FUNCTIONS OF THE RESPIRATORY TRUMPETS AND FIRST ABDOMINAL SPIRACLES IN *AEDES TRISERIATUS* (SAY) PUPAE (DIPTERA: CULICIDAE)[1]

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ABSTRACT. The respiratory trumpets can be forced to remain in the anterior, posterior or upright positions from larval-pupal ecdysis to adult emergence with no effect. Thus the constructed bases must not act as open-close valves as suggested by Christophers (1960).

We present evidence that pupae cannot respire solely by the first abdominal spiracles, i.e., at least one trumpet must be functional. Possible functions of the first abdominal spiracles are discussed.

Under dry conditions, the distal ends of the respiratory trumpets pinch partly or completely closed. Under moist conditions they remain open. This process is reversible and operates in excised as well as intact trumpets. Distal trumpet pinching is considered to be a mechanism of water conservation.

There are 2 possible major routes for gaseous exchange in a mosquito pupa, the respiratory trumpets and the 1st abdominal spiracles which open into the ventral air space.

The respiratory role of the trumpets is well-established. In reference to the constricted bases of the trumpets, Christophers (1960) stated: "The base is very loosely articulated allowing the trumpet to be easily swung into position by surface forces when brought to the surface. At the level of this articulation is a sharp bend which may possibly act as a valve closing the air passage when the trumpet is in certain positions."

The role of the first abdominal spiracles (Fig. 1) in respiration has not been studied although Christophers (1960) noted what appeared to be the opening and closing of these spiracles and hypothesized that the pressure in the ventral air space was thereby controlled. Romoser and Nasci (1979) showed that the gas in the ventral air space of *Aedes aegypti* is forced through the 1st abdominal spiracles from the tracheal system during larval-pupal ecdysis. Under conditions of forced submergence gas can be removed from the ventral air space via the spiracles; however, this gas, once removed, is not replaced.

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The integument is known to serve a respiratory function in young larvae (Christophers 1960), but its possible respiratory role in pupae, although unlikely, has not been investigated.

Pupae can survive under conditions where free, unabsorbed water is absent, e.g. in muck and on moist filter paper (see literature cited by Leung & Romoser 1979). The roles of the respiratory trumpets and 1st abdominal spiracles may be different under these conditions than under completely submerged conditions,

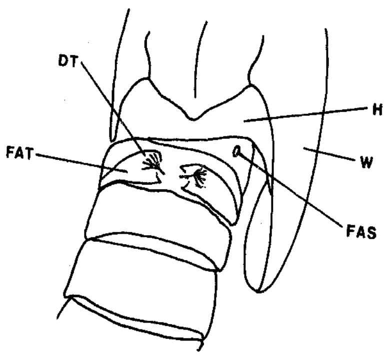

Fig. 1. Partial dorsal view of pupa with 1st abdominal tergum depressed to show right-hand spiracle. DT, dendritic tuft ("float hair"); FAS, first abdominal spiracle; FAT, first abdominal tergum; H, developing haltere; W, developing wing.

i.e., if the trumpets assumed the anterior or posterior positions and if there were an open-close valve, then a pupa would have to respire via the 1st abdominal spiracles.

Preliminary observations revealed that the distal ends of the trumpets pinch completely or partially closed when placed in a dry environment (Fig. 2A).

The purpose of this study was to determine if the constricted bases of the respiratory trumpets act as open-close valves; to examine the possible respiratory role of the 1st abdominal spiracles; and to examine the distal trumpet pinching described above.

MATERIALS AND METHODS

Rearing procedures were identical with those described by Leung and Romoser (1979). It was possible to obtain pupae of known ages.

Melted bioloid paraffin embedding compound (melting point = 50–52°C) was used to plug the trumpets and abdominal spiracles and to form casts to hold the trumpets in various positions. Molten paraffin was applied with a #2 insect pin, the tip of which was bent into a "U" shape. To prevent interference with paraffin application, the water film

