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EFFECT OF MECHANICAL SHOCK ON HYDROSTATIC BALANCE AND SURVIVAL OF MOSQUITO PUPAE

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ABSTRACT. The presence and distribution of ventral air space gas assures that mosquito pupae are positively buoyant and that they float, dive, and ascend in an upright, balanced orientation. Our objective was to test the effects of mechanical shocks of varying magnitude on mosquito pupae representing 3 genera. Forces that disrupt the pupa's buoyancy and/or hydrostatic balance are of a much lower magnitude than those that would cause tissue damage. Once hydrostatic balance and/or buoyancy are compromised, pupae are unable to restore them and eventually drown. This could represent a weak link in the mosquito life cycle. It is possible that mechanical shock or sound-generating devices could be designed that could break this link.

INTRODUCTION

Mosquito pupae are less dense than water and hence float, as has been known for many years. This positive buoyancy is due to the presence of gas in the ventral air space, a cavity formed by the developing wings, legs, and mouthparts (Romoser 1975, 1978; Romoser and Nasci 1979). In addition to providing for positive buoyancy, the ventral air space gas, due to the bilaterally symmetrical topography of the ventral air space itself, assures that the pupa floats, dives, and ascends in an upright, balanced position. The surface of the ventral air space cuticle is hydrophobic; the rest of the pupal cuticle is hydrophilic (Romoser and Nasci 1979). This hydrophobicity contributes to balance by maintaining the spatial integrity of gas distribution in the ventral air space.

While studying buoyancy and diving behavior in *Aedes aegypti* (Linn.) pupae, a small tube of water containing a pupa was accidently dropped and we observed that the mechanical shock had disrupted the pupa's hydrostatic balance and reduced its buoyancy. This suggested that perhaps even relatively slight mechanical disruption of hydrostatic balance in mosquito pupae might cause them to drown. If so, perhaps a device could be developed to deliver a mechanical shock of appropriate magnitude to kill pupae, particularly those breeding in discrete circumstances (e.g., container-breeding mosquitoes such as *Ae. aegypti*).

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The objectives of this study were to determine: 1) the relationship between different magnitudes of mechanical shock and pupal drowning, and 2) if the relationship between mechanical shock and drowning varies between males and females, among pupae of different ages, as a function of water depth, and among mosquitoes of different genera.

MATERIALS AND METHOD

We designed a simple apparatus that facilitated the controlled dropping of a small plastic tube, containing water and a pupa, from different heights. It consisted of a glass guide tube of slightly more than 100 cm in length held in a vertical position by a clamp and ring stand holder. This glass tube was marked in 10-cm increments starting at the bottom. The entire apparatus was set on a marble slab.

To expose a pupa to mechanical shock, we placed it in the drop tube with water and inserted a plastic cap that was weighted with a piece of plasticene. The drop tube was then placed in the guide tube and dropped the desired distance. After dropping, the tube was retrieved from the bottom of the guide tube by means of a thread that was taped to the tube containing the pupa. By dropping the tube from various heights, we could consistently produce a range of mechanical shocks.

We studied 3 mosquito species: *Aedes aegypti, Culex pipiens* Linn., and *Anopheles stephensi* Liston. After making several preliminary observations, we settled on the following experimental protocol: 1) Samples of pupae were exposed to a range of impact forces generated by dropping them from 0 to 100 cm. 2) The drop tube, which contained 10 ml of water, was dropped only when the test pupa was in the middle of the water-containing portion of the tube. 3) After exposure to a given mechanical shock, a test pupa was placed in a holding vial and checked 24 h later to see if it was still alive or if it had drowned.

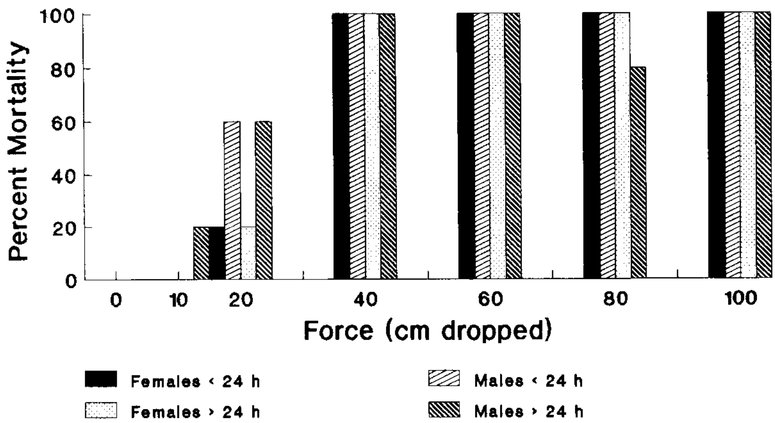

Fig. 1. Mortality as a function of distance dropped in *Aedes aegypti* pupae (n = 130; approximately evenly divided among the 4 groups, i.e., about 5 pupae/group/force.). Ten 4th-instar larvae were tested at 100 cm and mortality was 0.

