THE EFFECT OF *SIMULIUM VITTATUM* ZETT.

(DIPTERA:SIMULIIDAE)

LARVAL FEEDING BEHAVIOR

ON THE EFFICACY OF

BACILLUS THURINGIENSIS SEROTYPE H-14 (de Barjac)

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

© Kevin E. Nixon

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THE EFFECT OF *SIMULIUM VITTATUM* ZETT (DIPTERA SIMULIIDAE) LARVAL FEEDING BEHAVIOR ON THE EFFICACY OF BACILLUS THURINGIENSIS SEROTYPE H-14 (de Barjac)

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KEVIN E. NIXON

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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TABLE OF CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

Page

Page

APPENDIX 1.

Page

ABSTRACT

Nixon, Kevin Eric. M.Sc., The University of Manitoba, May 1988. The effect of Simulium vittatum Zett. larval feeding behavior on the efficacy of Bacillus thuringiensis serotype H-14 (de Barjac). Major Professor: Dr. M. M. Galloway. A study was conducted to determine the effect of S. vittatum larval feeding behavior on the efficacy of B. t. H-14 against black fly larvae at different temperatures, durations of exposure to B. t.H-14, and suspended solid loads. This laboratory study was carried out using a closed water bioassay system with a current velocity of 8.7 cm/sec. Mortality of S. vittatum larvae was 94.3, 45.1, and 40.7% at 22, 12, and 5 C respectively after a 15 min exposure to 1.95 ppm B. t. H-14. Mortality of S. vittatum larvae was 98.8, 87.0, and 34.4% at 22, 12, and 5 C respectively after a 30 min exposure to 1.95 ppm B. t. H-14. Reduced efficacy at the lower temperatures was attributed to reduced filter feeding of the larvae. Larval mortalities were 73.7, 10.1, and 10.9% at suspended solid loads of 10, 55, and 505 ppm respectively after a 15 min exposure to 1.95 ppm B. t. H-14. Mortalities were 94.2, 3.1, and 2.0% at suspended solid loads of 10, 55, and 505 ppm respectively after a 30 min exposure to 1.95 ppm B. t. H-14. The 30 min exposure to B.

t. H-14 resulted in significantly higher mortalities than the 15 min exposure at 12 C and 10 ppm suspended solids only. At 22 C most larvae were feeding rapidly enough to ingest a lethal dose of the toxin even at the 15 min exposure. At 5 C most larvae were feeding too slowly to ingest a lethal dose of the toxin even at the 30 min exposure. Most larvae were feeding too slowly in the presence of 55 and 505 ppm suspended solids to ingest a lethal dose of the toxin at the 30 min exposure. LC-50 values of B. t. H-14 against S. vittatum determined at suspended solid loads of 10, 25, 35, 45, and 55 ppm were 1.32, 2.84, 8.56, 8.62, and 10.93 respectively. Simulium vittatum larvae cleared 50% of their guts in 30.4, 16.6, 39.0, and 49.2 min at 10, 25, 35, and 55 ppm suspended solids respectively. Larval ingestion rates at 10, 35, and 55 ppm suspended solids were not significantly different. Ingestion rate at 25 ppm suspended solids was significantly faster than at 10, 35, or 55 ppm suspended solids. These results along with those of similar studies are used to discuss the mechanism involved in the decreasing efficacy of B. t. H-14 at increasing suspended solid loads.

INTRODUCTION

Black flies are a serious pest to man, domestic animals, and wildlife. Black flies are vectors of disease organisms. The species complexes of Simulium damnosum(Theobald) and Simulium neavei(Roubaud) are vectors of Onchocerca volvulus in Africa. Simulium ochraceum(Walker) and Simulium metallicum(Bellardi), among others, transmit onchocerciasis in South and Central America (Harwood and James 1979). Bovine and equine onchocerciasis as well as avian leucocytozoon infections are vectored by simuliids (Harwood and James 1979).

The bites of black flies cause considerable damage. Livestock have been reported killed by black fly attacks in periods of severe outbreaks. Outbreaks of Simulium colombaschense(Fabricius) in the Balkan states in 1923 and 1934 resulted in the deaths of 16,000 and 13,900 animals respectively (Harwood and James 1979). Between 1944 and 1948 more than 1,300 cattle were killed by Simulium arcticum (Malloch) in Saskatchewan (Fredeen 1977). In one area in Alberta 973 cattle were killed before the year 1971 by Simulium arcticum (Haufe 1980). Simuliid bites commonly cause effects ranging from general dermatitis to severe swelling and allergic asthma (Harwood and James 1979).

Pinheiro et al. (1974) reported a disease, hemorrhagic syndrome of Altamira, in the Amazon affecting children. Some deaths have been reported. The disease seems to be caused by a toxin associated with intense biting of simuliids.

The annoyance of simuliid attacks is important. Monetary losses in agriculture are due to reduced gain in weight of animals on pasture, reduced reproduction of livestock, and interruption of calving schedules and generally inefficient operations. In one area in Alberta containing about 13,000 cattle the average losses were estimated at $600,000 annually (Haufe 1980).

Humans suffer from the annoyance of simuliid attacks as well. The immense numbers of black flies that can occur in some areas may mean vacationing or seasonal occupation of summer homes is strongly discouraged. This is an important loss in tourism in these areas. The presence of large black fly numbers also may make it difficult or impossible for people to work in some localities (Harwood and James 1979).

Historically most black fly control has been done using chemical larvicides. The first pesticide to be widely used was DDT. However, DDT's unacceptabe environmental impact was responsible for its use being discontinued. Methoxychlor was subsequently used as the primary black fly larvicide in Canada (Sebastien 1986). Recently serious concerns have been raised concerning methoxychlor's

environmental impact. Methoxychlor is toxic to many non-target organisms. This topic was reviewed thoroughly by Sebastien (1986).

Bacillus thuringiensis H-14, a biological control agent for black fly and mosquito larvae was discovered in 1977. This pesticide is a stomach poison and must be ingested to be effective (Goldberg and Margalit 1977). As a result the feeding behavior of black fly larvae greatly effects the efficacy of B. t. H-14.

The purpose of this study was to investigate the effect of certain environmental factors on the feeding behavior of black fly larvae and how this in turn affects the efficacy of B. t. H-14. The environmental factors investigated were temperature, B. t. H-14 exposure period, and the concentration of suspended particles to which the larvae were exposed.

The null hypothesis was that temperature, B. t. H-14 exposure period, and the concentration of suspended particles has no effect on the efficacy of B. t. H-14. In the feeding studies the null hypothesis stated that ingestion rate remains constant as the concentration of suspended particles increase.

LITERATURE REVIEW

Introduction

Bacillus thuringiensis is a spore forming bacterium that was described by Berliner in 1915. This bacterium produces one or more crystals of toxic protein (delta-endotoxin) with each spore. Exotoxins or lytic enzymes are also produced. However, these exotoxins are less important than the delta-endotoxin (Faust and Bulla 1982). Most strains are specific to Lepidoptera and as a result the bacterium has been used to control agricultural and forestry pests for many years. Products containing B. t. are remarkably safe compared to other chemical pesticides. To date at a very conservative estimate, over 4500 metric tons have been used apparently without harm to non-target organisms. No harmful effects have been recorded in safety tests with bees, or mammals, including man. Most beneficial insects are unharmed even at enormous doses (Shadduck 1980, Thomas and Ellar 1983).

Until the late seventies no B. t. serotypes were known to be active against mosquitoes and black flies. However, in 1977 Bacillus thuringiensis serotype H-14, a powerful mosquito and black fly larval toxicant, was isolated (Goldberg and Margalit 1977). The crystals of this serotype

are insoluble in water and therefore spores and crystals constitute a particulate insecticide. B. t. H-14 is effective only when eaten by the insect and has no contact action.

In the search for safe and economical black fly and mosquito larvicides the entomopathogen B. t. H-14 has emerged as the most likely alternative to synthetic chemical larvicides. This pathogen is also referred to as B. t. (Berliner) variety israelensis (de Barjac).

Serotyping is used for the taxonomic classification of B. t. strains. The use of the antigenic properties of the flagella of the vegetative cell is the best available system for the classification of B. t. strains and their division into the 21 serotypes. Division into the 30 varieties is based on serological subfactors and biochemical properties (Burges 1984, Luthy and Ebersold 1981, Wld Hlth Org. 1979).

The delta-endotoxin is formed early in the sporulation phase. It is crystallized within the sporangium. The weight of these crystals reaches about one third of the whole cell. When sporulation is completed the sporangium is lysed and the heat resistant spore and the crystal are set free separately (Luthy and Ebersold 1981).

B. t. serotype H-14 delta-endotoxin is unusual among B. t. strains in several ways. Each sporulating cell forms on the average three inclusions which are surrounded by an envelope. This is in contrast to most other B. t. serotypes which form one bipyramidal-shaped crystal (Luthy and

Ebersold 1981, Wld Hlth Org. 1979). The crystals are serologically quite distinct from those of B. t. serotypes toxic to Lepidoptera (Krywienczyk and Fast 1980). The toxin has few if any antigenic determinants in common with the toxins active on other insect groups (Wld Hlth Org. 1979). The crystals also differ in amino acid composition (Guillet and de Barjac 1979, Larget and de Barjac 1981a, Tyrell et al. 1979).

No authors have attempted to explain what adaptive advantage production of the crystal gives to B. t. H-14. Bacteria often make up a large component of the diet of black fly larvae (Fredeen 1960 and 1964). Production of the toxic crystal may be an attempt to remove a predator from the bacteria's environment.

Histopathological Effects of B. t. H-14

B. t. serotypes commonly used against lepidopterans, and B. t. H-14 produce strikingly similar histopathological symptoms (Lacey and Federici 1979, Lahkim-Tsror et at. 1983). In general the symptoms include 4 stages: stage 0, appearance and locomotion normal but cessation of feeding; stage 1, slightly sluggish; stage 2, extremely sluggish; stage 3, complete paralysis (Nishhtsutsuji-Uwo and Yasuhisa 1980). Histopathological changes include the general loss of integrity of the gut epithelium as a result of the swelling, distortion and final bursting of the cells

(Charles and de Barjac 1981b, Lahkim-Tsror et al. 1983, Wld Hlth Org. 1979).

The histopathological effects of B. t. H-14 are similar against mosquitoes and black flies. In both groups healthy larvae have an anterior midgut zone with a monolayer of cuboidal cells without a brush border in the epithelium. The posterior midgut and the gastric caeca are composed of cuboidal epithelium cells with a well developed brush border. The midgut transitional zone is made up of cells from both zones (Lacey and Federici 1979, Lahkim-Tsror et al. 1983). The tissue most affected is the midgut epithelium in the regions of the gastric caeca and posterior midgut. Poisoned larvae fixed before death show cellular hypertrophy with swollen and sloughing cells exhibiting vacuolated cytoplasm. The nuclei show pycnotic characteristics and the brush borders are thinner and disrupted. There is a complete and undamaged peritrophic membrane. Larvae fixed after death show a completely damaged posterior midgut and gastric caeca with most of the cells sloughed off. The peritrophic membrane is also damaged (Lacey and Federici 1979, Lahkim-Tsror et al. 1983). Mosquito and black fly larvae with heavily damaged guts invariably die within a few hours. Death probably results from pore formation in cell membranes which causes disruption of the ionic regulation capacity of the midgut and the subsequent flow of toxic substances and ions into the haemocoel (Charles and de Barjac 1981a, Couch 1981,

Garcia et al. 1980, Sarjeet and Hornung 1987).

One of the earliest attempts to explain the mode of action of B. t. delta-endotoxin dealt with the possibility that the toxin was responsible for upsetting the midgut's ability to protect itself against gut enzymes. As a result self-digestion of the gut epithelium took place (Luthy 1973). Another hypothesis was that spore germination and vegetative cell growth was responsible for causing the pathogenicity (Prasertphon and Areekul 1973). However, even though death due to bacterial septicaemia can occur, especially in lepidopteran larvae (Wld Hlth Org. 1979), it is thought that mortality time is much too short to be explained by spore germination and massive multiplication of bacteria (Lahkim-Tsror et al. 1983). Studies of the larvicidal activity of isolated crystals, acrystalliferous mutants, and oligosporogenous but crystalliferous mutants of B. t. H-14 prove that it is in fact the delta-endotoxin crystal that is responsible for the toxicity of B. t. H-14 (Larget and de Barjac 1981b, Samasanti et al. 1982).

In spite of the similarities between the serotypes of B. t. used against lepidopterans and the H-14 serotype, B. t. H-14 is usually ineffective against lepidopterans (Tyrell et al. 1979). Some groups closely related to black flies and mosquitoes have a low susceptibility (Larget and de Barjac 1981b, Thomas and Ellar 1983). However, Ignoffo and co-workers (1981a) reported that unlike previous reports, he

found B. t. H-14 to be effective against certain lepidopterans. Further research needs to be done to clarify this discrepancy. Conversely the B. t. serotypes used against lepidopterans are usually ineffective against mosquito larvae (Ignoffo et al. 1981a, Larget and de Barjac 1981a, Tyrell et al. 1979).

