FEEDING RATE OF LARVAL *AEDES VEXANS* STIMULATED BY FOOD SUBSTANCES

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ABSTRACT. Feeding rates of fourth instar larvae of *Aedes vexans* were compared by counting substrate filled gut segments after exposure to food or inert particles. Food particles (wheat flour, fishmeal or yeast) were ingested approximately 3 times faster than inert particles (kaolin, pumice or synthetic cellulose). Aqueous fishmeal extract accelerated ingestion of inert particles to the level of ingestion of food particles, demonstrating gustatory stimulation of larvae. Absolute amounts of ingested materials were calculated on the basis of dry weights of guts completely filled with test substrates. At 21°C, 46–74 μg of food particles, and 15–33 μg of inert particles were ingested per larva during 10 min of exposure. In the subsequent time span, ingestion rates of food particles decreased continuously with satiation of larvae.

INTRODUCTION

In general, non-predaceous mosquito larvae are believed to be omnivorous and to filter particles indiscriminately out of the water of their breeding habitat (Clements 1963). This opinion is supported in that particles present in the breeding water can always be found in the gut of mosquito larvae inhabiting the water (Kühlhorn 1958, Gozhenko and Titova 1981). In addition, larvae also ingest inert materials such as dye particles, charcoal, diatomaceous earth or talc under experimental conditions (Schildmacher 1950). However, qualitative identification of a broad range of particles present in the gut does not prove that food intake is unregulated. Larvae of *Culex pipiens* Linn. were found to ingest cells of *Chlorella* and yeast more rapidly than inert materials like kaolin or brick dust (Dadd 1968). In addition, the presence of yeast extract stimulated the ingestion of inert particles, demonstrating that ingestion was influenced by chemical factors (Dadd 1970b). Thus, *Cx. pipiens* larvae are able, in effect, to select food substrates by differential rates of ingestion.

In recent years, feeding behavior of mosquito larvae has gained new interest due to the increasing use of the toxin(s) produced by *Bacillus thuringiensis* var. *israelensis* in larval mosquito control (Lacey 1985). The toxin acts as a stomach poison and is only effective when ingested. The purpose of this study was to quantify the effect of inert and food particles on the speed and level of particle ingestion by fourth instar larvae of *Aedes vexans* Meigen. This information should be useful in selecting suitable carrier materials for the formulation of *B. thuringiensis* var. *israelensis* toxin for use in mosquito control.

MATERIALS AND METHODS

Eggs of *Aedes vexans* were collected in soil samples at natural breeding sites in the Upper Rhine Valley, West Germany. Eggs were hatched by flooding samples with water (30°C), and larvae were reared at 20–25°C on detritus-fish food diet as described earlier (Aly 1985). Early fourth instar larvae were used in the experiments. Prior to exposure to test substrates, larvae were collected in a net and kept in distilled water (21–22°C) for 10–15 min. Tests were started by pipetting larvae into prepared suspensions within a time span of approximately 20 sec, and terminated by collecting and immobilizing larvae in ice cold 20% ethanol. Ingestion rates were evaluated by counting the substrate filled gut segments under a dissecting microscope, labelling the gut within the thorax as gut segment 1, within the first abdominal segment as gut segment 2, and so on (Fig. 1). This method was successfully employed to evaluate the feeding rate in larvae of the mosquito *Culex pipiens* (Dadd 1968).

INGESTION OF WHEAT FLOUR RELATIVE TO EXPOSURE TIME. Nine groups of 20 larvae each were exposed to an excess of wheat flour in 200 ml of water (21–22°C). After 10, 20, 30, 40, 60, 80, 100, 120 and 140 min, larvae from one beaker were removed and killed. The number of wheat filled gut segments/larva, and the

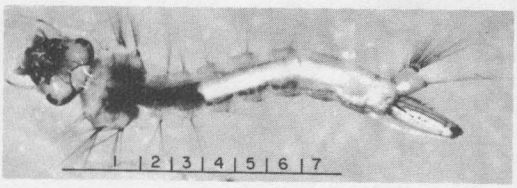

Fig. 1. *Aedes vexans* fourth instar larva. Gut segment 1–3 filled with dark organic debris, segments 4–7 filled with wheat flour. Note the sharp separation of organic debris and wheat flour, demonstrating that contents of gut are not mixed by peristalsis. Contents present in the 7th gut segment are already separated from the food column and will be defecated during the next minutes.

number of completely filled larvae/group was determined.

