observaciones tomadas durante la época lluviosa en Turrialba, indicaron que 10–40 por ciento de los huevos de *H. grandella* en una plantación de *Cedrela odorata* estaban parasitados por esta especie.

*Hypsipyla grandella* is considered the greatest detriment to the establishment of plantations of valuable meliaceous tree species. So far, no chemical or cultural treatments proved to be practical to keep the damage caused by this insect, below the threshold of economic importance.

The possibility of biological control has been suggested by Rao and Bennett (1).

Although *Trichogramma* has been reported in their list as an egg parasite of *Hypsipyla robusta* Moore, no publication has yet indicated, to the best of our knowledge, the existence of this genus as an egg parasite of *H. grandella* in Latin America.

This *Trichogramma* sp. was found recently in investigations carried out at the Research and Training Center of the Institute of Agricultural Sciences, in Turrialba, Costa Rica. It apparently has a preference for freshly laid eggs. After parasitation, the *H. grandella* eggs change color from red to black in two to four days. From the time of complete color change to emergence of the parasites, a lapse of five to six days was observed, indicating that the egg–larval cycle would be approximately between seven to ten days. The parasite adults live from two to four days, in the absence of presence of *H. grandella* eggs. Observations made during the rainy season on the natural parasitation in the Turrialba area, indicate that 10–40 per cent of the *H. grandella* eggs in a *Cedrela* plantation were parasitized in this period.

The parasitized *Hypsipyla* eggs show compartmentalization; little bumps on the egg wall indicate the presence of several parasites. Two to four minute wasps emerge generally from two emerging holes in the *H. grandella* egg. Some of the adult characteristics are: three tarsal segments, elbowed antennae with hairs, some pubescence on the wings, red eyes and three oceli. We have been successful in rearing this *Trichogramma* spp. on eggs of the Mediterranean flour moth *Anagasta kuehniella*. An indication of host specificity was shown by the fact that single freshly laid eggs of *Spodoptera* sp. were not parasitized by this *Trichogramma* sp.

* This work was supported by the U. S. Atomic Energy Commission under Contract AT(30–1):2043. Publication No. NYO–2043–227. Received for publication, Nov. 5, 1970

Received for publication November 5, 1970

## References

1. RAO, V. P. and BENNETT, F. D. Possibilities of biological control of the Meliaceous shoot borers *Hypsipyla* spp. (Lepidoptera: Phycitidae). Commonwealth Institute of Biological Control. Technical Bulletin 12:61–81, September, 1969.

# OBSERVATIONS ON A REARING TECHNIQUE AND ON HOST SELECTION BEHAVIOR OF ADULTS IN CAPTIVITY*

*P. Grijpma***

Silviculturist, Forestry Program
Inter–American Institute of Agricultural Sciences, Turrialba, Costa Rica
Attached to IICA through the Dutch Technical Assistance Program

## COMPENDIO

Se describe una técnica para la crianza en laboratorio de *Hypsipyla grandella* (Zeller) y un método para la producción masiva de huevos del insecto. Con base en los datos observados en dos tipos de dietas, una sintética y otra natural, se estimó una ecuación que relaciona el estado pupal y la emergencia en función del tiempo. De tres distribuciones probadas (Poisson, normal y binomial) la binomial se ajustó mejor a los datos observados.

Las generaciones de adultos de *H. grandella* criadas con dieta sintética y bajo las condiciones del experimento, continuaron ovipositando en plantas de *Cedrela odorata*. Se comprobó que los adultos criados con dieta sintética son de mayor tamaño que los adultos criados con dieta natural de hojas y brotes tiernos de *C. odorata*. El tamaño de la hembra de *H. grandella* criada con los dos tipos de dietas es significantemente mayor que el del macho. Hembras criadas con ambas dietas vivieron más tiempo que los machos.

Se encontró que adultos de *H. grandella* criados con dieta natural son atraídos durante la noche por la luz de un bombillo y por pupas u hojas deseçadas de *C. odorata*. Se sugiere un modelo de respuesta para una serie de estímulos que podrían causar la selectividad de *H. grandella* para las Meliáceas.

El autor

## Introduction

Availability of large numbers of adults and other life stages of *Hypsipyla grandella* (Zeller), is of importance for research on the lifecycle, host selection behavior, biological control and other related studies. Although an artificial diet for *Hypsipyla robusta* Moore, has been described by Achan (1), a complete rearing technique, which should include mass egg production, has not yet been reported.

In previous studies, executed at the Inter–American Institute of Agricultural Sciences (IICA), Turrialba, Costa Rica, *H. grandella* eggs were collected from field plots (2, 7). This was cumbersome and inefficient as eggs were not always available. Recent investigations have led to a rearing technique through which all life stages are more easily obtained throughout the year. This paper describes the research related to this method and compare the life cycle of *H. grandella* raised on an artificial diet developed by Hidalgo–Salvatierra (3) with those reared on a natural diet of *Cedrela* leaves and tender shoots.

* Received for publication May 31st, 1971.

** Study to be used by P. Grijpma for Ph.D. dissertation.

## Material and methods

The research was divided into two stages. In the first stage, oviposition of *H. grandella* in captivity was studied. Two wire screen cages (90 x 90 x 90 cm on 0.50 m long legs) were placed in the opne field at approximately 4 m distance from a building. Location of the cages in the open field was chosen because preliminary experiments, utilizing the same cages in the laboratory had never resulted in oviposition of fertile eggs. Inside the cages, six wooden frames (38 x 85 cm, two per wall) covered with ordinary corrugated kitchen paper towels were suspended on nails in such manner that three of the four vertical walls were covered (Fig. 1 and 2). The fourth wall had a door, wide enough to retrieve the frames. In each of the cages a potted plant of a host tree (*Cedrela odorata* L.) and a non–host (*Cananga odorata* (Lam,) Hook F. & Thoms.) were placed to determine if adults raised on artificial diet would still be host specific (Fig. 2). Periodically, these potted plants were removed for several days to establish if the presence of the plants influenced oviposition.

Unsexed *H. grandella* adults reared on natural and synthetic diets were released nightly at 20:00 hours after emergence in the laboratory in their respective cages. No

Fig. 1. Oviposition cage and frames covered with towel paper. The majority of eggs (circled) on the left frame, is deposited on the upper half the towel paper.

food was provided for the adults in the cages; during dry spells, however, the paper frames were wetted daily with approximately 30 cc of water to provide adults with moisture.

During a heavy rainy period (from April 4–13, 1971) the oviposition cages were relocated and placed under the leaves of the building in order to protect the towel paper from tearing and breaking at the perforations. The building had a night light (Osram, 50 W, 115–125 V, Exc.) located at 3 m distance from, and about 1 m above, the cage containing adults raised on the natural diet. The influence of this light on oviposition was determined during this period by comparing the numbers of eggs oviposited on the one lighted side with the others.

During the period of the rearing experiment (from December 10, 1970 – May 27, 1971), the frames in the cages were replaced every two days, and eggs oviposited on the towel paper frames, screen walls and the potted plants were counted. Sterile eggs were counted every two days. Counting of *H. grandella* eggs on towel paper was facilitated by using a light box containing two fluorescent tubes (Fig. 3). A Munsell Soil Color Chart (9) was used to define the color, under laboratory conditions, of recently deposited eggs and eggs 24 hours old. In the air conditioned laboratory temperature and humidity were recorded continuously.

Mating and oviposition behavior of adults was followed in the cages.

In the second stage of the experiment, a comparison was made between the life cycle of *H. grandella* raised in the laboratory on a natural diet (leaves and tender shoots of *C. odorata)* and on a syntetic diet developed by Hidalgo–Salvatierra (3). Fertile eggs, oviposited on the paper of the frames in the cages, were clipped out and put on small sheets of aluminum foil. These sheets were then put in hard, uncompartmented, polystyrene containers (size 28 x 13 x 4 cm) on top of the natural and artificial diets.

Previous trials had shown that if fresh eggs were placed directly on the synthetic diet (Ph: 5.7) hatching would not, or only rarely, occur. If soft plastic containers were used larvae bored holes into the lids and escaped.

The number of eggs placed inside each container varied with their availability. In general, between 20 to 60 eggs were used. Forty grams of fresh leaves and tender shoots of *C. odorata* were used each time the natural food was changed. The weight of the synthetic diet was 200 gr, which covered the lower fifth of each container. Synthetic diet often became contaminated with fungi and bacteria. When symptoms of contamination were observed, the larvae were removed and placed in clean containers with fresh diet. Data referring to

Fig. 2. Frames and potted host (*Cedrela odorata,* right) and non host (*Cananga odorata,* left) in oviposition cage.

Fig. 3. Counting of *H. grandella* eggs, facilitated by light box.

contaminated containers were exluded from the rearing results. However, the adults obtained from these transferred larvae were used in the oviposition cages in order to maintain the population.

The following data were recorded with respect to rearing: number of days after oviposition needed for hatching, pupation, and emergence.

An embryo was considered hatched, when it had left the chorion. Pupation was recorded as soon as a larva had spun a surrounding cocoon, including thus, the prepupal stage. An adult was considered emerged, when it had left the cocoon surrounding the pupa. When pupae had chitinized, they were removed from the containers and placed in marked glass jars covered with gauxe so that emergence could be traced back to the original batches of eggs.

To compare the yield of the two diets, the following data were noted with respect to each container: number of eggs hatched, number of larvae pupated and number of adults that emerged. These adults were subsequently used in the oviposition cages, to determine fecundity and sex ratio. Separate cages were used for adults reared on each diet. No attempt was made to determine the effect of the diets on the rearing of successive generations. Eggs oviposited in each cage were used for the continuously rearing of new adults on both diets.

To determine the quality of adults reared on both diets, the following properties of the insects were compared: longevity, size, average number of eggs oviposited, per female and percentage of eggs hatched in the containers. In the longevity test, executed in the laboratory, 24 recently emerged adults, of both diets were put in glass jars covered with gauze. No food nor water was provided. In order to avoid the possible influence of factors such as light (windows are only located in one of the walls of the laboratory) on longevity, the jars were placed in a rectangular block of 4 x 12, in which the adults reared on each diet alternated. Mortality, sex and size were determined at 07: 45 and 19:45 during this test. The size of the dead adults was established by measuring the distance between the fringes of the wing and the tips of the head with a vernier caliper, which measured to an accuracy of 0.01 cm. If the fringes of the wings or the wing tips were damaged size was not determined. In addition, sex and size were determined on samples of adults of both diets, taken randomly during the whole rearing period. Analysis of variance was used to help interpret results obtained from the rearing experiment and the longevity test. A mathematical equation for the distribution of pupation and emergence of *H. grandella* reared on both diets, with respect to time was determined. At this point Poisson, normal and binomial distribution were tested for best fit. Daily records of pupation and emergence were pooled together into 2–day intervals, so as to facilitate interpretation.

## Results and discussion

### Oviposition

The described method to obtain fertile *H. grandella* eggs from captive adults reared on synthetic and natural diets resulted in an ever increasing insect population. Egg production of adults from both diets during two periods (one at the beginning and one at the end of this evaluation), is presented in Tables 1–3. Although a strict comparison of these oviposition data cannot be made because of different number of insects, different ambient conditions and possibly different sex ratios, some valid information has been obtained. In none of these periods did oviposition take place until the third night after release of the first insects in the cages. Only once in the whole rearing period were eggs observed after the second night of release. If oviposition took place on the potted trees, adults of both diets by far preferred *Cedrela odorata*. Oviposition on the non host appears to be accidental and may have been influenced by the nearby host tree. Distance between the potted plants was only 35 cm (Fig. 2).

Location of eggs of synthetic and naturally reared adults on the potted *Cedrela* was similar to location of eggs found on host trees in the field; i.e. on leaf scars, near leaf veins, near lenticels and in small fissures of the bark. Larvae, hatching from these eggs behaved normally and bored into leaf–stems and axils of the topshoot until they pupated and emerged.

Presence of a host tree in the cages was not required to obtain fertile eggs.

The majority of eggs were deposited singly, in the pits of the corrugated towelpaper. Most of these eggs were located on the upper half of the frames (Table 4, Fig. 1 and 4). When female adults became old and were not able to fly or climb anymore, they oviposited fertile and sterile eggs on the wire screening of the cage bottom. This behavior could explain occasional oviposition of fertile *H. grandella* eggs on grass leaves or stones near host trees in field plots.

Freshly deposited *H. grandella* eggs are pale yellow to yellowish brown (Munsell color 2.5Y6/4 – 2.5Y7/4), whereas 24 hours old eggs are red (Munsell color 5R4/8). This difference in color is easily seen with the naked eye and facilitated recognition of fresh and one day old eggs, when the frames were retrieved every other day.

During the heavy rainy period, a remarkable effect of the night light on oviposition was observed. Ninety–one per cent of all eggs oviposited during this period were located on the two frames nearest to the light (Table 4).

TABLE 1. Number of adults, mortality, sex ratio and oviposition data of *H. grandella* in captivity, during the period of January 11 – February 11, 1971. Adults reared in the laboratory on natural diet.

| Date (night of) | No. of adults in cage | No. of adults added | Mortality | Number and sex of dead adults ♂ | Number and sex of dead adults ♀ | No.of fertile eggs oviposited | No.of sterile eggs oviposited | Oviposition on potted host and non host trees in the cage* |
|---|---|---|---|---|---|---|---|---|
| 11–12 Jan. 1971 | 0 | 1 | – | – | – | – | – | |
| 12–13 | 1 | 2 | – | – | – | – | | |
| 13–14 | 3 | – | – | – | – | 7 | 2 | |
| 14–15 | 3 | 2 | – | – | – | 101 | | |
| 15–16 | 5 | 2 | – | – | – | 37 | – | |
| 16–17 | 7 | 4 | – | – | – | 70 | | |
| 17–18 | 11 | 9 | – | – | – | 122 | 17 | |
| 18–19 | 20 | 2 | 2 | 1 | 1 | 125 | | |
| 19–20 | 20 | 3 | – | – | – | 144 | 49 | |
| 20–21 | 23 | 2 | 2 | 2 | – | 87 | | |
| 21–22 | 23 | 1 | 2 | – | 2 | 127 | 19 | |
| 22–23 | 22 | – | 6+2** | 3 | 3 | 96 | | 38 eggs on *Cedrela* |
| 23–24 | 14 | 7 | 2 | – | 2 | 161 | 9 | |
| 24–25 | 19 | 3 | 5 | 2 | 3 | 70 | | |
| 25–26 | 17 | 2 | 1 | 1 | – | 392 | 21 | |
| 26–27 | 18 | 10 | 4 | 1 | 3 | 94 | | 124 eggs on *Cedrela* |
| 27–28 | 24 | – | 4 | 1 | 3 | 101 | 14 | |
| 28–29 | 20 | 3 | – | – | – | 284 | | |
| 29–30 | 23 | 6 | 3 | – | 3 | 86 | 11 | |
| 30–31 | 26 | – | 2 | – | 2 | 89 | | 84 eggs on *Cedrela* |
| 31– 1 Feb. | 24 | 5 | – | – | – | 147 | 18 | |
| 1– 2 | 29 | – | 1 | – | 1 | 221 | | |
| 2– 3 | 28 | – | 1 | – | 1 | 222 | 12 | |
| 3– 4 | 27 | 2 | 4 | 1 | 3 | 65 | | |
| 4– 5 | 25 | – | 2 | 1 | 1 | 232 | 27 | |
| 5– 6 | 23 | – | 6 | 5 | 1 | 164 | | |
| 6– 7 | 17 | – | 4 | 3 | 1 | 16 | 43 | |
| 7– 8 | 13 | – | 4+2** | 3 | 1 | 35 | | |
| 8– 9 | 7 | – | 5 | 3 | 2 | – | – | |
| 9–10 | 2 | – | 1 | 1 | – | – | | |
| 10–11 | 1 | – | 1 | 1 | – | – | – | |
| Totals | | 66 | 66 | 29 | 33 | 3.295 | 242 | 264 eggs on *Cedrela odorata;* no eggs on *Cananga odorata* |

* Potted host and non–host trees were placed in the cages from January 20–31, 1971.
** Escaped or predated.

The fact that *H. grandella* is attracted to light was also observed repeatedly during the nightly releases of adults in the cages, when the recently emerged adults could be transferred from the jars to the cage with the help of a flashlight. This attraction to light of a lantern has also been observed by Ramírez Sánchez (10).

Another interesting observation was made during this period. If occasionally a jar containing pupae and dessicated *Cedrela* leaves was left in the cage of the adults raised on natural diet, *H. grandella* adults could be found the next morning on the gauze covering the jar (Fig. 5). If the gauze was removed during the night, the adults could be found repeatedly in the jars again. Although no formal experiment was executed with respect to this behavior, the response of *H. grandella* to the night light and the jars containing pupae and dessicated *Cedrela* leaves could fit into a hypothetical model for the chain of stimuli causing host selection of *H. grandella.*

Callahan (4, 5, 6) developed a theory for the intermediate and far infared sensing of nocturnal insects. Since common lightbulbs, such as the night light, are known to emit a great quantity of the infrared spectrum, the oviposition behavior of *H. grandella* during this period might well be a response to infrared. The attraction of *H. grandella* to the jars in the cages might possibly be caused either by an aggregation hormone or wavelength emission of the dessicated (darker) *Cedrela* leaves.

Taking into account these observations, a hypothetical model for *H. grandella* host selection could be the following:

Remote and intermediate orientation
a. wavelength emission of host
Intermediate and nearby orientation
b. a + chemoreception of host odors and aggregating hormones; sex attractants
Landing and oviposition
c. a+ b + copulation and tactile response.

With respect to mating behavior, observed in the cages during the releases at 20:00, observations gave evidence for the existance of a female sex attractant. *H. grandela* females often remained stationary on the oviposition frames, while lifting their abdomen and

TABLE 2. Number of adults, mortality, sex ratio and oviposition data of *H. grandella* in captivity, during the period of March 27 – May 22, 1971. Adults reared in the laboratory on natural diet.

| Date (night of) | No. of adults present | No. of adults added | Mortality | Number and sex of dead adults ♂ | Number and sex of dead adults ♀ | No. of fertile eggs oviposited | No. of sterile eggs oviposited | Oviposition on potted host and non host trees in the cage* |
|---|---|---|---|---|---|---|---|---|
| 27–28 March 1971 | 0 | 4 | – | – | – | – | – | |
| 28–29 | 4 | – | 1** | – | – | – | | |
| 29–30 | 3 | 5 | – | – | – | 10 | 20 | |
| 30–31 | 8 | 4 | 2 | 1 | 1 | 4 | | |
| 31– 1 April | 10 | 4 | – | – | – | 190 | – | |
| 1– 2 | 14 | 9 | 2 | 2 | – | 303 | | |
| 2– 3 | 21 | 3 | 1 | 1 | – | 82 | – | 22 eggs on *Cedrela* |
| 3– 4 | 23 | 3 | 3 | – | 3 | 274 | | |
| 4– 5 | 22 | 4 | – | – | – | 135 | – | |
| 5– 6 | 26 | 5 | 2 | 1 | 1 | 29 | | |
| 6– 7 | 29 | 1 | 4+4** | 2 | 2 | 78 | 3 | 5 eggs on *Cedrela* |
| 7– 8 | 22 | 2 | 5 | 4 | 1 | 138 | | |
| 8– 9 | 10 | – | – | – | – | 37 | 1 | |
| 9–10 | 19 | – | 2 | 1 | 1 | 11 | | |
| 10–11 | 17 | – | 1 | – | 1 | 75 | – | 15 eggs on *Cedrela* |
| 11–12 | 16 | – | 9 | 5 | 4 | 18 | | |
| 12–13 | 7 | 8 | 3 | – | 3 | 141 | – | |
| 13–14 | 12 | – | – | – | – | 32 | | |
| 14–15 | 12 | 11 | 1 | – | 1 | 17 | 5 | |
| 15–16 | 22 | 5 | – | – | – | 7 | | |
| 16–17 | 27 | 4 | 1 | – | 1 | 219 | 21 | 23 eggs on *Cedrela* |
| 17–18 | 30 | 5 | 7 | 2 | 5 | 146 | | |
| 18–19 | 28 | 1 | 6 | 3 | 3 | 75 | 46 | 3 eggs on *Cedrela* |
| 19–20 | 23 | 5 | 16 | 6 | 10 | – | | 2 eggs on *Cananga* |
| 20–21 | 12 | 8 | – | – | – | 57 | 50 | |
| 21–22 | 20 | 16 | 4 | 1 | 3 | 34 | | |
| 22–23 | 32 | 23 | 3 | 2 | 1 | 45 | 176 | |
| 23–24 | 52 | 11 | 10 | 4 | 6 | 63 | | |
| 24–25 | 53 | 21 | 3 | 2 | 1 | 146 | 161 | |
| 25–26 | 71 | 7 | 5 | 3 | 2 | 240 | | |
| 26–27 | 73 | – | 8 | 5 | 3 | 454 | 128 | |
| 27–28 | 65 | 10 | 16 | 9 | 7 | 286 | | |
| 28–29 | 59 | 21 | 9 | 4 | 5 | 128 | 215 | |
| 29–30 | 71 | 12 | 21 | 8 | 13 | 355 | | |
| 30– 1 May | 62 | 7 | 8 | 3 | 5 | 312 | 232 | |
| 1– 2 | 61 | – | 10 | 6 | 4 | 486 | | |
| 2– 3 | 51 | 10 | 5 | 2 | 3 | 304 | 131 | 7 eggs on *Cedrela* |
| 3– 4 | 56 | 9 | 11 | 8 | 3 | 193 | | |
| 4– 5 | 54 | 5 | 7 | 3 | 4 | 75 | 148 | |
| 5– 6 | 52 | 6 | 8 | 4 | 4 | 182 | | |
| 6– 7 | 50 | 8 | 8 | 3 | 5 | 330 | 277 | 5 eggs on *Cedrela* |
| 7– 8 | 50 | 16 | 18 | 12 | 6 | 234 | | |
| 8– 9 | 48 | 16 | 7 | 3 | 4 | 495 | 140 | 11 eggs on *Cedrela* |
| 9–10 | 57 | 4 | 11 | 7 | 4 | 349 | | |
| 10–11 | 50 | 3 | 6 | 2 | 4 | 91 | 165 | |
| 11–12 | 47 | 3 | 11 | 5 | 6 | 220 | | |
| 12–13 | 39 | – | 5 | 1 | 4 | 191 | 76 | |
| 13–14 | 34 | – | 11 | 6 | 5 | 26 | | |
| 14–15 | 23 | – | 15 | 9 | 6 | 105 | 25 | |
| 15–16 | 8 | – | – | – | – | 59 | | |
| 16–17 | 8 | – | 5 | 3 | 2 | 52 | – | |
| 17–18 | 3 | – | 1 | 1 | – | – | | |
| 18–19 | 3 | – | 1 | 1 | – | – | – | |
| 19–20 | 2 | – | 1 | – | 1 | – | | |
| 20–21 | 1 | – | – | – | – | – | – | |
| 21–22 | 1 | – | 1 | 1 | – | – | | |
| Totals | | 299 | 299 | 145 | 148 | 7.533 | 2.064 | 91 eggs on *Cedrela odorata*; 2 eggs on *Cananga odorata* |

* Potted host and non–host trees were placed in the cages from March 28 – April 19, and from May 1 – May 22, 1971.
** Escaped or predated.

