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Feeding Inhibition in Black Fly Larvae (Diptera: Simuliidae) and Its Effects on the Pathogenicity of *Bacillus thuringiensis* var. *israelensis*[1]

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ABSTRACT

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The normal feeding behavior of *Simulium vittatum* larvae was disrupted following exposure to high concentrations of suspended particles under simulated stream conditions. Virtually all particulates tested caused inhibition of feeding at a concentration of 50 mg/liter, indicating that inhibition is a nonspecific response. The percentage of larvae with inhibited feeding increased with increasing particle concentration. Our results indicate that inhibition was a consequence of rapid gut filling.

The pathogenicity of *Bacillus thuringiensis* var. *israelensis* against *S. vittatum* was diminished or enhanced depending on whether feeding inhibition was induced before or after bacterial exposure. These results were attributed to reduced rates of bacterial ingestion and voidance, respectively. Such effects may have important implications in laboratory bioassays and field control.

Introduction

Simulium larvae inhabit flowing fresh water where most species secure food by filter feeding while attached to rocks, vegetation or other stable substrates. Larvae filter feed continually by means of large, highly specialized, cephalic fans which strain suspended particles from the passing water. The fans are alternately extended for food capture and retracted for cleaning.

Larvae feed indiscriminately, ingesting sand and silt particles as well as algae and other organic matter (Anderson and Dicke, 1960; Wotton, 1977). Kurtak (1979) found that the material in larval guts was proportionally comparable to the types of suspended particles in stream water. Some selection for particle size does occur; Chance (1970) reported that *S. vittatum* tends to select particles of 25 μm diameter, within a range of 1–285 μm. Black fly larvae may also ingest particles of colloidal size (Wotton, 1976). In the laboratory, larvae have been reared on diverse diets, including bacteria (Fredeen, 1964), dog food (Fredeen, 1959), baker's yeast (Wood and Davies, 1966), and fish food (Colbo and Porter, 1979). Tarshis (1968) used 21 larval diets in attempts to culture black flies. Larval diets have also been added routinely to bioassay units as part of the test procedure (Lacey and Mulla, 1977; Undeen and Nagel, 1978).

Factors affecting the feeding rates of black fly larvae have been investigated by Kurtak (1978), Lacey and Mulla (1979) and Mulla and Lacey (1976). However, reduced ingestion due to the disruption of normal feeding behavior has been virtually unstudied. Such studies could have important implications, since, as Mulla and Lacey (1976) have suggested, the efficacy of an insecticide may be influenced by the rates of larval feeding and voidance. In this paper we report on feeding inhibition induced by commonly used larval diets. Evidence is also presented showing that inhibition can significantly alter the efficacy of an ingested control agent.

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Materials and Methods

Experimentation was completed at the Science Service's Biological Field Station in Cambridge, NY. Tests were conducted in the laboratory under simulated stream conditions, using water gravity-fed into bioassay units from a local pool. These units have the capability of being used in either the gravity flow-through (open) or pump recirculation (closed) mode. Test larvae were permitted to attach onto effluent lips, over which a laminar sheet of stream water continually flowed at a rate of 8 liters/min. The excellent optics provided by this thin water film allowed larval behavior to be closely observed. The downstream portion of the lip included a screen to prevent the loss of detaching larvae.

Simulium vittatum larvae (IS-7 sibling species) were field collected and allowed 48 h to attach and acclimate on the bioassay unit lips. Acclimatization occurred in the flow-through mode, permitting larvae to feed on particles naturally suspended in the stream water. One to two hours before testing the bioassay units were switched to the recirculation mode (6 liter volume). The lip screens were then cleaned of debris, and pupae and patently infected, or abnormally behaving larvae discarded. Exposures were ended by switching the units back to flow-through, thus flushing each unit out with fresh stream water.

Effects on Feeding Behavior

Many of the particulate materials examined in this study were selected for testing because of their previous use in laboratory rearing trials or bioassays. Materials were prepared for testing by grinding in a mortar and passing through a 125 μm aperture sieve. The resulting particles were then blended at high speed (5 min) with 100 ml of stream water before addition of appropriate concentrations to bioassay units. Treatment was ended after 5 h of exposure by continuous flushing and observations recorded for an additional 3 h. All tests except those examining the effects of food concentration were conducted at a concentration of 50 mg/liter. Each test was replicated at least twice; 30–50 late instar larvae were used in each replicate. The mean (± SD) postgenal

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length of the larvae used in these tests was 524 (± 42) μm.