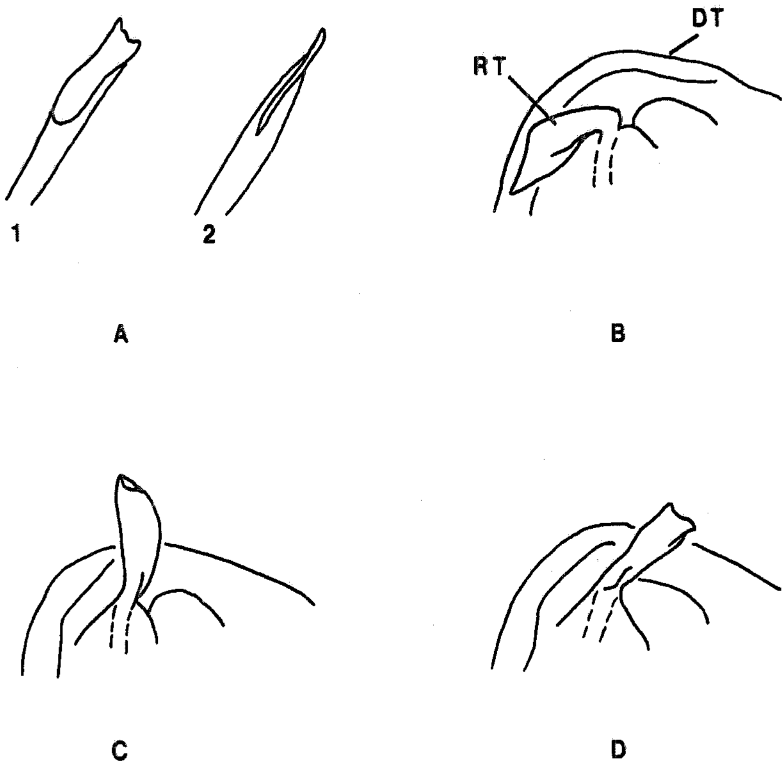

Fig. 2. A. Trumpet tip changes under wet and dry conditions; 1, wet; 2, dry. B.-D. Positions assumed by the trumpets; B, anterior; C, upright; D, posterior.; DT, dorsum of thorax; RT, respiratory trumpet.

adhering to the pupal body was removed with absorbent tissue. Treated pupae were placed on moist filter paper in small plastic cups and held in an incubator at 27±1°C. Leung and Romoser (1979) showed that greatest mortality occurs among pupae placed on moist paper during the 1st hr following ecdysis. Therefore, prior to all treatments, pupae were held for 4–5 hr in free water. All experiments were run at 27±1°C.

The following procedures were carried out on 4–5 hr old pupae. (1) To test for the possible "valve effect," trumpets were forced to remain in the anterior, posterior or upright positions (Fig. 2B-D). (2) To determine whether or not pupae could obtain sufficient air to survive to adult emergence via the first abdominal spiracles, both trumpets were plugged in pupae of 3 age groups, 4, 24 and 55 hr. (3) To see if pupae could survive by respiring solely by means of the trumpets, the 1st abdominal spiracles were plugged. (4) A single trumpet was plugged to see if introduction of paraffin is toxic. (5) To test for possible lethal effects, other than blockage of respiratory openings, small drops of paraffin were applied to the cuticle on both sides of the thorax near the trumpets and inside the ventral air space near the first abdominal spiracles. Three to five replicates with more than 20 pupae per replicate were used for each treatment.

To study distal trumpet pinching, groups of pupae 30–60 min and 4–5 hr post-larval-pupal ecdysis were put on dry filter paper in open containers exposed to the atmosphere within the incubator or on moist paper in closed containers. The degree of pinching under these conditions was checked at intervals.

To check the possible reversibility and repeatability of trumpet tip pinching, pupae of ages approximately 30–60 min, 24 and 48 hr post-ecdysis were subjected to alternating dry and moist conditions and after an interval of 30 or more min in a given condition, the degree of closure noted.

To determine if trumpet pinching involves mechanisms located outside the trumpets, 48 trumpets were excised from 30–60 min pupae, affixed to the edge of a strip of masking tape (1/32 in. wide) and attached to the inside of the lid of a plastic cup. By moving the lid, the trumpets could be exposed alternately to dry and moist conditions.

To determine the position assumed by the trumpets relative to the thorax under moist and dry conditions, 10 pupae were exposed to 5 wet (15 min)–dry (15 min) cycles. Trumpet position was noted at the end of each dry period and each wet period. All data were then grouped.

Arcsin transformation of all percent data was performed before statistical analysis.