TABLE 3. Number of adults, mortality, sex ratio and oviposition data of *H. grandella* in captivity, during the periods of December 14–20, 1970 and April 20 – May 18, 1971. Adults reared in the laboratory on a synthetic diet.

| Date (night of) | No. of adults present in cage | No. of adults added | Mortality | Number and sex of dead adults ♂ | Number and sex of dead adults ♀ | No. of fertile eggs oviposited | No. of sterile eggs oviposited | Oviposition on potted host and non host trees in the cage* |
|---|---|---|---|---|---|---|---|---|
| 14–15 Dec. 1970 | 0 | 2 | – | – | – | – | – | |
| 15–16 | 2 | – | – | – | – | – | | |
| 16–17 | 2 | – | – | – | – | 13 | – | 28 eggs on *Cedrela* |
| 17–18 | 2 | – | – | – | – | 89 | | |
| 18–19 | 2 | – | – | – | – | – | – | |
| 19–20 | 2 | – | – | – | – | 18 | | |
| 20–21 | 2 | 4 | – | – | – | 12 | – | |
| 21–22 | 6 | 1 | – | – | – | 23 | | |
| 22–23 | 7 | 1 | – | – | – | 93 | 8 | |
| 23–24 | 8 | – | – | – | – | 41 | | |
| 24–25 | 8 | – | 1 | 1 | – | 75 | 11 | |
| 25–26 | 7 | – | 1 | 1 | – | 162 | | |
| 26–27 | 6 | – | 1 | – | 1 | 48 | 17 | 15 eggs on *Cedrela* |
| 27–28 | 5 | – | 2 | 1 | 1 | – | | |
| 28–29 | 3 | – | 2 | 1 | – | – | – | |
| 29–30 | 1 | – | 1 | – | 1 | – | | |
| Totals | | 8 | 8 | 5 | 5 | 574 | 36 | 43 eggs on *Cedrela odorata* no eggs on *Cananaga odorata* |
| 20–21 April 1971 | 0 | 7 | – | – | – | – | – | |
| 21–22 | 7 | 11 | – | – | – | – | | |
| 22–23 | 18 | 4 | – | – | – | 3 | – | |
| 23–24 | 22 | 6 | 2 | 1 | 1 | 3 | | |
| 24–25 | 26 | 4 | – | – | – | 111 | 157 | |
| 25–26 | 30 | – | 5 | 4 | 1 | 125 | | |
| 26–27 | 25 | 7 | 4 | 2 | 2 | 176 | 93 | |
| 27–28 | 28 | 3 | 5 | 3 | 2 | 244 | | |
| 28–29 | 26 | 6 | 5 | 3 | 2 | 285 | 32 | |
| 29–30 | 27 | 6 | 1 | 1 | – | 127 | | |
| 30– 1 May | 32 | – | 6 | 3 | 3 | 128 | 13 | 3 eggs on *Cedrela* |
| 1– 2 | 26 | 1 | 10 | 5 | 5 | 136 | | |
| 2– 3 | 17 | 17 | 1 | 1 | – | 23 | 27 | |
| 3– 4 | 33 | 12 | 4 | 3 | 1 | 34 | | |
| 4– 5 | 41 | – | 3 | 1 | 2 | 358 | 138 | 1 egg on *Cedrela* |
| 5– 6 | 38 | – | 2 | 1 | 1 | 694 | | 22 eggs on *Cedrela* |
| 6– 7 | 36 | – | 3 | – | 3 | 947 | 197 | 1 egg on *Cananga* |
| 7– 8 | 33 | – | 2** | – | – | 777 | | 38 eggs on *Cedrela* |
| 8– 9 | 31 | – | 6 | 2 | 4 | 549 | 128 | 1 egg on *Cananga* |
| 9–10 | 25 | – | 1 | – | 1 | 170 | | 35 eggs on *Cedrela* |
| 10–11 | 24 | – | 4 | 1 | 3 | 192 | 41 | |
| 11–12 | 20 | – | 5 | 3 | 2 | 181 | | |
| 12–13 | 15 | – | 2 | 1 | 1 | 77 | 27 | |
| 13–14 | 13 | – | 1 | 1 | – | 88 | | |
| 14–15 | 12 | – | 6 | 4 | 2 | – | 8 | |
| 15–16 | 6 | – | 4 | 1 | 3 | – | | |
| 16–17 | 2 | – | 1 | 1 | – | – | – | |
| 17–18 | 1 | – | 1 | 1 | – | – | | |
| Totals | | 84 | 84 | 43 | 39 | 5,434 | 861 | 99 eggs on *Cedrela odorata;* 2 eggs on *Cananga odorata* |

* Potted host and non–host trees were placed in the cage from December 14–18; December 24–30, 1970 and April 29, May 10, 1971.

** Escaped or predated.

TABLE 4. Location of *H. grandella* eggs on towel paper, in a oviposition cage located near a common light bulb. Adults raised on natural diet.

| Date (night of) | Total No. of eggs oviposited | No. eggs located upper half of the frames | Percentage | No. eggs located on lower half of the frames | Percentage | No. eggs located on frames nearest to common lightbulb | Percentage |
|---|---|---|---|---|---|---|---|
| 4–5 Apr. 1971 | 113 | 88 | 78 | 25 | 22 | 108 | 96 |
| 5–6 | 29 | 16 | 55 | 13 | 45 | 23 | 79 |
| 6–7 | 56 | 35 | 63 | 21 | 37 | 52 | 93 |
| 7–8 | 125 | 96 | 77 | 29 | 23 | 120 | 96 |
| 8–9 | 37 | 22 | 59 | 15 | 41 | 37 | 100 |
| 9–10 | 6 | 6 | 100 | – | – | 6 | 100 |
| 10–11 | 77 | 43 | 57 | 34 | 43 | 57 | 74 |
| 11–12 | 20 | 5 | 25 | 15 | 75 | 20 | 100 |
| Totals | 463 | 311 | 67 | 152 | 33 | 423 | 91 |

Fig. 4. One day old fertile eggs on the upper half of the frames. The eggs are located in the pits of the towel paper.

Fig. 5. *H. grandella* adults in cage, on gauze of beaker containing paper and dessicated *Cedrela* leaves.

"fanning" their wings, supposedly to "fan away" a sex attractant. Fourteen out of 15 "fanning" adults sampled in the cages during the period of the rearing experiment proved to be females. Both males and females made flight exercises however, and also used their wings to climb up the frames.

In early hours of the morning (0.5:30–06:30) coupled pairs (tail to tail) were observed several times. Coupling itself has not been observed.

With respect to oviposition, a decrease in average egg production and an increase in the average number of sterile eggs per female of each diet can be noticed (Tables 1–3) when the two periods are compared. Average egg production per female reared on natural diet, was 100 fertile and 8 sterile eggs in the period of January 11 – February 11, 1971, whereas, these figures are 65 and 14 in the period of March 27 – May 22, 1971. Average egg production per female raised on the synthetic diet was: 191 fertile and 12 sterile eggs in the period of December 14 – 30, 1970, and 161 fertile and 22 sterile in the period of April 20 – May 18, 1971.

The main cause for these phenomena could possibly be attributed to the increased number of insects per cage, to sex ratio or to ambient conditions rather than degeneration of the insect population. Evidence regarding the possible effect of sex ratio was obtained when later (May 18 – May 27, 1971) 10 sexed adults (5 females and 5 males) raised on synthetic diet, were placed in a cage. These females produced 1062 eggs, of which only 61 were infertile; i.e. an average production of 200 fertile and 12 sterile eggs per female. Sex ratio during the rearing period was approximately 1:1 (Table 1–3).

## Rearing

Figure 6 represents the daily temperature and relative humidity fluctuations of the air–conditioned laboratory during the week of May 3–10, 1971. Although minor differences from this regular pattern occurred during the rearing period, daily maximum and minimum temperature never reached over 30°C or below 22°C, respectively. Relative humidity varied between 50 and 68 per cent during the rearing. Maximum temperature and minimum relative humidity in the laboratory occurred at approximately 14:30 hours.

Of three distributions tested, Poisson, normal and binomial, the latter fitted the observed frequencies best.

Figures 8 and 9 show the observed and expected frequencies of pupating larvae (A) and emerging adults (B) of both diets in relation to time (expressed in days after oviposition).

Observed maxima for pupation and emergence of *H. grandella* reared on synthetic diet occurred 26 and 37

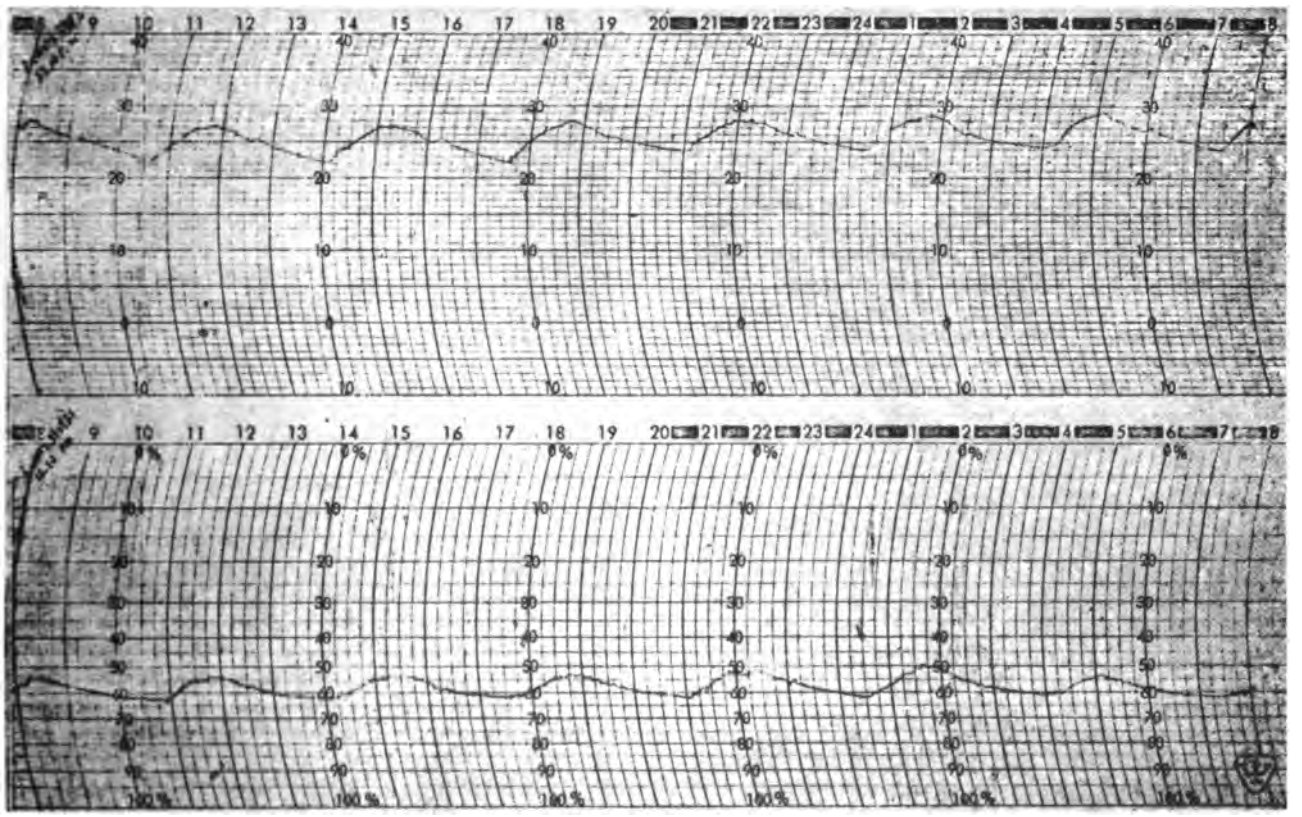


Fig. 6. Daily temperature and relative humidity fluctuations of the airconditioned laboratory, during the week of May 3–10, 1971. Maximum temperature and minimum relative humidity occurred approximately at 14.30 daily.

TABLE 5. Yield of *H. grandella* adults, reared under laboratory conditions on natural and synthetic diets in uncompartmented polystyrene containers.

| Type of diet | No. of eggs placed in containers | No. of eggs hatched | Percentage of eggs hatched | No. of larvae pupated | Percentage of larvae pupated: No. pupae / No. eggs hatched x 100% | No. of adults emerged | Yield (%): No. adults / No. eggs hatched x 100% | Observations |
|---|---|---|---|---|---|---|---|---|
| Natural | 379 | 339 | 89.4 | 182 | 53.7 | 154 | 45.4 | based on data from 11 containers |
| Synthetic | 389 | 319 | 82.0 | 150 | 47.0 | 133 | 41.7 | based on data from 8 containers |

days after oviposition, respectively. Observed maxima for pupation and emergence of *H. grandella* reared on natural diet occurred at 25 and 35 days after oviposition.

The difference in time needed for maximum pupation of larvae raised on both diets might be due to the spinning of a short of "community web" (Fig. 7) which occurred in the polystyrene containers with synthetic diet, but not in the natural diet.

This delaying effect, can also be noted from the observed and expected time needed for pupation of larvae from both diets (Fig. 8 and 9). The pupation–curve for larvae reared on artificial diet is flatter than the one for larvae reared on natural diet. Larvae reared on synthetic diet often crowded and bothered each other. Larval and pupal cannibalism was frequent in the containers with synthetic diet. Yield of adults (Table 5) was higher in natural than in synthetic diets.

Difference in duration of the prepupal and pupal stage in both diets, might be explained by the difference in size of adults reared on both diets. Adults reared on synthetic diet were generally bigger than those reared on natural diet. This could indicate a defficiency in the natural diet of leaves and young shoots, or differences in feeding response to each diet. In natural conditions *H. grandella* larvae will feed mainly on the pith of shoots.

The size of male and female adults reared on synthetic diet was based on 35 measurements for each sex, and ranged from 13.0 – 19.4 mm and 13.0 – 22.1 mm, respectively. Average size of these male and female adults was 16.7 ± 0.8 mm* and 17.9 ± 1.1 mm*, respectively.

The range in size of male and female adults reared on natural diet was from 11.8 – 18.0 mm for males and from 13.0 – 22.1 mm for females, based on 28 and 26 measurements respectively. Average size of these male and female adults was 14.6 ± 0.9 mm* and 15.1 ± 1.1 mm*, respectively.

* $x \pm t \frac{S}{0.01\ x}$

In the longevity test (Fig. 10), a significant difference at the one per cent level, was found between sexes; female *H. grandella* of both diets lived longer than males. It should be noticed however, that these observations refer to unmated adults under the specified test

Fig. 7. "Community web", spun by *H. grandella* larvae on synthetic diet. Pupae can be observed in the web.

conditions. In the cages, where only water was provided (either in the form of rain or offered water during dry spells) longevity of adults reared on synthetic and natural diet ranged from 3–15 and 2–10 days respectively. Adults drinking water could be observed frequently, particularly during the dry spells when water was offered.

## Mass rearing

The previously described techniques have led to a preliminary mass rearing program in which larvae are reared on synthetic diet in compartmented polystyrene containers, so as to avoid cannibalism. Oviposition cages are smaller (30 x 40 cm) and towel paper has been replaced by cheese cloth. Eggs are washed off in a 0.15 per cent solution of sodium hypochlorite which at the same time sterilizers the egg surface. This in turn, reduces contamination of the synthetic diet. Cocoons are removed with a 2.5 per cent solution of sodium hypochlorite and pupae are sexed (8). Some steps of the preliminary mass rearing procedures still have to be perfected in order to reach the same level of efficiency as rearing programs for other species of insects (11, 12, 13). Oviposition of fertile eggs in the laboratory has been obtained (at a relative humidity of 65 per cent and 25°C) but the technique needs further investigation. In addition, removal of eggs should be improved and yield of *H. grandella* adults should be increased.

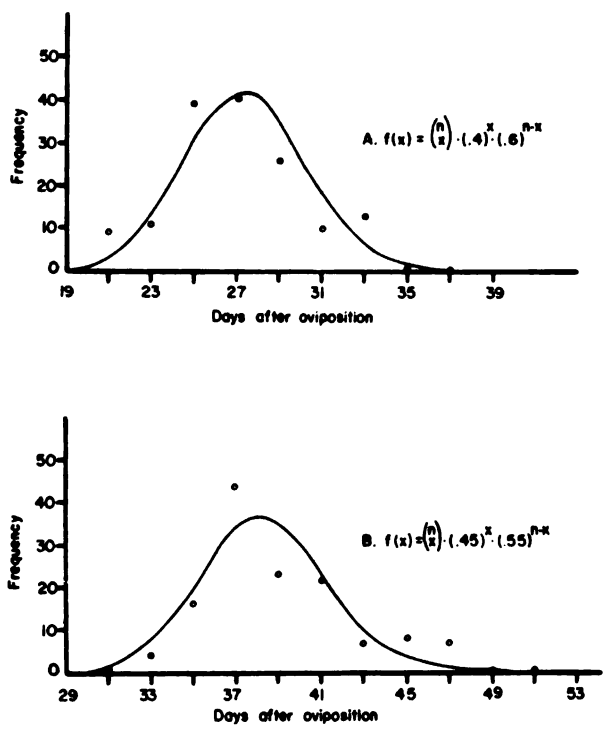



Fig. 8. Observed and expected frequencies of pupating larvae (A) and emerging adults (B) of *H. grandella* on synthetic diet, in relation to time.

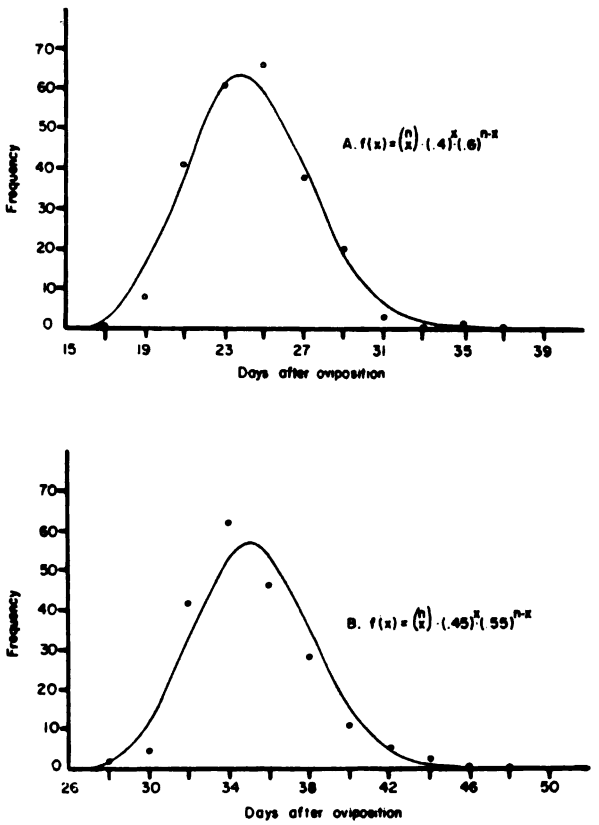



Fig. 9. Observed and expected frequencies of pupating larvae (A) and emerging adults (B) of *H. grandella* on natural diet, in relation to time.

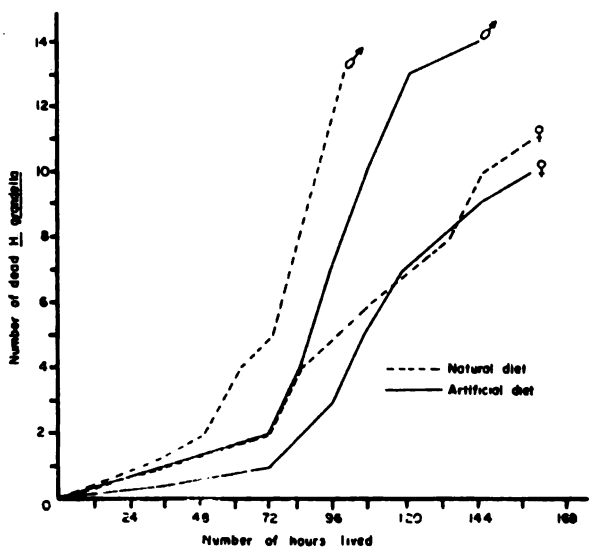



Fig. 10. Comparison of longevity of *H. grandella* adults (males and females) reared on synthetic and natural diet. Accumulated mortality per 12 hours under laboratory conditions; no food nor water provided.

## Acknowledgements

The author wishes to thank Dr. Gilberto Páez and Ing. Víctor Quiroga of the IICA–CTEI Computer Center for their assistance in the statistical evaluation of the results. Special thanks are due to Oscar Ovares, foreman of the Department of Tropical Forest Sciences, who dedicated many hours of his spare time on this investigation.

## Literature cited

1. ACHAN, P. D. Preliminary observations on the development of an artificial diet for *Hypsipyla robusta* Moore. Commonwealth Institute of Biological Control. Technical Bulletin No. 10:23–26. 1968.

2. ALLAN, G. G., GARA, R. I. and WILKINS, R. M. Studies on the shootborer *Hypsipyla grandella* (Zeller). III. The evaluation of some systemic insecticides for the control of larvae in *Cedrela odorata* L. Turrialba 20(4):478–487. 1970.

3. BERRIOS, F. and HIDALGO SALVATIERRA, O. Estudios sobre el barrenador *Hypsipyla grandella* (Zeller); VI. Susceptibilidad de la larva al hongo *Metarrhizium amisoplae* (Metch). Turrkalba 21(2):214–219. 1971.

4. CALLAHAN, P. S. Intermediate and far infrared sensing of nocturnal insects. Part. I: Evidences for a far infrared (FIR) electromagnetic theory of communication and sensing in moths and its relationship to the limiting biosphere of the corn earworm. Annals of the Entomological Society of America. 58(5):727–745. 1965.

5. _____ . Intermediate and far infrared sensing of nocturnal insects. Part II. The compound eye of the corn earworm, *Heliothis zea* and other moths as a mosaic optic–electromagnetic thermal radiometer. Annals of the Entomological Society of America 58(5):746–756. 1965.

6. _____ . The radiation environment and its relationship to possible methods of environmental control of insects. Proceedings Tall Timber Conference on Ecological Animal Control by Habitat Management. pp. 85–108. February 27–28, 1969.

7. GRIJPMA, P. and GARA, R. I. Studies on the shoot–borer *Hypsipyla grandella* (Zeller). II. Host preference of the larva. Turrialba 20(2):241–247. 1970.