Effects on the Pathogenicity of a Biocontrol Agent

The effects of feeding inhibition on the pathogenicity of the entomopathogenic bacterium, *Bacillus thuringiensis* var. *israelensis*, against black flies were studied using a concentration of 50 mg/liter of fish food (Tetra® Conditioning Food-Vegetable Diet) as the inhibitory agent. Accordingly, feeding inhibition was induced one hour before bacterial exposure in 5 of 14 bioassay units (Group I) by Tetra addition to the recirculating water. All 14 units, each with about 50 larvae, were then treated at a previously determined LD50 of 100 ppb with a primary powder of *B. t.* var. *israelensis* (#R153-78 produced by Roger Bellon-Biochem, Monts, France). Bacterial exposure was ended after 15 min by flushing all units with fresh water. After 5 min of flushing the units were returned to recirculation and Tetra was added immediately to another 5 of these 14 units (Group II). The remaining four units (Group III) did not receive Tetra. Groups I and III did, however, receive 50 mg/liter of chalk (Crayola®) immediately after flushing. Ingestion of these particles provided the mass needed to push the bacterial preparation out of the larval guts as might occur under stream conditions. Chalk was chosen because preliminary results showed it to interfere with normal feeding less than any other particulate tested, while Tetra was the most disruptive of normal feeding behavior. The fish food and chalk were flushed out after 24 h and the units again returned to recirculation. Control units, one corresponding to each of the above three groups, were treated similarly except that bacteria were not added. The tests were ended and mortality recorded at 48 h postexposure. *Simulium vittatum* larvae used in this test were late instars with a mean (± SD) postgenal length of 544 (± 35) μm.

Results and Discussion

Effects on Feeding Behavior

The feeding of *S. vittatum* larvae exposed to high concentrations of suspended particles was observed to be inhibited. (Inhibition was characterized as any atypical feeding behavior resulting in reduced ingestion. Feeding was considered to be inhibited, for example, when either or both cephalic fans remained partially or completely retracted). Microscopic observation of individual treated larvae revealed that feeding of treated larvae was discontinuous, fluctuating between normal feeding and complete inhibition. Thus, long periods of complete fan retraction were randomly interrupted by brief, 15–90 sec intervals of normal feeding or longer periods of partial fan retraction. For example, from 30–60 min postexposure, Tetra treated larvae fed normally 7.8% of the time, but were inhibited for 92.2% of the period (partial fan retraction, 17.2%; complete retraction, 75.0%). Thus, inhibition resulted in reduced feeding, but not an absolute cessation of ingestion. Larvae were also infrequently (<1%) observed that fully extended but seldom retracted their fans.

Feeding inhibition was induced by a variety of particulate materials (Table 1). Organic materials, and Tetra fish food in particular, tended to be the most inhibitory materials tested. Tetra also caused the greatest intensity of inhibition (degree and duration of fan retraction). It is likely that observed differences between materials are attributable to dissimilar particle size. That is, although Tetra and chalk, for example, were added at the same concentration, Tetra may have been several fold more inhibitory because it was of a size more efficiently ingested by *S. vittatum* than the smaller chalk particles. Kurtak (1978) established that particles larger in diameter than the spaces between the cephalic fan rays are ingested more efficiently than particles smaller than the ray spaces. He estimated the ideal particle diameter to be about 100–150 μm. The particle diameter of Tetra roughly corresponded to this range. Other materials not presented in Table 1, including brewers yeast, wheat germ and TetraMin® Stable fish food, also produced various degrees of inhibition. In fact, virtually all particulates tested disrupted normal feeding to some extent at a concentration of 50 mg/liter, indicating that inhibition is a nonspecific response unrelated to particulate type. Nonparticulate materials going into solution, such as yeast extract or nutrient broth, did not discernibly affect feeding behavior.

Table 1.—Effects of various particulates on the feeding response of late instar *Simulium vittatum* larvae[a].

Particulate material	Percent feeding inhibition at: 15 min	30 min	1 h	2.5 h	5.0 h
Controls[b]	0	0	0.9	0.9	1.8
Baker's yeast	1.5	19.1	43.3	68.4	51.1
Charcoal	18.8	20.4	26.4	11.3	36.8
Chalk	10.0	22.9	22.1	16.8	8.1
Clay	3.3	11.7	23.1	38.2	32.7
Stream detritus	30.8	46.5	37.8	52.1	34.7
Dog food	42.4	35.1	39.2	61.4	66.9
Rabbit chow	26.0	50.5	59.6	57.6	75.0
Tetra® fish food	23.3	50.8	80.5	87.1	95.3

[a] Exposed at 50 mg/liter.
[b] No particulate material added.