The serotype H-14 crystalline inclusion is a protoxin. This protoxin is very rapidly converted to smaller toxic subunits in the presence of high pH and suitable enzymes in the guts of susceptible hosts (Dadd 1975a, Charles and de Barjac 1981a, Lacey and Federici 1979, Lahkim-Tsror et al. 1983, Tyrell et al. 1979, Undeen 1979). The toxic moiety believed to be responsible for the larvicidal activity has a molecular weight in the vicinity of 25,000-28,000 Daltons (Aronson et al. 1982, Insell and Fitz-James 1985, Tyrell et al. 1981). A protease activity which is heat inactivated under alkaline conditions may be important for release of the toxic molecules from the protoxin of the B. t. H-14 crystal (Chilcott et al. 1981). The narrow spectrum of activity of the delta-endotoxin of B. t. H-14 is probably due to the absence in most invertebrates of the enzymatic system that transforms this protoxin into the actual toxin (Wld Hlth Org. 1979).

Alkaline solubilized delta-endotoxin crystals cause rapid cytological and cytopathological changes upon injection in a wide spectrum of host animals. These include insects from four orders, mice, and several tissue types.

Alkaline solubilized delta-endotoxin crystals also cause haemolysis in rat, sheep, horse, and human erythrocytes. Intravenous administration of these extracts are toxic to bab-c-mice and suckling mice. Conversely non-solubilized delta-endotoxin crystals had no affect on the above organisms and tissue types (Nishhtsutsuji-Uwo and Endo 1979, Roe et al. 1985, Thomas and Ellar 1983). Shadduck (1980) reported that B. t. H-14 was not harmful to mammals unless more than 10 million viable organisms were introduced directly into the brain. However, adult mosquitoes were killed by application of B. t. H-14 delta-endotoxin as an enema (Klowden et al. 1983). While the solubilized crystals had a greater toxicity than the non-solubilized crystals, the non-solubilized crystals were toxic to 50% of the larvae at levels of 0.21 ug/ml of mosquito (wet weight). The pH of the midgut of adult female mosquitoes is only slightly alkaline or slightly acid. Therefore a high gut pH is not necessary for toxin activation to occur (Klowden et al. 1983).

It is not known whether the primary site of action is the cell membrane or the interior of the cell. The primary effect is on cellular absorption (Fast and Donaghue 1971, Lacey and Federici 1979, Lahkim-Tsror et al. 1983, Nishhtsutsuji-Uwo and Endo 1979). The regions of the larval gut most effected by B. t. H-14 delta-endotoxin are the gastric ceca and posterior midgut which are the primary

sites of absorption (Lacey and Federici 1979, Lahkim-Tsror et al. 1983). Schnell and Nickerson (1983) found that the toxicity of purified B. t. H-14 crystals to Aedes aegypti (Linnaeus) larvae could be reversed 100-fold by levels of H_2CO_3 as low as 0.15%. Thus the toxin may act as an ionophore and stimulate ion absorption of the epithelial cells.

Unlike most biological control agents B. t. H-14 has not been demonstrated to replicate in the environment at levels required for toxic effects. As a result epizootics do not occur in mosquito breeding sites after application of B. t. H-14. Consequently B. t. H-14 must be reapplied whenever additional or continued control is necessary. Sinegre et al. (1980b) reported that the delta-endotoxin was rapidly inactivated within dead or dying larvae. They found that larvae which were fed cadavers of larvae freshly killed by the toxin were not affected. Replication and sporulation with toxin formation can occur under certain conditions in mosquito larvae cadavers (Aly 1985, Aly et al. 1985). However, because these cadavers are easily broken apart and acted upon by other microbials, which remove or disperse the substrates necessary for B. t. H-14 growth, there is insufficient toxin produced to cause death of other mosquito larvae that may be present.

Effect of B. t. H-14 against Mosquito Larvae

Filter Feeding Behavior of Mosquito Larvae

Mosquito larvae occur in a wide variety of habitats, including stream margins, brackish water, forest pools, discarded tires, tree and crab holes, plant axils and even pit latrines. Many mosquito groups feed in different microhabitats. For instance, anophelines feed primarily in the surface film while culicines feed mainly below the surface (Wallace and Merritt 1980).

Mosquito mouth parts consist of well developed mandibles and maxillae, a reduced labium and a modified labrum with attached brushes. Filter-feeding genera (e.g. Anopheles, some Culex and Mansonia) produce currents with their labral brushes, to bring food particles to the mouth (Wallace and Merritt 1980).

Mosquito larvae secrete a mucosubstance unto their palatal brushes which is involved in the capture of fine particulate matter (Merritt and Craig 1987). The substance may be homologous with a similar substance found in simuliids.

Most filter-feeding culicid larvae ingest particulate matter from colloidal to 50 microns in size (Dadd 1971, Dadd 1975b, Merritt et al. 1978). Optimum ingestion rate occurred with particles of 0.71-1.86 microns mean diameters in the case of Culex pipiens (Linnaeus) (Dadd 1971). Early

instar mosquitoes preferentially ingested smaller particles than did later instars (Dadd 1971, Dadd 1975b, Merritt et al. 1978). Pupal stages and late fourth instars or pre-pupae do not feed (Mulla et al. 1982b).

Little work has been done on the effect of concentration of suspended particles on the ingestion rate of mosquito larvae. Dadd (1971) found that the optimum ingestion rate of latex particles by Cx. pipiens larvae was at concentrations between 0.03 and 4.00% (weight/volume). Solutions such as yeast extracts or adenylic acid, which increased the overall rate of ingestion of various non-sapid particulates, increased the ingestion rate by Culex pipiens larvae whether there were particulate solids in suspension or not (Dadd 1970). Furthermore ingestion rate by Culex pipiens larvae in various dilute colloid solutions was rapid and comparable in rate to ingestion rates in water containing particulate solids (Dadd 1975b).

Microorganisms and particulate organic detritus generally constitute a major part of larval culicid diets (Ameen and Iversen 1978). However, several species can develop through all larval stages in sterile synthetic media with most of the essential nutrients in solution (Lea et al. 1956, Singh and Brown 1957). Fluctuations in microorganisms and particulate matter in mosquito habitats are generally reflected in the gut contents of mosquito larvae (Ameen and Iversen 1978). In conclusion, mosquitoes are more or less indiscriminate feeders.

Other factors influence mosquito larval feeding. Temperature may have an effect on the filter-feeding behavior of mosquito larvae. Feeding activity is lower at lower temperatures (Clements 1963, Mulla et al. 1980). On average early instars of mosquito larvae ingest smaller particles than do later instars (Merritt et al. 1978).

Laboratory Studies

Larvicidal activity of B. t. H-14 varies with the species of mosquito (Goettel et al. 1982a). In some cases activity can vary between strains of the same species. Dame et al. (1981) indicated that LC-50 values for Anopheles quadrimaculatus(Say) were five times greater than that required for Aedes aegypti. Ignoffo et al. (1981b) demonstrated that Ae. aegypti, with an LC-50 of 0.054 ug/ml, was more susceptible to B. t. H-14 than was Culex quinquefasciatus (Say), with an LC-50 of 0.11 ug/ml. Mulla et al. (1980) tested B. t. H-14 against larvae of eight mosquito species; Ae. aegypti, Ae. nigromaculis(Ludlow), Ae. taeniorhynchus(Wiedemann), An. quadrimaculatus, Cx. peus(Speiser), Cx. quinquefasciatus, Cx. tarsalis(Coquillett), and Psorophora columbiae(Dyar and Knab). They found a two to six fold variation in larvicidal activity when a given preparation of B. t. H-14 was used against these species. Sun et al. (1980) showed the LC-50's for five strains of Cx. quinquefasciatus to vary from 0.07-

0.15 ug/ml. In some cases this difference in the activity of B. t. H-14 to different species of mosquitoes can be attributed to differences in larval feeding behavior. Anopheles spp. tend to filter feed at the surface of the water while most other species filter feed below the surface. Anopheles spp. are often less susceptible to B. t. H-14 than are other species (Ali et al. 1984, Ramoska and Hopkins 1981). Cheung and Hammock (1985) studied the activity of B. t. H-14 against a surface feeding species, An. freeborni (Aitken), and a subsurface feeder, Ae. aegypti. Anopheles freeborni was noticeably less susceptible to the toxin then Ae. aegypti. However, when solubilized delta-endotoxin was used the species showed similar susceptibilities. Furthermore, when the toxic crystals were coated with lipophilic material, which increased their buoyancy, An. freeborni were more susceptible than Ae. aegypti.

The instar of the mosquito larvae has a significant effect on the efficacy of B. t. H-14. Earlier instars are always more susceptible then are later instars (Lahkim-Tsror et al. 1983, Mulla et al. 1982b, Sun et al. 1980, VanEssen and Hembree 1980, Wraight et al. 1981).

Water temperature influences the efficacy of B. t. H-14. Susceptibility of mosquito larvae declines with decreasing temperature (Lacey and Federici 1979, Sinegre et al. 1980b, Wraight et al. 1981). The precise mechanism by which temperature influences efficacy of B. t. H-14 against

mosquitoes is poorly understood. The rate of dissolution and absorption of the crystal toxin may change with changing temperature, with a subsequent change in endotoxin toxicity. However, since temperature influences filter feeding rates in mosquitoes (Clements 1963, Mulla et al. 1980), the quantity of toxin ingested must be temperature dependent. This is an area where more research is needed.

Water chemistry can effect B. t. H-14 efficacy against mosquito larvae. There is a very clear inverse correlation between free chlorine levels in the water and the larvicidal activity of B. t. H-14 (Sinegre et al. 1980b). For this reason it is important to use dechlorinated water in all laboratory tests of B. t. H-14 activity. However, other factors do not have an affect on B. t. H-14 efficacy. Water salinity of 0.5% has no apparent effect on the larvicidal activity of B. t. H-14 (Garcia and DesRochers 1979, Goettel et al. 1982a, Ignoffo et al. 1981b). A hydrogen ion concentration, between pH 4 and 10, does not influence the larvicidal activity of B. t. H-14 significantly in the laboratory (Garcia and DesRochers 1979, Ignoffo et al. 1981b, Mulligan et al. 1980, Sinegre et al. 1980a).

The amount of time mosquito larvae are exposed to B. t. H-14 influences the efficacy of the bacterium. Since mosquito larvae feed almost continuously the longer they are exposed to the toxin the more they ingest (Nugud and White 1982, Sun et al. 1980).

The presence of suspended particulate matter affects the efficacy of B. t. H-14. Ramoska and Pacey (1979) reported the efficacy of Bacillus sphaericus (Neide) (a bacillus similar to B. t. H-14 in that it is a particulate insecticide) was inversely related to the amount of food available to the larvae of Cx. quinquefasciatus and An. albimanus (Weidemann). Since B. t. H-14 is a particulate insecticide (Wld Hlth Org. 1979) and mosquitoes filter-feed more or less indiscriminately, suspended particulates have been said to "compete" with the B. t. H-14 particles. Ramoska and Pacey (1979) indicated that the amount of food available to mosquito larvae can result in as much as a four-fold difference in response to the bacterial treatment, which they attributed to differences in the relative amount of B. sphaericus consumed. They hypothesized that in environments containing a greater proportion of B. sphaericus, compared to other food sources, more bacteria will be consumed and result in higher larval mortality. Accordingly less larval mortality will result in environments containing a higher amount of other microbiota available for consumption. Purcell (1981) found that the presence of mud affected the efficacy of B. t. H-14 against Ae. taeniorhynchus larvae. LD-50's were higher for larvae tested with mud, which had a strong adverse effect on the activity of B. t. H-14. Mulligan et al. (1980) reported that B. t. H-14 was less active in raw and autoclaved sewage effluent than in tap water. However, supernatants of

centrifuged fractions showed similar activity as that of tap water. The suspended solids fraction resuspended in distilled water produced a marked reduction in efficacy. Thus, there appears to be a physical interaction with suspended solids which reduces B. t. H-14 efficacy. Ali et al. (1981) also reported that in bioassays of B. t. H-14 against mosquitoes and midges, the addition of food significantly reduced the effectiveness of all formulations tested and invariably resulted in higher LC-90 values. The authors suggested that since B. t. H-14 toxicity was exclusively mediated after ingestion of the parasporal crystal the presence of food particles most likely offered competition for ingestion of the toxic crystals. Margalit and Bobroglo (1984) found the efficacy of B. t. H-14 against second instar larvae of Cx. pipiens and of Ae. aegypti decreased when organic matter was present in the water. Efficacy was decreased when sterilized silt was added as compared to nonsterilized silt. Sludge from loess soil decreased the efficacy of B. t. H-14 more than did decomposing organic matter, or inorganic mud, or silica gel, respectively. These authors suggested that the B. t. H-14 toxin adsorbed onto silt particles. The toxin was then removed as the silt particles settled out of suspension. The particles used in this study differed in their reduction of B. t. H-14 efficacy due to their different sedimentation rates.

Ignoffo et al. (1981b) however, reported that the presence of food increased both the rate and extent of mortality of Ae. aegypti larvae treated with B. t. H-14. The presence of food evidently stimulated feeding and thus increased the probability that the larvae would ingest a lethal dose of the toxin. However, these authors reported in the same paper that B. t. H-14 was about 85 times more active against Ae. aegypti larvae in distilled water than in pond water. They found that an increase in the concentration of pond water sediment resulted in a corresponding decrease in the insecticidal activity of B. t. H-14. There was nearly a 45 fold difference in efficacy of the bacterium after three hours between pond water sediment concentrations of 0.0 and 5.0%. They suggested that the reduction in efficacy may be due to B. t. H-14 crystals binding with organic matter in the sediment rather than the toxin simply being diluted by sediment particles. B. t. H-14 efficacy was reduced by about one half by 2.0% pond water sediment. Ramoska et al. (1982b) suggested that the inactivation process was probably related to the particle charge on the crystal which acted (as do other proteins) in an amphoterical manner, adsorbing to the clay or soil in either acid or basic solution and not desorbing except at a precise isoelectric point (which they did not pursue). The crystal is apparently unable to dissolve in the larval gut while adsorbed to silt particles when consumed by mosquito larvae. Dr. J. Margalit, Center for Biological Control, Ben

Gurion University of the Negev, Israel (personal communication 1984) has conducted studies in the laboratory in which B. t. H-14 crystals were coated with certain polypeptides. This coating did not remove the B. t. H-14 crystals from competition with other particulates, however the coating might have blocked the ability of the crystals to adsorb onto other particulates. When Margalit conducted bioassays of this formulation he found that the activity was not influenced by other particulates. Apparently the reduction in the activity of B. t. H-14 in the presence of suspended particulates is not due to "competition" but the B. t. H-14 crystal's propensity to bind to these particulates. This binding then reduces the larvicidal activity of the crystals in some way. While it seems likely that suspended particles reduce the effect of B. t. H-14 by competing with the toxic crystals, and binding of the crystals to suspended particles is possible, it is not known which mechanism is the more important one.