8. HIDALGO–SALVATIERRA, O. Estudios sobre el barrenador *Hypsipyla grandella* (Zeller). VII. Determinación del sexo en pupas. Turrialba 21(2):221. 1971.

9. MUNSELL COLOR COMPANY, INC. Munsell soil color charts. Baltimore, Maryland 21218. USA. 1954 Edition.

10. RAMIREZ SANCHEZ, J. Investigación preliminar sobre biología, ecología y control de *Hypsipyla grandella* (Zeller). Boletín del Instituto Forestal Latinoamericano de Investigación y Capacitación. No. 16:54–77. 1964.

11. RAULSTON, J. R. and LINDGREN, P. D. A technique for rearing larvae of the bollworm and tobacco budworm in large numbers. Journal of Economic Entomology 62(4):959–961. 1969.

12. YAMAMOTO, R. T. Mass rearing of the tobacco hornworm. I. Egg production. Journal of Economic Entomology 61(1):171–174. 1968.

13. _____ . Mass rearing of the tobacco hornworm. II. Larval rearing and pupation. Journal of Economic Entomology 62(6):1427–1431. 1969.

# SUSCEPTIBILIDAD DE LA LARVA AL HONGO *METARRHIZIUM ANISOPLIAE* (METCH)*

*F. Berrios, O. Hidalgo–Salvatierra***

## ABSTRACT

Purified spores of *Metarrhizium anisopliae* (Metch.) were tested for pathogenicity against larvae of the Meliaceous shootborer *Hypsipyla grandella* (Zeller) reared on a newsynthetic diet. It was found that 50 per cent of the larvae tested were killed by the fungus at a concentration of $1.2 \times 10^7$ viable spores por ml. Greatest mortality occurred six days after bathing the larvae with the spore suspension. It is suggested that the green muscardine should be further evaluated as a potential agent in the integrated control of *H. grandella*.

The authors

## Introducción

El barrenador de las Meliáceas *Hypsipyla* spp. (Lepidoptera: Pyralidae) es una plaga seria en plantaciones de cedro (*Cedrela* spp.) y caoba (*Swietenia* spp.) en los bosques tropicales y subtropicales de las regiones Indo–Malaya, Australiana, Africana, de las Indias Occidentales y de Sur América. Ataca principalmente los brotes tiernos y en muchas especies también los frutos y semillas. Entwistle (3) considera que este insecto es uno de los factores detrimentales más importantes cuando se trata de establecer una plantación de Meliáceas tropicales.

Hasta el momento no se conoce una medida única y eficaz para controlar esta plaga. Rao y Bennet (7) sugieren posibilidades de control biólogico. Posiblemente la única forma de atacar este problema sea a base de un control integrado (1).

En su lista de enemigos naturales de *Hypsipyla* spp., Rao y Bennet (7) anotan que éste puede ser atacado por el hongo *Cordyceps* spp. Kandasamy (6) encontró que *Beauveria tenella* (Delacroix) fue capaz de infectar larvas de *Hypsipyla robusta* (Moore) cuando las esporas fueron inyectadas o administradas superficialmente. También se sabe desde hace algún tiempo (9) que larvas de *Pyrausta nubilalis* (Hbn.), el barrenador del maíz, perteneciente a la misma familia de *H. grandella*, pueden ser atacadas por el hongo *Metarrhizium anisopliae* (Metch.), comúnmente conocido como la muscardina verde. Esto sugiere que una investigación sobre el posible uso de patógenos, como *M. anisopliae*, en el control biológico de *H. grandella* debe ser parte de los estudios sobre un control integrado.

Zacharuk y Tinline (10) en pruebas de laboratorio con *M. anisopliae* y otros hongos, encontraron que todos los estados de la mayoría de especies económicas de elatéridos en Saskatchewan, Canadá, son susceptibles a infecciones por este hongo.

En los Estados Unidos, Brooks y Raun (2) encontraron que de nueve géneros de hongos aislados de ocho especies de insectos del maíz, *M. anisopliae* y *Beauveria bassiana* (Bals.) Vuill. eran los más patógenos.

Los resultados de la mayoría de estos experimentos muestran una gran variación en susceptibilidad de larvas de insectos a varios hongos entomopatógenos; los resultados favorables obtenidos con *M. anisopliae* sugieren la posibilidad de poder usar este hongo como agente de control de *H. grandella*.

El propósito de esta investigación fue determinar si el hongo *M. anisopliae* es capaz de infectar y matar larvas de *H. grandella* bajo condiciones de laboratorio.

## Materiales y métodos

### Microorganismo

El hongo *M. anisopliae* fue suministrado por el Dr. D. W. Roberts del Instituto Boyce Thompson de Nueva York. Este hongo fue cultivado rutinariamente en tubos inclinados con un medio de cultivo denominado SDAY (agar de Sabouraud con dextrosa y extracto de levadura) (8) e incubados a temperaturas de 28 a 30°C.

Las esporas fueron cosechadas añadiendo a cada tubo 6 ml de agua esterilizada más dos gotas de una suspensión de Triton X–100, agitando con un vibrador vortex y filtrando dos a tres veces a través de varias capas

* Recibido para publicación el 17 de mayo de 1971. Este trabajo se llevó a cabo bajo el contrato AT(30–1)–2043 entre la Comisión de Energía Atómica de los EE.UU. y el Instituto Interamericano de Ciencias Agrícolas de la OEA. Publicación No. NYO–2043–233.

** Estudiante graduado e investigador principal respectivamente del programa de Energía Nuclear del IICA–CTEI. Este trabajo es parte de la tesis de *Magister Scientiae* de F. Berrios.

de tela fina colocadas en un soporte para filtros Millipore. Cada filtrado se observó bajo el microscopio para asegurarse de la pureza de la preparación.

La concentración total de esporas se determinó con un hemocitómetro y la viabilidad, por dilución, sembrando éstas en platos Petri con el mismo agar.

## Insecto

La colonia de *H. grandella* se empezó a mediados del año 1970 con huevos y larvas traídas de parcelas experimentales de cedro y caoba del Departamento de Ciencias Forestales Tropicales del IICA. Las generaciones subsiguientes fueron mantenidas parte en dieta natural (hojas de cedro) y parte en dieta artificial preparada usando una licuadora Osterizer de la siguiente forma.

1. Licuar 80,7 g de soya en 450 ml de agua.
2. Disolver 8 g de agar en 400 ml de agua hirviendo y dejar enfriar hasta 60°C.
3. Continuar licuando y agregar:
   - 36 g de germen de trigo
   - 6 ml de KOH, 4 M
   - 10 g de sal–W
   - 1,8 g de p–hidroxibenzoato de metilo
   - 1,1 g de ácido sórbico
   - 5 ml de formaldehido al 10 por ciento
   - 13,3 ml de ácido acético al 25 por ciento
   - 20 g de mezcla de vitamina*, añadir el agar disuelto
   - 20 ml de aureomicina (166,7 mg)
   - 80 g de zuro molido y tamizado con una malla de 0,21 mm de diámetro

Esta fórmula es una modificación de una dieta para *Heliothis* spp. suministrada por el Dr. M. J. Lukefahr.**

## Inoculación

Sesenta larvas, criadas únicamente en dieta artificial, de 15 a 17 días de edad fueron divididas al azar en dos grupos de 30. Un grupo fue tratado con una suspensión de esporas, a una concentración de esporas viables de 1,2 x $10^7$ por mililitro, y el otro grupo, el control, fue tratado con agua más Triton X–100.

Cada grupo fue subdividido en tres subgrupos de nueve larvas y uno de tres. Cada subgrupo de larvas fue bañado durante un minuto con la suspensión correspondiente y después colocado en compartimentos en platos Petri conteniendo dieta artificial.

## Resultados y discusión

El experimento fue examinado cada dos días y las larvas muertas separadas y puestas en un plato Petri con un papel humedecido. Las larvas atacadas por el hongo fueron reconocidas por su decoloración, turgidez y rigidez. Dentro de las 24 horas subsiguientes el micelio blanquecino del hongo empezó a emerger a través de las seudopatas y espiráculos. Con el transcurso de la enfermedad el cadáver entero es cubierto por la muscardina que al esporular cambia a color verde obscuro (Fig. 1a). La larva muerta se transforma completamente en una masa de esporas que al final se desintegra con el más leve movimiento (Fig. 1b). Estos síntomas son parecidos a los descritos por Getzin y Shanks (4) para el sinfílido *Scutigerella inmaculata*, y a los descritos por Steinhaus (9) para varias especies de insectos.

La mortalidad de las larvas se muestra en el Cuadro 1. Se puede apreciar que bajo las condiciones del experimento 50 por ciento de las larvas fueron atacadas por el hongo. También se puede apreciar que el número

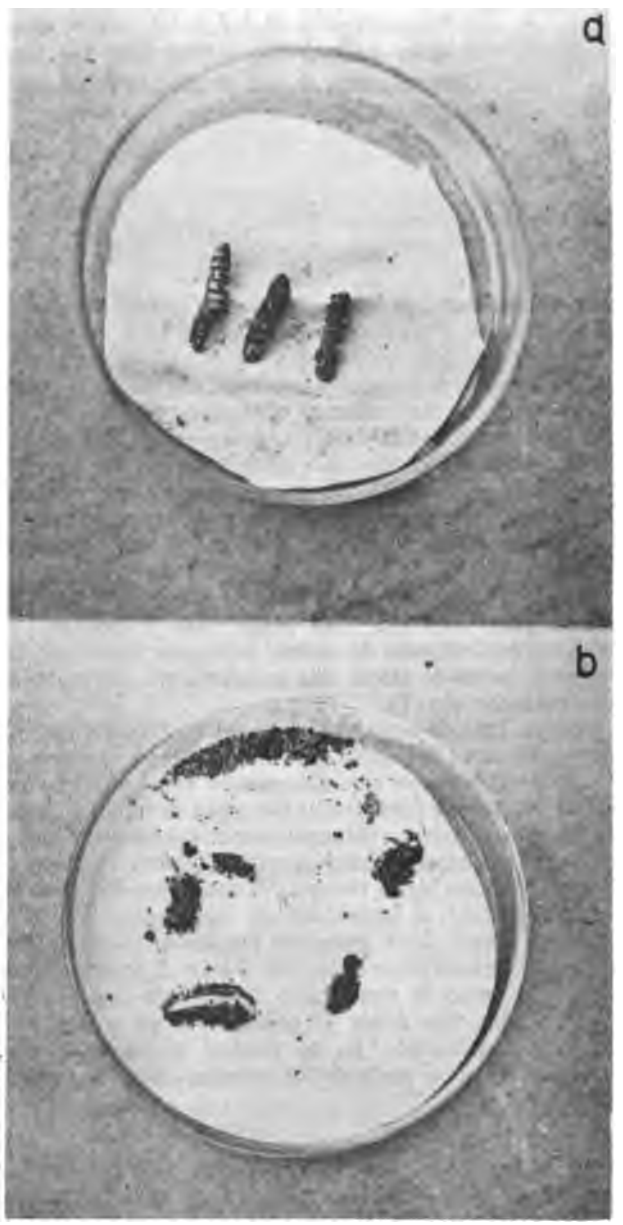



Fig. 1. Larvas de *Hypsipyla grandella* (Zeller) atacadas por el hongo *Metarrhizium anisopliae* (Metch), muscardina verde. Explicación en el texto.

* Esta es una mezcla vendida por Nutritional Biochemical Corporation, bajo el nombre de Vitamin Diet Fortification Mixture.

** M. J. Lukefahr, comunicación personal, 1970, USDA, Brownsville, Texas.

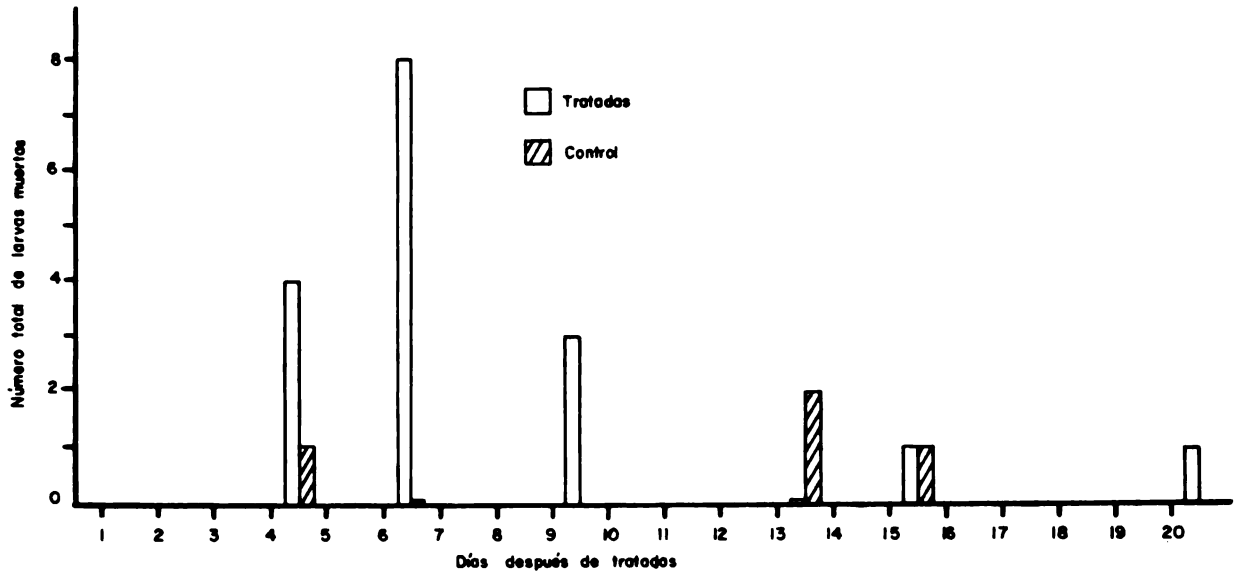



Fig. 2. Distribución de la mortalidad de larvas de *Hypsipyla grandella*(Zeller) atacada por el hongo*Metarrhizium anisopliae* (Metch). a una concentración de $1,2 \times 10^7$ esporas viables/ml.

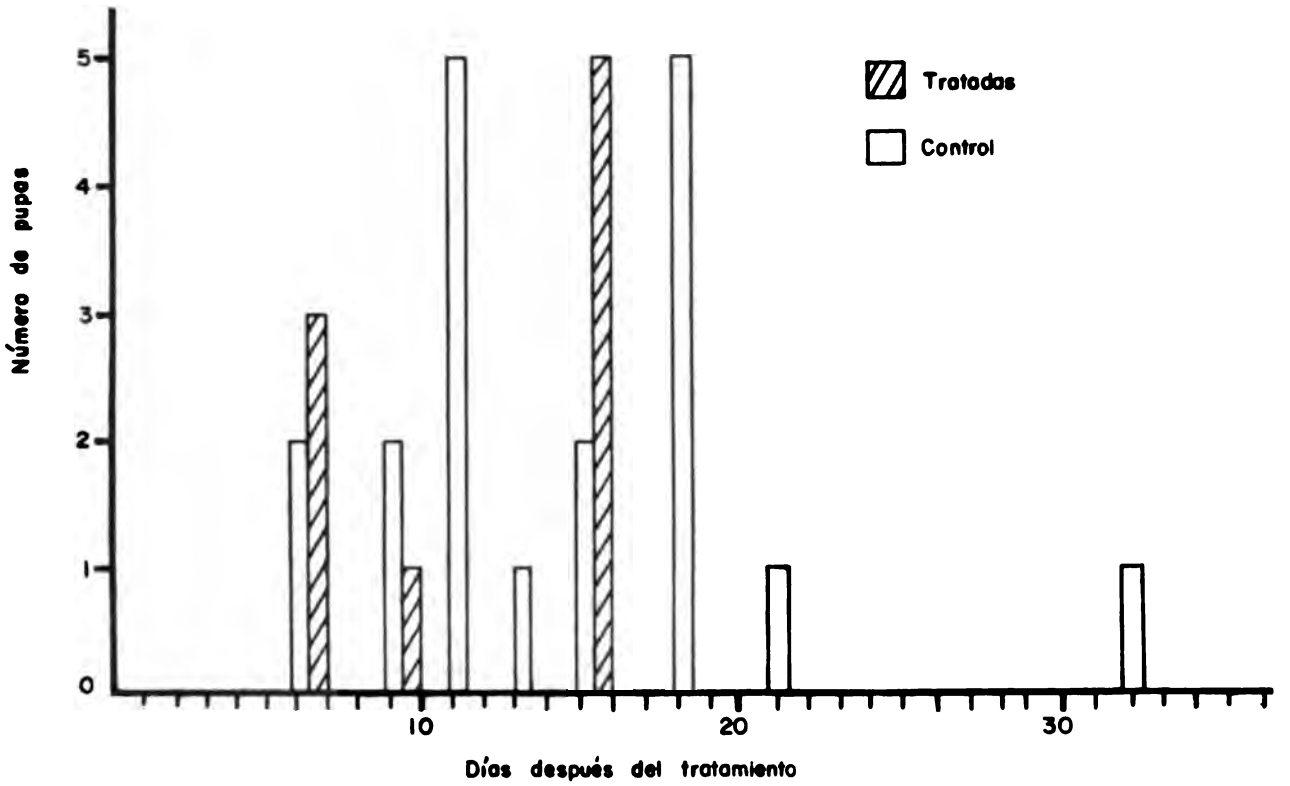



Fig. 3. Distribución de pupas de *Hypsipyla grandella*(Zeller) con respecto al tiempo transcurrido después que las larvas fueron tratadas con *Metarrhizium anisopliae* (Metch.) a una concentración de $1,2 \times 10^7$ esporas viables/ml.

de pupas, adultos y de larvas perdidas* en el grupo tratado con esporas se redujo aproximadamente al 50 por ciento del control como consecuencia de la reducción en la población. De las pupas formadas 90 por ciento llegó al estado de adulto en ambos grupos. El tiempo de emergencia fue también el mismo en ambos grupos demostrando que las larvas que no fueron atacadas lograron empupar y emerger normalmente.

La distribución del número total de larvas muertas con respecto al tiempo transcurrido después de la inoculación se presenta en Fig. 2. Se puede observar que el mayor número de larvas muertas se presentaron seis días después del tratamiento. En su trabajo con sinfílidos, Getzin y Shanks (4) también encontraron un período de máxima mortalidad a los seis días.

La distribución de pupas completamente formadas, con respecto al tiempo transcurrido después del tratamiento se muestra en Fig. 3. Se puede observar que las larvas usadas en ambos grupos eran uniformes fisiológicamente, y que la mayoría empupó de 11 a 18 días después del tratamiento con un período larval de 27 a 34 días, lo que es diferente a lo encontrado normalmente en la crianza de *H. grandella* en esta dieta sintética (5). Esto demuestra que las larvas que sobrevivieron el tratamiento se desarrollaron igual a las no tratadas.

* Larvas perdidas se refiere a larvas que desaparecieron porque escaparon o por canibalismo.

Cuadro 1. Mortalidad de larvas de *Hypsipyla grandella* (Zeller) atacadas por el hongo *Metarrhizium anisopliae* (Metch.) a una concentración de esporas viables de 1,2 x $10^7$ esporas/ml.

| | No. de larvas | Total de larvas muertas | Larvas muertas por el hongo | Total de pupas | Total adultos | Larvas perdidas |
|---|---|---|---|---|---|---|
| Control | 9 | 1 | 0 | 6 | 6 | 2 |
| | 9 | 1 | 1 | 6 | 5 | 2 |
| | 9 | 2 | 0 | 4 | 4 | 3 |
| | 3 | 0 | 0 | 3 | 2 | 0 |
| Total | 30 | 4 | 1 | 19 | 17 | 7 |
| Tratados | 9 | 5 | 3 | 2 | 1 | 2 |
| | 9 | 6 | 6 | 3 | 3 | 0 |
| | 9 | 6 | 6 | 1 | 1 | 2 |
| | 3 | 0 | 0 | 3 | 3 | 0 |
| Total | 30 | 17 | 15 | 9 | 8 | 4 |

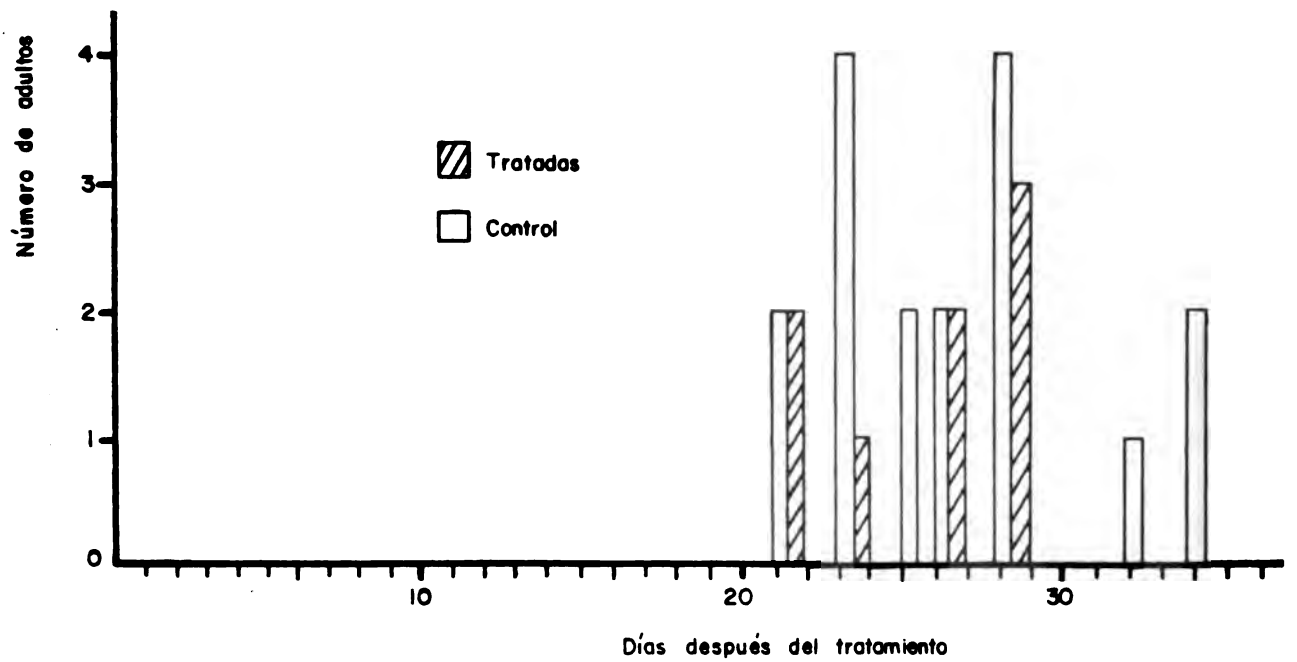



Fig. 4. Emergencia de adultos de *Hypsipyla grandella* (Zeller) con respecto al tiempo transcurrido después que las larvas fueron tratadas con *Metarrhizium anisopliae* (Metch.) a una concentración de 1,2 x $10^7$ esporas viables/ml.

