SOIL MOISTURE CONDITIONS THAT ARE MOST ATTRACTIVE TO OVIPOSITING FEMALES OF *PSOROPHORA COLUMBIAE* IN TEXAS RICELANDS [1]

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ABSTRACT. Moisture content of soil appears to be one of the more important factors that determine whether or not, and to what extent, *Psorophora columbiae* (Dyar and Knab) females will use a particular site for oviposition in Texas ricelands. Gravid females of this species exposed to various levee situations in the laboratory deposited the great majority of their eggs on soil that was relatively muddy (i.e., on soil whose moisture content ranged between 75% field capacity to just above field capacity). Conditions observed at field sites in Brazoria County, Texas, tend to support laboratory findings.

INTRODUCTION. *Psorophora columbiae* (Dyar and Knab), part of the *Ps. confinnis* complex, is sometimes referred to as the dark rice field mosquito because of its commonness in rice producing areas of the United States (Horsfall 1942, Schwardt 1939). The close association between the growing of rice and the breeding of large populations of *Ps. columbiae* is apparently because this form of agriculture provides an abundance of habitats considered optimum for deposition of eggs by females of this species. These same habitats subsequently support the needs of the larval and pupal stages. Some of the more important sources of oviposition sites used by *Ps. columbiae* populations in Texas riceland areas include levees on productive and fallow rice fields, various types of tire tracks that are made in fields by machinery during the harvesting of rice, and cattle hoofprints in fallow rice fields that are used as pastures (Meek and Olson 1976).

Moisture content of the soil appears to be one of the more important factors that determine whether or not and the degree to which floodwater mosquito species such as *Ps. columbiae* use a particular habitat for oviposition (Horsfall 1955, Horsfall et al. 1975). Horsfall (1955) stated that gravid *Ps. columbiae* females in Arkansas will generally deposit their eggs individually on soil between the time that the soil is in a firm moist state and when the surface layer of the soil begins to appear dusty. Horsfall (1963) also noted that the moisture content of the soil at the time of oviposition probably played a significant role in determining the number of eggs that would be deposited by *Ps. columbiae* females at a given location. The result was that *Ps. columbiae* eggs, like those of other floodwater mosquito species, came to lie in characteristic zones or horizons on soil below the waterline of maximum flood. Horsfall (1955) indicated that quantitative data concerning egg deposition by *Ps. columbiae* were generally lacking—particularly in terms of the exact placement of eggs within a given area by gravid females. A portion of our general study of the oviposition behavior of *Ps. columbiae* in Texas ricelands (Meek and Olson 1976) was devoted to the quan-

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titative determination of the location of egg horizons as influenced by soil moisture conditions. This particular study was accomplished by setting up profiles of levees in the laboratory. These profiles mimicked topographic, soil and moisture conditions observed in the field. Field-caught, gravid females of *P. columbiae* were exposed to the levee profiles; and the relative abundance and location of eggs deposited by these females on the profiles were assessed using techniques and equipment described herein.

METHODS AND MATERIALS. The container, or levee profile box, used in this study was constructed from 1/4 in. plexiglass sheeting (Fig. 1). The inside dimensions of the box were 5 x 2 x 0.5 ft (Fig. 1A). The sides, bottom and ends of the box were held together by metal screws (Fig. 1AA). Methylene chloride and window caulking were placed along the inside seams of the box to seal the box so that it would hold water. The plexiglass lid of the box was detachable. Five holes (1½ in. dia.) each equidistant from the other were cut into the lid. Four of the holes were covered with 16-mesh, plastic screen, and the fifth hole at one end of the lid was left unscreened and was fitted with a cork to provide a means of admitting mosquitoes into the profile box (Fig. 1A). A 12 x 6 in. plexiglass partition was placed in the bottom center of the box so that 2 profile experiments could be run simultaneously (Fig. 1A). The lower outside portions of the box were reinforced by a clamping device to prevent seam separation due to excess soil and water pressure on the box during a given experiment (Fig. 1C). The clamping device consisted of two 2 x 4 x 67 in. boards held in place on either side of the box by 2 fabricated bolts. The profile box fitted with the clamping device was placed on a 5 ft x 7 in. platform constructed from ½ in. plywood (Fig. 1B). The platform was equipped with 5 castors (2 on each end and 1 in the center) to facilitate movement of the profile box assembly.

