FIELD TEST OF MOSQUITO OVIPOSITIONAL CUES FROM VENEZUELAN PHYTOTELMATA

L. F. LOUNIBOS1 AND C. E. MACHADO-ALLISON2

1University of Florida,
Florida Medical Entomology Laboratory,
200 5th St. SE, Vero Beach, FL 32962
2Instituto de Zoología Tropical,
Universidad Central de Venezuela,
Apartado 47058, Caracas 1040A, Venezuela

ABSTRACT

Fluids held by four phytotelmata were compared for oviposition by mosquitoes in lowland rainforest in eastern Venezuela. Significantly more Wyeomyia ulocoma and Culex pleuristriatus were recovered in fluid from bracts of Heliconia caribaea, than in fluids collected from axils of Aechmea bromeliads, the aroid Alcocasía macrorrhiza, or internodes of Bambusa vulgaris. Wyeomyia ulocoma, whose immature stages occur uniquely in Heliconia bracts, was more specific to H. caribaea fluid than was the phytotelm generalist C. pleuristriatus. No preferences for oviposition site color were detected.

Key Words: Diptera, fluids, culex, Wyeomyia, Heliconia.

RESUMEN

Fluidos retenidos por cuatro fitotelmatas fueron comparados en una selva lluviosa de tierra al oriente de Venezuela con respecto a la frecuencia de oviposición por mosquitos. Significativamente más Wyeomyia ulocoma y Culex pleuristriatus fueron colectados en el fluido de las brácteas de Heliconia caribaea que en los fluidos de las axilas de bromelias del género Aechmea, de las axilas de Alcocasía macrorrhiza, o de los internodos del bambú Bambusa vulgaris. La W. ulocoma, cuales estados
preimaginales ocurren unicamente en las brácteas de Heliconia, fue más específica al fluido de H. caribaea que la C. pleuristriatus. No se encontraron preferencias por diferentes colores en las tazas de oviposición.

Mosquitoes use visual, olfactory, and tactile cues to determine appropriate egg laying sites, the relative importance of these stimuli being highly species-dependent (Bentley & Day 1988). Both site color and chemistry may affect the female's choice, and these cues may interact (e.g., Wilton 1988). Natural compounds that influence oviposition include some of plant origin (Bentley & Day 1988).

Phytotelmata are well suited for experiments on oviposition behavior because of their small size and replicability. These plant-held pools are particularly important as habitats for mosquitoes in the tropics (Frank & Lounibos 1983). For the few mosquito species studied, either phytotelm odor or color have been shown to affect oviposition site selection. Egg laying by both the fruit-husk specialist Eretrmapodites substipitipes (Edw.) and the pitcher-plant mosquito Wyeomyia smithii (Coq.) is enhanced by unidentified chemicals derived from the preferred habitats of these species (Lounibos 1978, Istock et al. 1983). Compounds that promote oviposition by the treehole mosquito Aedes triseriatus (Say) have been identified as phenolic derivatives from trees (Bentley et al. 1979, 1982). Chemical stimuli from their Tillandsia host plants did not affect oviposition choices of Wyeomyia michellii (Theobald) or Wyeomyia vanduzeei Dyar and Knab, but these bromeliad inhabitants did prefer yellow-green oviposition sites to other colors (Frank 1986).

The phytotelm fauna of Panaquire, Venezuela is relatively well known thanks to a series of studies by the authors and their students and colleagues in the 1980s (Machado-Allison et al. 1983, 1985, Lounibos et al. 1987). Mosquitoes encountered at Panaquire include specialists inhabiting only one type of water holding plant and generalists that occupy diverse phytotelmata (Machado-Allison et al. 1985). Oviposition behavior has been examined in only one mosquito resident of Panaquire, Trichoprosopon digitatum (Rondani), whose egg laying in cacao husks was influenced by fluid aroma and husk shape (Lounibos & Machado-Allison 1983). Here we report results from a field experiment in which choices of fluids from four phytotelmata were presented in colored containers to gravid females in nature.