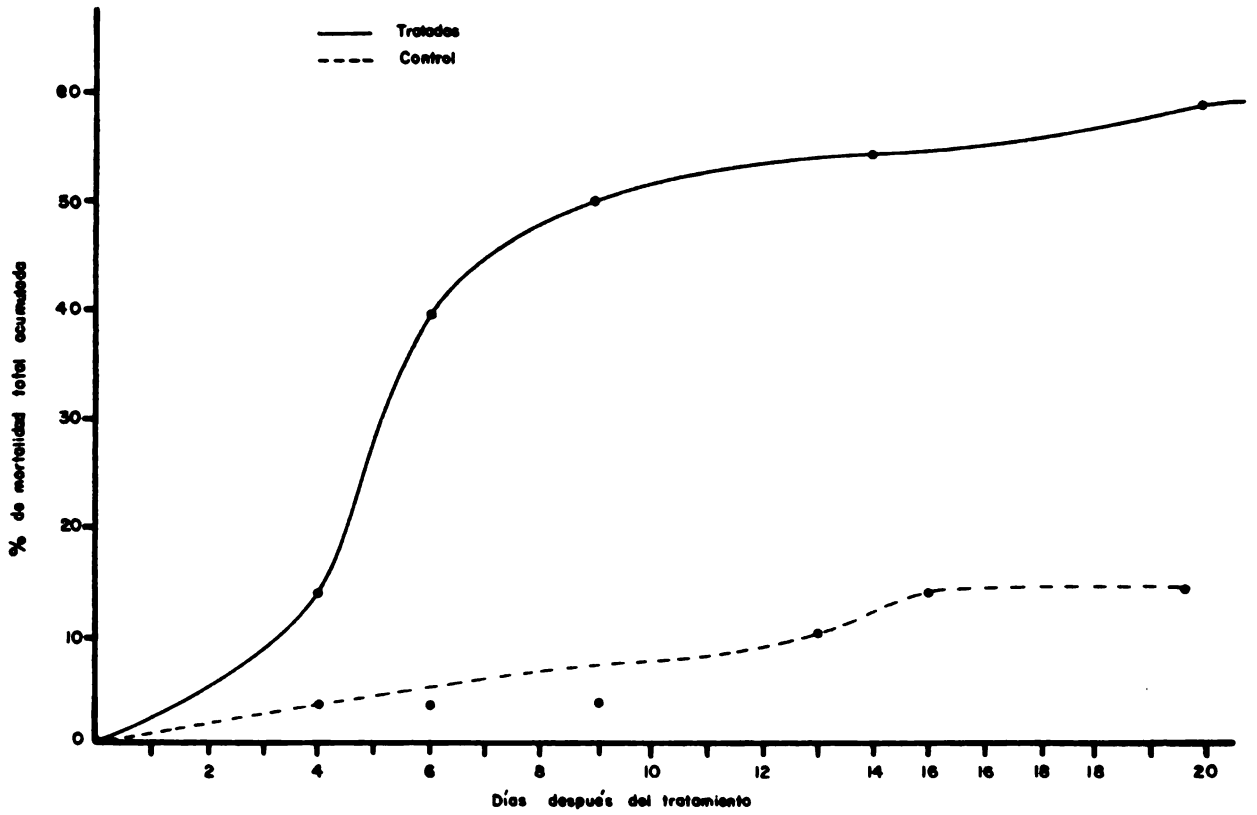



Fig. 5. Porcentaje de mortalidad total acumulada de larvas de *Hypsipyla grandella* (Zeller) expuestas a esporas de *Metarrhizium anisopliae* (Metch.).

La distribución de adultos mostrada en Fig. 4 parece indicar que el período pupal fue de 10 a 11 días.

La mortalidad total de larvas en ambos grupos se muestra en Fig. 5. El control muestra la mortalidad debida a causas ajenas al hongo bajo prueba o por contaminación. En este experimento una de las larvas del control fue contaminada accidentalmente por el hongo (Cuadro 1) con una contribución de 3 por ciento de la mortalidad total. La mortalidad total del control fue 13 por ciento y la del tratamiento 57 por ciento. La mortalidad total causada por el hongo fue 50 por ciento.

## Conclusiones

El experimento demostró la patogenicidad del hongo *Metarrhizium anisopliae* para larvas de *Hypsipyla grandella.*

Se concluye que este hongo debe ser considerado como un agente potencial de control en la lucha integrada contra el barrenador de las Meliáceas. Se sugiere que se amplíen los estudios de laboratorio incluyendo determinaciones sobre índices de patogenicidad, dosis requerida para alcanzar una mortalidad arriba del 50 por ciento, efectos del ambiente sobre la supervivencia de las esporas y la posible influencia del hongo en un habitat forestal.

## Agradecimiento

Se agradece la colaboración del Bachiller Luis G. Madrigal, y al Departamento de Ciencias Forestales Tropicales por la asistencia prestada.

## Literatura citada

1. BOLETIN INFORMATIVO DEL GRUPO DE TRABAJO SOBRE *Hypsipyla.* IICA, Turrialba, Costa Rica. 1970. 4 p.

2. BROOKS, D. L. y RAUN, E. S. Entomogenous fungi from corn insects in Iowa. Journal of Invertebrate Pathology 7(1):79–81. 1965.

3. ENTWISTLE, P. F. The current situation on shoot, fruit and collar borers of the *Meliaceae.* Ninth British Commonwealth Forestry Conference. 1968. 15 p.

4. GETZIN, L. W. y SHANKS, C. H. Infection of the garden Symphylan, *Scurigerella immaculata.* Journal of Insect Pathology 6(3):542–543. 1964.

5. GRIJPMA, P. Studies on the shootborer *Hypsipyla grandella* (Zeller). V. Observations on a rearing technique and on host selection behavior of adults in captivity. Turrialba 21(2):202–213. 1971.

6. KANDASAMY, D. *Hypsipyla robusta* Moore, a new host for *Beauveria tenella* (Delacroix) Siemaszko. Journal of Invertebrate Pathology 13:149–150. 1969.

7. RAO, V. P. y BENNET, F. D. Possibilities of biological control of the Meliaceous shoot borer *Hypsipyla* spp. (lepidoptera: Phycitidae). Commonwealth Institute of Biological Control Technical Bulletin No. 12:61–81. 1969.

8. ROBERTS, D. W. Toxins from the entomogenous fungus *Metarrhizium anisopliae*. I. Production in submerged and surface cultures, and in inorganic and organic nitrogen media. Journal of Invertebrate Pathology 8:212–221. 1966.

9. STEINHAUS, E. A. Principles of insect pathology. New York Hafner, 1967. 757 p.

10. ZACHARUK, R. Y. y TINLINE, R. D. Pathogenicity of *Metarrhizium anisopliae* and other fungi for five Elaterids (Coleoptera) in Saskatchewan. Journal of Invertebrate Pathology 12(3):294–309. 1968.

# DETERMINACION DEL SEXO EN PUPAS

*Oscar Hidalgo–Salvatierra*

Programa de Energía Nuclear, Instituto Interamericano de Ciencias Agrícolas
Turrialba, Costa Rica

## SUMMARY

The pupae sex characteristics of the shootborer *Hypsipyla grandella*(Zeller) are described. The female genital opening is located in the eigth abdominal segment, extending from the caudal margin of the seventh segment to the caudal margin of the eigth segment, entering into the ninth segment but without dividing it. The male genital opening is found entirely in the ninth segment.

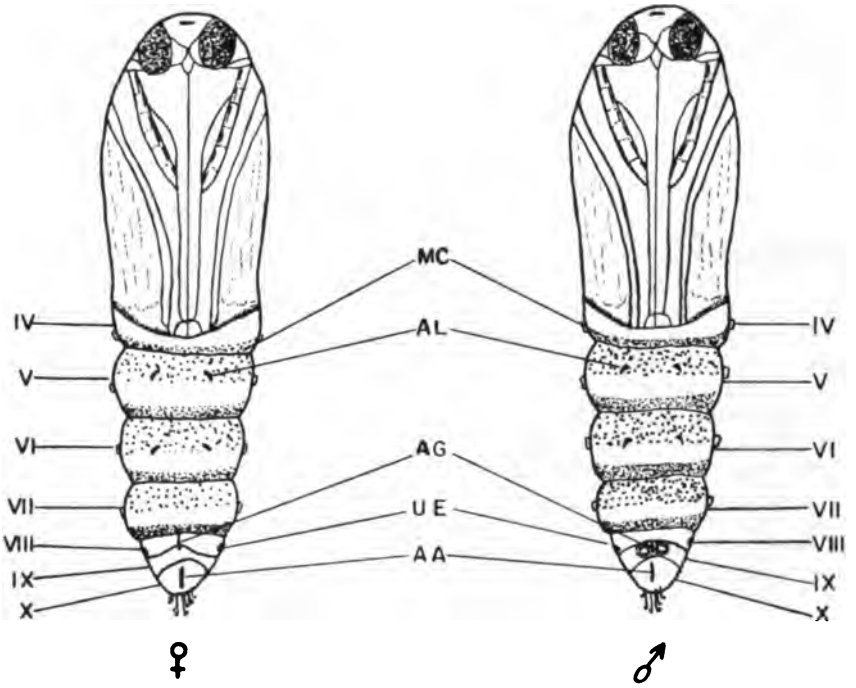



**Fig. 1.** Pupas de *Hypsipyla grandella* Zeller mostrando las características ventrales de la hembra (izquierda) y el macho (derecha): AL, alvéolos; AA, abertura anal; AG, abertura genital; MC, margen caudal; UE, último espiráculo. Los números romanos indican los diferentes segmentos abdominales.

En 1969 Maddox (1) publicó las características principales usadas para diferenciar los sexos en las pupas del barrenador *Vogtia malloi* Pastrana. En esta ocasión se comunica sobre características similares observadas en una especie relacionada, *Hypsipyla grandella*(Zeller),el barrenador de las Meliáceas.

Puede observarse en Fig. 1 que la abertura genital de la hembra divide completamente el octavo segmento, extendiéndose desde el margen caudal del sétimo segmento hasta un poquito más allá del margen cuadal del octavo segmento pero sin dividir el noveno segmento.

En el macho, la abertura genital se encuentra localizada en la línea media ventral del noveno segmento mostrando a cada lado los pequeños y característicos abultamientos genitales. Esto hace que la abertura genital y la abertura anal se encuentren en mayor proximidad en el macho que en la hembra.

El octavo segmento se localiza fácilmente por la presencia del último espiráculo. El margen cuadal de los segmentos octavo y noveno no es tan visible como el de los otros segmentos abdominales por falta de acumulación de pigmento. Estas últimas características son idénticas a las de *V. malloi.*

Este trabajo fue auspiciado por la Comisión de Energía Atómica de los EE.UU. bajo contrato AT(30–1)–2043. Publicación No. NYO–2043–234.

## Referencias

1. MADDOX, D. M. Sex determination of pupae of *Vogtia malloi* (Lepidoptera: Phycitidae). Annals of the Entomological Society of America 62:1212–1213. 1969.

Recibido para la publicación el 26 de mayo de 1971.

# SUSCEPTIBILIDAD DE LA LARVA A LOS HONGOS *BEAUVERIA BASSIANA* (BAL.) Y *BEAUVERIA TENELLA* (DEL.)*[1]

*F. Berrios, O. Hidalgo–Salvatierra*

Estudiante graduado e investigador principal, respectivamente
del Programa de Energía Nuclear del IICA–CTEI, Turrialba, Costa Rica

## ABSTRACT

Purified spores of *Beauveria bassiana* (Bal.) and *Beauveria tenella* (Del.) were tested for pathogenecity against larvae of the Meliaceous shootborer *Hypsipyla grandella* (Zeller) reared on a synthetic diet. Larvae immersed for one minute in a suspension of spores showed a mortality of 13.9 per cent with *B. bassiana* at a concentration of $1.4 \times 10^6$ viable spores/ml and 12.7 per cent with *B. tenella* at a concentration of $2.9 \times 10^6$ viable spores/ml. Greatest mortality occurred eigth days after infection with *B. bassiana*, and 10 days after infection with *B. tenella*.

The authors

## Introducción

Entre los patógenos de insectos conocidos, *Beauveria bassiana* (Bal.) y su especie relacionada *Beauveria tenella* (Del,) han sido extensivamente investigadas (5).

Trabajando con *B. tenella* (Del.), Kandasamy (4) encontró que larvas de *Hypsipyla robusta* (Moore) eran infectadas al ser inyectadas o administradas superficialmente con esporas del hongo. Getzin (2) en pruebas comparativas de virulencia de hongos parásitos encontró un 50 por ciento de mortalidad en larvas del medidor del repollo *Trichoplusia ni* (Hübner) asperjadas con esporas de *B. bassiana* a una concentración de $1{,}0 \times 10^7$ esporas/ml.

En nuestros estudios preliminares sobre la susceptibilidad de *Hypsipyla grandella* (Zeller) a hongos entomopatógenos hemos encontrado que es susceptible al hongo *Metarrhizium anisopliae* (Metch.) (1).

El propósito de esta investigación preliminar es determinar si larvas de *H. grandella* son susceptibles a los hongos *B. bassiana* y *B. tenella* bajo condiciones de laboratorio.

## Materiales y métodos

### Microorganismos

Los hongos *B. bassiana* y *B. tenella* fueron suministrados por Gerald M. Thomas del Laboratorio de Patología de Insectos de la Universidad de California, Berkeley. *B. bassiana* fue cultivado en tubos inclinados con un medio de cultivo denominado SDAY (Agar de Sabouraud con dextrosa y extracto de levadura) (8), e incubados a condiciones ambientales. *B. tenella* fue cultivado en el mismo medio, pero fue incubado a 28°C. En experimentos anteriores se encontró que bajo estas condiciones se observó buena esporulación. Resultados similares han sido descritos por Walstad *et al.* (10).

Las esporas de ambos hongos fueron cosechadas, añadiendo a cada tubo 6 ml de agua esterilizada más dos gotas de una suspensión de Triton X–100, agitando con un vibrador Vortex y filtrando dos o tres veces a través de varias capas de tela fina colocada en un soporte para filtros Millipore. Cada filtrado se observó bajo el microscopio para asegurarse que las esporas estaban libres de micelio.

### Insecto

La crianza y mantenimiento de la colonia se efectuó de la manera descrita anteriormente (1).

### Inoculación

*Beauveria bassiana:* setenta y dos larvas, criadas únicamente en dieta artificial, de 17 a 20 días de edad, fueron divididas al azar en dos grupos de 36. Un grupo fue tratado con una suspensión de esporas, a una concentración de esporas viables de $1{,}4 \times 10^6$ por mililitro, y el otro grupo, el control, fue tratado con agua más Triton X–100.

Cada grupo fue dividido en cuatro sub–grupos de nueve larvas cada uno. Cada sub–grupo de larvas fue bañado durante un minuto con la suspensión correspondiente y después colocado en compartimientos en platos Petri con dieta artificial.

*Beauveria tenella:* Ciento veintiséis larvas, criadas únicamente en dieta artificial de 17 a 20 días de edad,

* Recibido para la publicación el 1° de octubre de 1971.

1. Este trabajo se llevó a cabo bajo el Contrato AT(30–1)–2043 entre la Comisión de Energía Atómica de los EE.UU. y el Instituto Interamericano de Ciencias Agrícolas de la OEA. Publicación No. NYO–2043–240.

fueron divididas al azar en dos grupos de 63. Un grupo fue tratado con una suspensión de esporas viables de 2,9 x $10^6$ por mililitro y el otro grupo, el control, fue tratado con agua más Triton X–100.

Cada grupo fue subdividido en siete sub–grupos de nueve larvas cada uno. Cada sub–grupo de larvas fue bañado durante un minuto en la suspensión correspondiente y después colocado en compartimientos en platos Petri con dieta artificial.

## Resultados y discusión

Ambos experimentos fueron examinados periódicamente y las larvas muertas separadas y puestas en un plato Petri con un papel humedecido. Las larvas atacadas por los hongos fueron reconocidas por su decoloración y rigidez. La mortalidad causada por el ataque de *B. bassiana* empezó a manifestarse a los tres días de iniciado el experimento; resultado similar fue encontrado por Paschke (6) en el escarabajo de la hoja de cereales, *Oulema melanopa*. La mortalidad causada por *B. tenella* empezó a manifestarse a los ocho días; Kandasamy (4) encontró que la mortalidad de larvas de *Hypsipyla robusta* (Moore) bañadas con suspensión de esporas de *B. tenella* comenzó a manifestarse a los 12 días (Figs. 1 y 2).

La presencia del micelio blanquecino de *B. bassiana* o blanquecino cremoso de *B. tenella* que emergía a través de las pseudopatas y espiráculos, 2–3 días después que las larvas momificadas se han separado sobre el papel humedecido, confirma la etiología de la enfermedad. El micelio crece abundantemente sobre el cadáver de la larva dándole el aspecto característico de la muscardina blanca (Fig. 3).

Estos síntomas son parecidos a los descritos por Steinhaus (9) para el taladrador europeo del maíz *Pyrausta nubilalis* (Hbn) y otras varias especies de insectos; a los señalados por Jacques y MacLellan (3) en *Carpocapsa pomonella* (Lin) y a los indicados por Kandasamy (4) en *Hypsipyla robusta* (Moore).

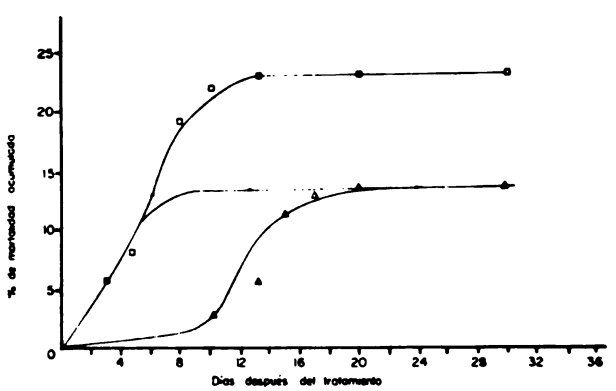



Fig. 1. Porcentaje de mortalidad total acumulada de larvas de *Hypsipyla grandella* (Zeller) expuestas a esporas de *Beauveria bassiana* (Bal.) (1,4 x $10^6$ esporas viables/ml). □ = mortalidad total en las larvas tratadas; △ = mortalidad total en las larvas de control; • = mortalidad causada por el hongo en las larvas tratadas.

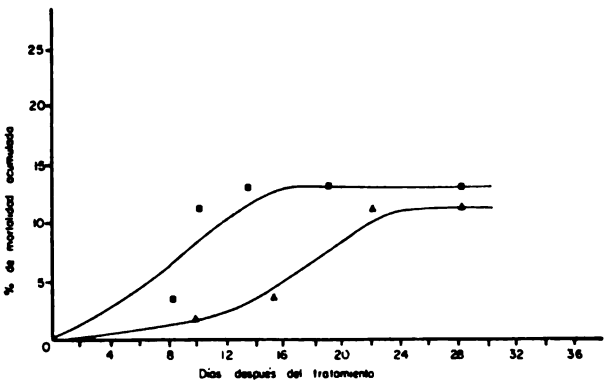



Fig. 2. Porcentaje de mortalidad acumulada de larvas de *Hypsipyla grandella* (Zeller) expuestas a esporas de *Beauveria tenella* (Del.) (2,9 x $10^6$ esporas viables/ml). □ = mortalidad total en las larvas tratadas; △ = mortalidad total en las larvas de control; • = mortalidad causada por el hongo en las larvas tratadas.

Fig. 3. Larvas de *Hypsipyla grandella* (Zeller) atacadas por el hongo *Beauveria tenella* (Del.). Explicación en el texto.

La mortalidad de las larvas en ambos experimentos se muestra en el Cuadro 1 y la Figura 1 para *B. bassiana* y en el Cuadro 2 y la Figura 2 para *B. tenella*. Se puede deducir de los Cuadros 1 y 2 que en las condiciones del experimento el 13,9 por ciento de las larvas fueron atacadas por *B. bassiana* y el 12,7 por ciento lo fueron por *B. tenella*.

Cuadro 1. Mortalidad de larvas de *Hypsipyla grandella* (Zeller) atacadas por el hongo *Beauveria bassiana* (Bal.) a una concentración de esporas viables de 1,4 x $10^6$ esporas/ml.

| No. de larvas | Total de larvas muertas | Larvas muertas por el hongo | Total de pupas | Total de adultos | Larvas perdidas |
|---|---|---|---|---|---|
| Control | | | | | |
| 9 | 0 | 0 | 9 | 9 | 0 |
| 9 | 3 | 0 | 5 | 3 | 1 |
| 9 | 1 | 0 | 8 | 3 | 1 |
| 9 | 1 | 0 | 6 | 6 | 2 |
| Total 36 | 5 | 0 | 28 | 21 | 4 |
| Tratadas | | | | | |
| 9 | 2 | 1 | 6 | 6 | 1 |
| 9 | 4 | 3 | 4 | 2 | 1 |
| 9 | 1 | 1 | 7 | 5 | 1 |
| 9 | 2 | 0 | 5 | 4 | 2 |
| Total 36 | 9 | 5* | 22 | 17 | 5 |

* Mortalidad causada por el hongo 13,9 por ciento.

Cuadro 2. Mortalidad de larvas de *Hypsipyla grandella* (Zeller) atacadas por el hongo *Beauveria tenella* (Delacroix) a una concentración de esporas viables de 2,9 x $10^6$ esporas/ml.

| No. de larvas | Total de larvas muertas | Larvas muertas por el hongo | Total de pupas | Total de adultos | Larvas perdidas |
|---|---|---|---|---|---|
| Control | | | | | |
| 9 | 2 | 0 | 5 | 5 | 2 |
| 9 | 0 | 0 | 8 | 6 | 1 |
| 9 | 0 | 0 | 7 | 2 | 2 |
| 9 | 1 | 0 | 5 | 5 | 3 |
| 9 | 1 | 0 | 6 | 3 | 2 |
| 9 | 1 | 0 | 6 | 2 | 2 |
| 9 | 2 | 0 | 6 | 1 | 1 |
| Total 63 | 7 | 0 | 43 | 24 | 13 |
| Tratadas | | | | | |
| 9 | 0 | 0 | 7 | 2 | 2 |
| 9 | 1 | 1 | 5 | 2 | 3 |
| 9 | 2 | 2 | 5 | 3 | 2 |
| 9 | 1 | 1 | 5 | 4 | 3 |
| 9 | 1 | 1 | 7 | 4 | 1 |
| 9 | 1 | 1 | 5 | 2 | 3 |
| 9 | 2 | 2 | 4 | 3 | 3 |
| Total 63 | 8 | 8* | 38 | 20 | 17 |

* Mortalidad causada por el hongo 12,7 por ciento.