RESULTS

Mean percent survival in the anterior and posterior trumpet position groups was lower than the control group (Fig. 3) ($P \leq 0.05$; C-P: $t = 4.26$, d.f. = 4; C-A: $t = 5.35$, d.f. = 4). There was no difference between the control (C) and upright (U) groups (Fig. 2) ($P < 0.05$; $t = 1.18$, d.f. = 4). There was no difference in mean percent survival in the single-trumpet-plugged group (T) and the control (C) ($P \geq 0.05$; $t = 1.53$, d.f. = 5). There were no differences among the various age groups with both trumpets plugged (TT4-TT24: $t = 0.61$, d.f. = 4; TT4-TT55: $t = 1.47$, d.f. = 4; TT24-TT55: $t = 0.42$, d.f. = 4; $p \geq 0.05$). Mean percent survival in the group with the 1st abdominal spiracles plugged was lower than the control group ($P \leq 0.05$; $t = 2.306$, d.f. = 8), but still comparatively high.

Figure 4 shows the effect of constant dry conditions on the trumpets of 12 pupae 30–60 min post-ecdysis when first put on dry paper. Pupae with both trumpets pinched closed, one closed and one partially closed, one closed and one open or both partially closed are treated as a group. All trumpets were open initially. Within 15 min, most were partially or completely closed and remained so. Variability occurred in 2 or 3 of the pupae and was not randomly scattered. Figure 4 also shows the effect of constant dry conditions on eleven 4-5 hr old pupae. Most responses occurred within 15 min. In comparison with the 30–60 min pupae a greater percentage of the trumpets in the 4-5 hr pupae remained open.

The nine 30–60 min and eleven 4–5 hr pupae which were placed on moist paper kept their trumpets open all of the time.

Figure 5 shows the effect of alternating dry and moist conditions on trumpet tip pinching. Trumpet tips can be reopened after once being closed and this cycle can be repeated.

Excised trumpets responded to alternation of dry and moist conditions in a manner similar to intact trumpets in pupae of the same age.

Under dry conditions most trumpets remain upright (Fig. 6). Under moist conditions the majority are found in the anterior or posterior positions. It appears that when the trumpets are moved close to the body surface, they adhere to the water film and are held in place. If the

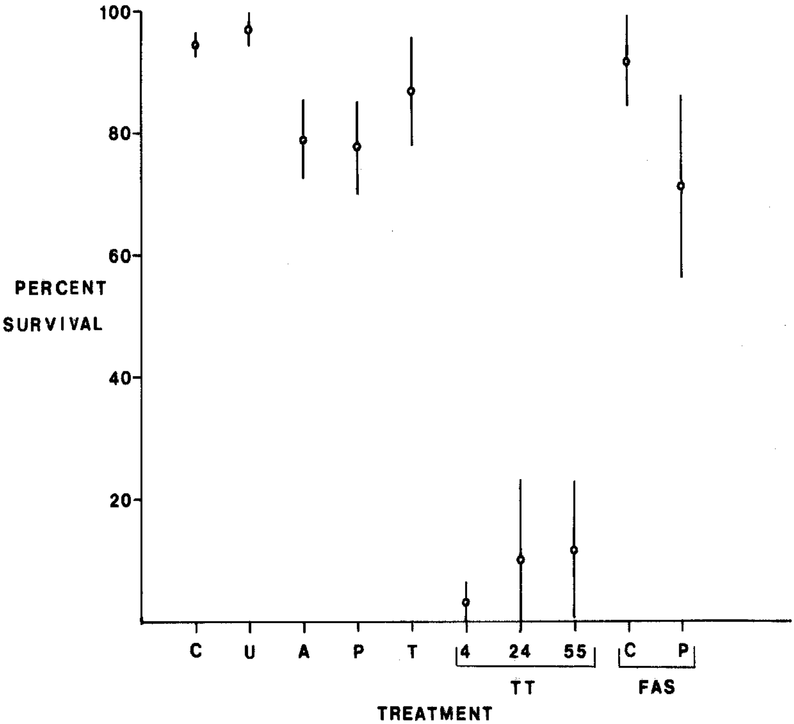

Fig. 3. Effect of plugging trumpets and 1st abdominal spiracles amd forcing trumpet position. C, control, paraffin applied to thorax (number/replicate = 13, 25, 17); U, A & P, trumpets forced to remain in the upright (number/replicate = 20, 20, 20), anterior (number/replicate = 20, 23, 21) and posterior (number/replicate = 20, 20, 23) positions respectively; T, single trumpet plugged (number/replicate = 20, 25, 21, 18); TT, both trumpets plugged at 4 hr (number/replicate = 20, 21, 21), 24 hr (number/replicate = 20, 20, 20), 55h (number/replicate = 20, 20, 19); FAS-C, control, paraffin applied near 1st abdominal spiracles (number/replicate = 40, 20, 20, 20, 20); FAS-P, 1st abdominal spiracles plugged (number/replicate = 20, 20, 20, 20, 41); circle, mean; vertical line, standard deviation.

body surface loses the water film the trumpets have nothing to hold them down and body movement shifts the trumpets into the upright position.