PARTICLE INGESTION IN A NATURAL HABITAT. Since in the laboratory experiments ingestion rates varied broadly among individuals, the particle ingestion of larvae which were not removed from the habitat was assessed. Two temporary water bodies in the redundation zone of the river Rhine (Federal Republic of Germany) were selected, each holding approximately 1 m^3 of water and more than 1,000 fourth instars larvae of *Ae. vexans*. In one pond, a suspension of 200 g fishmeal was distributed by gentle stirring; the other pond was similarly treated with 200 g kaolin. After 20 min, approximately 30 larvae were collected with a net, and transported in ice cold diluted ethanol to the laboratory. Ingestion rates were determined as described above.

INGESTION OF DIFFERENT MATERIALS. Three groups of 20 larvae each were exposed to an excess of fishmeal, wheat flour, yeast, pumice, kaolin or Sigmacell® (a synthetic cellulose) suspended in 200 ml of water (21–22°C). After 10, 20, and 34 min, one group of larvae was collected and killed; the number of gut segments filled with the offered substrate was determined. To estimate the weight of ingested material, 3 groups of 10 larvae each were exposed to one of the test materials listed above until larvae had filled their entire gut with the offered substrate. Larvae were killed and dissected. The gut content enveloped in the peritrophic membrane was dried at room temperature for 3 days under vacuum (22°C) on calibrated pieces of aluminum foil. Dry weights were determined on an electronic balance with an accuracy of ± 2 μg.

STIMULATION OF INGESTION BY FISHMEAL EXTRACTS. In this series of experiments, the influence of fishmeal extracts on the ingestion of inert materials was determined. Five grams of fishmeal were extracted in 100 ml acetone, or distilled water, by grinding the fishmeal in a mortar with the liquid for 5 min. Extracts were filtered twice through Whatman No. 1 filter paper. Ten ml of the acetone extract were mixed with 500 mg kaolin, talc or Sigmacell. The solvent was evaporated at 60°C, and the dry materials were suspended in 15 ml water and centrifuged for 5 min at 8,000 rpm. The pellet was resuspended in 200 ml distilled water (test suspension). Ingestion of materials pretreated with acetone extract was compared with ingestion of fishmeal (positive control) and ingestion of untreated materials (negative control). Aqueous extract was directly added to 500 mg kaolin, talc or Sigmacell, suspended in 200 ml distilled water. Ingestion of these materials in extract concentrations of 0.1, 1 or 10 ml extract/200 ml water was compared with the ingestion of fishmeal, and ingestion of the test materials in the absence of extract. All experiments were conducted with 10 larvae/200 ml suspension, exposed for 10, 20 or 30 min. With 0 and 10 ml of aqueous extract, experiments were replicated 3 times.

RESULTS

INGESTION OF WHEAT FLOUR RELATIVE TO EXPOSURE TIME. Exposed to an excess of wheat flour, larvae ingested particles to fill the first 3 gut segments within 10 min, 4 segments within 20 min and 5 segments within 40 min (Fig. 2). Larvae with guts completely filled with wheat flour were observed after 60 min (10%), 80 min (20%), 100 min (35%), 120 min (65%) and after 140 min (100%) of exposure. Thus, food was not ingested at a constant rate over time, but was ingested more slowly as larvae became satiated.