## Conclusiones

Se ha demostrado que larvas de *Hypsipyla grandella* (Zeller) son susceptibles a los hongos *Beauveria bassiana* y *Beauveria tenella*. El primero causó mayor mortalidad aún cuando se encontraba a menor concentración. De esto se infiere que *B. bassiana* es más patogénico hacia *H. grandella* que *B. tenella*. Pruebas definitivas, sin embargo, requieren la utilización de larvas de estadios conocidos. Teóricamente, si se aumenta la concentración de esporas, se aumentaría la mortalidad total.

Se recomienda determinar el por ciento de mortalidad a concentraciones 10 ó 100 veces mayores.

Este experimento no es comparable al efectuado anteriormente con *Metarrhizium anisopliae* (1) por haber diferencias en la concentración de esporas de ambos.

## Agradecimiento

Se agradece la colaboración del señor Luis G. Madrigal, así como al Departamento de Ciencias Forestales Tropicales por la asistencia prestada y al Gobierno de Holanda por el suministro de materiales.

## Literatura citada

1. BERRIOS, F. e HIDALGO-SALVATIERRA, O. Estudios sobre el barrenador *Hypsipyla grandella* (Zeller). VI. Susceptibilidad de la larva al hongo *Metarrhizium anisopliae* (Metch.). Turrialba 21(2):214-219. 1971.

2. GETZIN, L. W. *Spicaria rileyi* (Farlow) an entomogenous fungus of *Trichoplusia ni* (Hübner). Journal of Insect Pathology 3(1):2-10. 1961.

3. JACQUES, R. P. y MacLELLAN, C. R. Fungal mortality of overwintering larvae of the codling moth in apple orchards in Nova Scotia. Journal of Invertebrate Pathology 7(3):291-296. 1965.

4. KANDASAMY, D. *Hypsipyla robusta* (moore) a new host for *Beauveria tenella* (Delacroix) Siemaszko. Journal of Invertebrate Pathology 13:149-150. 1969.

5. MacLEOD, D. M. Investigations on the genera *Beauveria* Vuill and *Tritirachum* Limber. Canadian Journal of Botany 32:818-890. 1954.

6. PASCHKE, J. D. Infection of the cereal leaf beetle *Oulema melanopa* (Linnaeus) by *Beauveria bassiana* (Bal.) Vuill. Journal of Invertebrate Pathology 7(1):101-102. 1965.

7. RAO, V. P. y BENNET, F. D. Possibilities of biological control of the Meliaceous shoot borer *Hypsipyla* spp. (Lepidoptera: Phycitidae). London, Commonwealth Institute of Biological Control. Technical Bulletin No. 12:16-81. 1969.

8. ROBERTS, D. W. Toxins from the entomogenous fungus *Metarrhizium anisopliae*. I. Production in submerged and surface sultures and inorganic and organic nitrogen media. Journal of Invertebrate Pathology 8:212-221. 1966.

9. STEINHAUS, E. A. Principles of insect pathology. New York. Hafner. 1967. 757 p.

10. WALSTAD, J. D., ANDERSON, R. F. y STAMBAUCH, W. J. Effects of environmental conditions on two species of muscardine fungi *(Beauveria bassiana* and *Metarrhizium anisopliae)*. Journal of Invertebrate Pathology 16:221-226. 1970.

# UN NUEVO MICROGASTERINO NEOTROPICO *(HYMENOPTERA, BRACONIDAE)* PARASITO DE LA LARVA*

*Luis de Santis*

Facultad de Ciencias Naturales y Museo, La Plata, Argentina

## ABSTRACT

A new species of the genus *Hypomicrogaster* parasitizing the larva of *Hypsipyla grandella* is described. The parasite was reared from a prepupal *H. grandella* larva found in a fruit of *Cedrela oaxacensis* C.DC. & Rose (Synonym: *C. tonduzii* C.DC.), growing in a natural stand near Santa Cruz, on the slopes of the Turrialba volcano at 1.500 m altitude. About 15 parasites pupated externally on the host larva within its cocoon. All of the adult parasites which emerged, were females.

La nueva especie de microgasterino que se describe en esta comunicación, parásito del barrenador de las Meliáceas, ha sido encontrada en Costa Rica por Pieter Grijpma.

*Hypomicrogaster hypsipylae* sp. n. (Figura 1).

**Hembra:** Negro. Labro, mandíbulas, palpos, escapo excepto en el lado externo y en el ápice, una mancha ínfero–anterior en el protórax, tégulas , patas anteriores e intermedias excepto en el ápice, coxas posteriores, fémures posteriores excepto en la extremidad apical, tibias posteriores excepto en el tercio apical, base del primer tarsito posterior, márgenes laterales de los tres primeros urotergitos y vientre, amarillo o amarillento. Alas hialinas, las anteriores con pterostigma y nervaduras negruzcas excepto la costal que es amarillenta.

Cara, clípeo, frontovértice, sienes, superficie externa de las coxas y fémures posteriores, mesepisterno y parte superior del mesepímeron, con punteado más o menos separado; mesoescudo y con punteado denso y profundo en la mitad anterior, en la parte posterior es más brillante y el punteado es gradualmente menos profundo y más separado; escudete liso y brillante con unos pocos puntos débilmente marcados y esparcidos; sutura preescutelar con débiles rugas longitudinales; propodeo rugoso–punteado sobre todo en la mitad anterior, con aréola central bien aparente y también rugoso–punteada; mitad apical del escudo del primer urotergito con puntos esparcidos, los restantes casi lisos.

Cabeza y tórax recubiertos por setas blanquecinas; ojos profusamente pestañosos.

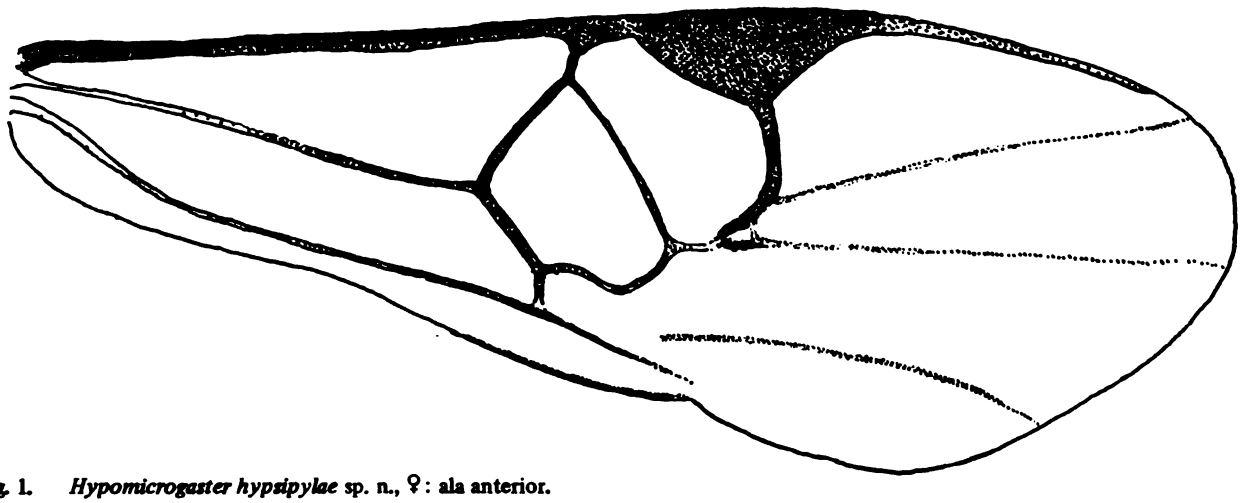


Fig. 1. *Hypomicrogaster hypsipylae* sp. n., ♀: ala anterior.

* Recibido para la publicación el 28 de febrero 1972.

Cabeza un poco más estrecha que el tórax; cara algo más ancha que larga con una elevación mediana y longitudinal; clípeo confusamente delimitado; antenas casi tan largas como el cuerpo.

Nervación de las alas anteriores tal como presentada en Figura 1; coxas posteriores largas; espolón interno de las tibias intermedias un poco más largo que el basitarso correspondiente, el de las tibias posteriores no mucho más largo que el externo y de una longitud igual a las dos terceras partes del basitarso correspondiente.

Escudo del urotergito I gradualmente estrechado hacia la base, más largo que ancho y de una longitud que es algo mayor que la tercera parte de la longitud total del gáster; urotergito II transverso, con margen posterior convexo, cuatro veces más ancho que largo y tan largo como la tercera parte de la longitud del urotergito III; hipopigio prominente, sin sobrepasar el ápice del gáster; vainas del oviscapto tan largas como las tibias posteriores, ensanchadas hacia el ápice y con setas negras y largas.

Longitud del cuerpo, excluida la proyección del oviscapto, 3 mm.

**Macho**: Desconocido.

**Distribución geográfica**

Turrialba, Costa Rica. Localidad tipo: Santa Cruz.

**Bionomía**

Los ejemplares examinados fueron obtenidos de una larva prepupal de *Hypsipyla grandella* (Zeller), encontrada en un fruto de *Cedrela oaxacensis* C.DC. & Rose. (Sinónimo: *C. tonduzii* C.DC. ) recolectada por P. Grijpma en un bosquete natural que se encuentra a 1.500 m de altura en las faldas del Volcán Turrialba. Alrededor de 15 parásitos se empuparon externamente sobre la larva hospedante dentro de su capullo. Todos los parásitos que emergieron fueron hembras. El material fue enviado por Vitor O. Becker del grupo Interamericano de Trabajo sobre *Hypsipyla*, IICA–CTEI, Turrialba, Costa Rica.

## Observaciones

Esta nueva especie debe referirse al grupo *zonaria* de Nixon (1). Se ubica cerca de *H. jocarae* (Muesebeck, 1958) de Puerto Rico y Cuba. Se diferencia por la coloración del gáster y el mesoescudo con punteado menos denso y también por las estructuras del propodeo. De *H. diaphaniae* (Muesebeck, 1958), de México, El Salvador, Costa Rica y Brasil, se diferencia por la coloración del tórax y gáster, las estructuras del propodeo y ofrecer el escudete con punteado débil y esparcido (2).

## Material estudiado

1 ♀ holotipo y 3 ♀ ♀ paratipos, Santa Cruz (Turrialba, Costa Rica), 6. XII. 1971, P. Grijpma col., depositados en las colecciones del Museo de La Plata, Argentina.

## Referencias


1. NIXON, G. E. J. Bulletin of the British Museum (Natural History) Entomology Supplement 2:209, 212–215. 1965.
2. MUESEBECK, C. F. W. Proceedings of the United States National Museum, 107:412–416. 1958.

# OBSERVATIONS ON THE EGG PARASITE *TRICHOGRAMMA SEMIFUMATUM* (PERKINS) (HYM.: TRICHOGRAMMATIDAE)*

*P. Grijpma***

Silviculturist, Forestry Program
Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica
Attached to IICA through the Dutch Technical Assistance Program

## COMPENDIO

En este artículo se hace mención de los siguientes registros nuevos relacionados a los estudios sobre el barrenador de las meliáceas: 1) la oviposición de *Hypsipyla grandella* (Zeller) sobre el cedro australiano *Toona ciliata* M. Roem var. *australis* (F.v.M.) C.DC. fue observada por primera vez el 20 de mayo de 1971 en una parcela experimental en Puente Cajón, Turrialba, Costa Rica; 2) mortalidad de larvas del primer instar de *H. grandella* que atacan el cedro australiano. Estas larvas mueren dentro de uno a tres días, sugiriendo la existencia de un compuesto tóxico o la ausencia de un compuesto vital para las larvas en los tejidos del cedro australiano; 3) parasitismo de *Trichogramma semifumatum* (Perkins) sobre los huevos de *H. grandella*. Esta es la primera vez que se registra la presencia de este parásito en Costa Rica y de un nuevo hospedante *Hypsipyla grandella* (Zeller); 4) según la determinación del Dr. S. Nagarkatti, del Commonwealth Institute of Biological Control en India, el material original colectado contenía también *Trichogramma pretiosum* Riley y posiblemente una otra especie no registrada *Trichogramma* cerca de *pretiosum*.

Se estableció una cría pura de *T. semifumatum* sobre huevos de *Hypsipyla grandella* y se hicieron observaciones con respecto al ciclo de vida, emergencia, copulación y comportamiento de este parásito.

Un inventario de la plantación recién establecida del cedro australiano reveló un alto porcentaje de parasitación de los huevos de *H. grandella* que podría ser consecuencia de una época seca anormal.

El autor

## Introduction

Oviposition of *Hypsipyla grandella* (Zeller) on the resistant Australian red cedar, *Toona ciliata* M. Roem. var. *australis* (F.v.M.) C.DC., was recorded by the author for the first time on May 20, 1971, in a field plot in Puente Cajón, Turrialba, Costa Rica. First instar larvae of *H. grandella* boring into this species die within 1–3 days, suggesting the existence of a toxic, or the absence of a vital component for the larvae in the plant tissues of *T. ciliata* var. *australis*.☆

A 100 per cent survey, conducted in a plot of recently planted Australian cedar not only revealed a high incidence of *H. grandella* eggs on the seedlings but also a high percentage of parasitization of these eggs by *Trichogramma*. Subsequent rearing of the *Trichogramma* population on *H. grandella* eggs was initiated and resulted in a series of laboratory experiments on the parasitization of *H. grandella* eggs by this parasite, which are reported here.

## Materials and methods

A 100 per cent survey on the incidence of parasitized and non-parasitized *H. grandella* eggs was conducted on December 30, 1971 in a two months old plantation of the Australian cedar. Height of the seedlings was measured and the number of eggs deposited on each was determined. The plantation consisted of 480 trees planted between approximately 1.50 m high *Cedrela angustifolia* Sessé & Moc. ex. DC. trees (provenance

* Received for publication September 27, 1972.
** Study to be used by P. Grijpma for Ph.D. dissertation.

☆ Studies on the shootborer *Hypsipyla grandella* (Zeller) (Lep., *Pyralidae*). On the resistance of *Toona ciliata* M. Roem. var. *australis* (F.v.M.) C.DC. (in preparation).

Barinitas, Venezuela). Of the Australian cedar seedlings, 432 had been planted with naked roots, the remainder as potted seedlings (4). Precipitation data in the plantation area were recorded. Survival of the *Toona* seedlings was determined and living as well as dead trees were checked for oviposition. Planting distance between seedlings was 1 x 1 m. The plantation was established in an open field, covered with vegetation consisting mainly of grasses and weeds, on property of the Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica. The neighboring vegetation consisted of a pure *Cedrela angustifolia* plantation and an abandoned coffee plantation, heavily shaded by 15 m high *Erythrina* trees. A sugar cane plantation was located at about 100 m distance from the meliaceous plantations, behind the abandoned coffee field. Successful *H. grandella* attacks, indicated by fresh frass, were observed on the *C. angustifolia* plants in the pure plantation as well as in the part of the plantation mixed with the Australian cedar seedlings. Two rows of the pure *C. angustifolia* plantation, consisting of 46 and 41 plants respectively, were also surveyed to determine the incidence of parasitized and non-parasitized *H. grandella* eggs.

Parasitized eggs were removed from the *T. ciliata* var. *australis* and *C. angustifolia* trees with a razor blade and a preliminary rearing method was developed for the *Trichogramma* parasites utilizing *H. grandella* eggs (5). Hereto, the parasitized eggs were put in small polystyrene boxes (12 x 9 x 3.5 cm) and provided with eggs of the shootborer each time upon emergence. Observations were made on the life cycle, emergence, and mating behavior of this *Trichogramma* sp.

An experiment was conducted to determine and compare the pre-emergence and incubation periods respectively of parasitized and non-parasitized *H. grandella* eggs. Fifty parasitized eggs of which the time of parasitization had been recorded and 50 fertile, non-parasitized, fresh *H. grandella* eggs were put in one of the polystyrene boxes. The age of these eggs may have varied between 2-14 hours. Temperature and humidity in the air-conditioned laboratory were recorded continuously. No water was added to the polystyrene box. Upon hatching, first instar *H. grandella* larvae were removed from the box, to avoid consumption of the parasitized eggs by the first instar *H. grandella* larvae which have been observed frequently feeding on *H. grandella* eggs in the absence of other feeding material.

Samples of the original *Trichogramma* population were sent to Dr. B. D. Burks of the USDA Entomology Research Division in Beltsville, Maryland: to Dr. L. De Santis, Museum of La Plata, Argentina, and to Dr. S. Nagakatti of the Commonwealth Institute for Biological Control, Bangalore, India, for determination. Samples of the subsequent rearing were forwarded to Dr. Nagakatti for determination.

Preference of the *Trichogramma* sp. for fresh eggs (1, 6), was tested in an experiment in which 20 fresh, 20 one-day old and 20 two-days old *H. grandella* eggs were exposed simultaneously to 20 female parasites in one of the polystyrene boxes. The three age groups of *H. grandella* eggs had been obtained employing a newly developed technique (8), and were distributed randomly on 9 $cm^2$ of towel paper. Behaviour of *Trichogramma* with respect to preference and the parasitization *per se* was followed through a microscope.

The sex-ratio of the egg parasite, reared under prevailing laboratory conditions, was established by counting male and female populations of eight polystyrene rearing boxes after they had died.

Finally, observations were made on the parasitization of unfertilized *H. grandella* eggs by this *Trichogramma*.

## Results

The 100 per cent survey for *H. grandella* oviposition in the *T. ciliata* var. *australis* plot of 480 seedlings showed that 123 seedlings planted with naked roots had died. No oviposition of *H. grandella* was observed on these dead seedlings. Of the remaining 357 plants, 93 has a total of 221 *H. grandella* eggs, of which 217 were black, indicating parasitization, and 4 were red. The only other category of *H. grandella* eggs found, consisted of 12 empty, transparent chorions which were collected on 7 seedlings. No further count was made of these hatched eggs, in view of the great difficulties encountered in locating them. No damage to the *Toona* seedlings was noticed, although *H. grandella* larvae had apparently hatched on at least 7 plants. Average height of the Australian cedar seedlings was 55.4 cm.

Microscopic examination of the four red *H. grandella* eggs, showed that they were also parasitized.

The inventory of the two rows of the neighboring *Cedrela angustifolia* plantation presented the same picture. Here, 30 parasitized eggs were found on 13 of a total of 87 plants. Average height of these plants was 1.75 m. Two of the collected eggs were red but also proved to be parasitized when examined under the microscope. The number of parasitized eggs on the *T. ciliata* var. *australis* seedlings varied between 1 and 10, averaging 2.4 per tree. The number of parasitized eggs on *C. angustifolia* plants varied between 1 and 9, averaging 2.3 per tree.

The apparent high incidence of parasitization observed in these meliaceous plantations may have been influenced by abnormally dry weather conditions. Figure 1 compares the average precipitation of 26 years with the rainfall measured during the first two months after planting of the Australian cedar in the field.

Laboratory rearing of the *Trichogramma* population on *H. grandella* eggs proved to be easy, and was obtained by offering fresh eggs of the shootborer as long as female parasites were alive in each box. About 33 generations have been reared meanwhile and the parasite population is still increasing. The daily fluctuations of temperature and humidity in the air conditioned laboratory varied between 22 and 28°C and 50-60 per cent R.H., respectively.

The incubation period of *H. grandella* eggs and the developmental period of *Trichogramma*, under the indicated laboratory conditions, varied between 84-116 h and 231-240 h, respectively. The greater variability in the incubation period of *H. grandella* eggs is most probably due to differences in time of oviposition. The age of white, "fresh" *H. grandella* eggs may have varied from 2-14 hours under the conditions of this experiment. The *H. grandella* eggs utilized for parasitization by the *Trichogramma* sp. were all exposed at the same time for parasitization by the parasite.

Observations made during this experiment indicated that *H. grandella* eggs become reddish-black approximately 3 days after parasitization and are

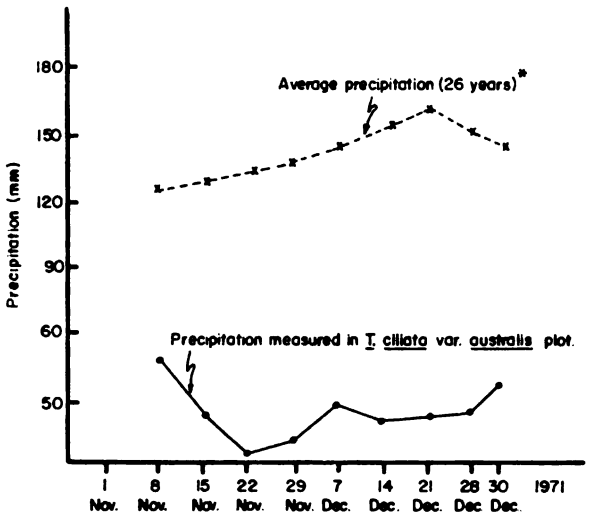



Fig. 1. Weekly precipitation registered in *T. ciliata* var. *australis* plot as compared to precipitation averages of 26 years (1944–1970).
* Source: Meteorological observation station at IICA–CTEI, Turrialba, Costa Rica. Location approximately 1 km from *T. ciliata* var. *australis* plantation.

completely black 4 days after having been parasitized by the *Trichogramma* (Table 1). Since most *Trichogramma* emerged three days after having been collected from the field plot, it can be taken for granted that all *H. grandella* larvae hatching from un–parasitized eggs had already emerged on the seedlings. The empty chorions encountered in the field gave further evidence to this reasoning. It may therefore be assumed that the encountered high percentage of parasitization of *H. grandella* eggs was probably not at all representative of the efficiency of the parasite nor did it give a correct picture of the actual rate of parasitization in this case.

Burks* and De Santis* identified the specimens of the originally collected population as *T. semifumatum* and *T. pretiosum* respectively. Nagarkatti*, Nagarkatti y Nagaraja (7) indicated that *T. semifumatum, T.* near *pretiosum* and possibly *T. pretiosum* were present in the specimens of the original population. The data presented on the lifecycle, emergence, copulation and behavior only refer to *T. semifumatum* of which pure populations were established in the laboratory and later identified by Nagarkatti.*

The preference of *Trichogramma semifumatum* for fresh *H. grandella* eggs was demonstrated in the preference test (Fig. 2). The lapse of time needed to parasitize 20 eggs of each age group (fresh, 1–day, and 2–days old) by 20 females of *T. semifumatum* was taken as an indication of preference. Microscopic observation of the parasitization of these 60 *H. grandella* eggs demonstrated the preference of the parasite for fresh eggs. The female egg parasite could be seen walking over 1–day and 2–days old eggs to reach and oviposite in the fresh *H. grandella* eggs. This is in agreement with the records of Clausen (1), Hidalgo–Salvatierra and Madrigal (6).

* Personal communication.