The heavy, clay soil used to construct simulated levees in the laboratory was taken from our field research sites on the J. T. Garrett Ranch, Brazoria County, Texas. Prior to its use in experiments, the soil was put into enamel pans and flooded with water for 2 to 3 days to stimulate hatching of contaminant mosquito eggs. Subsequently, the soil was placed on plastic sheeting and allowed to dry in the sun to a cracking, crumbling state. The dry soil was then broken up by hand so that there were no clods of soil larger than ¾ in. in diameter. The conditioned soil was placed in boxes and stored in the laboratory until it was used in a given experiment.

Two types of experiments were performed. In one experiment, *Ps. columbiae* females were exposed simultaneously to both rice field and pasture levees. In this case, half of the profile box was devoted to the construction of levee slopes typical of the ones found in pasturelands in the Texas rice belt (Fig. 2B). Conditioned soil was poured into the pasture side of the box, and a levee having a 34° slope was contoured by hand to a vertical height of 9¾ in. above a levee ditch constructed at the bottom of the levee. Following the construction of the pasture levee and associated ditch, dry plant debris from riceland pastures was loosely scattered over the levee surface to a depth of ca. ¼ in.

A rice field levee, and associated ditch, was constructed in the other half of the profile box (Fig. 2A). The rice field levee measured 9½ in. in height and had an approximate slope of 25°. Two-foot high rice plants from the Garrett Ranch were transplanted on the simulated levee at the rate of 5 plants per 6 in. square.