The size of suspended particles influences the efficacy of B. t. H-14. There is an inverse relationship between suspended particle size and B. t. H-14 larvicidal activity for particles between 147-840 microns (Margalit and Bobroglo 1984, Ramoska et al. 1982b). Mosquitoes can ingest particles of colloidal to 50 microns in size (Dadd 1971, Dadd 1975b, Merritt et al. 1978). The size of the toxic particles of several early formulations of B. t. H-14

were in the range commonly ingested by mosquito larvae (Molloy and Jamnback 1981). The sizes ranged from a mean of 2.1 microns (range of 0.5-28.0 microns) to 3.9 microns (range of 0.5-98.8 microns).

Field Studies

B. t. H-14 has great versatility against mosquito larvae in the field. Trials of B. t. H-14 efficacy have been successful in many areas of the world (Cheyne 1981, Goettel et al. 1982b, Majori and Ali 1984, Pantuwatana and Youngvanitsed 1984, Rettich 1983, Sudomo et al. 1981), and in many different habitats, including salt marshes, rice fields, flood waters, tree holes and tire habitats, snow pools, pasture lands and muskeg ponds (Anonymous 1984, Eldridge et al. 1985, Fanara et al. 1984, Garcia and DesRochers 1980, Hembree et al. 1980, Lacey et al. 1984, Lake et al. 1980, de Maio et al. 1981, Mclaughlin and Billodeaux 1983, Mclaughlin and Vidrine 1984a and 1984b, Merriam and Axtell 1983, Mulla et al. 1982a and 1982b, Mulla et al. 1985, Purcell 1981, Ramoska et al. 1982a, Stark and Meisch 1983).

Generally the results of field trials are similar to those found in the laboratory. Different species of mosquitoes differ in their susceptibility to B. t. H-14 in the field (Goettel et al. 1982b, Silapanantakul et al. 1983). Temperature decreases the efficacy of the bacillus in field trials (Mulla et al. 1980). Earlier instars are

more susceptible to the bacillus than are later instars (Hembree et al. 1980, Mulla et al. 1982b, Sebastien and Brust 1981, Standaert 1981). Salt concentrations in the range 0.07 ppt to 32.00 ppt have minimal if any effect on the bacillus in the field (Garcia and DesRochers 1980, Garcia et al. 1981, Goettel et al. 1982b, Lake et al. 1980, Merriam and Axtell 1983, Purcell 1981, Sudomo et al. 1981). As the concentration or the duration of exposure to B. t. H-14 increases, so does the efficacy of B. t. H-14 against mosquito larvae (Goettel et al. 1982b, Hembree et al. 1980, Merriam and Axtell 1983).

The pH of mosquito breeding sites may affect the efficacy of B. t. H-14 in the field. Mulla et al. (1980) showed that activity levels of B. t. H-14 were lower against the same species where water pH was higher than 8. Mulla et al. (1982b) studied the efficacy of B. t. H-14 against mosquitoes in two different ponds. They found that the pond with vegetation and a high pH (9.4) necessitated application of higher rates of B. t. H-14 (about two-fold) as compared to the pond which was devoid of rooted plants and in which the pH was 8.2. However, these results cannot be regarded as conclusive as the presence of vegetation alone can affect the efficacy of B. t. H-14 (Goettel et al. 1982b, Mulla et al. 1982b, Mclaughlin et al. 1982).

Vegetation, especially algal mats, can influence the efficacy of B. t. H-14 treatments. In brackish lagoons

where heavy vegetation and floating algae were present, larval mortality was reduced compared to non vegetated lagoons following B. t. H-14 application (Sudomo et al. 1981). The presence of grass may also greatly effect the dispersal and efficacy of B. t. H-14 (Goettel et al. 1982b). Conversely vegetation or debris covered areas are more likely to enhance success of B. t. H-14 for the control of anopheline mosquitoes (Mclaughlin et al. 1982). Apparently a water meniscus forms around surface material such as emergent vegetation or plant debris. This meniscus probably retains the toxic crystals near the surface longer than if they were applied to open water. Anopheline larvae tend to feed in these debris covered areas and as a result are exposed to a greater quantity of B. t. H-14 (Mclaughlin et al. 1982).

Larval abundance has a significant effect on the efficacy of B. t. H-14 formulations in the field. In habitats where larvae are extremely abundant the level of control, with a given rate of application, is lower than in similar habitats with less abundant larvae (Mulla et al. 1982a). Mulla and co-workers pointed out that this relationship is most likely due to lower rates of toxin consumption per larva in populations of high density, in comparison with those where densities are low. This is supported in laboratory studies where the average amount of active material per larva is the most important factor in determining efficacy between different bioassays when all

other conditions are equal (Sinegre et al. 1980b).

Efficacy of B. t. H-14 decreases with increasing water depth in the field A dosage of 4 oz/acre B. t. H-14, in water less than 0.30 meters deep gave 100% control of Ae. increpitus (Dyar) after 18 hours (Cheyne 1981). In water 0.30 to 0.46 meters deep, control was 75-80% at 18 hours posttreatment. In water over 0.46 meters deep control was only 50-60% after 18 hours. However, at 30 hours posttreatment control at all depths was complete (Cheyne 1981). Cheyne did not discuss the possible reasons for this. It would seem that the deeper water diluted the concentration of B. t. H-14 and hence larvae in this deeper water took longer to ingest a lethal dose.

Water quality and the presence of suspended solids adversely affect the efficacy of B. t. H-14 in the field. (Eldridge and Callicrate 1982, Garcia et al. 1981, de Maio et al. 1981, McLaughlin and Fukuda 1982, Mulla et al. 1982b, Purcell 1981, Silapanuntakul et al. 1983, Sinegre et al. 1980a, Sudomo et al. 1981, VanEssen and Hembree 1982). Control was reduced whenever suspended solids or organic material were suspended in the water.

Another problem encountered in the field is the lack of persistence of the toxic crystals once applied. These crystals are more dense than water, and tend to settle out of the water column. Once incorporated into the mud or silt at the bottom of the habitat they are broken down by

microorganisms and unavailable to mosquito larvae (Sinegre et al. 1980a, VanEssen and Hembree 1982). As a result the effects of B. t. H-14 applications last for less than 24 hours up to 1 week (de Barjac et al. 1980, Dame et al. 1981, Davidson et al. 1981, Eldridge and Callicrate 1982, de Maio et al. 1981, Mulligan et al. 1980, Sebastien and Brust 1981, Standaert 1981). However, greater persistence has been obtained with improved formulations with activity lasting up to 15 weeks (Goettel et al. 1982b, Mulla et al. 1982b, Ramoska 1982, Silapanuntakul et al. 1983).

It is probable that most problems with B. t. H-14 can be overcome by using special formulation techniques. A vegetative canopy can cause problems by intercepting the B. t. H-14 particles when they are aerially applied. However, when the toxic crystals are incorporated onto a granule such as sand or corn cob granules, they have sufficient momentum to get through a vegetation canopy (Anonymous 1984, Clarke and Rowley 1984, Fanara et al. 1984, Lacey and Inman 1985, Mulla and Darwazeh 1985, Mulla et al. 1985, Ramoska et al. 1982a, Stark and Meisch 1983). Cheung and Hammock (1985) reported that it is possible to encapsulate B. t. H-14 crystals, and even solubilized endotoxin, in certain materials. Coating of these materials could serve to protect the toxin from degradation by microorganisms, which could dramatically increase the toxin's residual activity. These authors also demonstrated that it is possible to increase the buoyancy of the B. t. H-14 crystals. This

increased buoyancy would function to keep the toxin at or near the surface of the water, which is useful for control of anopheline mosquitoes. It may be possible to make formulations with very precise buoyancies to enable the toxin to be placed at precise depths in the water column. Another important advancement has been the development of slow release formulations to increase field persistence (Lacey et al. 1984).

Effect of B. t. H-14 Against Black Fly Larvae

Filter-feeding Behavior of Black Fly Larvae

Black fly larvae are adapted for life in lotic waters by their size and streamlined shape, method and sites of attachment, and feeding behavior. Simuliids have a characteristic feeding position that allows them to minimize energy expenditure. They attach to the substrate and twist their bodies longitudinally, from 90-180 degrees, so that the ventral surface of the head and fans face the current (Colbo and Wotton 1981).

When the larvae are in the feeding position they orient themselves with their posterior ends upstream. The broadest part of the streamlined body, which is about one-third the overall length from the posterior end, directs the water to flow closely along the larval surface and on to the cephalic fans. The proleg, which is situated ventro-posteriorly to the head capsule, serves to "split" the flow of water which

will pass to left and right and sweep over the aboral surface of the fans (Colbo and Wotton 1981).

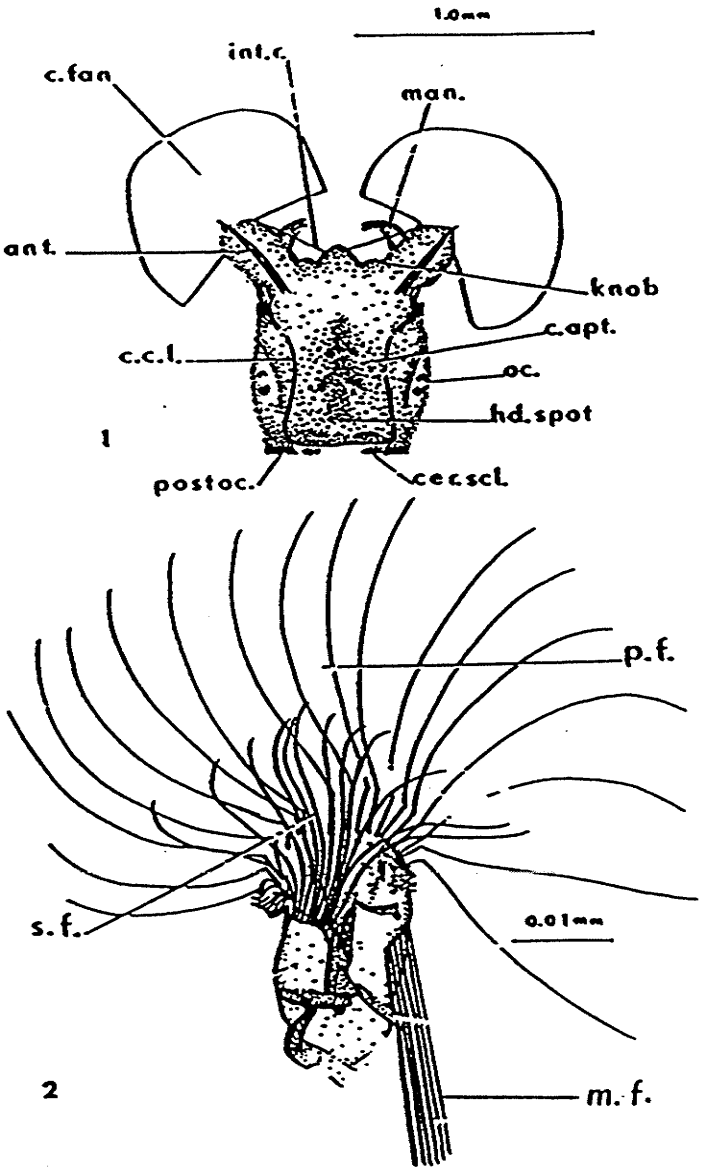
The shape and behavior of black fly larvae induce laminar flow. However, the presence of the cephalic fans causes vortices that influence larval feeding. These vortices are particularly important to larvae close to one another. The effect of larval density on larval feeding is reviewed by Craig and Chance (1982) and Chance and Craig (1986).

The filtering organs have been studied in great detail by Chance (1970) and Craig (1974). What follows is a summary of their findings.

The cephalic fans used in filtering are paired structures consisting of a fan stem and a series of fans (Figure 1). The fan stem serves as a support for the fans and arises from the anterolateral corners of the cephalic apotome. The dorsal surface of the cephalic fan stem is sclerotized. The ventral wall of the fan stem is concave and membranous. This ventral wall is reinforced by a system of sclerotized rods which are involved with the movement of the cephalic fans.

There are three well developed fans supported by each fan stem. The primary fan (p.f.) arises from the apex of the fan stem and is the largest. The secondary fan (s.f.) lies laterobasal to the primary fan. The medial fan (m.f.) lies on the medial side of the fan stem. The rays of the

Figure 1. Head capsule and cephalic fan of S. vittatum. 1, head capsule dorsal view; 2, cephalic fan ventral view. ant. = antenna, c. apt. = cephalic apotome, c. c. l. = cephalic cleavage lines, cer. scl. = cervical sclerite, c. fan = cephalic fan, hd. spot = head spot, int. r. = intermediate ray, man. = mandible, m. f. = medial fan, oc. = ocelli, p. f. = primary fan, postoc. = postocciput, s. f. = secondary fan. (Figure from Chance 1970).