SITE AND METHODS

The experiment was performed on a cacao plantation in lowland tropical rain forest near Panaquire (10° 13'N, 66° 14'W), Miranda State, Venezuela. Our field test was conducted in July 1983 in the middle of the long rainy season (Machado-Allison et al. 1983).

The aquatic contents of four classes of phytotelmata growing naturally at the plantation were poured or pipetted with a turkey baster into separate plastic buckets. Phytotelmata sampled were (1) axils of Alcacia macrorrhiza (L.) (Araaceae); (2) bracts of Heliconia caribaea Lamark (Heliconiaceae); (3) internodes of Bambusa vulgaris (Schrad.) (Gramineae); (4) axils of Aechmea aquilega (Salisb.) and Aechmea nudicaulis (L.) (Bromeliaceae)—collections from these two bromeliads were pooled.

The contents of buckets were passed separately through 80-mesh/cm² sieves to separate detritus and invertebrate fauna from fluid. Disposable plastic cups of 20 ml capacity were used to expose the four fluids for mosquito oviposition. This capacity is slightly larger than that of an average H. caribaea bract or aroid or bromeliad axil, but consid-
erably smaller than the average volume held by a *R. vulgaris* internode. To simulate the colors of phytotelmata, cups were spray-painted matte red (*H. caribaea*), green (*A. macrorrhiza* or *Aechmea* sp.), or off-white or black (controls).

Oviposition choices were arranged in a 4 × 4 factorial design of the colors and fluids. Cups were half-filled with fluid (10 ml) and suspended by hooks on a crosswire 1.5 m above ground in shade under the canopy because sites with more light were not available. The relative position of each fluid/color combination was determined for each replicate by selection of random permutations of 16 (Cochran & Cox 1957). Twelve replicates of the 16 treatments were separated by one-meter distances on the crosswire.

Cups were set at dusk on the same day as fluids were collected, and the experiment terminated after 86-88 hours exposure by preserving the contents of each cup in separate bags with 70% ethanol. Larvae were counted and identified in the laboratory with keys (Lounibos et al. 1987). Eggs were not counted because of the lack of specific keys. Based on an estimate of 1.5 to two days from oviposition to hatch, our methods recorded only egg-laying occurring within the first 48 hours after setting of cups.

**RESULTS**

Numbers of mosquitoes per cup were treated by two-way ANOVA with fluid origin and cup color as independent variables. An $F_{max}$ test revealed no significant differences among variances ($F_a = 6.06; F_{(12,11),0.05} = 7.48$) after numbers were transformed by square root of $(Y + 0.5)$. Fluid source, but not cup color, significantly influenced mosquito oviposition, with no significant interaction between the factors (Table 1).

Four species of mosquito were identified from 601 larvae: *Culex pleuristriatus* Theobald and *Wyeomyia ulocoma* (Theobald) accounted for, respectively, 77.9% and 15.3% of identified specimens. Three larvae of *Wyeomyia portinana* (Williston) were recovered in three separate cups of *A. macrorrhiza* fluid and 38 larvae of *T. digitatum* were found in one cup of *A. macrorrhiza* fluid.

For the two commonest mosquito species captured, numbers of larvae per cup were compared among the four fluids. As the numbers of *W. ulocoma* among fluids were heteroscedastic even after transformation, the Kruskal-Wallis (KW) statistic (H) was used in place of ANOVA to measure preference (Lounibos 1981).

Larvae of *C. pleuristriatus* were recovered in all four fluids, but the mean number in *H. caribaea* extracts was more than twice that in alternative plant fluids, yielding the significant difference in location by the KW test (Fig. 1A). *Wyeomyia ulocoma* also preferred to oviposit in *H. caribaea* fluid and more selectively than *C. pleuristriatus*, as reflected by the higher H value from the KW test (Fig. 1B); 96.7% (88/92) of the identified *W. ulocoma* were recorded from this plant fluid.