In the other experiment, *Ps. columbiae* females were exposed only to a pas-

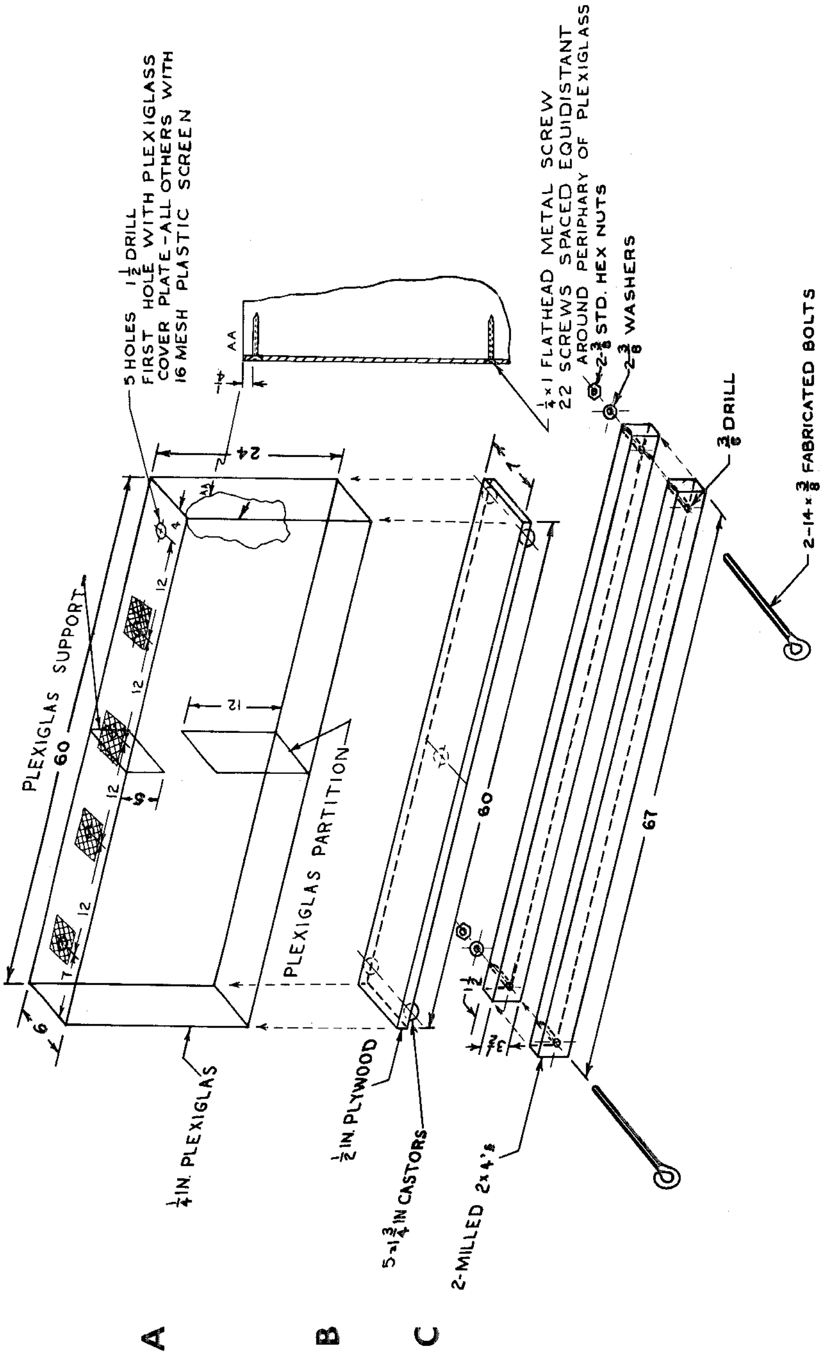

Fig. 1. Diagram of soil profile box with descriptions and measurements (in inches) of various components: (A) soil profile box; (B) platform equipped with castors; (C) clamping device.

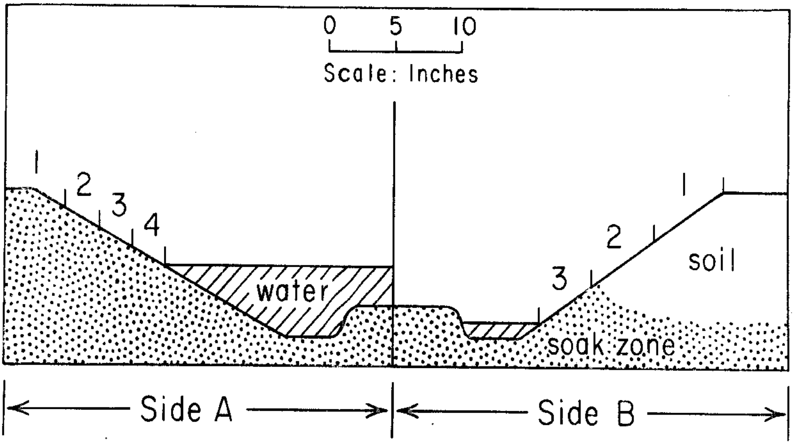

Fig. 2. Diagram of the rice field levee (A) and pasture levee (B) profiles used during experiments involving simultaneous exposures of gravid females of *Psorophora columbiae* to rice field and pasture levees: (A_1–A_4) position of soil samples taken from rice field levee; (B_1–B_3) position of soil samples taken from pasture levee.

ture levee. The levee profile constructed in this case was 8¾ in. in vertical height, and had a slope of 25° (Fig. 3).

Two days prior to a given test involving the exposure of mosquitoes to pasture levee profiles, deionized water was added to the pasture side of the box (Fig. 2B,3) to a level that filled the levee ditch to its maximum depth (2–2½ in.). The water in the ditch was allowed to soak into the soil and the ditch was then refilled. This procedure was repeated several times until a discernible moisture gradient (or soak zone) was established along the slope of the pasture levee profile and until at least some water remained standing in the levee ditch (Fig. 2B,3). In tests where mosquitoes were exposed simultaneously to pasture and rice field levee profiles, water was added to the rice field levee side of the box until the levee was flooded to ca. half its vertical height (i.e., ca. 4½ in. in depth). At this depth ca. half of the levee slope was covered by water. Again, water was allowed to soak into the levee soil and water was added periodically until the depth of standing water and the soak zone on the levee stabilized (Fig. 2A). After each addition of water, the lid of the profile box was placed on top of the container and sealed along its edges with masking tape. This retarded evaporation and prevented any mosquitoes that were loose in the laboratory from ovipositing on the soil profiles.

Two replicates of each experiment were performed. At the start of a given replicate, gravid *Ps. columbiae* females caught at our field research sites in Brazoria County, Texas, were transferred from laboratory holding cages to the levee profile box using mechanical aspirators (Carver 1967). A total of 85 and 69 mosquitoes were used respectively in the 2 replicates of the experiment involving simultaneous exposure of mosquitoes to rice field and pasture levees (Fig. 2). Ninety-five mosquitoes were used in each of the 2 replicates involving mosquito exposure to only pasture levees. In each case, the mosquitoes were allowed to oviposit over night in the profile box (ca. 15 hrs). During the test period, the profile