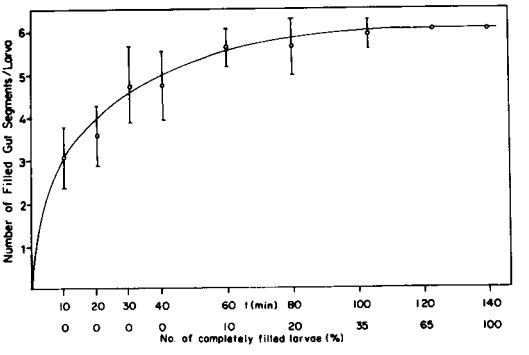

Fig. 2. Feeding rate of larvae on wheat flour, expressed as mean number of wheat filled gut segments of 20 larvae. Bars represent standard deviation.

PARTICLE INGESTION IN A NATURAL HABITAT. Broad variability in ingestion rates of individuals was also observed after treatment of larvae with fishmeal or kaolin in a natural breeding pond (Fig. 3). Treatment with kaolin resulted in an average of 2.87 gut segments filled with this substrate, with a standard deviation of 1.07. Larvae treated with fishmeal had an average of 4.47 (standard deviation 1.06) gut segments filled. Distribution of ingestion rates was normal. Although standard deviations overlapped, it appeared that fishmeal was ingested more rapidly than kaolin.

INGESTION OF DIFFERENT MATERIALS. Food substances (wheat flour, fishmeal and yeast) were ingested with similar velocity, although particle size differed; wheat flour consisted of oval spheres with diameters ranging between 4 and 30 μm, whereas yeast and fishmeal aggre-

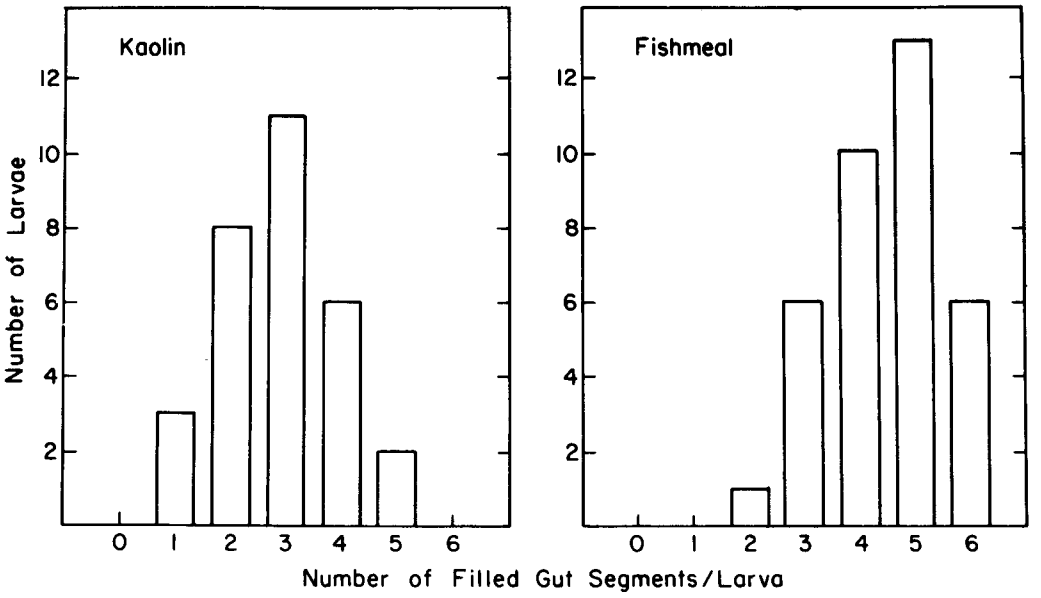

Fig. 3. Distribution of ingestion rates of larvae treated in natural breeding ponds with a suspension of kaolin or fishmeal.

gated in clusters with diameters up to 140 μm. Inert particles (cellulose, kaolin and pumice) were ingested significantly slower than food substances (paired *t*-test, $p \leq 0.01$) (Fig. 4). Among the different inert materials, there was no significant difference in velocity of ingestion, although the test materials were different in chemical and physical nature; e.g., Sigmacell consisted of uniform, elongated particles of 40 × 140 μm, kaolin and pumice of edged crystals with 2–40 μm diameter.