Using our experimental protocol, we tested each of the following in each of the 3 mosquito species: 1) female pupae, 2) male pupae, 3) pupae younger than 24 h old, and 4) pupae older than 24 h postpupation. In addition, we tested the effect of holding *Ae. aegypti* and *Cx. pipiens* pupae in very shallow water (0.5 ml) following exposure to mechanical shock.

In order to determine if the magnitude of mechanical shock necessary to cause a pupa to drown would have any effect on aquatic organisms that lack a ventral air space and associated gas, we tested 4th-instar larvae at the maximum force (i.e., dropped 100 cm).

RESULTS

We observed a variety of outcomes ranging from no effect; to no apparent immediate effect, but with the pupa drowning by 24 h later; to immediate loss of balance and/or positive buoyancy. In a few cases, pupae that had begun to undergo the increases in buoyancy associated with pupal–adult ecdysis (Walker and Romoser 1987) were less vulnerable to mechanical shock.

Figures 1–3 show the influence of increasing magnitude of mechanical shock on *Ae. aegypti, Cx. pipiens,* and *An. stephensi* pupae, respectively. Four groups of pupae are included: females less than 24 h old, females more than 24 h old, males less than 24 h old, and males more than 24 h old. After exposure, all specimens were held in vials containing 10 ml of water. It is apparent that most pupae, regardless of sex or age, ultimately drown if exposed to the shock generated by dropping them 40 or more centimeters. Lower numbers are killed by the shock generated by dropping them less than 40 cm, but

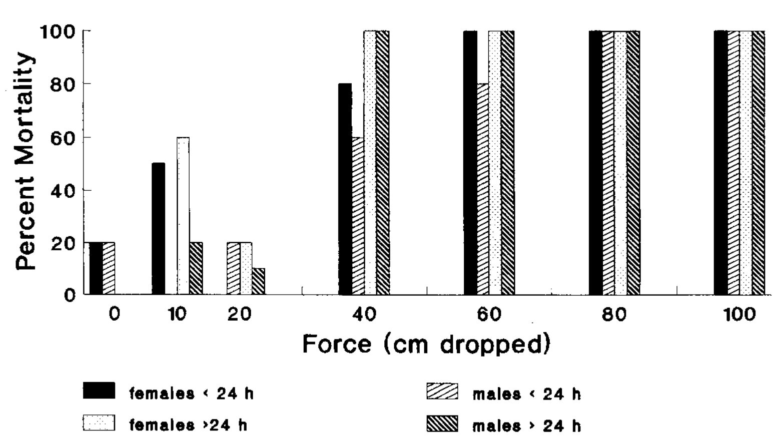

Fig. 2. Mortality as a function of distance dropped in *Culex pipiens* pupae (n = 139; approximately evenly divided among the 4 groups, i.e., about 5 pupae/group/force.) Ten 4th-instar larvae were tested at 100 cm and mortality was 10%.

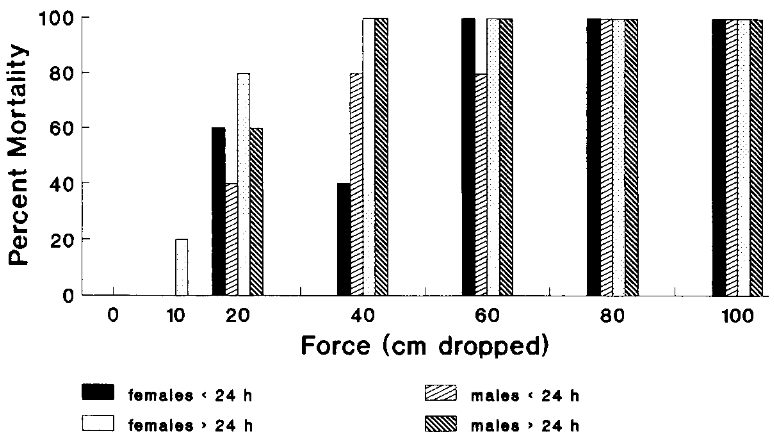

Fig. 3. Mortality as a function of distance dropped in *Anopheles stephensi* pupae (n = 134; approximately evenly divided among the 4 groups, i.e., about 5 pupae/group/force.). Five 4th-instar larvae were tested at 100 cm and mortality was zero.

no consistent patterns are apparent. No *Ae. aegypti* and *An. stephensi* and only one in 10 *Cx. pipiens* 4th-instar larvae were killed by dropping them the maximum distance.