DISCUSSION

When trumpets were forced to remain in a given position, there was greater mortality among the anterior and posterior groups. However, survival in these groups was quite high and the paraffin may have interfered with emergence in some individuals. It appears that there is no open-close valve effect relative to trumpet position. Pupae can respire adequately with the trumpets in any position.

Most of the pupae with both trumpets plugged died. The few pupae which survived are considered to have been inadequately plugged. That inadequately plugged pupae can survive is consistent with the fact that most of the pupae with only a single trumpet plugged survived to

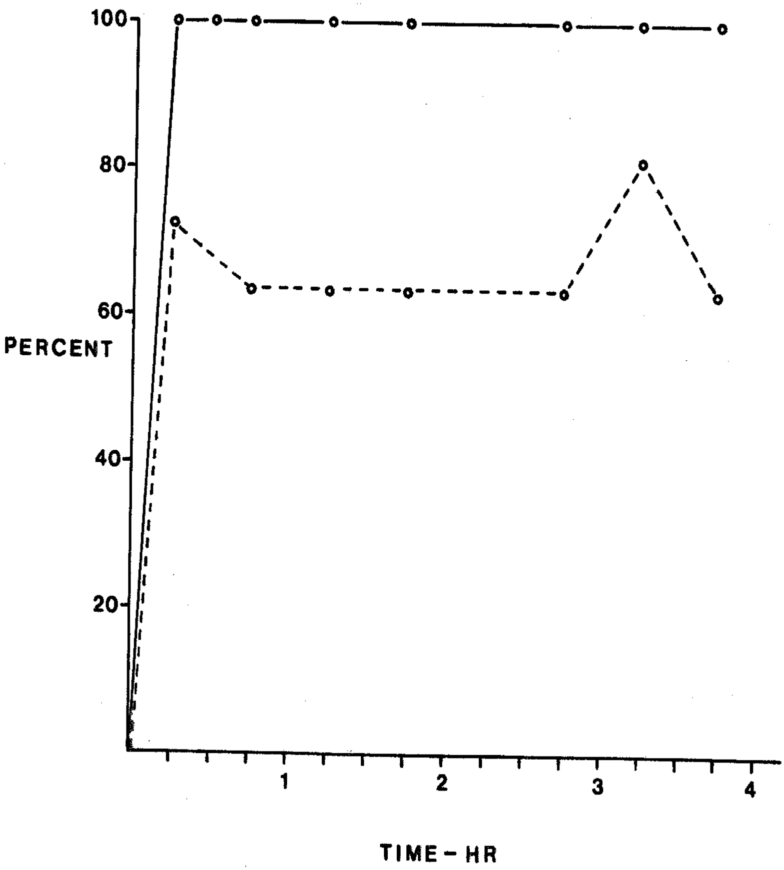

Fig. 4. Trumpet pinching under constant dry conditions. Ordinate, percent with at least one trumpet closed or both partially closed; abcissa, time in hr; solid curve, pupae 30–60 min post-ecdysis (n = 12); dashed curve, pupae 4–5 hr post-ecdysis (n = 11).

emergence. The 1st abdominal spiracles (+integument?) apparently do not supply sufficient air for survival, at least over an extended period.

Plugging the 1st abdominal spiracles causes only a slight decrease in survival when compared with controls. We consider this reduction to be due to trauma and not blockage of the spiracles. The 1st abdominal spiracles could conceivably function in the release of carbon dioxide which is highly soluble and would rapidly diffuse into surrounding water. However, this must not be a critical function since pupae survive with these spiracles plugged. Although more work is needed, evidence to date suggests that the major role of the 1st abdominal spiracles is the filling of the ventral air space at pupation and in the withdrawal of gas only under extreme conditions, i.e. forced submergence (Romoser & Nasci 1978).