1.0mm
int.c.
c.fan
man.
ant.
knob
c.apt.
c.c.l.
oc.
hd.spot
1
postoc.
cec.scl.
p.f.
s.f.
0.01mm
2
m.f.

primary fan are arranged in a semicircle around the apex of the fan stem. When fully abducted the rays cover an angle of 200 to 250 degrees. The bases of the secondary fan rays lie in a curved line that connects with the primary rays medially. With the fan fully abducted the secondary rays cover an angle of about 270 degrees, and overlie the basal quarter of the medial primary rays. The secondary rays are weaker than the primary rays. The medial fan differs from the other fans in that its rays lie in a straight line and are not curved. These rays are parallel to each other and do not spread out when the fan is abducted.

The individual rays of all fans are hollow and except for the medial fan rays, are sickle-shaped. All rays bear microtrichia on their inner surfaces. The number of rays of each fan and the number and arrangement of microtrichia vary with instar and species.

The filtering behavior of black fly larvae consists of adducting the cephalic fans into the current for a period of time. The cephalic fans are then abducted and cleaned by the mandibles, labrum, and maxillae. The fans are closed, cleaned, and reopened very rapidly. This operation is frequently referred to as "flicking" (Chance 1970).

The frequency of flicking is irregular. Larvae extend their fans for several seconds and then flick them continually for several seconds. Fans are generally flicked alternately. Chance (1970) reported the frequency of flicking did not vary between instars or between larvae with

full guts or empty guts. However, Schroder (1980) found that the frequency of the fan movements increased with each successive instar.

Loss of particles either from the fans or the mouthparts is frequent. Larvae do not always flick the fans immediately on catching a particle and they may flick them without having caught a particle (Chance 1970). Hart and Latta (1986) reported Prosimulium spp. larvae flicked their fans after a fixed number of particles had accumulated.

The sizes of particles that filter-feeding black fly larvae ingest are well known. Black fly larvae are capable of ingesting particles of colloidal size to about 350 microns. The most commonly ingested particles range from 10-100 microns in diameter (Carlsson et al. 1977, Chance 1970, Kershaw et al. 1965, Kurtak 1978, Kurtak 1979, Williams et al. 1961, Wotton 1976 and 1977). Black fly larvae remove fine particulate matter from stream water using a mucosubstance which coats their filtering organs. This allows simuliid larvae to capture fine particles that might otherwise escape between the fan rays. Direct interception is probably the predominant mode of fine particle filtration for black flies (Ross and Craig 1980).

The diet of black fly larvae in the field is extremely variable. Larval simuliids are capable of living on bacteria alone at concentrations of 1,300,000 to 34,900,000 cells/ml (Fredeen 1960, 1964). Bacterial levels in this

range are commonly found in streams and rivers (Fredeen 1964). Some species of simuliid larvae can feed exclusively on filaments of the green algae Oedegonium inconspicuum (Hirn) and O. moniliform (Wittrock) (Burton 1973). Gut contents may include diatoms, mineral particles, aquatic mites and other arthropods, as well as pollen (Kurtak 1979). Black fly larvae are generally indiscriminate feeders within their preferred size range of particles. In gut contents of black fly larvae the proportions of their constituents, within the preferred size range, generally coincide with the proportions in the stream water (Kurtak 1979, Thompson 1987, Wotton 1977).

The suspended particle concentration affects the feeding rate of simuliid larvae. Fredeen (1964) found that black fly larvae developed faster in higher concentrations of bacteria than in lower concentrations and that a higher percentage of larvae developed into adults in the higher concentration of bacteria. Kurtak (1978) studied the efficiency of black fly larvae feeding by exposing them to synthetic particles. He reported that while only a small percentage of the particles passing through an area equal to that of the cephalic fan rays of a single larva was ingested (1-10% by weight), the feeding efficiency was higher at lower particle concentrations. Two groups of Simulium pictipes(Hagen) larvae were exposed to the same quantity of dye particles over different time periods. One group was exposed to 0.027 g/min for 15 min (0.4 g total). A second

group was exposed to 0.0027 g/min for 150 min (0.4 g total). The larvae offered the low concentration for a longer time ingested more particles by the end of the experiment. Lacey and Mulla (1979) reported that as particle density increased up to 50 ppm feeding rate increased. This relationship however, was not a linear one over the entire range of concentrations. At some concentration less than 50 ppm, the filtering process was operating at maximum efficiency and an increase in particulate density beyond this level did not result in an increased feeding rate. Also, above the optimal particle concentration, an inhibitory effect would undoubtedly be noted with the addition of more particulate matter. Gaugler and Molloy (1980) also demonstrated that normal feeding of black fly larvae was inhibited by exposure to high concentrations of suspended solids. Virtually all particle types tested inhibited at 50 ppm and the percentage of larvae with inhibited feeding increased with increasing particle concentration. Gaugler and Molloy (1980) suggested that inhibition is a result of rapid gut filling.

The effect of instar on feeding behavior of simuliid larvae is unclear. Some authors have found no difference in the size of particles ingested, or in the rate of gut filling, by early or late instars (Ladle et al. 1972, Williams et al. 1961). Other workers have found that feeding rate was greater in earlier instars (Mulla and Lacey 1976, Wotton 1978). Schroder (1980) found frequency of fan

movements to be faster in later instars. Merritt et al. (1978) reported that more coarse particles relative to fine particles were retained by small instars as compared to large instars. Simuliid pupae or larvae approaching pupation do not feed. However, Hinton (1958) reported that Simulium pupae actively feed and defecate until about the time that they begin to spin their cocoon.

Temperature has an effect on the feeding behavior of black fly larvae. Black fly larvae in chalk streams did not feed at temperatures between 5 and 8 C. Above 8 and up to 21 C there was no significant difference in feeding rate (Ladle et al. 1972). Reisen (1974), reported the number of particles removed from a creek by black fly larvae was independent of water temperature. In experiments with S. pictipes at temperatures of 15, 18, and 23 C, there was a significant decrease in feeding efficiency at 23 C (Kurtak 1978). Mulla and Lacey (1976) however, showed that early and late instars of S. tescorum (Stone and Boreham) voided a dye marker faster at 30 C than at 12.8 C. Lacey and Mulla (1979) showed that temperature of the water affected feeding rates in S. vittatum larvae exposed to dye particles at 4, 10, 15, 19, 24, and 28 C. The feeding rates at 4 and 10 C were significantly lower than those at 15-28 C. Schroder (1980 and 1981) reported that both the frequency of fan movements and ingestion rate of Odagmia ornata Meigen increased with increasing temperature.

Feeding behavior can vary with species of black fly

larvae. However, the sizes of particles in the guts of S. ornatum s.l., S. reptans, and S. variegatum are similar (Williams et al. 1961). Ladle et al. (1972) found that species did not differ measurably in the rate of filling of the guts. On the other hand, other species differ in the size of particles, the time taken to void a dye marker, and feeding efficiency (Carlsson et al. 1977, Chance 1970, Kurtak 1978, Mulla and Lacey 1976, Schroder 1986).

Reisen (1974) reported that current velocity had no effect on the number of particles removed from a stream by black fly larvae. Kurtak (1978) found that mean efficiency of ingestion for all species decreased slightly as velocity increased for all particle types tested. However, this decrease in efficiency of ingestion, with increasing velocity, was not significantly different. He pointed out that individual species responded differently to current velocity. Cnephia dacotensis (Dyar and Shannon) was most efficient at 50 cm/sec. At higher or lower velocities, efficiency was lower. Other species showed a decrease in efficiency with an increase in velocity from 30-70 cm/sec. Lacey and Mulla (1979) reported that feeding rates of S. vittatum increased with an increase in current velocity up to a point. They described a curvilinear relationship between current velocity and feeding rate over the whole range. After 50 min of feeding on dye particles at 26, 39, and 53 cm/sec, on average, the guts were 42, 52, and 30%

filled respectively. Larvae of Simulium bivittatumMalloch exposed to water velocities of 3.2-47.0 cm/sec consumed a maximum of particles at velocities of 10.0-25.0 cm/sec. Larvae consumed significantly fewer particles at velocities above or below this range (Braimah 1987). Some authors have noted that black fly larvae can feed more efficiently in eddies (Chance and Craig 1986). Kurtak (1978) suggests that this gives them more opportunities to capture each particle.

Black fly larvae are capable of feeding by grazing as well as filter feeding. Burton (1973) reported a population of S. hargreavesi (Gibbons) that were feeding exclusively by grazing on filamentous algae and on organic detritus trapped among the filaments. Kurtak (1978) reported that filter feeding species can graze as well. He noted that S. vittatum from one location were filter feeders while individuals of the same species from another location were predominantly grazers. Chance (1970) pointed out that some black fly larvae are exclusively grazers and that mouth parts of filtering and grazing species are well adapted for their respective mode of feeding.

Black fly larval feeding can vary between seasons and geographically. Mulla and Lacey (1976) showed that S. vittatum feeding rates in the Colorado River were essentially the same as that found in small streams. However, Carlsson et al. (1977) noted that three dominant species present at a lake outlet were virtually absent from other localities where, conversely most larvae belonged to

species not present at the lake outlet. Quantity and quality of suspended material varies greatly between sites and season (Kurtak 1979). He noted that the type of food to which larvae will be exposed, is dependent on the timing and site of oviposition of the adult female black fly.

Larval feeding is a very important consideration in all black fly suppression programs using agents which must be ingested to be effective. Any condition that causes less material to be ingested by the larvae is important and could drastically reduce the efficacy of any control program. A change in the ingestion rate will be reflected in a change in the length of time material is present in the gut. A longer gut retention time will allow any toxin present in the gut more time to act upon the larvae. Simuliid larvae have been reported to clear their guts in 10 to 65 min, depending on species, instar, temperature, current velocity, and amount of particulate matter available (Chance 1970, Fredeen 1964, Kurtak 1978, Lacey and Lacey 1983, Ladle et al. 1972, Mulla and Lacey 1976, Wotton 1978).

Laboratory Studies

Black fly larval susceptibility to B. t. H-14 has similarities with mosquito larval susceptibility to B. t. H-14. Black fly larval susceptibility varies with species (Lacey et al. 1978, Molloy et al. 1981, Undeen and Nagel 1978). There is a strong positive correlation between temperature and efficacy of B. t. H-14 against black fly

larvae in the laboratory (Lacey et al. 1978, Lacey and Federici 1979, Molloy et al. 1981). Most of the reduced efficacy at lower temperatures is likely due to simuliid larvae feeding more slowly than at higher temperatures. Efficacy of B. t. H-14 is consistently higher against earlier instars of simuliid larvae (Molloy et al. 1981). Lacey and Mulla (1977) confirmed that there is a direct and positive relationship between concentration of B. t. H-14 and larval mortality.

Controlling black fly larvae with B. t. H-14 differs from controlling mosquito larvae in that black fly larvae live in flowing water. It is difficult to expose simuliid larvae to the toxin for long periods of time. There is a direct and positive relationship between the duration of exposure and mortality up to about one hour (Frommer et al. 1980, Lacey and Mulla 1977, Lacey and Federici 1979, Undeen and Nagel 1978). Exposure times longer than 1 to 3 hours did not increase mortality of simuliid larvae significantly (Frommer et al. 1980, Lacey and Federici 1979).

The presence of suspended solids affects the efficacy of B. t. H-14 (Gaugler and Molloy 1980). Efficacy of B. t. H-14 was reduced to insignificant levels by the addition of suspended solids before larval exposure to the toxin. Black fly larvae which were not feeding or were feeding at a diminished rate, cannot ingest the same quantity of inoculum as larvae feeding normally. Thus, lower mortality results.

They also found that the efficacy of B. t. H-14 was increased by nearly 90% over the LD-50 level when feeding inhibition was induced after larval exposure to the bacterium. They attributed this to increased bacterial retention time in the gut resulting from feeding inhibition.

Field Studies

Many field trials using B. t. H-14 for the control of black fly larvae have been successful (Car and de Moor 1984, Colbo and O'Brien 1984, Gaugler et al. 1983, Guillet and Escaffre 1979a and 1979b, Guillet and de Barjac 1979, de Joux 1979, Lacey and Undeen 1984, Pistrang and Burger 1984, Undeen and Colbo 1980, Undeen et al. 1981, White and Morris 1985). However, as with the use of B. t. H-14 for control of mosquito larvae, there are a number of problems. Simuliid species differ in their response to B. t. H-14 (Lacey et al. 1982b). Earlier instars are more susceptible than late instars (Gaugler et al. 1983, Molloy et al. 1981). Cold water temperatures demand the use of greater concentrations and/or prolonged treatment periods of B. t. H-14 to achieve reasonable results (Colbo and O'Brien 1984). This agrees with what has been reported in the laboratory, and with the effect of temperature on feeding of black fly larvae.

An important consideration when using B. t. H-14 against black fly larvae is the variation in toxicity between batches or formulations of the pesticide.

International Toxicity Units/mg (ITU/mg) are used as a measure of the potency of various formulations. However, Frommer et al. (1981c) pointed out that the validity of comparing ITU/mg when working with simuliids is questionable, since these units are established from bioassays using the mosquito larvae Ae. aegypti and not black fly larvae. It is unwise to assume that these formulations will affect black fly larvae in the same manner as mosquito larvae.