STIMULATION OF INGESTION BY FISHMEAL EXTRACTS. Results from the previous experiment indicated that differences in chemical composition, rather than particle size and shape, influenced velocity of ingestion of particles by larvae. The addition of aqueous extract increased the velocity of ingestion of inert materials (Table 1). There was a tendency for increasing extract concentrations to increase ingestion, with a concentration of 10 ml/200 ml

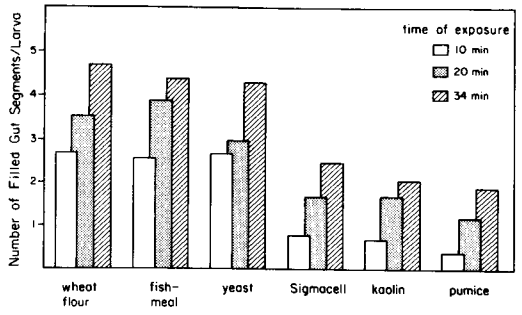

Fig. 4. Feeding rate of larvae on different substrates, expressed as mean number of filled gut segments of 10 larvae.

Table 1. Ingestion of inert particles, as related to presence and concentration of aqueous fishmeal extract.[a]

Substrate	Extract concentration (ml/200 ml water)	Gut segments filled[b] Time of exposure (min) 10	20	30
Fishmeal	—	2.9	4.0	5.0
Kaolin	0	1.0**[c]	2.4*	2.9*
	0.1	1.6*	2.4*	3.5*
	1.0	1.1**	2.6*	4.9
	10.0	2.9	3.8	5.4
Talc	0	0.5**	1.1**	1.7**
	0.1	0.7**	0.7**	2.2**
	1.0	1.5*	3.1*	—
	10.0	1.8	3.2	4.3
Sigmacell[d]	0	1.0**	1.5**	2.9**
	0.1	1.0**	3.1*	3.7*
	1.0	1.6*	3.3	4.0
	10.0	2.5	4.0	4.4

[a] 500 mg fishmeal extracted in 10 ml distilled water.

[b] Counted in intact larvae, separation of gut segments see Fig. 1; mean of 10 larvae.

[c] Significance of difference between individual substrate ingestion rates and ingestion rates of fishmeal (paired *t*-test within column): **: $P \leq 0.001$, *: $P \leq 0.01$.

[d] Synthetic cellulose.

water eliciting the highest response. In subsequent tests, therefore, the influence of this concentration was compared with a negative (inert particles without extract) and a positive (fishmeal) control. In the presence of 10 ml extract/200 ml water, kaolin, talc, and cellulose were ingested as quickly as fishmeal (Fig. 5). Pretreatment of inert materials with acetone extract did not increase the rate of ingestion (Table 2).

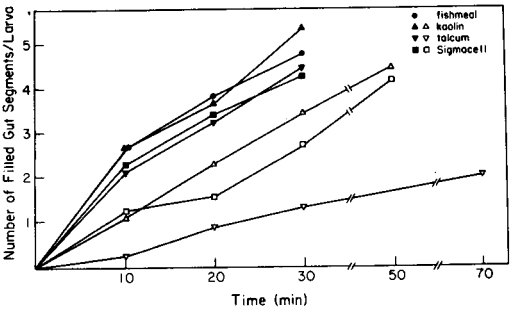

Fig. 5. Stimulation of feeding rate of larvae on inert particles with aqueous fishmeal extract; data points represent mean number of filled gut segments of 30 larvae, tested during 3 replicates. Open symbols: inert particles without extract; closed symbols: fishmeal (●), or inert particles in the presence of 10 ml extract/200 ml water.

Table 2. Ingestion of inert particles, as related to pretreatment of particles with acetone fishmeal extract.

Substrate	Pretreatment[a] + = pretreated − = not pretreated	Gut segments filled[b] Time of exposure (min) 10	20	30
Fishmeal	−	2.6	3.7	4.5
Kaolin	−	1.3*[c]	2.2*	3.1*
	+	1.6*	2.1**	2.5**
Talc	−	0.3**	0.8**	0.5**
	+	0.2**	0.4**	0.3**
Sigmacell[d]	−	1.6*	1.7**	3.1*
	+	2.1	2.3**	2.5*

[a] Pretreated particles: 500 mg mixed with 10 ml acetone extract from 500 mg fishmeal, solvent evaporated.