Tests with Tetra showed that feeding inhibition was a function of particle concentration (Fig. 1). Consequently, the percentage of larvae inhibited at the highest concentrations tested (200 mg/liter) was two to three times greater than that of the lowest (10 mg/liter). Although not easily determined from Figure 1 (since several points between 0–30 min were deleted for clarity), the onset of inhibition was generally fastest at higher food concentrations, where the rate of gut filling would be the most rapid. A plateau was usually reached within 30 min posttreatment. This plateau was irregularly maintained until exposures were ended by flushing the units with fresh stream water. Inhibition then dropped quickly to preexposure levels. A similar postflush drop was observed with particulates listed in Table 1. No decrease in inhibition, however, was noted in tests where no flush occurred and exposure was continued for 48 h.

Particle concentration would be expected to decrease with time because of ingestion, thus decreasing inhibition. Nevertheless, even when tests were conducted in 500 ml of water using Colbo and Thompson's stirring bar method (1978), no reduction in inhibition was observed during a 5 h test period.

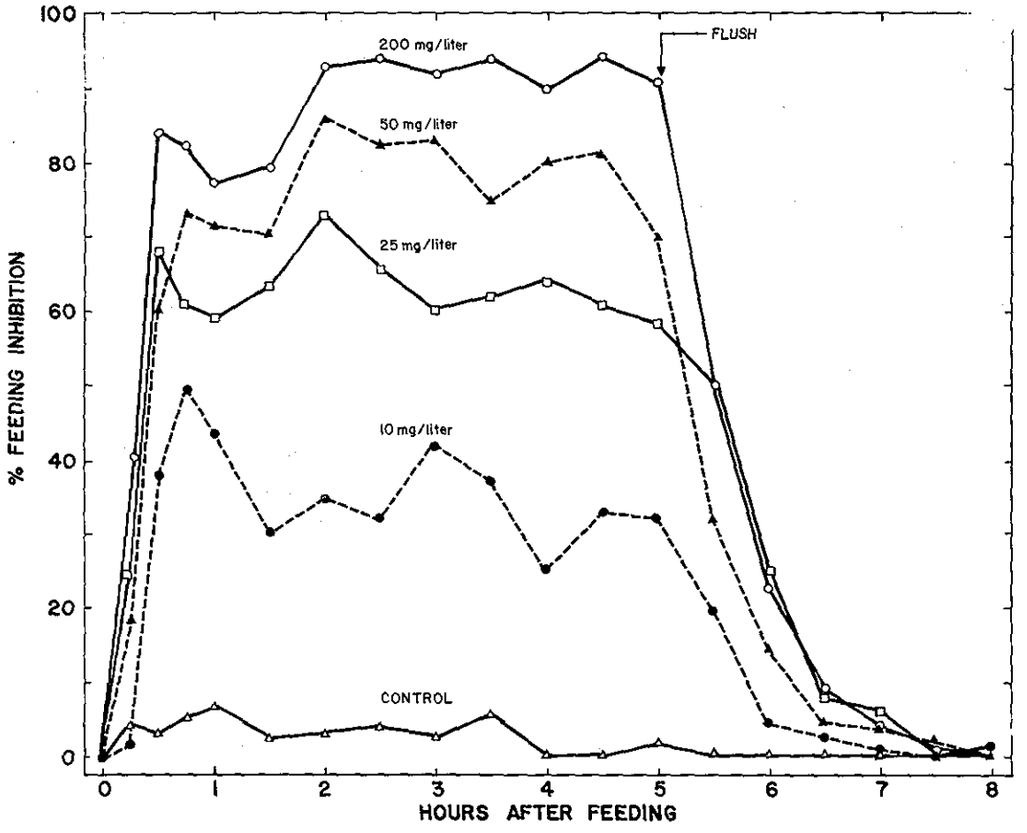

FIG. 1.—Effects of particulate concentration (Tetra® fish food) on the feeding response of late instar *Simulium vittatum* larvae.

Feeding inhibition was not limited to *S. vittatum* late instars. In tests with late instar *S. pictipes* and *S. tuberosum*, and early to mid-instar *S. vittatum*, feeding was also inhibited by Tetra addition.

Kurtak (1978) reported that the efficiency of ingestion was reduced at high particle concentrations. He attributed this observation to the limited rate at which the fans could be cleaned. Our observations suggest that feeding inhibition may have been a contributing factor. Kurtak further noted that high concentrations of large particles caused feeding to stop because larvae were "disturbed by the bombardment." This assessment implies that the inhibition observed in our study might have been the result of larvae being disturbed by striking particles. However, we quickly obtained feeding inhibition when high concentrations of particles were delivered by micropipette directly to the fans, without particles striking the larval body.