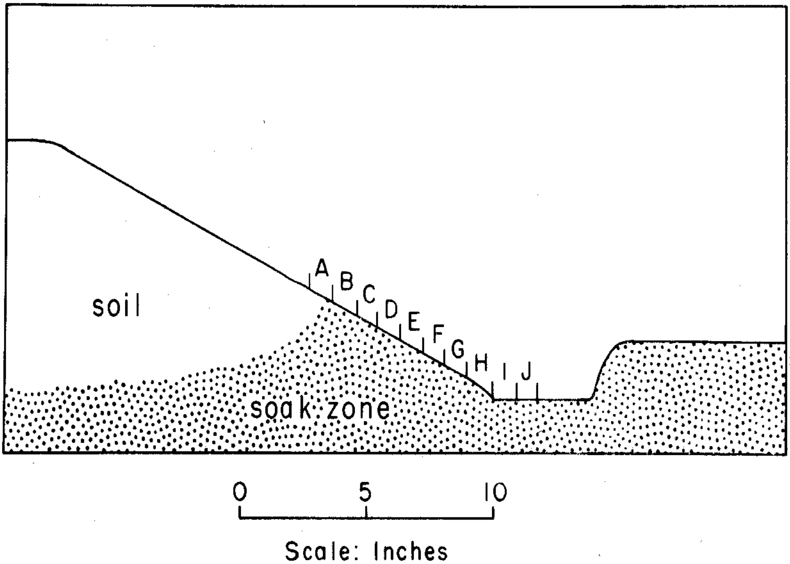

Fig. 3. Diagram of the pasture levee profile used during experiments involving the exposure of gravid *Psorophora columbiae* females to only pasture levees: (A–J) position of 1 x 6 x 1 in. soil samples taken from the levee.

box was held in a "walk-in" incubator set at 82°C), 50% relative humidity and a 14:10 hr light: dark regimen. The twilight hours of the light cycle were simulated by a crepuscular lighting system comprised of 8 fluorescent lights which turned on or off sequentially in pairs by means of time clock switches. The simulated crepuscular periods lasted for approximately 1 hr each.

On the morning following a given replicate, the mosquitoes were aspirated from the box, and soil samples were taken from the levee profile(s). The methods used in retrieving soil samples were similar to those described by Horsfall (1956). In the case of rice field levees, 3 x 1 in. soil samples were taken sequentially beginning at the waterline and ending at the top of the levee (Fig. $2A_1$–A_4). The width of each soil sample (6 in.) was equal to the width of the levee profile box. Soil samples taken from the pasture levee profiles in tests involving the simultaneous exposure of mosquitoes to both kinds of levees measured 6 x 6 x 1 in. and were taken sequentially up the entire expanse of the levee slope (Fig. $2B_1 - B_3$). Soil samples taken from the soil profile box after tests involving the exposure of mosquitoes to only pasture levees measured 1 x 6 x 1 in. and were taken primarily from the water-soaked zone of the levee slope (Fig. 3A–J).

During the course of taking soil samples following these latter tests, the relative moisture content of the soil was estimated using the hand-squeeze method described by Box and Bennett (1959). This method involved the taking of soil masses (each 1½ in. diameter) from various points on the levee and squeezing them in the hand. The events that took place upon squeezing the soil mass were compared to a chart adapted from the article by Box and

Bennett (1959), and the relative level of soil moisture at a given point on the levee was estimated in terms of percent "field capacity." Field capacity is the maximum amount of water that a given soil type can hold against drainage by gravity (Box and Bennett 1959). Soil moisture conditions were assessed at 4 points along the pasture levee slope: (1) at the bottom of the levee (Fig. 3H); (2) in the middle of the soak zone (Fig. 3E); (3) at the top of the soak zone (Fig. 3B) and 1–2 in. above the soak zone (Fig. 3A).

Each soil sample taken from the profile box was placed in a separate plastic bag and labeled as to replicate number, type of levee, and relative position on the levee. Each sample was subsequently processed individually using soil washing and salt flotation techniques described by Horsfall (1956) for the separation of floodwater mosquito eggs from soil samples. Mosquito eggs retrieved from each sample were confirmed as to species and counted. The number of eggs was recorded in accordance with the particular sample processed.

RESULTS AND DISCUSSION. Distribution of eggs deposited by *Ps. columbiae* females on levees in the laboratory are summarized in Tables 1 and 2 for tests involving the simultaneous exposure of mosquitoes to rice field and pasture levees (Fig. 2) and in Table 3 for tests involving the exposure of mosquitoes only to pasture levees (Fig. 3). On the basis of egg distributions, it appears that the moisture content of the soil must be high in order for a given site to be attractive to gravid *Ps. columbiae* females for oviposition. In each replicate, the vast majority of the eggs were deposited on soil in the wetter regions (or soak zones) of the experimental levees. In tests involving the simultaneous exposure of mosquitoes to rice field and pasture levees, between 92 and 99% of the total eggs deposited

Table 1. Distribution of *Psorophora columbiae* eggs on rice field levees for levee profile box tests involving the simultaneous exposure of gravid female mosquitoes to pasturel and rice field levees.