TABLE 1. Development of *Thrichogramma semifumatum* in *Hypsipyla grandella* eggs. The most important observable changes in 50 parasitized eggs are recorded in hours after parasitization; parasitization took place between 15:30 and 16:30 h.

| Time lapse after parasitization | Observations |
|---|---|
| 29 h | Yellowish "cells" have become visible within red colored *H. grandella* eggs. One or two dents can be noticed in the egg shell. |
| 38 h | Net like concentration of red pigment in eggs. |
| 45 h | Walls between developing *Trichogramma* visible; red pigment concentrated near walls. |
| 51 h | Further delineation of walls within *H. grandella* eggs. Red pigment is concentrated near the walls and edges of the parasitized egg. |
| 68 h | Concentration of red pigment in the developing embryos (reverse situation of the records made from 29 h – 51 h after parasitization). Developing *Trichogramma* can be easily counted now. |
| 72 h | Individual humps for each *Trichogramma* visible. Walls are yellowish colored, red pigment inside. |
| 75 h | Eggs are turning reddish–black; walls are still yellowish colored. |
| 78 h | A few eggs have turned black. |
| 88 h | Majority of the eggs are black. |
| 93 h | All eggs are completely black now. No further changes noticed until emergence. |
| 232 h | First *Trichogramma emerged.* Cleaned antenae and unfolded their wings employing last pair of legs. Emergence took approximately 10 minutes; cleaning of antennae and unfolding of wings also. |

Parasitization of fresh (1–14 h old) 1–day old eggs, age between 26–38 h, was 100 per cent succesful, *i.e.* larval development of *H. grandella* was halted completely whereas parasitization of the 2–days old eggs, age between 50–62 h, still resulted in hatching of 30 per cent of the *H. grandella* larvae.

Superparasitism was observed frequently on fresh and 1–day old eggs, but to a far lesser degree on the 2–days old *H. grandella* eggs.

During the 11–month period in which approximately 33 successive generations of *T. semifumatum* were reared, observations were made on egg development, emergence and behavior of the parasite. Table 1 represents the changes noticed during the pre–emergence period. The number of developing *Trichogramma* per egg was determined on 128 eggs which had been exposed for a period of 24 hours to 79 males and 79 females of this parasite.

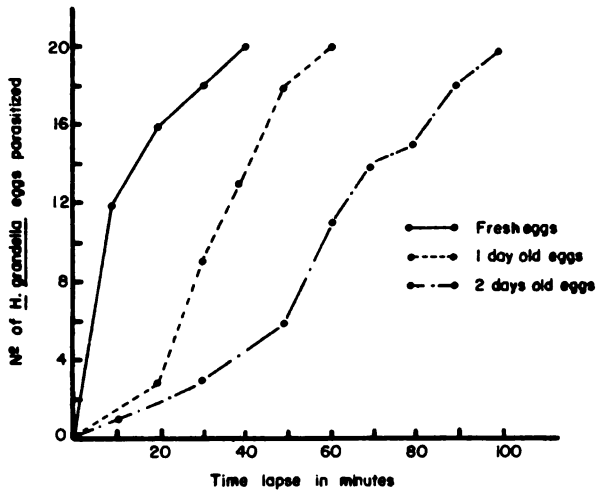



Fig. 2. Time needed for parasitization of 20 fresh, 20 one day, and 20 two days old eggs of *Hypsipyla grandella* distributed randomly on 9 $cm^2$ towel paper and exposed simultaneously to 20 female *Trichogramma semifumatum* (parasitization accumulated in period of 10 minutes).

A counting of developing embryos of the parasite per *H. grandella* egg, 3 days after initiation of the experiment resulted in 11 eggs containing 1 embryo; 62 eggs containing 2; 36 eggs containing 3; 13 eggs containing 4, and 6 eggs containing 5 developing embryos.

The majority of the adults emerged through one exit. Of 1918 parasitized eggs, 1762 or 91.9 per cent used the same exit hole; 151 eggs or 7.9 per cent had two exit holes and only 5 eggs or 0.2 per cent had three exit holes. Adults when emerging, faced away from the oviposition stratum and were either of the same sex, only males or females, or of mixed sex. All three possibilities were observed during the rearing period. On a number of occasions male parasites could be observed sitting on top of parasitized eggs from which *Trichogramma* were emerging. Mating or mating attempts by these males, took place as soon as a female or male had actually emerged. Occasionally attempted oviposition on empty chorions from which first instar *H. grandella* larvae had already hatched was observed. Form and texture of eggs are probably important factors for parasitization by the female *Trichogramma*. Parasitization behavior is similar to that described by other authors (1, 2, 3).

Determination of the sex of 1742 *T. semifumatum* adults emerging in 8 polystyrene boxes indicated a sex ratio of approximately 1:1 (877 ♂♂ versus 865 ♀♀).

Parasitization of unfertilized *H. grandella* eggs took place any time they were offered. However, the majority of these *H. grandella* eggs collapsed and dried out within a day.

Nevertheless, several well developed egg parasites were obtained from unfertilized shootborer eggs. It is assumed here that unfertilized *H. grandella* eggs are permeable to water and will dry out or swell depending on the availability of water either in the air in the form of vapor or on the surface on which they were oviposited. Succesful parasitization of these *H. grandella* eggs may depend on the time needed by the parasite to form the inner layer (1), or may take place in those cases in which the availability of water in the air or on the substrate is enough to permit the normal formation of the inner layer of the parasite in the *H. grandella* eggs. Once this layer has been formed, protection of the egg against changes in its water balance may have been secured and normal developmment of the parasite may take place.

Some evidence supporting the assumption of the permeability of unfertilized *H. grandella* eggs to water was obtained by moistening the towel paper on which unfertilized eggs had been oviposited. These eggs swelled and collapsed within 24 hours when they dried. Apparently, fertile *H. grandella* eggs do not dry out and collapse.

Succesfully parasitized unfertilized *H. grandella* eggs are black with a yellowish tinge, indicating that the black color of parasitized eggs is the result of parasitization by *Trichogramma*.

## Acknowledgements

The author wishes to thank Dr. S. Nagarkatti, CIBC, Bangalore, India, for the determination of the *Trichogramma* species and for her valuable comments. Helpful criticism on the content of the paper was also received from Dr. F. D. Bennett, CIBC, Trinidad. The cooperation of Dr. B. D. Burks, USDA Entomology Research Division, Beltsville, Maryland, USA, and Dr. L. De Santis of the Museum of La Plata, Argentina, with respect to the determination of specimens, has also been highly appreciated.

## Literature cited

1. CLAUSEN, C. P. Entomophagous insects. New York, McGraw-Hill, 1940. pp. 106–117.

2. DE BACH, P. Biological control of insect pests and weeds. London, Chapman and Hall Ltd., 1964. 844 p.

3. BAERG, W. J. Introduction to appliced entomology. Minnesota, Burgess Publishing Co. 1951. p 175.

4. CHACON, G. L. Ensayo comparativo de tres métodos de plantación a raíz desnuda de *Toona ciliata* M. Roem. var. *australis* (F.v.M.) C.DC. Tesis Mag.Sci. Turrialba, Costa Rica, Instituto Interamericano de Ciencias Agrícolas, 1972. 56 p.

5. GRIJPMA, P. Studies on the shootborer *Hypsipyla grandella* (Zeller). V. Observations on a rearing technique and on host selection behavior of adults in captivity. Turrialba 21(2):202–213. 1971.

6. HIDALGO-SALVATIERRA, O. and MADRIGAL, L. R. Estudios sobre el barrenador *Hypsipyla grandella* (Zeller). IV. *Trichogramma* sp. parásito de los huevos. Turrialba 20(4):513. 1970.

7. NAGARKATTI, S. and NAGARAJA, H. Redescriptions of some species of *Trichogramma* (Hym, *Trichogrammatidae*), showing the importance of male genitalia as a diagnostic Character. Bulletin of Entomological Research 61:13–31. 1971.

8. SAMANIEGO, A. y STERRINGA, J. T. Estudios sobre el barrenador *Hypsipyla grandella* (Zeller) (Lep., *Pyralidae*). XV. Un nuevo método para obtener oviposisión en cautividad. (In preparation).

# GROWTH OF LARVAE REARED ON A SYNTHETIC DIET*

*O. Hidalgo Salvatierra, F. Berrios***

Physiologist and graduate student
Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica

## COMPENDIO

Se ha encontrado que larvas de *Hypsipyla grandella* (Zeller), criadas con dieta sintética, pueden pasar por siete fases con duración de 28,7 ± 2,4 días, o por seis fases con duración de 26,6 ± 2,8 días. La longitud del cuerpo y la edad de larvas de la misma fase son más variables que el tamaño de la cápsula cefálica. Se recomienda que al seleccionar larvas de una fase dada se use como criterio el tamaño de su cabeza. El factor de Dyar, que relaciona tamaño de cápsula cefálica con edad al tiempo de mudarla, fue como promedio de 1,5. El tamaño de la cabeza no tiene correlación con la longitud del cuerpo.

Los autores

## Introduction

Knowledge of the life cycle of *Hypsipyla grandella* under various experimental conditions is extremely important if it is desired to select the stage of the insect most suitable for the bioassay of insect pathogens.

Ramirez (3) in his preliminary studies of natural populations was able to detect six instars of the insect.

Working with larvae reared on synthetic diet, Grijpma (2) determined the larval and pupal periods of *H. grandella* but did not determine the number and duration of the different stadia.

It is the purpose of this study to follow a group of individually reared larvae, fed on synthetic diet, through their complete life cycle, to determine the number of instars through which they pass, their growth rate, and head capsule size distribution.

## Materials and methods

**Diet**: the larvae used in this experiment were reared on a synthetic diet consisting of the following ingredients: water, 670 ml, cracked soybeans, 53 g; granulated agar, 6.6 g; brewer's yeast, 33 g; wheat germ, 53 g; ascorbic acid, 3.5 g; methyl parahydroxybenzoate, 2.1 g; sorbic acid, 1.1 g; formaldehyde 10% solution, 12.0 ml; Kanamicine, 250 mg; Nutritional Biochemical Co. Vitamin Mixture, 13.0 g; corncob frits, 53 g.

**Rearing**: rearing cells were individually constructed by sandwiching a lamina of formica strips, containing slits, between coverslides which act as viewing windows. The number of strips could be increased during the experiment to allow the growing larvae freedom of movement. The rearing cells simulated to a certain extent, the natural environment of the larvae while still facilitating observation of the time of ecdysis and localization of the head capsule. Desiccation of the diet was prevented by enclosing within a closed plastic box (26 x 19 x 10 cm) a 2.5 cm layer of moist sand covered by a paper towel upon which the cells rested. The diet was changed daily during observation and measurement periods, allowing regulation of the feeding level by larval size.

The experiment was started with 50 newly hatched larvae but only 31 completed the cycle from eclosion to adult emergence. The main problem was escaping of the very young larvae.

**Measurements**: small larvae were measured, after immobilization with ether, with the calibrated micrometer of a microscope. A 12 cm ruler was needed for larger larvae.

---

* Received for publication September 29th, 1972.
This work was supported by the United States Atomic Energy Commission under Contract No. AT(11-1):3217. Publication No. C00-3217-10.
The data presented herein are taken in part from the junior author's thesis presented to the graduate school of IICA as partial fulfillment for the Master of Science degree.

** Berrios' present address: Escuela Nacional de Agricultura y Ganadería, Km. 12, Carretera Norte, Managua, Nicaragua.

TABLE 1. Days from eclosion to ecdysis, pupation and adult emergence.

| Larva No. | Eclosion | 1st ecd. | 2nd ecd. | 3rd ecd. | 4th ecd. | 5th ecd. | 6th ecd. | Pupae | Adults |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 3 | 8 | 15 | 19 | 27 | – | 30 | – |
| 2 | 0 | 3 | 7 | 10 | 13 | 18 | 21 | 29 | 43 |
| 3 | 0 | 3 | 6 | 10 | 13 | 17 | 26 | 28 | 38 |
| 4 | 0 | 3 | 7 | 11 | 14 | 17 | – | 26 | – |
| 5 | 0 | 3 | 7 | 10 | 13 | 17 | 23 | 30 | 42 |
| 6 | 0 | 3 | 6 | 9 | 12 | 17 | 20 | 26 | 36 |
| 7 | 0 | 3 | 7 | 10 | 13 | 17 | – | 26 | 31 |
| 8 | 0 | 3 | 7 | 11 | 16 | 18 | 23 | 29 | 41 |
| 9 | 0 | 3 | 7 | 10 | 13 | 18 | – | 31 | – |
| 10 | 0 | 3 | 7 | 10 | 13 | 17 | 23 | 27 | 38 |
| 11 | 0 | 3 | 7 | 9 | 12 | 15 | 21 | 31 | 43 |
| 12 | 0 | 3 | 9 | 12 | 15 | 18 | 23 | 26 | 44 |
| 13 | 0 | 3 | 7 | 11 | 14 | 18 | – | 27 | 38 |
| 14 | 0 | 3 | 7 | 9 | 12 | 15 | – | 26 | 36 |
| 15 | 0 | 3 | 9 | 17 | 21 | 26 | – | 33 | 44 |
| 16 | 0 | 3 | 7 | 10 | 15 | 18 | – | 26 | 36 |
| 17 | 0 | 3 | 7 | 9 | 12 | 15 | – | 23 | 35 |
| 18 | 0 | 3 | 7 | 9 | 11 | 14 | – | 22 | 35 |
| 19 | 0 | 3 | 6 | 10 | 14 | 16 | 20 | 31 | 43 |
| 20 | 0 | 3 | 6 | 9 | 12 | 16 | 19 | 30 | 42 |
| 21 | 0 | 3 | 6 | 9 | 14 | 18 | – | 28 | 42 |
| 22 | 0 | 3 | 7 | 10 | 14 | 17 | – | 27 | 42 |
| 23 | 0 | 3 | 7 | 10 | 14 | 19 | 24 | 32 | 43 |
| 24 | 0 | 3 | 7 | 11 | 14 | 18 | – | 25 | 36 |
| 25 | 0 | 3 | 6 | 9 | 12 | 16 | – | 26 | 36 |
| 26 | 0 | 3 | 6 | 10 | 14 | 18 | – | 25 | 37 |
| 27 | 0 | 3 | 7 | 10 | 13 | 16 | 19 | 26 | 40 |
| 28 | 0 | 3 | 6 | 9 | 13 | 17 | 22 | 25 | – |
| 29 | 0 | 3 | 8 | 12 | 19 | – | – | 33 | 43 |
| 30 | 0 | 3 | 6 | 10 | 13 | 17 | – | 25 | 36 |
| 31 | 0 | 3 | 6 | 9 | 12 | 17 | 22 | 32 | – |

## Results and discussion

### Larval growth

Data in Table 1 show the lapse of time from eclosion to ecdysis, pupation,* and adult emergence for each of the remaining 31 larvae and indicate that *Hypsipyla* may have from five to seven instars during normal development. More specifically, one larva had five instars, 45 per cent of the larvae had seven instars with a larval period of 28.7 ± 2.4 day and 52 per cent had six instars with a larval period of 26.6 ± 2.8 days. A t–test shows that these means are significantly different at the 5 per cent level ($P < 0.05$) but not at the 2 per cent level ($P < 0.02$).

The lengths of time of each stadium are compared in Table 2. These data indicate that the last stadium of a larva is both the longest and the most variable.

Average length of the larvae at the time of ecdysis is shown in Table 3. The growth of each larva was modeled with the logistic function (equation 1), and the parameters were

$$L = \frac{B_0}{1 + B_1 e^{-B_2 t}} \quad [1]$$

calculated using an IBM–1130–8K research computer. From these data a theoretical growth curve was drawn in Figure 1, and shows that older larvae are more variable in size than younger larvae. If we compare the observed average length with the theoretical average length at the time of ecdysis, as shown in Table 3, we find excellent agreement, indicating that the logistic function describes the growth of *H. grandella* larvae.

### Head capsule size

The head capsules were measured very accurately to one hundredth of a millimeter on each day of ecdysis. The average width and average length are also shown in

* A larva completely covered by a silk case was considered a pupa.

TABLE 2. Average length, in days, and standard deviation of the six of seven different stadia of *Hypsipyla grandella* (Zeller). Roman numerals indicate ecdysis number.

| Instar | Stadium | $\bar{x}$ | s |
|---|---|---|---|
| 1 | Eclosion – I | 3.0 | 0.0 |
| 2 | I – II | 3.9 | 0.8 |
| 3 | II – III | 3.5 | 1.3 |
| 4 | III – IV | 3.3 | 0.9 |
| 5 | IV – V | 3.9 | 1.2 |
| 6 | V – Pupation | 8.5 | 2.2 |
| 6 | V – VI | 4.9 | 1.5 |
| 7 | VI – Pupation | 6.9 | 2.9 |

TABLE 3. Average measured body length (MBL), theoretical body length (TBL); head capsule width (HCW), head capsule length (HCL), and standard deviations (in mm) of *Hypsipyla grandella* larvae immediately after ecdysis.

| Ecdysis number | $\overline{MBL}$ | s | TBL | $\overline{HCW}$ | s | $\overline{HCL}$ | s |
|---|---|---|---|---|---|---|---|
| I | 2.39 | 0.01 | 2.4 | 0.28 | 0.01 | * | * |
| II | 4.62 | 0.75 | 4.6 | 0.44 | 0.03 | 0.37 | 0.03 |
| III | 7.60 | 1.90 | 7.4 | 0.73 | 0.06 | 0.63 | 0.07 |
| IV | 10.90 | 1.60 | 11.0 | 1.05 | 0.12 | 0.91 | 0.09 |
| V | 15.6 | 2.20 | 15.4 | 1.73 | 0.14 | 1.42 | 0.17 |
| VI | 20.3 | 3.10 | 20.3 | 2.18 | 0.16 | 1.80 | 0.15 |

* Not available

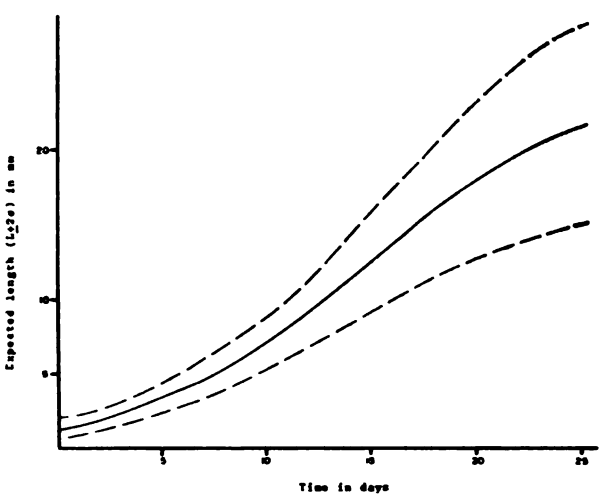



Fig. 1. Theoretical growth rate, and 96.4 per cent confidence band, of *Hypsipyla grandella* larvae.

Table 3. The standard deviations indicate that the variability in head capsule size is greater in the older instars than in the younger ones.

The average head capsule width or length, as a function of average time of ecdysis, is plotted in Figures 2 and 3, and show linear correlation. In both cases we calculated an average Dyar's factor (1) of 1.5. There was no correlation, however, between head capsule size and larval size.

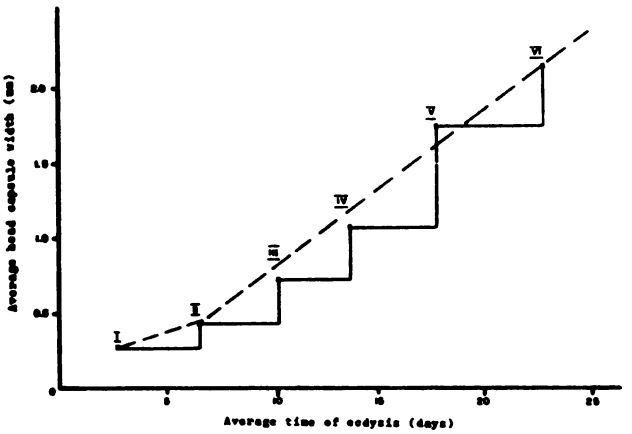



Fig. 2. Correlation between average time of ecdysis and average head capsule width.

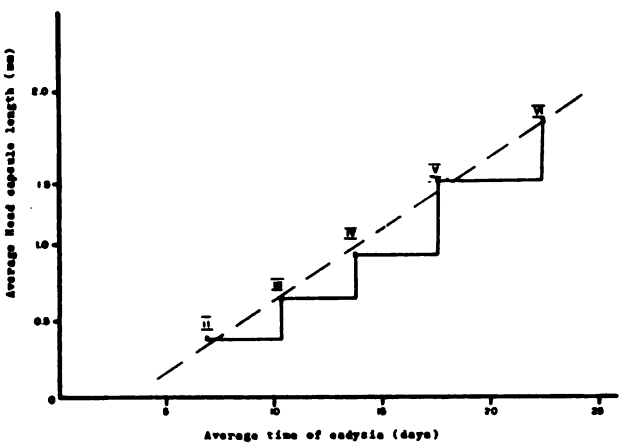



Fig. 3. Correlation between average time of ecdysis and average head capsule length.

## Conclusions

This study shows that both the size of a larval instar and the period of its stadium are highly variable. Therefore, it is more reliable to select a larva of a given instar by looking at its head size than at its body length or age.

It is also shown that in a given population of larvae reared under the same conditions the number of total instars may range from 5 to 7. This finding points out that it may be difficult to determine the number of instars of an insect by solely taking head capsule measurements of a number of insects collected at random in the field.

## Acknowledgement

The cooperation of the Department of Forestry Sciences of IICA–CTEI is deeply appreciated.

## References

1. GHENT, A. W. Linear increment in width of the head capsule of two species of sawflies. Canadian Entomologist 88:17–23. 1956.

2. GRIJPMA, P. Studies on the shootborer *Hypsipyla grandella* Zeller. V. Observations on a rearing technique and on host selection behavior of adults in captivity. Turrialba 21(2):202–213. 1971.

3. RAMIREZ, J. Investigación preliminar sobre biología, ecología y control de *Hypsipyla grandella* Zeller. Boletín del Instituto Forestal Latinoamericano de Investigación y Capacitación (Venezuela) 16:55–17. 1964.

# DETERMINATION OF THE $LC_{50}$ OF *METARRHIZIUM ANISOPLIAE* (METCHNIKOFF) SOROKIN SPORES ON FIFTH INSTAR LARVAE*

*O. Hidalgo–Salvatierra, F. Berrios***

Physiologist and graduate student, respectively

## COMPENDIO

Se ha determinado que la quinta fase larval de *H. grandella* (Zeller) es la más susceptible al hongo *M. anisopliae*. En la evaluación del índice de patogenicidad de este hongo, usando solamente larvas de quinta fase, se encontró que la concentración que mata al 50 por ciento de la población tratada fue de $3,6 \times 10^7$ esporas viables por mililitro, cuando las larvas son bañadas en la suspensión por un minuto.

Los autores

## Introduction

The importance of microorganisms as natural insect control agents has received limited consideration in Latin America. Some countries are field testing commercial bacterial and viral preparations, but rely heavily on the manufacturer's instructions and data. Few people are working on such basic aspects as isolation, identification and biology of insect pathogens. But the evaluation of a pathogen in terms of its pathogenicity index, host range, most susceptible insect stage, resistance to weathering conditions, mutability, and a spectrum of other considerations that help to decide whether a pathogen could be useful or not, are aspects that are practically forgotten (7).