The effect of concentration and exposure time on B. t. H-14 efficacy in the field is similar to that found in the laboratory. Frommer et al. (1981a) found that a 35 min exposure at 3.10 ppm resulted in little overall reduction in the number of larvae throughout the test area except for a 25% reduction at 312 m below the application point. Treatment at 1.55 ppm for 70 min resulted in 50-70% reductions in numbers of larvae along 312 m of the test stream after 24 hours. Lacey and Undeen (1984) however, reported that a 1 min application at 10 ppm gave significantly higher mortality than a 20 min application at 0.5 ppm. They report doubling and trebling the concentration of the bacteria increased the mortality over that of the 10 ppm/1 min application but that these increases in mortality were not significant.

It is important to note that other factors, such as the presence of vegetation and pools, can influence the movement

of inoculum downstream. These factors in turn effect exposure time and concentration. Every stream is different and the presence of vegetation and pools along the length of a stream can effect the performance of B. t. H-14. One of the most important parameters in treating black fly infested streams or rivers with B. t. H-14 is the distance downstream in which the inoculum is effective (downstream carry). Undeen and Colbo (1980) tested B. t. H-14 in small Newfoundland streams. The dosage time relationship changed with passage downstream. There were some areas in which an optimum concentration of B. t. H-14 was present for the optimum length of time. Upstream near the application site the bacterial concentration was high but the time of exposure to the toxin short and the larval mortality poor. Farther downstream the concentration was reduced providing a longer exposure time with a still adequate dose. Eventually by removal and dilution the bacterial concentration was below the lethal level, even for prolonged exposures. They also reported that the flow rate of the stream appeared to be a major variable in determining the maximum downstream carry. When the stream volume was high the downstream carry was greater than when the volume was low. Undeen and Colbo suggested this was probably related to the surface/volume ratio of the streams. Attached organisms, which filter fine particulate matter from the water, have less total volume of water to filter and clear the water in a shorter distance when the stream volume is low. Horosko and Noblet (1984)

demonstrated that black fly larval mortality was reduced substantially at 1100 to 1200 m downstream from the application point in streams with discharges of 2-8 cubic meters per min. Chilcott et al. (1982) treated three streams, with discharges of 7.02, 3.34, and 1.67 cubic meters per min, with B. t. H-14. The effective carry was very similar among the three. However, they used different concentrations and the physical parameters of the streams (pools, etc.) were different. As a result it is difficult to compare B. t. H-14 carry to stream discharge in this study.

Lacey and Undeen (1984) found that the presence of vegetation can markedly decrease the downstream carry of B. t. H-14. However, Frommer et al. (1981b) found that an application of B. t. H-14 to a stream with a flow rate of 18,269 l/min without vegetation was less successful than an application at the same concentration (3.10 ppm/35 min) in the same stream, but with a flow rate of 23,900 l/min, with extensive aquatic vegetation (Frommer et al. 1981c). The greater discharge was likely partially responsible for the increased success of the latter treatment. However, Frommer et al. (1981c) suggested that this effect can be also explained by the vegetative growth, which acted to retard the rate at which the inoculum moved through the test area. This was supported by the delays in the anticipated arrival of the treatment of 2-6 min for each downstream sampling

station up to 152 m. The delay at 312 m was 11 min. There was greater spore dispersion in the vegetated stream. These authors concluded that the concentration-time response is heavily dependent on environmental and physical conditions of the test stream at the time of treatment. These conditions must be known prior to treatment. Frommer et al. (1981a and d) conducted detailed studies on spore distribution in flowing water with and without vegetation. Frommer et al. (1981a) found that in the absence of extensive vegetation spore recovery profiles remained relatively uniform through the first three downstream sampling stations (37 m, 91 m and 152 m). The most significant difference from the first three sampling stations, to that of the last (312 m) was the appreciable reduction in overall recovered spores. These authors recovered 50 to 80% (1.5 to 2.5 ppm) of the desired 3.10 ppm treatment concentration over a 20 to 22 min time interval, with peak recovery occurring approximately midway through the 35 min exposure time. The remaining 13 to 15 min consisted of the leading and trailing edges of the treatment suspension which were at noticeably reduced spore levels. The authors hypothesized that the difference between initial treatment concentration levels from that recovered may be a result of statistical variability in sample collection procedures, i.e. single point samples instead of multiple sample per time-distance, and in laboratory spore determination procedures. Two things could account for the

sudden drop in peak concentration at 312 m following stable profiles to 152 m: losses of B. t. H-14 spores through settling or attachment, and dilution of the spore suspension by mixing with greater quantities of water. Frommer et al. (1981d) studied spore distribution in flowing water with extensive aquatic vegetation. These authors conducted two treatments, one in July and one in September, both with the same concentration (3.10 ppm/35 min) of B. t. H-14. The July treatment resulted in the recovery of 24 to 90% (0.8 to 2.8 ppm) of the 3.10 ppm treatment concentration over an 18 to 28 min period. This spore profile was quite similar in its overall pattern to the distribution study conducted in May (Frommer et al. 1981a) when extensive aquatic vegetation was absent. Extensive aquatic vegetation did not significantly impair spore movement, (i.e. spore profile remained close to a 35 min application interval) or larvicidal activity (as demonstrated by the field efficacy test conducted in July 1980 where a 27 to 92% reduction in larvae was achieved) (Frommer et al. 1981c).

In the September field study 3.10 ppm/35 min (98,800 spores/ml) of B. t. H-14 was also used, but the stream volume was now 43,890 l/min as compared to 23,900 l/min in July. For this treatment spore recovery profiles varied drastically between each sample station. At 37 m the level of spores recovered was more than twice the amount initially applied. The peak level of recovered spores did not fall

below the level initially dispensed until 312 m. Dissimilar profiles in recovered spore counts in the September field trial, as compared to the July trial, is the result of the increased volume of water in conjunction with extensive aquatic vegetation. This combined physical effect apparently kept the inoculum from being completely mixed with the whole volume of stream water. As a result much of the spore suspension was channeled into a smaller volume of water throughout the first 2 sample stations (37 and 91 m). This produced a higher recovered spore count from that initially calculated prior to treatment. The gradual drop in recovered spores at 152 m was most likely caused by greater spore dispersion as the channeling effect lessened.

So far no workers have reported on the effects of suspended solids on B. t. H-14 efficacy against black fly larvae in the field. However, it has been indicated that suspended solids in the field will have a profound effect. Suspended solids above 50 ppm drastically reduce the efficacy of B. t. H-14 (Gaugler and Molloy 1980). Suspended solid loads greater than 50 ppm are common in rivers where control of black flies is needed in Canada (Fredeen 1964).

Since simuliid larvae are only exposed to B. t. H-14 for a short length of time, the limited field persistence of B. t. H-14 is not a problem. However, there is a problem of the bacterial suspension settling out in the slower sections of the stream or river. Cheung and Hammock's (1985) work on

micro-lipid-droplet encapsulation of B. t. H-14 for control of mosquito larvae may have some application to the control of black fly larvae. Such a formulation used in streams and rivers for black fly control might have the necessary buoyancy to stay suspended even in the presence of pools. This buoyancy might dramatically increase the downstream carry of the inoculum. This technique has great promise and needs to be field tested.

Effect of B. t. H-14 on Non-targets

A great deal of work has been done to assess the impact of B. t. H-14 on non-target organisms. The only aquatic insects susceptible to B. t. H-14, other than simuliids and mosquitoes, are other Diptera in the same suborder, the Nematocera. The most susceptible are the Chironomidae (Ali 1981, Ali et al. 1981, Burton 1984, Car and de Moor 1984, Garcia et al. 1980, Mulligan and Schaefer 1981, Sinegre et al. 1979). Dixidae are also susceptible to B. t. H-14 (Garcia et al. 1980). Ceratopogonidae may (Garcia et al. 1980), or may not be susceptible to B. t. H-14 (Lacey and Kline 1983, Larget and de Barjac 1981a, de Maio et al. 1981). However, the species of Ceratopogonidae used in all these studies were not identified. The differing efficacy of B. t. H-14 could be a result of different species being used in these studies. Some Sciaridae are also susceptible (Cantwell and Cantelo 1984).

In general no mortality of non-target organisms, with the exception of nematoceran Diptera, has been reported from studies for mosquito control (Garcia et al. 1980, Miura et al. 1980, Mulla et al. 1982a, Purcell 1981, Sebastien and Brust 1981). No direct mortality has occurred in non-target organisms, excluding nematoceran Diptera, in tests of B. t. H-14 in the field against black fly larvae (Burton 1984, Chilcott et al. 1982, Colbo and Undeen 1980, de Joux 1979, Lacey et al. 1982b, Molloy and Jamnback 1981). However, Pistrang and Burger (1984) reported increased drift of two Ephemeroptera and two Trichoptera species following treatment of a stream with B. t. H-14.

Two things must occur in order for B. t. H-14 to become toxic to an organism: it must be ingested by that organism, and it must be changed from the protoxin into the toxin in the organism's digestive tract. The latter is presumably caused by the presence of alkaline conditions and or the appropriate lytic enzymes in the guts of susceptible organisms.

It has been demonstrated in certain studies that organisms have ingested B. t. H-14. Lacey and Kline (1983) demonstrated ingestion of B. t. H-14 by culturing B. t. H-14 spores recovered from the guts of Culicoides spp. and Leptoconops spp. exposed to B. t. H-14 formulations. These dipterans were unaffected by the toxin. Brazner and Anderson (1986) demonstrated B. t. H-14 ingestion by the

amphipod, Gammarus lacustris(Sars). Gammarus lacustris was not affected at concentrations up to 35 ppm for 96 hr exposure periods. On the other hand, Reish et al. (1985) reported that the amphipod Elasmopus bampo(Barnard) was susceptible to B. t. H-14, with an LC-50 of 12.8 ppm. This concentration is still many times higher than that used for mosquito control. This concentration is within the range used in black fly control programs. However, the organisms in a stream or river would be exposed to the toxin for a fraction of the time E. bampo was in the above study.

Future of Bacillus thuringiensis H-14

Several subjects would benefit from further research. More work should be carried out to assess the efficacy of B. t. H-14 against non-target organisms that can be shown to ingest the toxin. This research would be even more valuable if it were directed at species that are likely to be exposed to B. t. H-14 during mosquito or black fly control programs in order to get a better understanding of B. t. H-14's environmental acceptability.

Additional work should be carried out to investigate further the influence of stream discharge, vegetation and other physical characteristics of streams on the downstream carry of B. t. H-14. The relatively short downstream carry of B. t. H-14 is one of the major drawbacks. Improving the downstream carry of B. t. H-14, with improved formulation

techniques, will help make it competitive with traditional chemical black fly control agents.

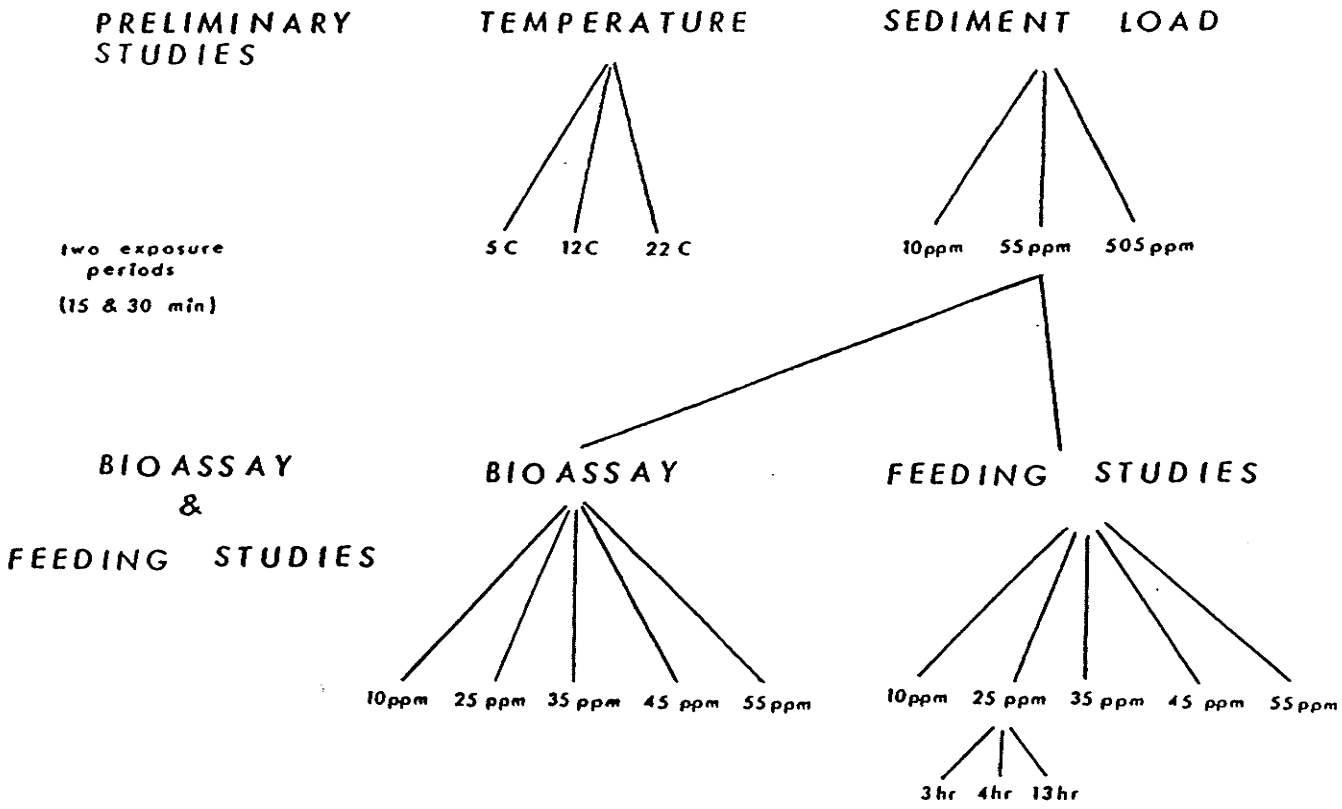
The effect of suspended particles on B. t. H-14 efficacy should be further investigated. It is important to know how suspended particles cause feeding inhibition. To ascertain this relationship a better understanding of black fly filter feeding behavior is needed.