Figures 4 and 5 allow comparison of the results of holding *Ae. aegypti* and *Cx. pipiens* pupae previously exposed to different degrees of mechanical shock in very shallow water (i.e., 0.5 ml vs. 10 ml of water in the holding vials). Measurements were made only using the forces associated with dropping the tube 20, 60, and 100 cm. The curves represent the results generated by holding pupae in shallow water and the bars represent holding in deeper water. Easier access to the air–water interface in the shallow water may have enabled a few pupae to survive that would otherwise have drowned. However, the results do not depart dramatically from those obtained from pupae held in deeper water and it thus appears that the depth of water in which pupae are held after exposure to mechanical shock does not have a major impact on survival.

DISCUSSION

Using a simple apparatus to generate mechanical shocks of varying magnitude, we have provided a crude demonstration that the mechanism by which mosquito pupae are positively buoyant and by which they maintain hydrostatic balance makes them vulnerable to mechanical forces that disrupt this mechanism. This vulnerability exists in males and females and in young and old pupae. Once their hydrostatic balance and buoyancy are compromised, pupae are unable to re-

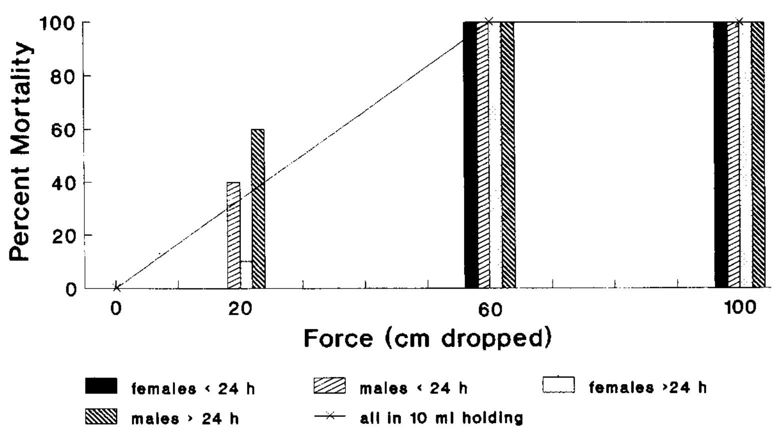

Fig. 4. Mortality as a function of distance dropped in *Aedes aegypti* pupae. Curves represent pupae held in shallow (0.5 ml) of water in holding tube following mechanical shock (n = 80 divided among the groups and forces, 5–10 pupae/group/force). Vertical lines represent pupae held in deep (10 ml) of water (n = 79).

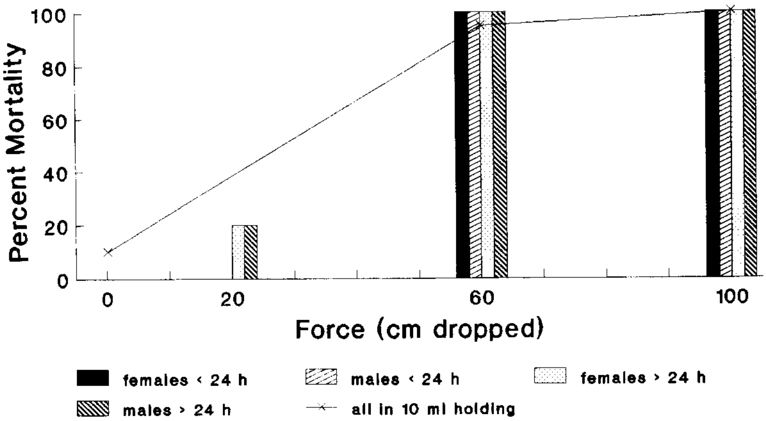

Fig. 5. Mortality as a function of distance dropped in *Culex pipiens* pupae. Curves represent pupae held in shallow (0.5 ml) of water in holding tube following mechanical shock (n = 80 divided among the groups and forces, 5–10 pupae/group/force). Vertical lines represent pupae held in deep (10 ml) of water (n = 80).

store them and eventually drown. Fourth-instar mosquito larvae are similar in mass to mosquito pupae, but lack a ventral air space and associated gas. We used these larvae as controls to represent nontarget aquatic arthropods. Our results show that mosquito larvae are much less vulnerable to mechanical shock than mosquito pupae.

We present this phenomenon as indicative of a potential weak link in the mosquito life cycle, namely the pupa. It seems possible that effective ways, such as the use of mechanical shock or a sound-generating device, could be designed to break this link. We view the experiments reported here as very simple and preliminary and our results are presented only to illustrate the phenomenon of pupal vulnerability to mechanical shock. We are currently initiating a more rigorous series of experiments in order to physically characterize the reported phenomenon under a variety of conditions.

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