[b] Counted in intact larvae, separation of gut segments see Fig. 1; mean of 10 larvae.

[c] Significance of difference between individual substrate ingestion rates and ingestion rates of fishmeal (paired *t*-test within column) **: $P \leq 0.001$, *: $P \leq 0.01$.

[d] Synthetic cellulose.

DISCUSSION

The present study demonstrates phagostimulation of *Ae., vexans* larvae by chemical compounds associated with food substrates. Larvae ingested wheat flour, fishmeal and yeast approximately 3 times faster than inert particles like kaolin, cellulose or talc. In another study, the same food substances, but not the inert materials, acted as arrestants for *Ae. vexans* larvae (Aly 1983). Thus larvae behaviorally select suitable food substances. In the absence of gustatory stimulants, larvae interrupt feeding frequently and search for food sources; in the presence of stimulants, searching decreases (Aly 1983), and feeding activity is increased, as demonstrated here. A similar behavioral pattern was found in larvae of *Cx. pipiens* (Dadd 1970b).

Phagostimulant compound(s) are polar. They were present in food substrates as different as wheat flour, fishmeal and yeast, and were extractable with water. Detailed studies with *Cx. pipiens* larvae (Dadd et al. 1982) demonstrated phagostimulation by amino acids, sugars and nucleic acids. However, deletion of one phagostimulant compound in a complex diet did not result in decreased feeding of exposed larvae. Dadd et al. (1982) concluded that a variety of components, rather than one particular compound, stimulates ingestion in *Cx. pipiens* larvae. A similar situation might be true for the larvae of *Ae. vexans*. But it is also possible that a single, ubiquitous compound is a phagostimulant for *Ae. vexans* larvae, as has been demonstrated in other omnivorous insects (Lindstedt 1971).

In all experiments, particle filtration varied broadly among individuals. Similar levels of variation have been observed in the ingestion rates of other filter feeding larval Diptera (*Cx. pipiens:* Dadd 1968; Simuliidae: Kurtak 1978). Jorgensen (1975) reviewed the physiology of suspension feeding and regarded high variability in filtration rates often to be a laboratory artifact. In the present study, however, larval *Ae. vexans* showed the same level of variation in their feeding rates, when ingestion was assessed in a natural breeding pond. Therefore, individual variation is unlikely to be of artificial origin; instead, phases of active feeding alternate most likely with resting phases. Such a behavior was directly observed in larval *Cx. pipiens* (Dadd 1970a).

Ingestion of food by the larvae was dependent on increasing satiation of larvae. After the filling of 3–4 gut segments within 10 min, velocity of ingestion was reduced to 0.5–1 segment filled in the subsequent 10 min period. Similarly, satiated larvae stop diving for food located at the bottom of their habitat and rest at the water surface (Aly and Schnetter 1985).

In laboratory experiments, formulations of *Bacillus thuringiensis* var. *israelensis* toxin as food

bait have been more effective against *Ae. vexans* larvae than with toxin suspensions (Aly 1983). Results presented here demonstrate that replacement of gustatory food materials in baits by inert materials may not be appropriate since under field conditions larvae will tend to ingest particulate material only on those regions of the pond that have high concentrations of nutrient particles. Since the stimulatory component(s) are water soluble, successful impregnation of inert materials with extract seems difficult, unless a formulation of toxin, inert carrier and phagostimulant extract also includes some material that delays diffusion of extract from the bait.

ACKNOWLEDGMENTS

The author acknowledges support of the study by Dr. H. W. Ludwig and Dr. W. Schnetter (both University of Heidelberg/FRG), and critical review of the manuscript by Dr. B. Mullens and Dr. R. H. Dadd (both University of California/USA, at Riverside and Berkeley). This work was partly supported by UNDP/World Bank/WHO Special Programme for Research & Training in Tropical Diseases.

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