**TABLE 1. ANALYSIS OF VARIANCE (TWO-WAY) OF THE EFFECTS OF FLUID SOURCE AND CUP COLOR ON MOSQUITO OVIPOSITION**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluids</td>
<td>3</td>
<td>14.65</td>
<td>11.54***</td>
</tr>
<tr>
<td>Colors</td>
<td>3</td>
<td>0.78</td>
<td>0.61</td>
</tr>
<tr>
<td>Fluids X Colors</td>
<td>9</td>
<td>0.40</td>
<td>0.31</td>
</tr>
<tr>
<td>Error</td>
<td>176</td>
<td>1.27</td>
<td></td>
</tr>
</tbody>
</table>

*Number of larvae per cup transformed by square root of $(Y + 0.5)$.

*** = P < 0.001.
Fig. 1. Mean numbers of the two most common mosquito species identified in experimental cups containing fluids from four phytotelmata. Error bars show standard error of the mean, n is the total number of larvae and H the Kruskal-Wallis statistic evaluated for significance against chi-square.
DISCUSSION

It is conceivable, but unlikely, that differential mortality or, in the case of *C. pleuristratius* differential egg raft size, caused the observed distributions of larvae (Fig. 1) instead of differential oviposition. A differential mortality hypothesis would require that egg or larval survival be significantly higher in the *H. caribaea* fluid. However, we observed no evidence of mortality such as decaying corpses or the occurrence of predators in cups, at the time that our experiment was terminated. A differential egg raft size hypothesis would require that *C. pleuristratius* had laid egg masses in *H. caribaea* fluid that were more than twice the size as those in the other phytotelm fluids. While it is impossible to discount this possibility without counting eggs in rafts, we know of no precedence for such site-specific variation in mosquito egg raft size. Therefore, we feel it safe to conclude that the distributions of larvae among phytotelm fluids reflect ovipositional preferences.

Because no behavioral observations were made of the choice by *C. pleuristratius* and *W. ulocoma* of *H. caribaea* fluid, we have avoided use of the terms ‘attractant’ and ‘stimulant’ which have been defined in relation to specific behaviors elicited (Dethier et al. 1960).

Fluids of our chosen phytotelmata have different origins. Rainfall or throughfall probably accounts for most fluid held by axils of *A. macrorrhiza* and *Aechmea* bromeliads. In contrast, at least some of the fluid in *H. caribaea* bracts is of plant origin, because newly opened bracts already contain liquid. These young bracts are highly attractive to ovipositing *C. pleuristratius* and *W. ulocoma*, as evidenced by the superabundance of early instar larvae in them (Machado-Allison et al. 1983). It seems likely that these two mosquito species are responding to phytochemical cues in their choices of *H. caribaea* fluid, although our experiment does not rule out other sources of chemicals. In this respect *H. caribaea* resembles the North American pitcher plant *Sarracenia purpurea* whose newly opened pitchers are favored oviposition sites of the inquiline mosquito *W. smithii* (Fish & Hall 1978) owing to a water-soluble chemical secreted by the plant (Istock et al. 1983).

Of the two commonest mosquitoes recovered from cups, *W. ulocoma* showed the stronger preference for *H. caribaea* fluid, as indicated by the higher H value (Fig. 1). The higher selectivity of this species agrees with the habitat valency of the two in nature. *W. ulocoma* being a specialist in bracts of *H. caribaea*, and *C. pleuristratius* occupying diverse phytotelmata, including bamboo internodes and the axils of aroids and bromeliads (Machado-Allison et al. 1985, Lounibos et al. 1987).