*Metarrhizium anisopliae* has been isolated from many species of insects (8). It was one of the more pathogenic fungi, along with *Beauveria bassiana*, isolated by Brooks and Raum (2) from several corn insect pests, using the European corn borer, *Ostrinia nubilalis*, as test insect. *M. anisopliae* has been used with success in Brazil, in the integrated control of the sugarcane froghoppers (8).

The potency of a pathogen varies according to the test insect used and the stage of the insect used for the bioassay (6, 9). Thus, any program dealing with the selection of more pathogenic strains must standardize its biological assay in order to obtain meaningful results. In the present case, we have selected *Hypsipyla grandella* (4) as our test insect, which was shown to be susceptible to *M. anisopliae* (1). The purpose of this investigation was to determine the most susceptible instar to the pathogen, and to quantify the effect in terms of the lethal concentration that kills 50 per cent ($LC_{50}$) of the treated population.

## Materials and methods

*H. grandella* larvae were supplied by the mass rearing laboratory of the Forestry Department of the Tropical Training and Research Center of the Inter–American Institute of Agricultural Sciences. The different instars were separated by head capsule size (5).

The fungus was grown on Sabouraud Dextrose Agar plus yeast extract, and the spores collected as powder.

To prepare a spore suspension, five grams of spores were added to 50 cc of sterile water plus one drop of sterile Triton–X–100, and dispersed for 2 minutes with a Sorval Omnimixer. The concentration of total spores was determined with a hemocytometer, and viable spores by dilution and plating.

Inoculation was performed in small groups either by spraying or submerging the larvae for one minute in the spore suspension. Mortality was calculated dividing the number of larvae killed by the fungus by the number of live larvae and pupae in the control (1).

For the determination of the most susceptible instar we used 63 larvae of each instar and inoculated them by spraying with a suspension of $9.2 \times 10^7$ viable spores/ml. Sixth and seventh instars were grouped together because not all larvae completed the seven stages. An equal number of larvae of each instar was used as control.

* Received for publication October 3rd, 1972.
This work was supported by the United States Atomic Energy Commission under Contract AT(11–1)–3217. Publication No. C00–3217–08.
The data presented herein are taken in part from the junior author's thesis presented to the Graduate School of IICA as a partial fulfillment for the Master of Science degree.

** Berrios' present address is: Escuela Nacional de Agricultura y Ganadería, Km. 12, Carrera Norte, Managua, Nicaragua.

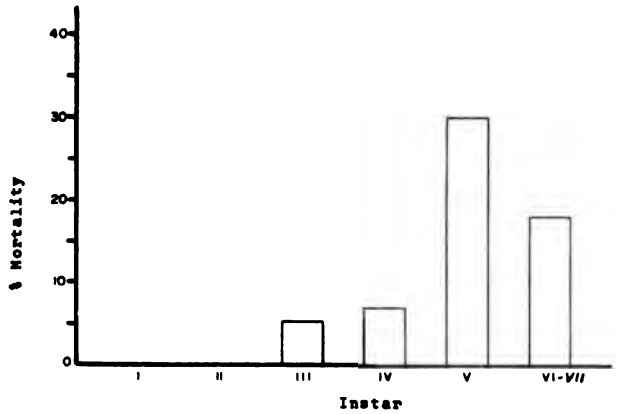



Fig. 1. Susceptibility of the different instars of *Hypsipyla grandella* sprayed with a spore suspension of $9.2 \times 10^7$ viable spores per ml.

TABLE 1. Mortality of 5th instar *Hypsipyla grandella* larvae 15 days after immersion in a suspension of *Metarrhizium anisopliae*. Number of larvae per treatment: 99.

| Viable spores per mL | Total dead larvae | No. of larvae killed by fungus | Lost larvae | Live larvae | Live pupae | % mortality | Probit |
|---|---|---|---|---|---|---|---|
| 0 | 1 | 0 | 3 | 68 | 27 | 0 | – |
| $0.39 \times 10^7$ | 25 | 25 | 2 | 59 | 13 | 28.7 | 4.43 |
| $0.89 \times 10^7$ | 22 | 22 | 10 | 53 | 14 | 23.4 | 4.27 |
| $1.59 \times 10^7$ | 41 | 40 | 6 | 24 | 28 | 42.6 | 4.81 |
| $1.59 \times 10^8$ | 56 | 56 | 1 | 22 | 20 | 59.6 | 5.24 |
| $6.19 \times 10^8$ | 78 | 78 | 2 | 4 | 15 | 82.8 | 5.95 |

## Results and discussion

The results of the most susceptible instar are shown in Figure 1. It can be seen that the fifth instar gives the highest mortality, 30 per cent, followed by the sixth and seventh instars with 17.8 per cent, and then the fourth and third instars with 7.0 and 5.3 per cent, respectively.

We attempted to induce the disease in first and second instar larvae in several independent trials, but were unsuccessful except for one larva of the 2nd instar which was attacked in one trial, (Figure 2). Thus the first instar is resistant, whereas the second instar shows a very small degree of susceptibility.

We inoculated by immersion and used only 5th instar larvae to determine the $LC_{50}$ of *M. anisopliae*. The results are tabulated in Table 1. We can see that mortality is a function of the concentration of spores in the inoculum. A plot of percent mortality on a probabilistic scale or probit units on a linear scale against concentration of spores on a logarithmic scale, Figure 3, gives a linear function from which we obtain the $LC_{25}$, $LC_{50}$ and $LC_{75}$. Table 2 gives the $5.4 \times 10^6$, $3.6 \times 10^7$ and $2.7 \times 10^8$ values for $LC_{25}$, $LC_{50}$ and $LC_{75}$, respectively, plus their fiducial limits at the 95 per cent level.

Comparing the results of Figure 1 against the results of Figure 3, we can see that, at a concentration of $9.2 \times 10^7$ viable spores per ml, for fifth instar larvae, inoculation by immersion gives a higher mortality, 63 per cent, than inoculation by spraying, 30 per cent.


Fig. 2. Different instars of *Hypsipyla grandella* covered with spores of *Metarrhizium anisopliae*.

## Summary

This study shows that the fifth instar of *H. grandella* Zeller larvae is the most susceptible to infection by spores of *M. anisopliae*, followed by the sixth and seventh instars. We were unable to obtain infection of the first instar under our experimental conditions.

When fifth instar larvae were bathed for one minute with a spore suspension, the concentration that killed fifty per cent of the treated population was $3.6 \times 10^7$ viable spores per milliliter.

TABLE 2. Concentration of *Metarrhizium anisopliae* viable spores per ml required to induce 25, 50 and 75 per cent mortality of 5th instar larvae *H. grandella* when these are bathed in the suspension during one minute.

| Per cent mortality | Viable spores per ml. | 95% fiducial limits | |
|---|---|---|---|
| | | Higher | Lower |
| 25 | $5.4 \times 10^6$ | $7.2 \times 10^6$ | $2.9 \times 10^6$ |
| 50 | $3.6 \times 10^7$ | $7.5 \times 10^7$ | $2.3 \times 10^7$ |
| 75 | $2.7 \times 10^8$ | $7.7 \times 10^2$ | $2.0 \times 10^8$ |

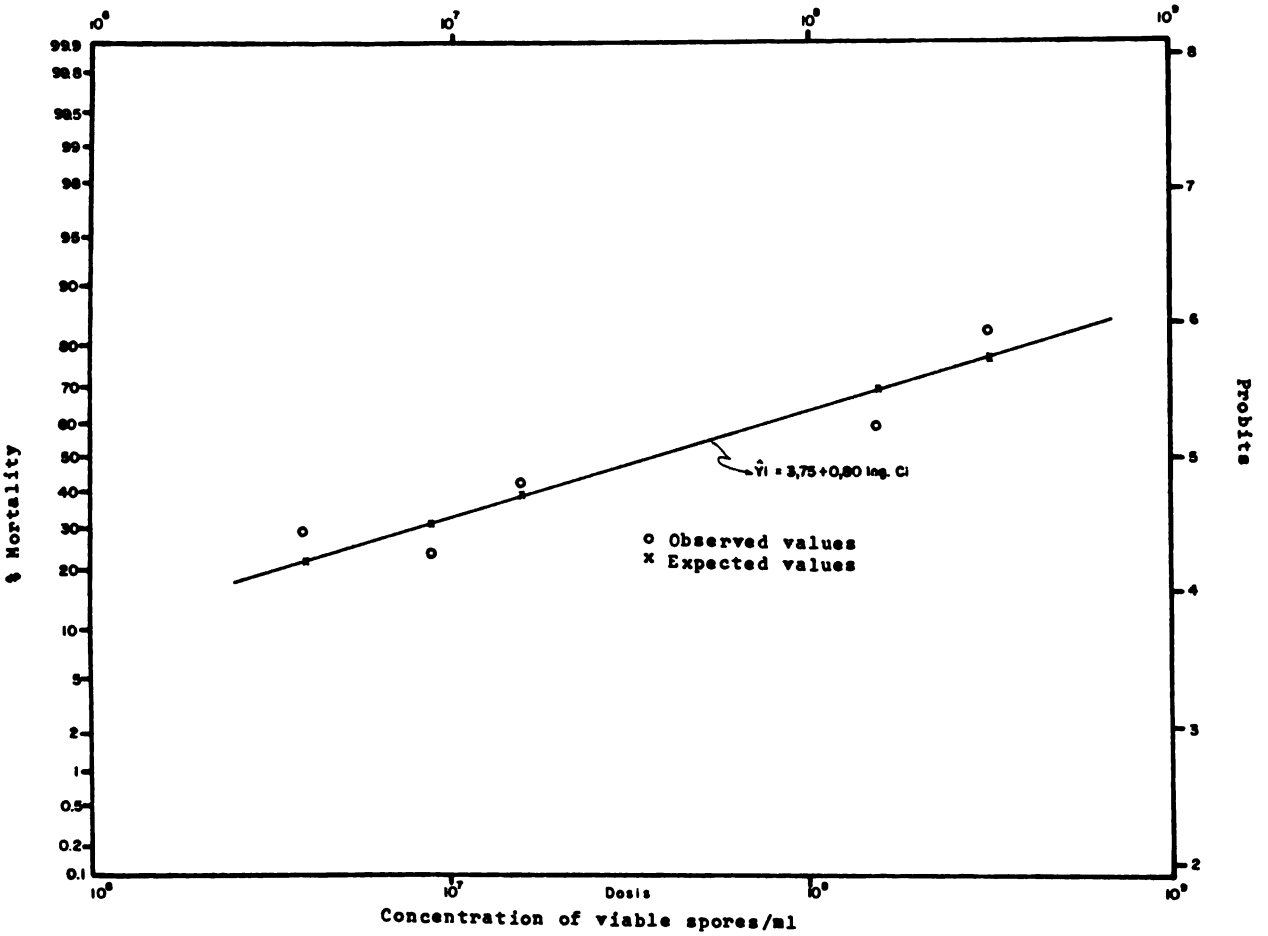



Fig. 3. Survival of 5th instar *Hypsipyla grandella* larvae bathed for one minute with different concentrations of spores of *Metarrhizium anisopliae.*

Ci: concentration of viable spores/ml x $10^6$
Y: Probits unit

## Acknowledgement

The cooperation of Mr. Pieter Grijpma is appreciated.

## Literature cited

1. BERRIOS, F. and HIDALGO SALVATIERRA, O. Estudios sobre el barrenador *Hypsipyla grandella* (Zeller). VI. Susceptibilidad de la larfa al hongo *Metarrhizium anisopliae* (Metch) Sor. Turrialba 21(2):214–219. 1971.

2. BROOKS, D. L., and RAUN, E. S. Entomogenous fungi from corn insects in Iowa. Journal of Invertebrate Pathology 7(1):84–81. 1965.

3. GUAGLIUMI, P. Lucha integrada contra las 'cigarrinhas' (Hompft:Cercopidae) en el noroeste del Brasil. Revista Peruana de Entomología 14(2):361–368. 1971.

4. GRIJPMA, P. Studies on the shootborrer *Hypsipyla grandella* (Zeller).V. Observations on a rearing technique and on host selection behavior of adults in captivity. Turrialba 21(2):202–213. 1971.

5. HIDALGO–SALVATIERRA, O. and BERRIOS F. Studies on the shootborer *Hypsipyla grandella* (Zeller). XI Growth of larvae on synthetic diet. Turrialba 22(4):431–434. 1972.

6. KILGORE, W. W. Pest control: biological, physical, and selected chemical methods. New York, Academic Press, 1967. 474 p.

7. KUNO, G. Regional correspondent for the Society of Invertebrate Pathology. Newsletter, April, 1972.

8. STEINHAUS, E. A. Fungus infection. *In* Principles of insect pathology. New York, McGraw–Hill, 1942. pp. 318–398.

9. ZACHARUK, R. Y. and TINLINE, R. D. Pathogenicity of *M. anisopliae* and other fungi for five elaterids(Coleoptera)in Saskatchewan, Journal of Invertebrate Pathology 12(3):294–309. 1968.

# METHODS FOR BREEDING PARASITES FOR RELEASE AGAINST *HYPSIPYLA* SPP. IN LATIN AMERICA*

*M. Yaseen, F. D. Bennett*

West Indian Station
Commonwealth Institute of Biological Control, Gordon Street, Curepe, Trinidad

## SUMARIO

Se describen métodos para la crianza en laboratorio del hospedante secundario *Corcyra cephalonica* y los parásitos *Apanteles* spp., *Phanerotoma* sp., *Flavopimpla latiannulata, Tetrastichus spirabilis, Antrocephalus renalis* y *Trichogrammatoidea nana,* todos parásitos de *Hypsipyla robusta* Moore de la India, introducidos por el Instituto para Control Biológico de la Comunidad Británica, en Trinidad. Además se incluyen datos sobre el ciclo de vida y la parasitación del hospedante por los parásitos. Finalmente se indica la manera más apropiada para el envío de estos parásitos a otros países.

## Introduction

In connection with a project sponsored by the Overseas Development Administration of the United Kingdom for the biological control of *Hypsipyla* spp. (*H. grandella* (Zeller) and *H. ferralis* Hmps.) attacking Meliaceous trees, mahogany, cedar and carapa in the Lesser Antilles and British Honduras and a similar project financed by the Trinidad Government, several species of parasites –*Apanteles leptoura* Cam., *Apanteles* sp. *puera* group, *A.* sp. *vitripennis* group, *Phanerotoma* sp. (Braconidae), *Flavopimpla latiannulata* Cam. (Ichneumonidae), *Tetrastichus spirabilis* Wtston. (Eulophidae), *Antrocephalus renalis* Wtston., (Chalcididae) and *Trichogrammatoidea nana* (Zehntner) (Trichogrammatidae)– which attack *Hypsipyla robusta* Moore in India were obtained from the Indian Station, Commonwealth Institute of Biological Control (CIBB), and have been released in the various territories over the past 3 to 4 years (1). From nucleous cultures some of these parasites have been massreared in Trinidad at the W. I. Station, CIBC, and the breeding techniques for others have been worked out at the Indian Station. As the Netherlands Bureau for International Technical Assistance has provided funds to CIBC to breed and ship nucleus cultures of certain of these parasites to centres in Latin America to be designated by Pieter Grijpma, Coordinator, Inter–American Working Group on *Hypsipyla,* the following notes on breeding techniques of the parasites have been prepared. As certain species of the *Hypsipyla* parasites can be readily bred on the stored products moth *Corcyra cephalonica* Staint, the method utilized for culturing this species is given first.

* Received August 10, 1972

## Methods for breeding *Corcyra cephalonica* in the laboratory

To initiate a culture of this stored products moth which we have used successfully to breed the egg parasite *Trichogrammatoidea nana* and the egg–larval parasite *Phanerotoma* sp., adults collected from a nearby feed mill were confined in a 6" diameter 5" deep plastic dish (Fig. 1). The eggs which are laid on the bottom of the dish or adhere lightly to the sides and top are readily dislodged by means of a camel hair brush whereupon they are transferred to other plastic dishes which contain around ½ lb of corn meal, 300–500 per dish and have ventilated tops. These may be left undisturbed until the adults of the next generation begin to emerge or the contents of several containers transferred to a large sleeve cage *i.e.* a 3' x 1½' wooden boxlike structure with a glass top, two cloth sleeves fitted to 6" diameter circular holes in the front of the cage –which permit the entry of the hands and collecting equipment,– and a cloth panel for ventilation let into the back of the cage (Figs. 2, 3). The moths are collected daily and placed in the plastic oviposition dish and the cycle repeated. Food in the form of honey droplets on strips of wax paper is provided as a precautionary measure although moths will oviposit in the absence of this food.

The life cycle of *C. cephalonica* ranges from five to six weeks dependent on the diet and temperature: the egg stage is usually less than 48 hours, the larval stage about four weeks and the pupal stage 7–10 days.

Once cultures of *C. cephalonica* and the parasites being reared on it are well established, all eggs are glued onto 3" x 1" cardboard strips and these egg cards exposed to the parasites. In the cultures of *Phanerotoma*

sp. (see below) after the parasites emerge the unparasitised individuals are allowed to develop and the contents of the rearing dishes containing these are transferred to the sleeve cage from which the adults of *C. cephalonica* are removed daily as they emerge.

Fig. 1. Adults of *Corcyra cephalonica* confined in a plastic dish for oviposition.

Fig. 2. Cage for rearing *Corcyra cephalonica* (front view).

## Methods for rearing *Hypsipyla* parasites

a) ***Apanteles*** spp.

All of the *Apanteles* parasites of *Hypsipyla* mentioned in the introduction are larval parasites and attack young, preferably second instar, larvae. The adults mate readily after emergence and the females usually commence oviposition the following day. For laboratory breeding 8–10 mated females are kept in a 4" x 1" glass vial and provided with honey droplets on a strip of wax paper. A second–instar larva held on the tip of a camel–hair brush is introduced into the vial. As soon as a parasite female locates the larva it inserts its ovipositor and deposits eggs within the host. In the gregarious species several eggs may be laid during each attack.

As soon as it is parasitised each larva is removed and fresh larvae are successively introduced into the vial for stinging. The parasitised larvae are placed in small artificially constructed tunnels in succulent portions of cedar or mahogany shoots and kept individually in glass vials stoppered with cotton wool. The shoots are replaced as they become completely tunnelled usually every 3rd or 4th day. The larvae usually stop feeding 8–10 days after parasitisation. The parasite larvae emerge from the host body about 2 weeks after oviposition and spin cocoons. The adults which emerge about 7–10 days later usually live for about 2 weeks.

Fig. 3. Cage for rearing *Corcyra cephalonica* (rear view).

Fig. 4. *Phanerotoma* sp. ovipositing in eggs of *Corcyra cephalonica* glued to index card, placed in a plastic dish containing corn meal.

b) *Phanerotoma* sp.

This braconid is a solitary egg–larval parasite. Mating takes place readily in captivity, frequently within 24 hours of emergence and females commence oviposition (in the host egg) shortly thereafter.

For mass–production eggs of *C. cephalonica* are glued onto strips of index cards about 500 per 2" x 3" strip by lightly coating it with an adhesive paste and sprinkling the eggs onto it. Each card is then placed on a half pound of corn meal contained in a plastic dish 5" deep and 6" in diameter (Fig. 4). The lid of the dish contains two 1" diameter circular holes one being covered with a fine mesh to permit ventilation and the other, once the parasite adults have been added and a strip of a paper covered with honey droplets partially inserted, being closed by a cork. Usually 10 mated female parasites as well as 10 males are placed in a container for 24 hours and then transferred to another dish containing fresh eggs.

Alternatively the successive cards of eggs can be exposed in an empty container and transferred daily to dishes containing corn meal.

The *Corcyra* eggs hatch normally and the larvae after completing development on the corn meal spin their pupation chambers whereupon they are killed by the developing *Phanerotoma* larvae which in turn spin their cocoons within these chambers. On this host, egg–larval development of the parasite is 20–22 days, and the pupal stage 7–8 days. If circles of corrugated cardboard are placed on the corn meal about 15 days after parasitised eggs are added, many of the *Corcyra* larvae both parasitised and unparasitised leave the corn meal, and spin their sheltering webs between the corrugations. Parasite cocoons can be obtained in this manner for shipment to other countries.

c) *Flavopimpla latiannulata*

This ectoparasitic ichneumonid has not been mass–bred in Trinidad, although preliminary experiments utilizing the following technique developed at the Indian Station, CIBC indicated that *H. grandella* is a suitable host.

Small pieces of meliaceous shoots about 4" long are split longitudinally. A small depression just large enough to contain a larva is made on the split surface of one half and a minute aperture of about 1 mm in diameter is drilled in the other half opposite to the depression in the first. A fourth–instar host larva is placed in the depression and the two halves of the shoot are fitted together and held in position by rubber bands. About 5 such pieces each containing a larva are placed in a wooden framed 1' x 1' x 1' screened rearing cage with sliding glass or perspex front. Mated parasite females are then introduced through a cotton sleeve let into one side of the cage and provided with honey. As soon as a searching female senses the presence of a larva she inserts the ovipositor through the hole drilled earlier, paralyses the host larva and deposits an egg on or near it. The whole process takes about 10–15 minutes. If a larva is exposed for prolonged periods several eggs are sometimes deposited on it. As only one parasite per host develops, additional eggs can be removed with the aid of a fine brush and placed on other larvae paralysed by the female or on larvae placed in stems after they have been paralysed by dipping them briefly in hot water. The hatching parasite larva feeds externally and after consuming most of its host pupates within the shoot.

d) *Tetrastichus spirabilis*

This eulophid is a gregarious endoparasite developing in the host pupae. For lab rearing several emerging adults are kept in glass vials provided with honey on wax paper as food and allowed to mate. Females oviposit a day afterwards. About 8–10 host pupae, within their cocoons, are glued to a strip of index card about 3½" x 1" in size and placed in a 4" x 1" glass or plastic vial (Fig. 5). Pupae of *Hypsipyla* are seldom attacked unless they are within their cocoons. About 10 mated females with about as many males (for repeated mating) are introduced in the vial. The parasites are provided with honey applied to a small strip of wax paper and the tube is plugged with cotton and left for two days to allow the females time to oviposit, after which the strip containing the pupae is replaced with a fresh one. The parasitized pupae are kept undisturbed in glass vials until the adults emerge 20–21 days later. The species is gregarious and over a hundred adults are frequently produced from a single host pupa. The adults live for about a week. In Trinidad during periods when *Hypsipyla* spp. are scarce in the field, especially in the early part of the dry season, *T. spirabilis* has been successfully bred on pupae of the cassia pod–borer *Trachylepidia fructicassiella* Rag. Again these have to be exposed within their cocoons.