MATERIALS AND METHODS

A liquid formulation (SAN 402 WDC Identification no. T15-2A) of*B. t.* H-14, Teknar® from Zoecon-Sandoz Inc., San Diego was used. This formulation had a potency of 1500 AA (*Aedes aegypti* units) per milligram. Fisher Scientific laboratory grade bentonite powder (<90 microns) was used as the inert suspended solid.

A diagrammatic summary of the study is shown in Figure 2. Two preliminary studies were conducted to investigate the effect of temperature (5, 12, and 22 C), duration of exposure to *B. t.* H-14 (15 min and 30 min), and the presence of suspended solids (10, 55, and 505 ppm), on the efficacy of *B. t.* H-14 against *S. vittatum* larvae. After analysis of these results it was decided to investigate further the influence of suspended solids on the efficacy of *B. t.* H-14. Two further studies were conducted: a bioassay study to determine the toxicity of *B. t.* H-14 (LC-50 and LC-90 values) against *S. vittatum* larvae in the presence of different suspended solid loads (10, 25, 35, 45, and 55 ppm) and a feeding study to determine the ingestion rate of *S. vittatum* at these same suspended solid loads.

Figure 2. A diagrammatic summary of the study showing the relationships between the two preliminary studies and the bioassay and feeding studies. All concentrations (ppm) are of suspended solid loads. Temperature trials were run with 5 ppm Tetra-min®.


PRELIMINARY STUDIES
two exposure periods
(15 & 30 min)
TEMPERATURE
5 C
12C
22 C
SEDIMENT LOAD
10ppm
55ppm
505ppm
BIOASSAY & FEEDING STUDIES
BIOASSAY
10ppm
25 ppm
35 ppm
45 ppm
55ppm
FEEDING STUDIES
10ppm
25 ppm
35 ppm
45 ppm
55ppm
3hr
4hr
13hr

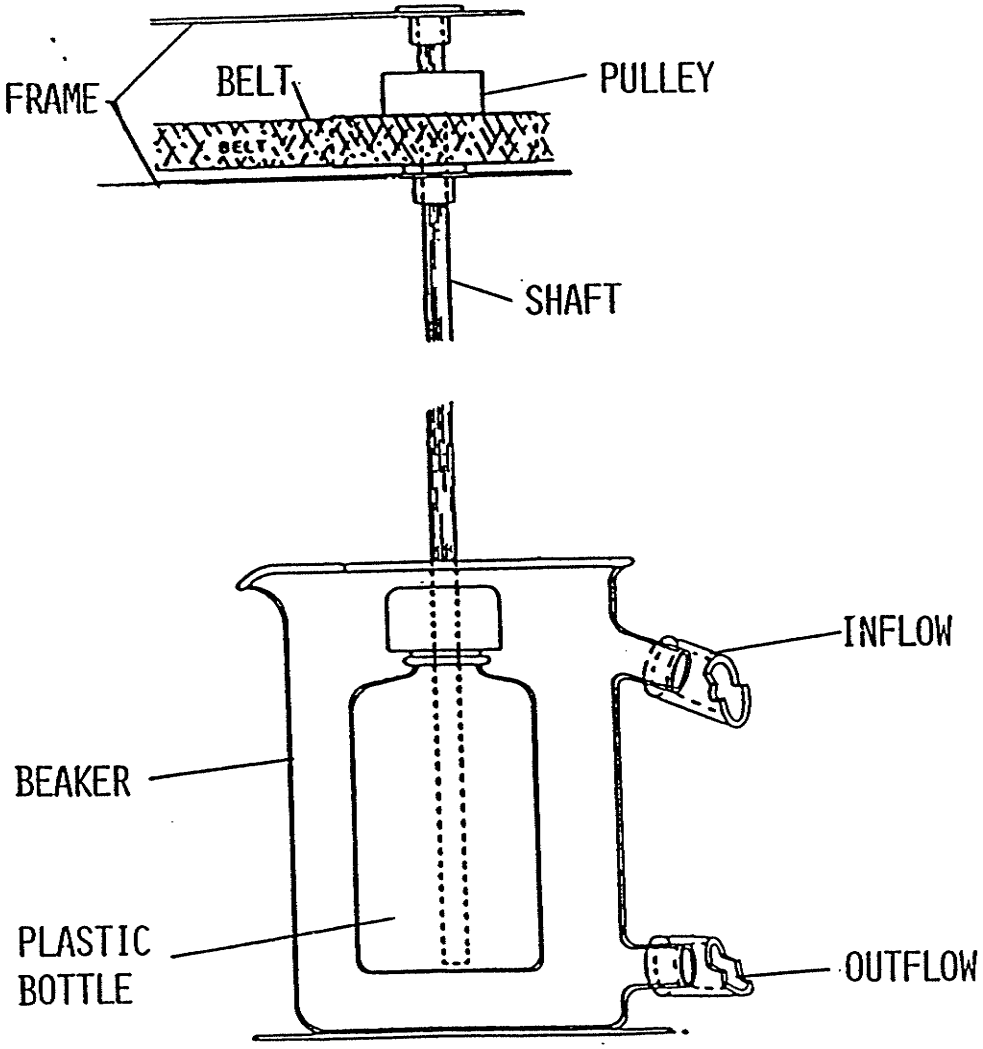
Bioassay Machine and Protocol

All studies were performed using the bioassay machine and protocol of Lacey et al. (1982a), except for the following changes. From 15 to 30 penultimate instars were used in each experimental unit (beaker) depending on the larval supply at the time and the acclimation mortality. Penultimate instars are the last instar before the formation of pupal gill filaments, which signal imminent pupation. An acclimation period of 12-24 hours was used. Finely ground (<90 microns) Tetra-min vegetable diet was used for larval food instead of rabbit chow.

The bioassay machine of Lacey et al. (1982a) consists of 10 glass beakers into which are suspended 10 small plastic bottles (Figure 3). Eight of the beakers are treated and two are controls. The plastic bottles do not touch the glass beakers and are rotated inside the beakers by an electric motor and a system of pulleys and a belt. Each beaker has a water inflow and outflow tube to allow the water in the beakers to be replaced without removing the beakers and the larvae from the machine.

The rotating bottles produce a current in the water contained in the glass beakers. Each beaker held approximately 250 ml of water. A method was developed to estimate current in the bioassay beakers. Small pieces of tissue paper were introduced into each beaker. The tissue paper was saturated in oil to increase its buoyancy, and to

Figure 3. Diagram of the bioassay machine (in part) of Lacey *et al*. (1982a).


FRAME
BELT
PULLEY
BELT
SHAFT
INFLOW
BEAKER
PLASTIC
BOTTLE
OUTFLOW

insure it remained suspended in the water column at mid depth. Current was estimated by counting the number of revolutions made by the pieces of tissue paper in each beaker in one minute. This was repeated 5 times for each beaker and a mean was calculated. These means differed significantly among the beakers. The means were averaged to get an estimate of 8.7±0.09 cm/sec for the current velocity in the beakers. Treatments were assigned randomly to the beakers at all times to minimize this bias.

The general protocol of Lacey et al. (1982a) consisted of adding larvae to the bioassay beakers randomly. Larval food was added at a concentration of 5 ppm. After the acclimation period beakers were removed and dead or moribund larvae were removed. Remaining larvae were counted and the beakers were returned to the assay machine. After a period of one hour, to allow larvae to resume normal behavior, the bioassay was begun. Toxicant was added to the eight treatment beakers for the correct exposure period. At the end of the exposure period all ten beakers were flushed at the rate of 1 l/min for 5 min to remove any trace of the toxicant. After the flush 5 ppm of larval food were reintroduced. The beakers were flushed at 24 hours to remove larval waste products. After 48 hours the bioassay machine was stopped, beakers removed, and the living larvae in each beaker counted. Larvae were considered dead when they showed no response when probed with forceps.

Lacey et al. (1982a) recommended a statistical protocol to determine LC-50 and LC-90 values. A minimum of 5 toxicant concentrations is recommended to give mortalities within 10-95%. A minimum of 3 replications should be conducted per concentration. Treatment mortality was corrected for control mortality by Abbott's formula when control mortality was 20% or less. When control mortality was over 20% that test run was rejected.

Preliminary Studies of the Effects of Temperature, Concentration of Suspended Solids, and B. t. H-14 exposure time, on the Efficacy of B. t. H-14 against S. vittatum Larvae

S. vittatum larvae used in the preliminary studies were field collected from the downstream face of dams at an irrigation canal located just north of St. Adolphe, Manitoba, and the LaSalle River in Labarrière Park, Manitoba. Field collected larvae were transported to the laboratory in plastic trays with tight fitting lids. The trays were transported in ice water to minimize the possibility of thermal stress to larvae. Larvae were put into 15 gallon aquaria, with air stones to generate current. Temperature of the water was brought gradually up to 19 C where it was maintained. Larvae were fed in the aquaria by the addition of finely ground (<90 microns) Tetra-min every two days.

The LC-90 value at 19 C using a 15 min exposure time

was estimated at 1.95 ppm. Subsequent analysis using log probit analysis (SAS Institute Inc. 1985) revealed the actual LC-90 value to be 2.11 ppm. All preliminary studies were conducted using a concentration of B. t. H-14 of 1.95 ppm. The value of 1.95 is not significantly different, using fiducial limits, from the actual LC-90 of 2.11 ppm ($P \geq .05$).

Effects of Temperature on Efficacy of B. t. H-14 against S. vittatum Larvae

The influence of temperature on the efficacy of B. t. H-14 against S. vittatum was determined by conducting two trials each at 22, 12, and 5 C. All larvae were held at the correct test temperature from the time of collection until treatment. A trial consists of 8 treatment beakers each treated with 1.95 ppm B. t. H-14 and 2 control beakers all tested at the same time over 48 hours. At each temperature, one trial was made with a 15 min exposure and the other, with a 30 min exposure.

Effect of Suspended Solids on the Efficacy of B. t. H-14 against S. vittatum Larvae

The influence of suspended particle loads on the efficacy of B. t. H-14 against S. vittatum larvae was determined by conducting two trials each at 10, 55, and 505 ppm suspended solids.

Trials dealing with the influence of suspended solids

necessitated a change in the general protocol. A second acclimation period of 4 hours was added after the 12-24 hour acclimation period. This was necessary to allow the larvae to become accustomed to the sudden increase in suspended solid load. The 4 hour acclimation period was begun when bentonite powder was introduced to the bioassay beakers, as a slurry, after the 12-24 hour acclimation period when larvae were counted. At the end of the B. t. H-14 exposure period the containers were flushed with bentonite-free water. Thus the suspended solids was present before and during larval exposure toB. t. H-14 but not after. In all cases 5 ppm of the suspended solid load were finely ground (<90 microns) Tetra-min. The rest of the suspended solids was bentonite (<90 microns). All trials were run at 19 C.

After analysis of the above trials it was decided to investigate further the effect of suspended solids, in the range 10-55 ppm, on the efficacy of B. t. H-14.

Effect of B. t. H-14 Exposure Time on the Efficacy of B. t. H-14 against S. vittatum Larvae

At each temperature and suspended solid load, one trial was conducted with a 15 min exposure and the other with a 30 min exposure.

Toxicity Studies

An alternate site located just downstream from the dam that forms Minnedosa Lake north of Brandon, Manitoba, yielded a large number of S. vittatum eggs. Consequently

larvae reared from these eggs were used exclusively in the bioassay and feeding studies.

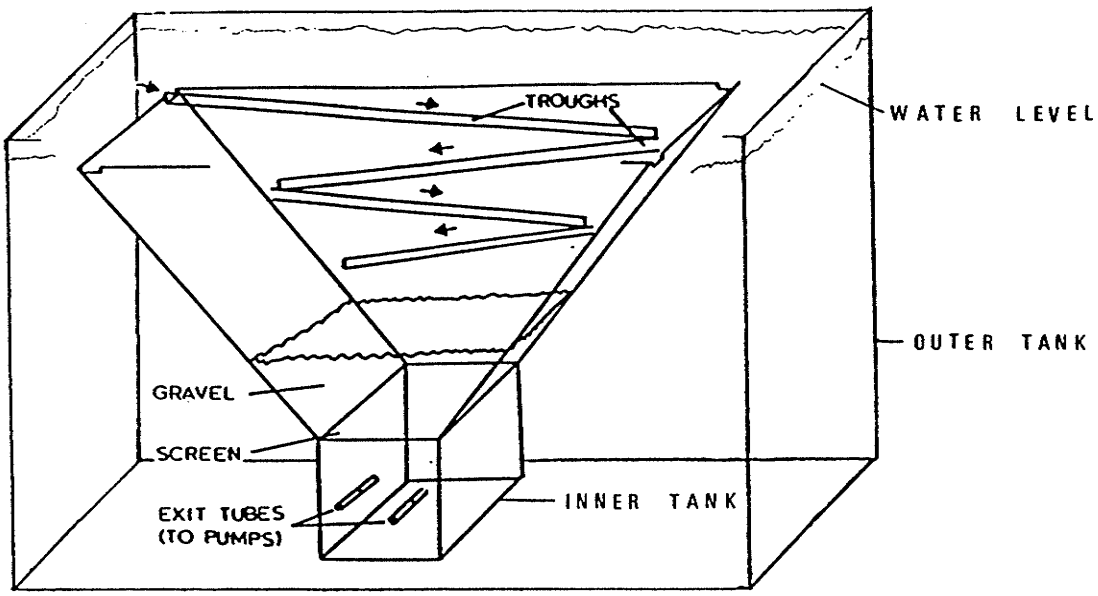
Field collected eggs were transported to the laboratory in plastic trays with tight fitting lids. The trays were transported in ice water to reduce thermal stress to the eggs. Eggs were reared using the apparatus developed by Simmons and Edman (1982) (Figure 4). Vegetation with attached eggs was placed into troughs and allowed to hatch. Larvae were fed finely ground Tetra-min vegetable diet (<90 microns) added to the outer tank every two days. On days that Tetra-min was not added, the gravel filter in the inner tank was agitated to release partially degraded Tetra-min and silt. Larvae were used for bioassay and feeding studies as they reached the penultimate instar. Larvae were reared at 19 C.