The two species, *W. pertinans* and *T. digitatum*, recorded infrequently from oviposition cups, were both regarded as exploiting diverse phytotelmata by Machado-Allison et al. (1985). Although our experiment recovered the former only in fluid of *A. macrorrhiza*, in nature it inhabits both bromeliad and aroid axils (Machado-Allison et al. 1985). The one cup with *T. digitatum* larvae contained *A. macrorrhiza* fluid, although bamboo internodes and cacao husks are the preferred habitats of this species at Panaquira (Machado-Allison et al. 1985). Curiously, *T. digitatum* occurs commonly in *Heliconia* bracts at Rancho Grande, Venezuela (Seifert 1980).

Oviposition behaviors of the mosquito species recorded in cups fall into two categories: raft formation by *C. pleuristratius* and *T. digitatum* and aerial oviposition by *Wyeomyia* spp. which eject eggs singly on to the water surface. Since the raft-formers 'decide' upon one oviposition site for a complete egg clutch, whereas *Wyeomyia* 'inspect' the fluid surface prior to ejecting each egg (Frank, unpublished), it could be argued that the five-fold fewer larvae of *W. ulocoma* still represent a greater number of oviposition 'decisions' by that species compared to *C. pleuristratius*. 
While Frank (1986) showed that the bromeliad specialists W. mitchelli and W. vanduzei discriminated colors of oviposition choices in cages, Istock et al. (1983) could not detect color preferences for egg sites of W. smithii. It would be premature to conclude from our experiment that neither C. pleuristriatus nor W. ulicorna use color as an oviposition site cue. In nature H. caribaea grows in open sunlight and Aechmea spp. and A. macrorrhiza occur in semi-shade, but our experiment was set in shade, where light intensity may have been too low for diurnal Wyeomyia spp. (Frank et al. 1985) to use visual cues. Also, our green and red spray paints were not calibrated to conform spectrally with the colors of phytotelmata intended for simulation.

ACKNOWLEDGMENTS

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INFLUENCE OF CORN PHENOLOGY AND PLANTING DATE ON DAMAGE BY THE BLACK CUTWORM (LEPIDOPTERA: NOCTUIDAE)

L. A. RODRIGUEZ-DEL-BOSQUE AND J. LOERA-GALLARDO
Campo Experimental Río Bravo, INIFAP-SARH
Apartado Postal 172, Río Bravo, Tamaulipas, México 88900

ABSTRACT

Damage by the black cutworm, Agrotis ipsilon (Hufnagel), in relation to corn growth stage and planting date, was studied in commercial cornfields near Río Bravo, Tamaulipas, Mexico, during the 1990, 1991, and 1992 spring seasons. Fields were sampled during each of the 1- to 7-leaf stages to estimate the percentage of plants damaged by A. ipsilon. Planting dates ranged from 15 January to 15 March. Regardless of planting date, 81% of the attacks by A. ipsilon occurred during the first 3 leaf stages. Fields planted during March sustained the highest damage, in contrast to the January and February plantings, when damage was generally low.

Key Words: Agrotis ipsilon, Mexico, corn damage, agroecosystems.

RESUMEN

Se estudió el efecto de la etapa de desarrollo del maíz y la fecha de siembra sobre el daño del gusano trozador, Agrotis ipsilon (Hufnagel), en parcelas comerciales de maíz en Río Bravo, Tamaulipas, México, durante los ciclos de primavera de 1990, 1991, y 1992. Las parcelas fueron muestreadas en cada una de las etapas de 1 a 7 hojas para estimar el porcentaje de plantas dañadas por A. ipsilon. Las parcelas fueron sembradas desde el 15 de Enero hasta el 15 de Marzo. El 81% del total de plantas dañadas se observaron durante las etapas de 1 a 3 hojas, independientemente de la fecha de siembra. El daño más severo ocurrió en las siembras de Marzo, en contraste con las siembras en Enero y Febrero, cuando los niveles de daño fueron en general bajos.

*Current address: Apartado Postal 133, Tecomán, Colima 28130, México.