Soil sample # and location on levee[a]	Replicate 1	Replicate 2	Both replicates
	% total eggs	% total eggs	% total eggs
1+2 (Top 6 in.)	4.7	7.5	6.4
3 (Mid 3 in.)	31.9	43.6	39.1
4 (Lower 3 in.)	63.4	48.9	54.5
Total eggs deposited	636	999	1365

[a] See Fig. 2 for relative position of each soil sample taken from the levee slope.

by female *Ps. columbiae* were located in soil samples taken from the first 6 in. of exposed levee slope above the water line (Tables 1 and 2, respectively).

Estimates of soil moisture conditions on the pasture levees using the technique described by Box and Bennett (1959) indicated that the majority of *Ps. columbiae* eggs will be deposited on heavy clay soil whose moisture content

Table 2. Distribution of *Psorophora columbiae* eggs on pasture levees for levee profile box test involving the simultaneous exposure of gravid female mosquitoes to pasture and rice field levees.

Soil sample # and location on levee[a]	Replicate 1	Replicate 2	Both replicates
	% total eggs	% total eggs	% total eggs
1 (Top 6 in.)	2.0	0.3	0.8
2 (Mid 6 in.)	0.3	0.3	0.3
3 (Bottom 6 in.)	97.7	99.4	98.9
Total eggs deposited	388	1016	1404

[a] See Fig. 2 for relative position of each soil sample taken from the levee slope.

Table 3. Distribution of *Psorophora columbiae* eggs on the soak zone area of pasture levees for levee profile box tests involving the exposure of gravid females to only pasture levees.

Location of soil sample on levee[a]	Both replicates % of total eggs	Soil moisture condition[b]
A—Above soak zone	0.2	No ball formed; dry; crumbly (0.0% field capacity)
B—Top of soak zone	0.7	Soil ball formed; wetted hand; strong soil ribbon (75% of field capacity —field capacity)
C—	2.7	
D—	9.9	
E—	19.1	No ball formed; gluey, tacky (field capacity to above field capacity)
F—	15.5	
G—	30.1	
H—Bottom of levee soak zone	17.0	No ball formed; muddy (above field capacity)
I—Levee ditch	4.6	
J—	0.3	Free water on soil surface of ditch bottom; oozing (above field capacity)
Total eggs deposited	3132	

[a] See Figure 3 for position of each soil sample on the experimental levee.
[b] Based on technique described by Box and Bennett (1959).

Table 4. Relative abundance of *Psorophora columbiae* eggs in different soil mositure zones on rice field levees in Brazoria County, Texas, as determined by the hand squeeze soil ball technique.

Condition of soil ball and estimated % of field mositure capacity[a]	No. of soil samples taken	No. of eggs per sample and range	Frequency of samples containing eggs
No soil ball; (0.0% field capacity)	3	0.0	0.00
Weak soil ball; did not wet the hand; weak soil ribbon (50% field capacity)	3	0.0	0.00
Strong soil ball; wetted hand; strong soil ribbon; (75% field capacity)	4	4.8 (0–12)	0.75
Soil ball formed; small amount of water squeezed out of soil; weak soil ribbon; (above field capacity)	3	9.3 (1–26)	1.00
Weak soil ball formed; moderate amount of water squeezed out of soil; no soil ribbon; (above field capacity)	3	4.3 (2–9)	1.00
Weak soil ball formed; large amount of water squeezed out of soil; no soil ribbon; (above field capacity)	3	0.5 (0–1)	0.67

[a] Based on techique described by Box and Bennett (1959).

ranges between 75% field capacity or just above field capacity (Table 3). Approximately 95% of the total eggs recovered during the course of the 2 replicates of the pasture levee experiment were from sample regions B-H (Table 3; Fig. 3). Soil in this region of the levees ranged from that which could be formed into a distinct ball when squeezed in the hand and pinched out into a ribbon between the index finger and thumb to that which could not be formed into a ball or ribbon due to the presence of too much water (Table 3).

To check our laboratory findings with conditions that exist in the field, we estimated the soil moisture content on 4 separate occasions at egg sampling sites on levees in our riceland study sites in Brazoria County, Texas (Meek and Olson 1976). The results of these checks are summarized in Table 4. Again, regions of the field levees having soil moisture content that ranged between 75% field capacity to just above field capacity proved to be the ones where *Ps. columbiae* eggs were concentrated.

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