Fig. 5. *Tetrastichus spirabilis* ovipositing in pupae of *Hypsipyla grandella*.

e) *Antrocephalus renalis*

This chalcid is a solitary endoparasite attacking freshly formed pupae. Mating occurs 3–4 days after emergence. There is a preoviposition period of 4–5 days. Breeding in the laboratory is fairly easy. Freshly formed host pupae and parasite adults are introduced into the 5" x 6" plastic dishes and furnished with honey as described above. Fresh pupae are introduced every 48 hours, development from egg to adult is completed in the host in about 3 weeks, and adults live for 20 days or

longer. The chalcid can also be bred in the laboratory on *Ancylostomia stercorea* (Zeller) and *T. fructicassiella*. These hosts as well as *Hypsipyla* have to be offered to the parasites while still within their cocoons.

f) ***Trichogrammatoidea nana***

This egg parasite of *Hypsipyla* can easily be produced on a large scale on eggs of *C. cephalonica*. Two to three hundred freshly laid eggs glued to a 3½" x 1" size card are killed by exposing them to 27–28°F temperatures for 24 hours. The card is placed in a 4" x 1" rearing vial and about 20 freshly emerged adults including both sexes, are introduced into the tube (Fig. 6). They also are provided with honey droplets on a small strip of wax paper.

Fig. 6. Eggs of *Corcyra cephalonica* stuck on a strip of index card and placed in a plastic vial containing *Trichogrammatoidea nana* adults ready to oviposit.

The exposed eggs are removed after two days and a new strip with sterilized eggs is introduced. The parasite completes development with 8–9 days and the adults live for 4–5 days. For field releases cards of parasitised eggs can be pinned on the leaves or stems of meliaceous trees or alternatively the parasite adults can be released onto the trees.

Other egg parasites, *e.g. Trichogramma* spp. can be mass produced in the same manner.

## General

The methods described require a minimum of specialized equipment and personnel. It should be feasible to streamline and at least to partially automate them if large scale production is contemplated. These techniques while developed for the Indian *Hypsipyla* parasites can also be used or adapted for breeding certain of the Neotropical parasites of *Hypsipyla grandella*. For example the egg parasites *Trichogramma* spp. and *Trichogrammatoidea* sp. have been bred on eggs of *C. cephalonica*. As *C. cephalonica* is cosmopolitan there would not be the same risks involved in sending cultures of *Hypsipyla* egg parasites comprised of live parasite adults dispatched with live eggs of *C. cephalonica* from one geographical region to another as there would be if for example similar cultures containing eggs of *H. grandella* were sent from Central America to Asia. It is now common practice to sterilize the lepidopterous eggs by subjecting them to low temperatures or exposing them to UV light prior to exposing them to *Trichogramma* etc.

For the shipment of species other than trichogrammatids, it is preferable to ship the parasites while they are in the pupal stage when they require no attention, or food. As freshly formed cocoons glued to cardboard strips which are stapled to the side of a 6" x 4" x 3-1/3" wooden box do not emerge for at least a week, it is possible to dispatch material by airmal or iar express to most parts of the world. If food in the form of split raisins, and sponge soaked in diluted honey is added, emerging adults will also survive for several days. Similarly adults of the smaller parasites, *e.g. T. spirabilis* survive for several days in glass or plastic vials when provided with honey droplets as food and if they are kept out of direct sunlight and away from extreme heat or cold. At present CIBC has only three species of the Indian *Hypsipyla* parasites, *Phanerotoma* sp., *Tetrastichus spirabilis* and *Trichogrammatoidea nana* under culture in Trinidad. Other species of *Hypsipyla* parasites can be obtained from India and possibly also from Africa if there is sufficient interest and provided funds to cover the costs of investigations can be obtained.

## Reference

1. BENNETT, F. D., and YASEEN, M. Parasite introduction for the biological control of three insect pest in the Lesser Antilles and British Honduras, PANS 18(4) (in press).

# SUSCEPTIBILITY OF FIRST INSTAR LARVAE TO *BACILLUS THURINGIENSIS**

*O. Hidalgo Salvatierra, J. D. Palm*

Nuclear Energy Program
Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica

## SUMARIO

Esta comunicación muestra que las larvas de la primera fase de *H. grandella* (Zeller) son susceptibles al *Bacillus thuringiensis*.

The use of bacterial pathogens of insects as microbial insecticides has been extensively reviewed by Angus (1, 2), Jaques (5), Heimpel (3) and Rogoff (6). Specifically, *Bacillus thuringiensis* has been tested in many different insect species. However, to the best of our knowledge, no one has tested it against the *Meliaceae* shootborer *Hypsipyla grandella* (Zeller). This communication is a preliminary report on the susceptibility of *H. grandella* to *B. thuringiensis*.

*B. thuringiensis* was kindly supplied by G. Thomas, Insect Pathology Laboratory, University of California, Berkeley, and maintained in nutrient agar slants.

*H. grandella* eggs were supplied by the mass rearing laboratory of the Forest Department of IICA-CTEI. The composition of the insect diet is found in a previous paper (4).

Treatment A (control) consisted of 50 g diet plus 2 g of 2 per cent agar solution plus 50 ml sterile water. Treatment B, 50 g of diet plus 2 g of 2 per cent agar solution plus 50 ml of a bacterial overnigth culture in nutrient broth. Treatment C, D, and E were 1/10, 1/100 and 1/1000 dilutions of B, respectively. Each treatment was tested against 50 first instar larvae individually placed small, tightly closed plastic boxes.

The mortality induced by the contamination of the diet is shown in Table 1. It is clear that *H. grandella* first instar larvae are susceptible to *B. thuringiensis* var. *thuringiensis* and var. *entomocidus*. As expected, the highest concentration of bacteria gave the highest mortality. Microscopic examination of blackish dead larvae revealed the presence of the crystalliferous bacteria. A more detailed investigation is under way to determine the $LC_{50}$ of preparations of different varieties of *B. thuringiensis* to determine the most pathogenic strain against *H. grandella* Zeller.

This work was supported by the U. S. Atomic Energy Commission under Contract AT (11-1)-3217. Publication No. COO-3217-9.

Table 1. Mortality of *Hypsipyla grandella* first instar larvae fed on synthetic diet contaminated with *Bacillus thuringiensis*.

| Bacteria | Treatment | Dead Larvae | Lost Larvae |
|---|---|---|---|
| *B. thuringiensis* var. *thuringiensis* | B | 48 | 5 |
| | C | 24 | 7 |
| | D | 28 | 17 |
| | E | 0 | 2 |
| *B. thuringiensis* var. *entomocidus* | B | 10 | 5 |
| | C | 11 | 11 |
| | D | 5 | 40 |
| | E | 1 | 17 |
| Control | A | 0 | 4 |

## References

1. ANGUS, T. A. Bacterial pathogens of insects as microbial insecticides. Bacteriological Review 29(3):364-372. 1965.

2. _____ . The use of *Bacillus thuringiensis* as a microbial insecticide. World Review of Pest Control 7(1):11-26. 1968.

3. HEIMPEL, A. M. A critical review of *Bacillus thuringiensis* var. *thuringiensis* Berliner and other crystalliferous bacteria. Annual Review of Entomology 12:287-329. 1967.

4. HIDALGO-SALVATIERRA, O. and BERRIOS, F. Studies on the shootborer *Hypsipyla grandella* (Zeller). XI. Growth of larvae on synthetic diet. Turrialba 22(4):431-434. 1972.

5. JAQUES, R. P. Insect control by bacterial pathogens. Annals of the Entomological Society of Quebec 9:17-29. 1974.

6. ROGOFF, M. N. Crystal forming bacteria as insect pathogens. Advances in Applied Microbiology 8:291-313. 1966.

* Received October 13, 1972.

# REVERSAL OF THE LIGHT–DARK CYCLE IN RELATION TO ADULT EMERGENCE UNDER LABORATORY CONDITIONS*

*E. H. Holsten and R. I. Gara*

Department of Tropical Forest Sciences
Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica

College of Forest Resources, University of Washington
Seattle, Washington, U.S.A. (respectively)

## SUMARIO

Se describe una técnica para obtener emergencia de adultos de *Hypsipyla grandella* en las horas de la mañana. Se obtuvo el 100 por ciento de emergencia de adultos, cambiando en las pupas el período de luz por dos o más días.

*Hypsipyla grandella* is a serious problem in the establishment of *Cedrela* spp. and *Swietenia* spp. plantations in the American tropics. Accordingly, much time and effort is being devoted to research on the biology and possible control measures of this insect pest. Laboratory studies of adult behavior have to date been limited to evening hours. Although night time studies are, at times, an inconvenience to the investigator, they are necessary due to the nocturnal behavior of the adult insect.

It is fairly common practice to observe and study nocturnal animals during daylight hours by means of a shift in the light–dark cycle. Fatzinger and Asher (1), in their studies of the mating behavior and pheromone attraction of *Dioryctria abietella* (Lep.:Pyralidae), pre-conditioned adult moths to shifted 12 hour–light and 12 hour–dark (12L:12D) cycles by conditioning the pupal stage. Grijpma** in a preliminary trial obtained adult emergence of *H. grandella* during early morning hours by rearing first instar larvae to adults under a shifted 12L:12D cycle.

Ramírez–Sánchez (4) has described partially the biology of *H. grandella*. Adult insects have been found to be most active during the evening hours. Under laboratory conditions, Sliwa and Becker (5) determined adult emergence to be at it's maximum between 1800–1900 hours, when light conditions approach zero lux. *H. grandella* females assume a "calling–position" at approximately 0100–0200 hours and mating occurs within one to two hours. It is of importance in this study to note that the pupal stage, under laboratory conditions, is approximately ten days (2).

Many additional tests on mating behavior and pheromone production of *H. grandella* could be initiated throughout the day, if adult emergence could be obtained at predicated intervals. Consequently, the objective of this study was to evaluate the possibility of obtaining adult emergence in morning hours by a twelve hour shift in the light–dark cycle.

## Materials and methods

Using a General Electric "Precision Scientific" incubator (Model 805), a 12L:12D cycle was set as follows: 0800–2000 hours:dark and 2000–0800 hours:light. Temperature was set at 25°C and remained constant throughout the cycle. Relative humidity, regulated by means of a water bath, fluctuated between 75 and 85 per cent. Pupae were obtained from larvae reared on artificial diet under laboratory conditions. Three tests, using different aged pupae, were simultaneously run. Test 1 consisted of ten pupae (five males and five females) placed in the incubator after four days of pupal age. Tests 2 and 3 likewise contained ten pupae, five of each sex, each placed in the incubator after five and six days respectively of pupal stage. Starting with the seventh day of the pupal stage, observations of adult emergence were taken between 0600–0800 hours, 0800–1000 hours, 1800–2000 hours, and 2000–2200 hours. The mating behavior of the emerged adults, kept under the same 12L:12D cycle as the pupae, was observed in the afternoon.

At a later date, 118 pupae (56 males and 62 females) were placed in the incubator under the 12L:12D conditions described above. Pupae, 1, 2, 3, 7, 8, and 9 days of age, were tested.

* Received por publication, January 1st, 1973.
** 1972. Personal communication, Grijpma P.

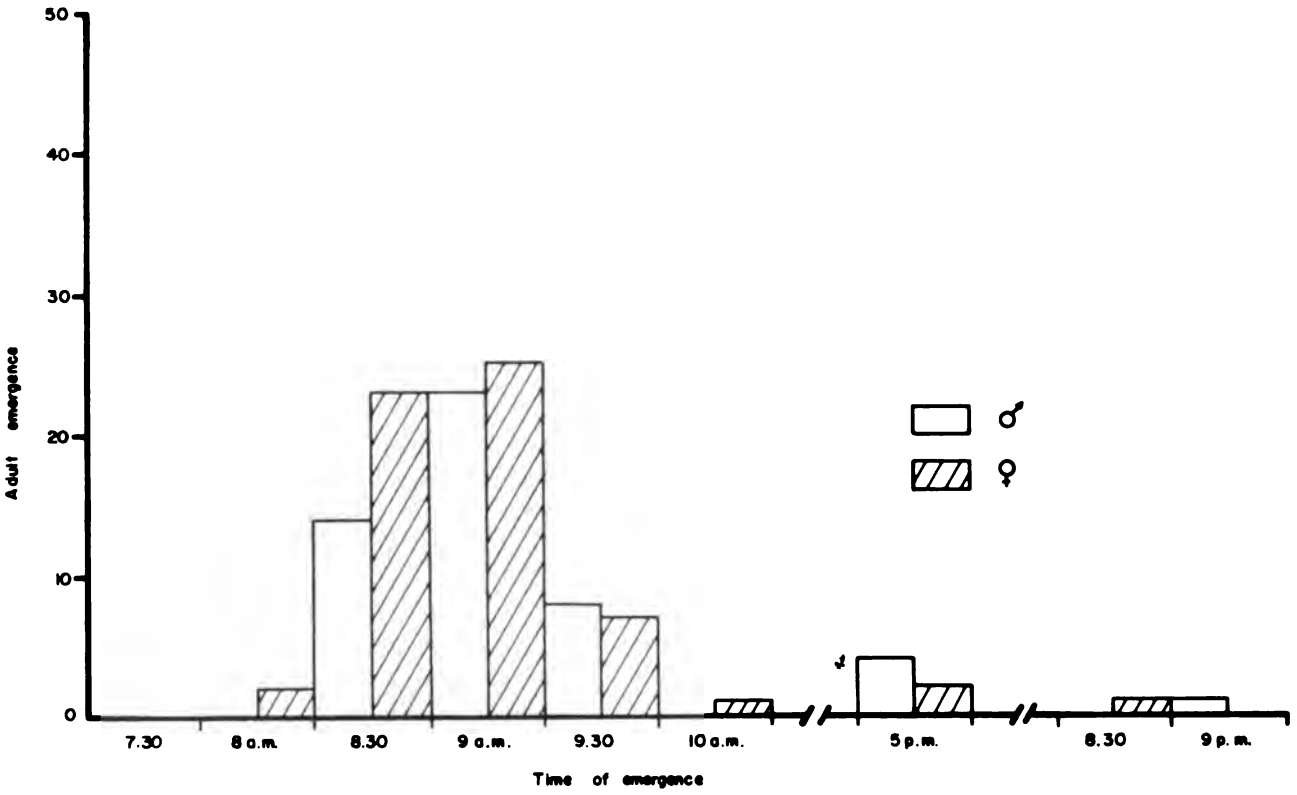



Fig. 1. Number of *H. grandella* moths preconditioned to a 12–hr shift in the 12L:12D cycle as related to time of emergence.

## Results and discussion

In Test 1, 90 per cent of the pupae emerged and in Test 2 and 3, 100 per cent of the pupae emerged. Adult emergence, both males and females took place between 0800 and 1000 hours. Concerning adult behavior under the shifted 12L:12D cycle, approximately 20 per cent of the female moths were observed in "calling–position" between 1400 and 1530 hours, a shift of approximately twelve hours over natural environmental behavior.

Of the 118 pupae that were later tested, 94 per cent emerged, whereas the remaining 6 per cent died. Fig. 1 shows the number of emerged moths as related to time of emergence. The majority of the moths, 81 per cent, emerged between 0830 and 0930 hours. There seems to be no difference in time of emergence between pupal sexes. It is of interest to note that eight adults, three females and five males, out of a group of fifteen, emerged between 1700–2100 hours. The remaining nine adults emerged between 0830 and 0930 hours. These fifteen pupae were originally placed into the incubator after approximately nine days of pupal age, or one day before adult emergence. It appears that two days of shifted 12L:12D treatment should be given to the pupae to obtain adult emergence in the morning hours.

## References

1. FATZINGER, C. W. and ASHER, W. C. Mating behavior and evidence for a sex pheromone of *Dioryctria abietella* (Lep.:Pyralidae (Phycitinae)). Ann. of the Ent. Soc. of America. Vol. 64:3. 1971. pp. 612–620.

2. GRIJPMA, P. Studies on the shootborer *Hypsipyla grandella* (Zeller).V. Observations on a rearing technique and on host selections behavior of adults in captivity. Turrialba 21(2):202–213. 1971.

3. RAMIREZ–SANCHEZ, J. Investigación preliminar sobre biología, ecología y control de *Hypsipyla grandella* Zeller. Boletín del Instituto Forestal Latino Americano de Investigación y Capacitación No. 16:54–77. 1964.

4. SLIWA, D. and BECKER, V. O. Studies on the shootborer *Hypsipyla grandella* (Zeller) (Lep.:Pyralidae (Phycitinae)). Observations on the emergence, activity patterns, mating, and oviposition behavior of adults in captivity. Turrialba (to be published). 1973.

# APPENDIX

# DOS DIETAS APTAS PARA LA CRIA DE *HYPSIPYLA GRANDELLA* (ZELLER)

A. Dieta para Lepidópteros (recibido del Dr. M. J. Lukefahr, Cotton Research Laboratory ARS–USDA, Brownsville, Texas, USA).

1. 80,7 gramos de harina de soya en 550,0 ml de agua; licúe.

2. Disuelva 8,0 gramos de agar en 450,0 ml de agua hirviendo; deje enfriar hasta 60°C.

3. Continúe licuando y añada:

   36,0 gramos de germen de trigo
   6,0 ml de KOH (4M)
   10,0 gramos Sales de Wesson
   14,7 gramos Sucrosa
   1,1 gramos Acido ascórbico
   1,8 gramos p–hidroxibenzoato de metilo
   1,1 gramos Acido sórbico
   4,0 ml de suspensión de vitaminas*
   8,3 ml de Colina HCl (15%)
   5,0 ml. Formaldehído (10%)
   13,3 ml Acido acético (25%)
   20,0 ml Auromicina (166,7 mg/20 ml)
   80,0 gramos de Olote (zuro) molido

* Suspensión de vitaminas (mg/ml):

| | |
|---|---|
| Pantotenato de calcio | 12 |
| Niacina | 6 |
| Riboflavina | 3 |
| Acido fólico | 3 |
| Thiamina HCl | 1,5 |
| Piridoxina HCl | 1,5 |
| Biotina | 0,12 |
| $B_{12}$ | 0,00625 |

B. Dieta para Lepidópteros (Lukefahr modificado por O. Hidalgo–Salvatierra). Turrialba 21(2):214–219, 1971.

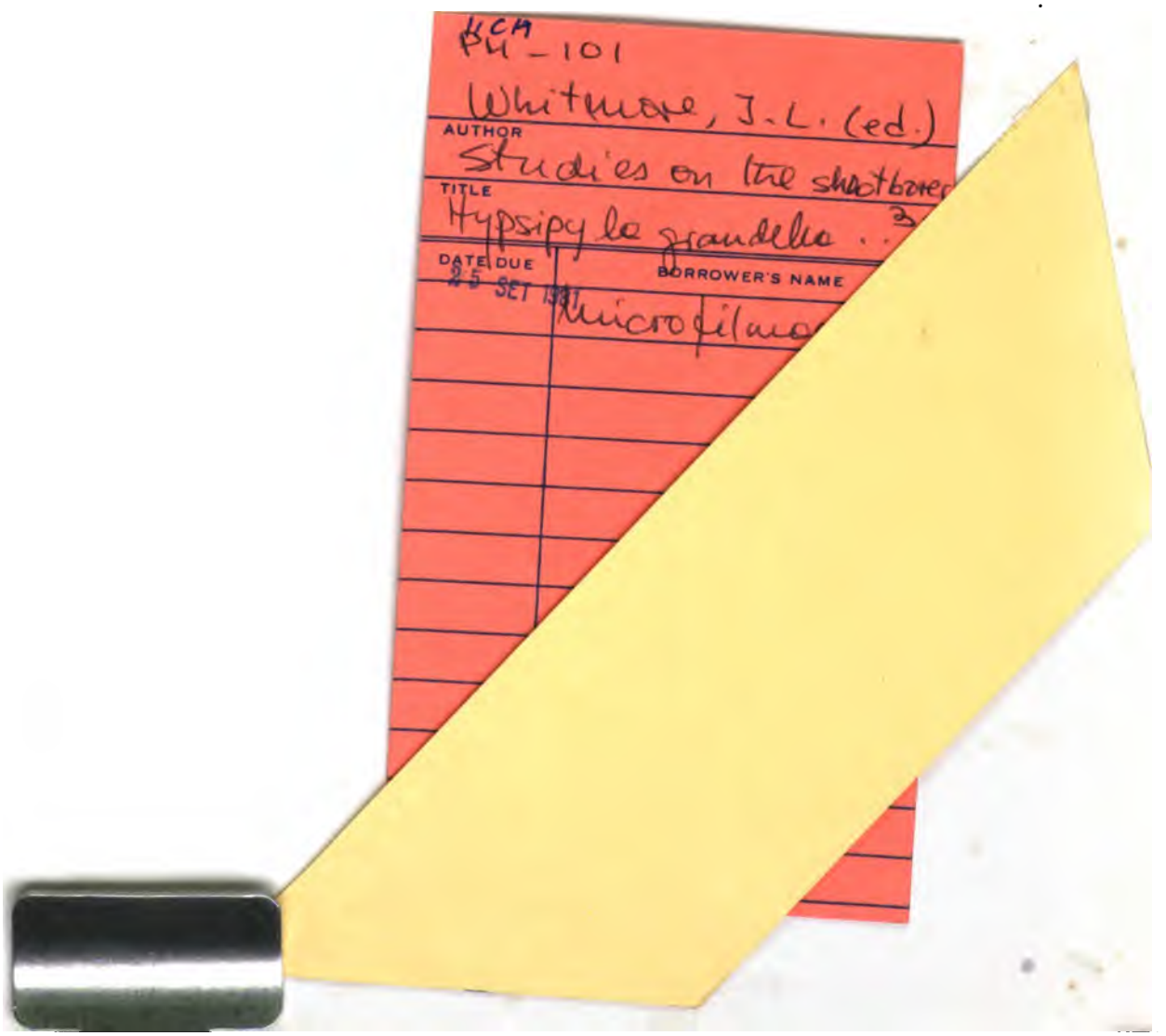
1. 80,7 gramos de harina de soya en 450,0 ml de agua; licúe.

2. Disuelva 8,0 gramos de agar en 400 ml de agua hirviendo; deje enfriar hasta 60°C.

3. Continúe licuando y agregue:

   36,0 gramos de germen de trigo
   6,0 ml de hidróxido de potasio (4 M)
   10,0 gramos de Sales Wesson
   1,8 gramos de p–hidroxibenzoato de metilo
   1,1 gramos Acido Sórbico
   5,0 ml de Formaldehído (10%)
   13,3 ml de Acido Acético (25%)
   20,0 ml Auromicina (166,7 mg/20 ml)
   20,0 gramos de Mezcla de vitaminas (Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corp. International, 21010, Miles Ave. Cleveland, Ohio 44128, USA)
   80,0 gramos Olote (zuro) molido.

| FECHA DE DEVOLUCION | | | |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |


BCA
PH-101
Whitmore, J.L. (ed.)
AUTHOR
Studies on the shortbeak
TITLE
Hypsipyla grandella ..
DATE DUE
BORROWER'S NAME
25 SET 19
Microfilm