Under moist conditions, trumpet tips remain open. Under dry conditions, they become partially or fully pinched closed. Opening and closing repeat as conditions alternate between moist and dry. Excised trumpet tips undergo the same closing

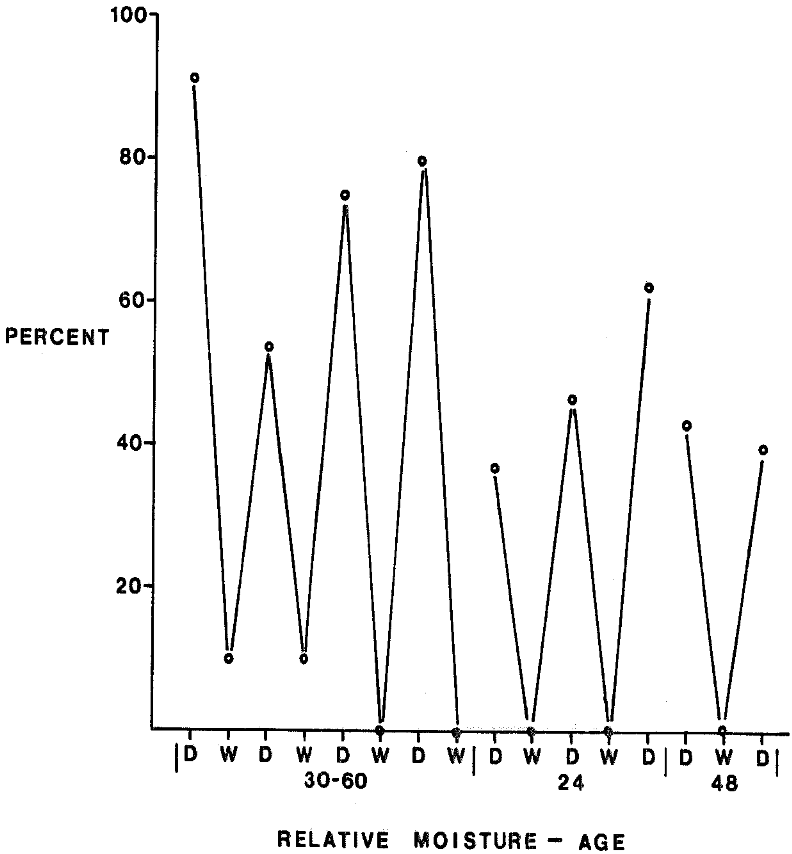

Fig. 5. Trumpet pinching under alternating dry (D) and moist paper (W) conditions. Ordinate, as in Fig. 4; abcissa, ages, 30–60 min (n= 10), 24 hr (n= 10) and 48 hr (n= 10) post-ecdysis.

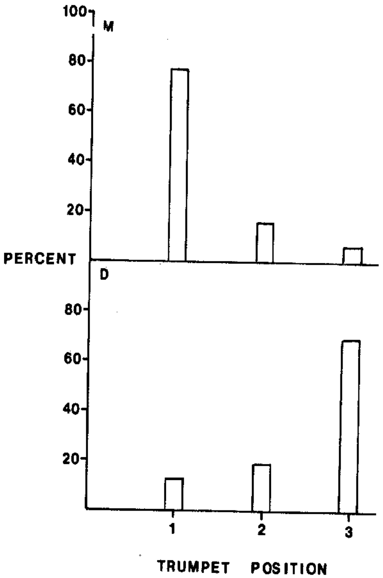

Fig. 6. Trumpet position (abcissa) under dry (D) and moist paper (M) conditions (n=10). 1, trumpets in anterior, posterior or combination of anterior and posterior positions; 2, 1 trumpet upright; 3, both trumpets upright.

and opening as intact trumpets. Pinching appears to be physical as opposed to neuromuscular.

Trumpet tip pinching probably retards water loss in pupae exposed to drying conditions. It is well-known that insects can lose large amounts of body water through spiracular transpiration. This would seem to be especially true of the pupal stage since there is no feeding or drinking and hence no major intake of water.

Under moist conditions the trumpets usually assume an anterior, posterior or combination of anterior and posterior positions. Under dry conditions the trumpets usually assume an upright position. We have presented evidence that there is no open-close valve effect relative to the constricted trumpet bases and respiration. The fact that the trumpets assume an upright position under relatively dry conditions suggests that such a valve effect relative to moisture retention is unlikely.

Literature Cited

Christophers, S. R. 1960. *Aedes aegypti* (L.) *The Yellow Fever Mosquito.* Cambridge University Press, Cambridge, England, xii + 739 pp.

Leung, M. L. and W. S. Romoser. 1979. Survival of *Aedes triseriatus* (Say) pupae in the absence of free water. Mosquito News 39, 477–482.

Romoser, W. S. and R. S. Nasci. 1979. Functions of the ventral air space and first abdominal spiracles in *Aedes aegypti* pupae (Diptera: Culicidae). J. Med. Entomol. 15:109–114.