Lacey and Mulla (1979) determined that the feeding rate of *S. vittatum* larvae increased at particle concentrations up to 50 mg/liter, where larvae were feeding at maximum efficiency. These investigators predicted that particle concentrations in excess of an "optimal plateau" would have an inhibitory effect on feeding rate, but did not propose possible mechanisms. Our observations suggest that the changes in feeding rate predicted by Lacey and Mulla would result, at least in part, from changes in feeding behavior induced by high particle concentrations.

Because of the time lag before the onset of inhibition and the observations that inhibition is nonspecific and discontinuous, it appears almost certain that this response is merely a consequence of rapid gut filling. If particulate concentration is so high that food is ingested faster than it can be voided, then termination of feeding must result until additional gut space is created by egestion. Examination of treated larvae showed that particles were densely packed in the gut. Dethier and Bodenstein (1958), in experiments with the blow fly, *Phormia regina*, have shown that mechanoreceptors in the foregut respond to the stimulus of gut filling by terminating further ingestion. Abdominal distension is believed to regulate food intake in blood-sucking mosquitoes and hemipterans (Dethier, 1976). The mechanisms controlling the feeding response of black flies are undetermined.

Effects on the Pathogenicity of a Biocontrol Agent

Our observations prompted us to investigate the possibility of using feeding inhibition to modify the pathogenicity of an ingested control agent. Assays demonstrated that despite treatment at the LD50 level the

Table 2.—Effect of inducing feeding inhibition on the pathogenicity of *Bacillus thuringiensis* var. *israelensis* to late instar *Simulium vittatum* larvae[a].

Group	Tetra® added[b]:	No. insects dead/treated	Corrected % mortality[c] ± SE	95% confidence limits
I	Pretreatment	8/287	2.8±0.97	0.9–4.7
II	Posttreatment	230/244	93.9±3.60	83.9–100.0
III	None	111/196	55.7±3.50	48.8–62.6

[a] 15 min exposure to a previously determined LC50 of 100 ppb of Bellon primary powder of 13°C.
[b] Feeding inhibition induced by addition of Tetra fish food (50 mg/liter) either 1 h before (Group I) or immediately after (Group II) exposure to *B. thuringiensis* var. *israelensis*.
[c] Corrected with Abbott's formula.

efficacy of *B. t.* var. *israelensis* could be reduced to insignificant levels by the addition of Tetra *before* larval exposure to the pathogen (Table 2). Clearly, black flies which are not feeding or are feeding at a diminished rate, can not ingest the same quantity of inoculum as larvae feeding normally. Thus, lower mortalities must result.

Of greater practical importance, the pathogenicity of *B. t.* var. *israelensis* was increased by nearly 90% over the LD50 level when feeding inhibition was induced with Tetra *after* larval exposure to the bacterium (Table 2). In contrast, posttreatment with chalk, which was much less inhibitory than Tetra, did not cause a significant increase in pathogenicity. This Tetra induced increase is probably attributable to increased bacterial retention time in the larval gut resulting from feeding inhibition. Black fly larvae usually retain ingested particles for only short periods of time and have a correspondingly low rate of assimilation (Ladle et al., 1972). However, when feeding activity is reduced, the rate of displacement and subsequent voidance of ingested particles also decline (Lacey and Mulla, 1979). In our studies, inhibition of feeding following exposure probably permitted the parasporal crystals of the bacterium to undergo greater dissolution and thereby release more endotoxin.

Ramoska and Pacey (1979) also found food availability to significantly alter the efficacy of the mosquito pathogen, *B. sphaericus*. Similarly, Undeen and Nagel (1978), citing unpublished data, mentioned that increases of food reduced *B. thuringiensis* pathogenicity against black flies. Conversely, Lacey et al. (1978) observed that food concentration did not affect the pathogenicity of *B. thuringiensis* against *S. vittatum*. However, the lengthy exposure time used (24 h as compared to 15 min in the present study) may have negated any effects of altered feeding behavior.

Lacey and Federici (1979) reported that exposure of late instar *S. vittatum* to *B. thuringiensis* causes partial fan retraction within one hour. We have not observed this response following treatment with *B. t.* var. *israelensis* and suggest that their finding was a result of the addition of charcoal (50 mg/liter) to the test water.

Workers conducting laboratory bioassays should be aware that feeding inhibition may reduce or enhance the efficacy of an ingested control agent, depending on whether food is added before or after treatment. The effects of adding food and *B. t.* var. *israelensis* simultaneously were not determined here. Inhibition has obvious application in field control strategies since increasing the efficacy of a control agent would reduce the quantity of inoculum required for black fly control. Since it is unfeasible to dump large quantities of particulates into a stream, efforts might be directed toward screening for compounds which inhibit feeding by a different mode of action, perhaps by fouling the fans or causing detachment.

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