LC-50 and LC-90 determinations were conducted at 10, 25, 35, 45 and 55 ppm suspended solids to determine the toxicity of B. t. H-14 at these suspended solid loads. In all cases 5 ppm of the suspensions were Tetra-min, the rest was bentonite powder. All trials were run at 19 C.

Feeding Studies

The feeding studies were carried out using the same apparatus and general protocol as the bioassays. However, three different second acclimatization periods of 3, 4, and 13 hours were tried using 25 ppm suspended solids. The 4 hr period gave the most rapid ingestion rate. The 4 hr

Figure 4. Simuliid rearing apparatus of Simmons and Edman (1982).


TROUGHS
WATER LEVEL
OUTER TANK
GRAVEL
SCREEN
INNER TANK
EXIT TUBES
(TO PUMPS)

period was used for all the other suspended solid loads. Five ppm finely ground charcoal (<90 microns) was introduced to each beaker in turn to start the experiment. The charcoal provided a marker in the larval guts showing the start of the exposure period. After a five min period the beakers were flushed with clean water for five min at one l/min. Immediately after the flush, 5 ppm Tetra-min and the appropriate concentration of bentonite were reintroduced. It was at this time that the exposure time was deemed to begin.

There were ten different exposure periods, 4, 12, 20, 28, 36, 44, 52, 60, 68, and 76 min. Each beaker was randomly assigned an exposure period. The exposure periods were started at eight min intervals and all treatments were stopped at the same time. At the end of the exposure period the beakers were emptied and rinsed with 95% ethanol to kill the larvae. Two replicate feeding trials were carried out for each concentration of suspended solids of 10, 35, 45, and 55 ppm.

Larvae were examined after being cleared for one half hour in warm lactophenol. Each larva was measured for total gut length and the length of the gut traversed by the charcoal marker. Total gut length was measured from the posterior margin of the head capsule to the anterior margin of the anal sclerite. The distance traversed down the gut by the charcoal marker was measured from the posterior

margin of the head capsule to the anterior margin of the charcoal plug. Examples of cleared larvae with charcoal markers are illustrated in Figure 5.

At any one time a proportion of the larvae did not feed. Hence some larvae did not ingest the charcoal marker during the five min that they were exposed to it. Of the larvae that did ingest the charcoal marker, some fed long enough to have voided the charcoal marker and completely cleared their guts during the exposure period. The problem was to estimate which larvae not having the charcoal marker in their guts were those that failed to ingest the charcoal marker. These larvae could then be omitted from the analysis, and remaining larvae not showing charcoal in their guts could be assumed to have voided the marker and be counted as having completely cleared their guts.

According to gut clearance times recorded in the literature, the larvae could not have completely cleared their guts in under 12 min under these experimental conditions (Fredeen 1964, Kurtak 1978, Lacey and Lacey 1983, Ladle et al. 1972, Mulla and Lacey 1976, Wotton 1978). Therefore all larvae not having charcoal in the first two exposure periods (4 and 12 min) could only be larvae that failed to ingest the charcoal marker. The proportion of larvae not having charcoal in the 4 and 12 min exposure times for each trial was determined. This proportion was then multiplied by the number of larvae in each exposure period to give the expected number of larvae that did not

Figure 5. S. vittatum larvae after being cleared with lactophenol. Charcoal markers are visible in the guts.

ingest the marker. These were then omitted from further analysis. Remaining larvae not having charcoal in their guts in that exposure period were assumed to have completely cleared the gut.

Using this procedure it was possible to measure only the proportion of the gut filled up to the time that the charcoal marker was voided. At this time the proportion of the gut filled would be 1.00. However, it was impossible to measure any further ingestion. As a result at longer exposure times, when a high percentage of the larvae had voided the marker, the proportion of the gut filled could be drastically under estimated. One way to avoid this bias would be to omit exposure periods in which a high proportion of the larvae had greater than 100% gut clearance. Once the mean of the proportion gut filled for any exposure period reached .85, that and any following exposure period, was dropped from further analysis.

The data from each trial were analyzed using regression analysis. Subsequently the two trials in each sediment load were tested for equality of slopes (Sokal and Rohlf 1969). If the two trials were not significantly different from each other the data were pooled. These data were reanalyzed using linear regression to estimate the slope of the line for each sediment load. These lines were then tested for equality of slopes (Sokal and Rohlf 1969). Sediment loads with significantly different slopes represent significantly different ingestion rates by larvae exposed to those

sediment loads. Finally the time required to clear 50% of the gut was estimated for each sediment load (Sokal and Rohlf 1969). These estimates give an idea of the ingestion rate of the larvae expressed in minutes.

RESULTS

Effects of Temperature, B. t. H-14 Exposure time, and Concentration of Suspended Particles on the Efficacy of B. t. H-14 against S. vittatum

Toxicity of B. t. H-14 to S. vittatum Larvae

The LC-50 and LC-90 values of the Teknar formulation to penultimate instars of S. vittatum larvae were 0.43 and 2.11 ppm respectively. Fiducial limits (95%) were 0.34-0.53 and 1.66-2.87 respectively. Data for these determinations, corrected for control mortality, are shown in Table 1.

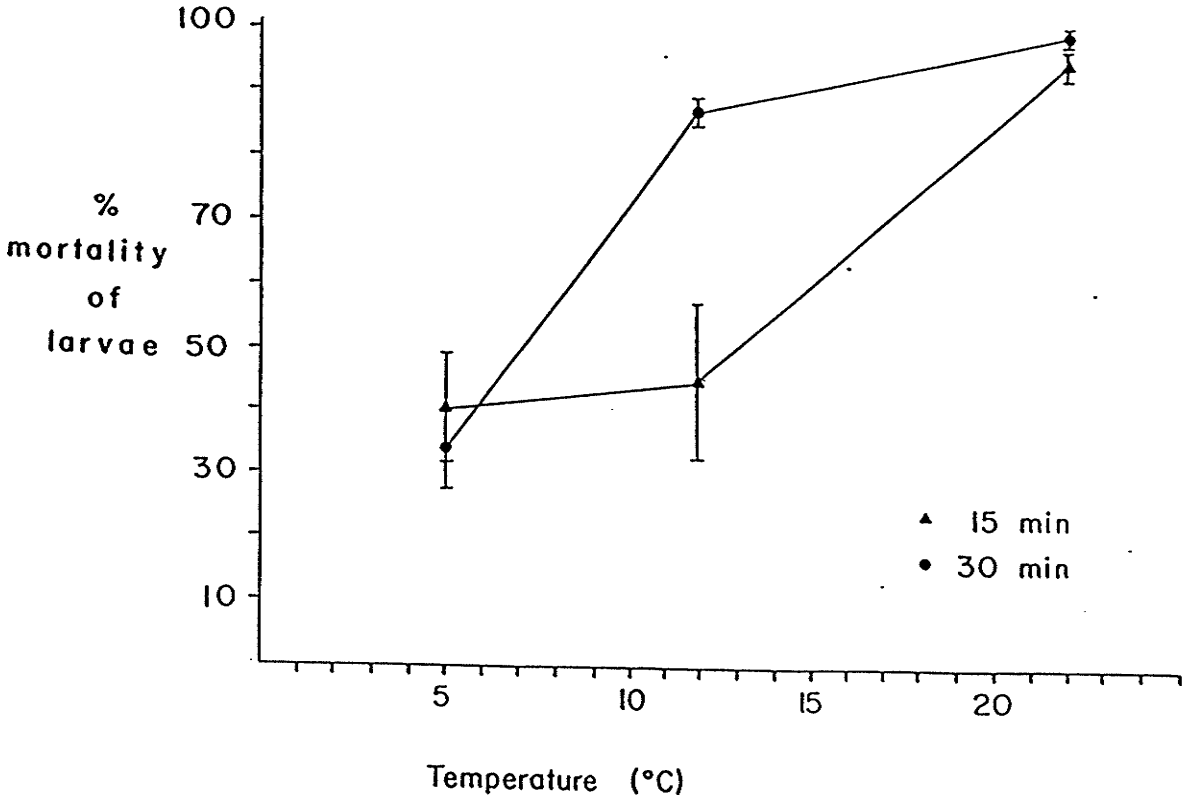
Influence of Temperature on Efficacy of B. t. H-14

Efficacy of B. t. H-14 diminished at lower temperatures after both the 15 min and 30 min exposures (Figure 6). Mortality was reduced by about half at 12 C after the 15 min exposure time. The 30 min exposure time provided better than 85% mortality at 12 C. The 15 min exposure mortality at 22 C was significantly different from that at 12 C and 5 C ($P \leq .01$) but mortality at 12 and 5 C was not significantly different from each other ($P \geq .05$). Mortalities after the 30 min exposure under the three temperatures were all significantly different from each other ($P \leq .01$).

Table 1. Toxicity of *B. t.* H-14 against *Simulium vittatum* larvae after a 15 min exposure at 19 C. Data corrected for control mortality using Abbott's formula.

B. t. H-14 conc. (ppm)	Total number larvae	Number dead larvae	% Mortality
0.125	44	6.0	13.6
0.250	43	14.0	32.6
0.500	47	27.9	59.3
1.000	47	36.5	77.7
2.000	49	41.7	85.0
3.000	48	46.0	95.8
4.000	68	63.8	93.9
5.000	49	49.0	100.0

Figure 6. The effect of temperature on the efficacy of B. t. H-14, at 1.95 ppm, against S. vittatum larvae using 15 min and 30 min exposure periods. The verticle lines illustrate the standard errors of the means.


100
70
50
30
10
% mortality of larvae
5
10
15
20
Temperature (°C)
15 min
30 min

Influence of B. t. H-14 Exposure time on Efficacy of B. t. H-14

At 10 ppm suspended solids, mortality after the 30 min exposure was significantly greater than mortality after the 15 min exposure ($P \leq .05$) (figure 7). However there was no significant difference between mortalities at 55 and 505 ppm between the two exposure periods ($P \geq .05$) (figure 7). Mortality between the two exposure periods was not significantly different at 22 C and 5 C ($P \geq .05$). It was however significantly different at 12 C ($P \leq .01$) (figure 6).

Influence of Suspended Solids on Efficacy of B. t. H-14

Efficacy of B. t. H-14 was affected by the presence of suspended particles (Figures 7). At suspended particle loads of 55 ppm or greater, efficacy at both exposure times was greatly reduced. After 15 min exposure, mortality at 10 ppm suspended solids was significantly greater than that at 55 and 505 ppm ($P \leq .01$). Mortalities at 55 and 505 ppm were not significantly different from each other ($P \geq .05$).

After the 30 min exposure, mortality at 10 ppm suspended solids was significantly greater than that at 55 or 505 ppm ($P \leq .01$). The 55 and 505 ppm mortalities were not significantly different from each other ($P \geq .05$).

Toxicity Studies

The LC-50 and LC-90 values for larvae exposed to B. t. H-14 and suspended solids are given in Figures 8 and 9 respectively. Fiducial limits (95%) for the LC-50 and LC-90 values are included in these figures. The slope of the linear

Figure 7. The effect of, concentration of suspended solids and exposure time to B. t. H-14, on efficacy of B. t. H-14, at 1.95 ppm, against S. vittatum larvae at 19 C.

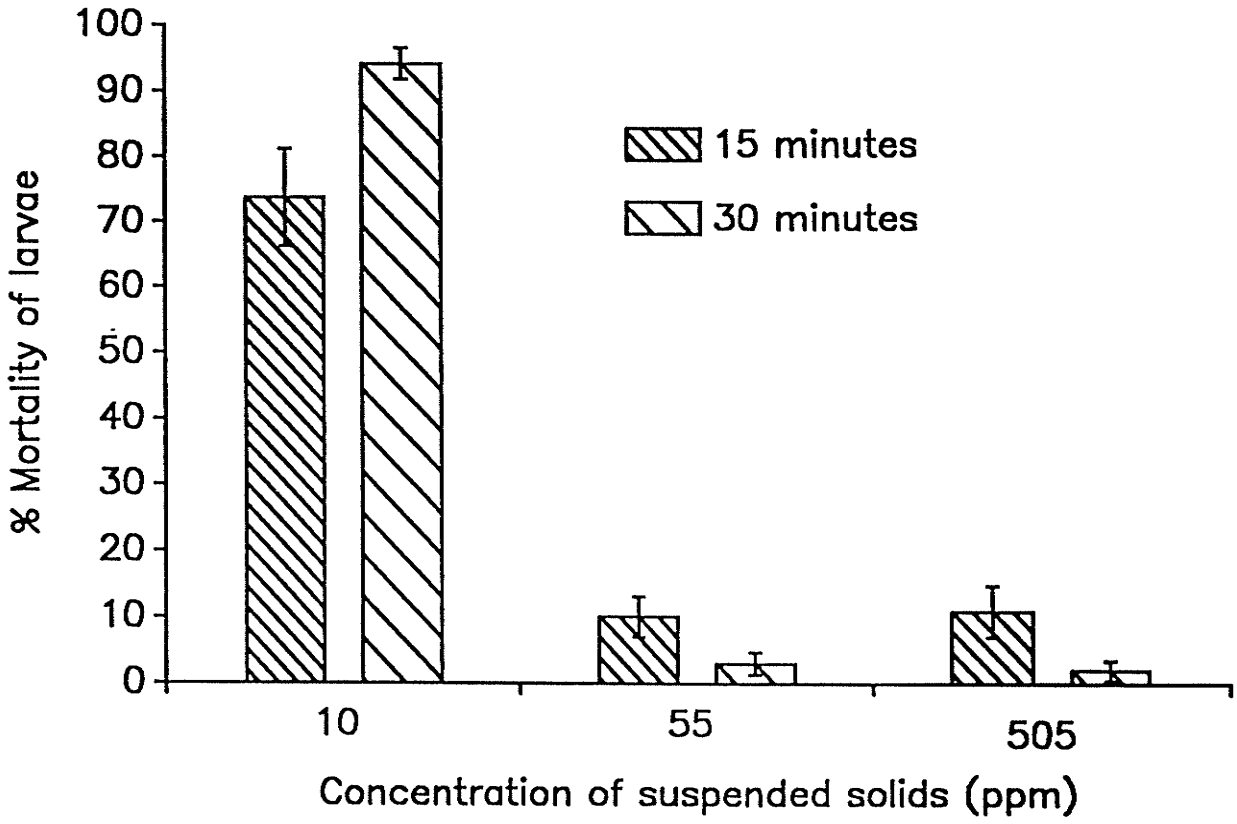

100
90
80
70
60
50
40
30
20
10
0
% Mortality of larvae
15 minutes
30 minutes
10
55
505
Concentration of suspended solids (ppm)

Figure 8. The effect of concentration of suspended solids on efficacy of B. t. H-14 against S. vittatum larvae at 19 C. LC-50 values with 95% fiducial limits.

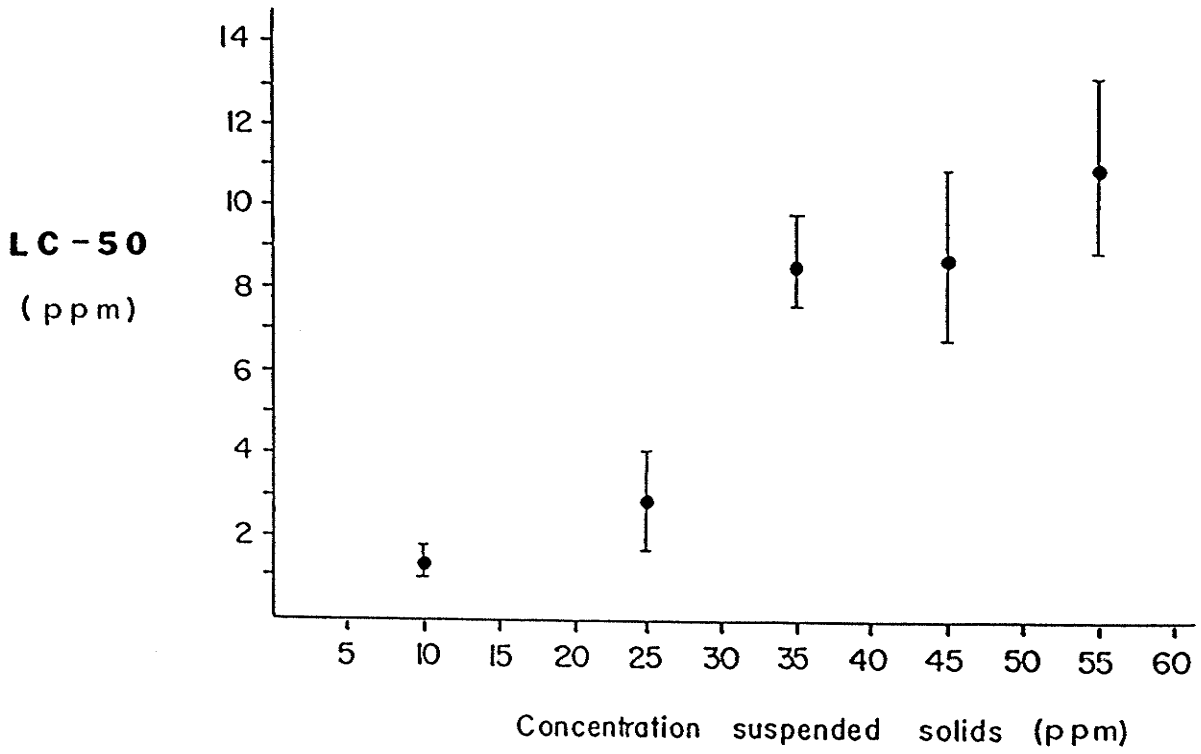
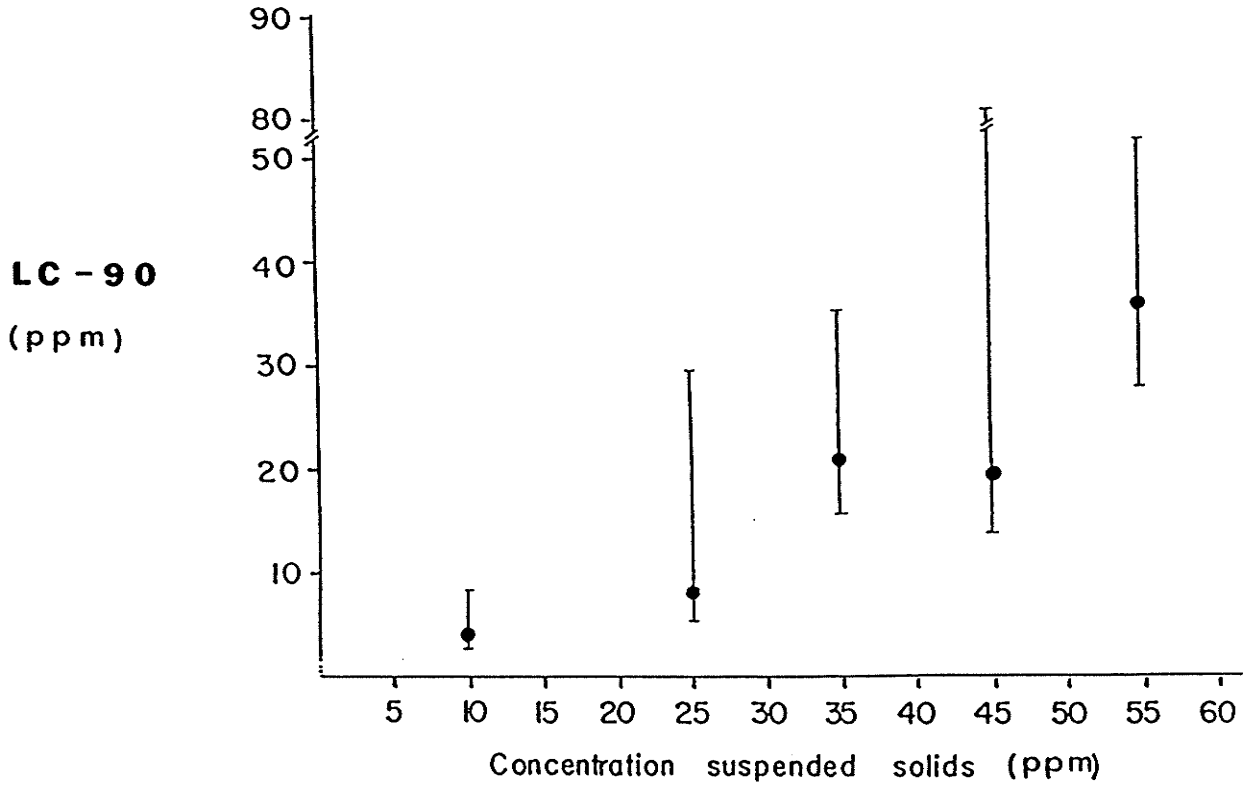

LC-50
(ppm)
14
12
10
8
6
4
2
5
10
15
20
25
30
35
40
45
50
55
60
Concentration suspended solids (ppm)

Figure 9. The effect of concentration of suspended solids on efficacy of B. t. H-14 against S. vittatum larvae at 19 C. LC-90 values with 95% fiducial limits.


LC - 90
(ppm)
90
80
50
40
30
20
10
5
10
15
20
25
30
35
40
45
50
55
60
Concentration suspended solids (ppm)

regression lines of both LC-50 and LC-90 values on suspended solid concentration were significantly different from zero ($P \leq .05$).

Feeding Studies

The mean per cent of larvae not showing charcoal after the 4 and 12 min combined exposure periods for each trial ranged from 0.0 to 18.2% with an overall mean of 5.7±2.1 (S.E.)% (Table 2.). Means of the proportion of the gut filled for each exposure time in each trial are shown in Table 3. The exposure times omitted from regression analysis are underlined in Table 3.

The slopes of the linear regression lines, of mean proportion gut filled with exposure time, were significantly different from zero ($P \leq .01$) in all trials. These lines along with their 95% confidence limits are shown in Figures 14 to 22 (Appendix 1.).

The slopes of both trials at each sediment load of 10, 35, and 55 ppm were not significantly different ($P \geq .05$). Data from both trials at each of the above sediment loads were pooled to give a final regression analysis for each sediment load. The slopes of the lines at 25 ppm, representing three trials with different second acclimation periods, differed significantly. The slope of the line of the 4 hr acclimation period was significantly greater than that for the trials with 3 or 13 hour acclimation periods. The lines representing trials at 3 and 13 hours second

Table 2. The mean per cent of S. vittatum larvae without the charcoal marker in the 4 and 12 min exposures combined. Total number of larvae in the two exposure periods combined is also included. Numbers in brackets indicate trials with different second acclimation periods.

Suspended Solid Load	Replicate	Mean % not Showing Charcoal	Total Number
10 ppm	1	13.9	43
10 ppm	2	0.0	39
25 ppm	1 (4hr)	3.8	53
25 ppm	1 (3hr)	18.2	33
25 ppm	1 (13hr)	4.8	42
35 ppm	1	0.0	47
35 ppm	2	0.0	47
55 ppm	1	5.4	37
55 ppm	2	5.0	40

Table 3. Mean proportion of the guts filled by S. vittatum larvae for each exposure period in each trial. Values underlined represent means that are equal or greater than 0.85 or are preceded by such a mean. Exposure periods with means underlined are omitted from regression analysis. Numbers in brackets indicate different second acclimation periods.

Suspended Solid Load	Exposure Time (min)									
	4	12	20	28	36	44	52	60	68	76
10 ppm Rep 1	0.34	0.33	0.51	0.43	0.57	0.50	0.76	0.73	0.79	0.95
10 ppm Rep 2	0.22	0.35	0.61	0.50	0.49	0.56	0.56	0.73	0.67	0.81
25 ppm Rep 1 (4hr)	0.31	0.35	0.58	0.73	0.83	0.90	0.94	0.95	0.94	0.88
25 ppm Rep 1 (3hr)	0.23	0.30	0.34	0.42	0.41	0.62	0.62	0.54	0.78	0.63
25 ppm Rep 1 (13hr)	0.26	0.34	0.37	0.56	0.53	0.57	0.82	0.85	0.82	
35 ppm Rep 1	0.26	0.27	0.38	0.38	0.43	0.46	0.64	0.59	0.73	0.85
35 ppm Rep 2	0.20	0.29	0.35	0.46	0.48	0.65	0.53	0.77	0.88	0.87
55 ppm Rep 1	0.23	0.32	0.27	0.33	0.38	0.41	0.53	0.66	0.52	
55 ppm Rep 2	0.15	0.29	0.33	0.33	0.36	0.46	0.50	0.51	0.64	0.83

acclimation periods were not significantly different from each other ($P \geq .05$). All other trials at the other sediment loads had a second acclimation period of 4 hours. Therefore the trial at the 4 hr acclimation period alone was used to represent gut clearance rates at the 25 ppm sediment load. The regression lines and 95% confidence limits for the sediment loads of 10, 35, and 55 ppm after pooling of both trials are shown in Figures 10-12. The 4 hr trial at 25 ppm sediment load is shown in Figure 16 (Appendix 1). The slopes of the regression lines at 10, 35, and 55 ppm sediment load are not significantly different ($P \geq .05$). The slope of the regression line at 25 ppm was significantly different from all others ($P \leq .05$).

The estimated time needed for the larvae to clear 50% of their gut contents at 10, 25, 35, and 55 suspended solid loads was 30.4, 16.6, 39.0, and 49.2 minutes respectively.

Figure 10. The mean proportion gut filled in S. vittatum larvae versus time at 10 ppm suspended solids. Trials 1 and 2 pooled. Circles = mean proportion gut filled at each exposure time. The solid line = the regression line and the dotted lines = 95% confidence limits for the mean predicted values.

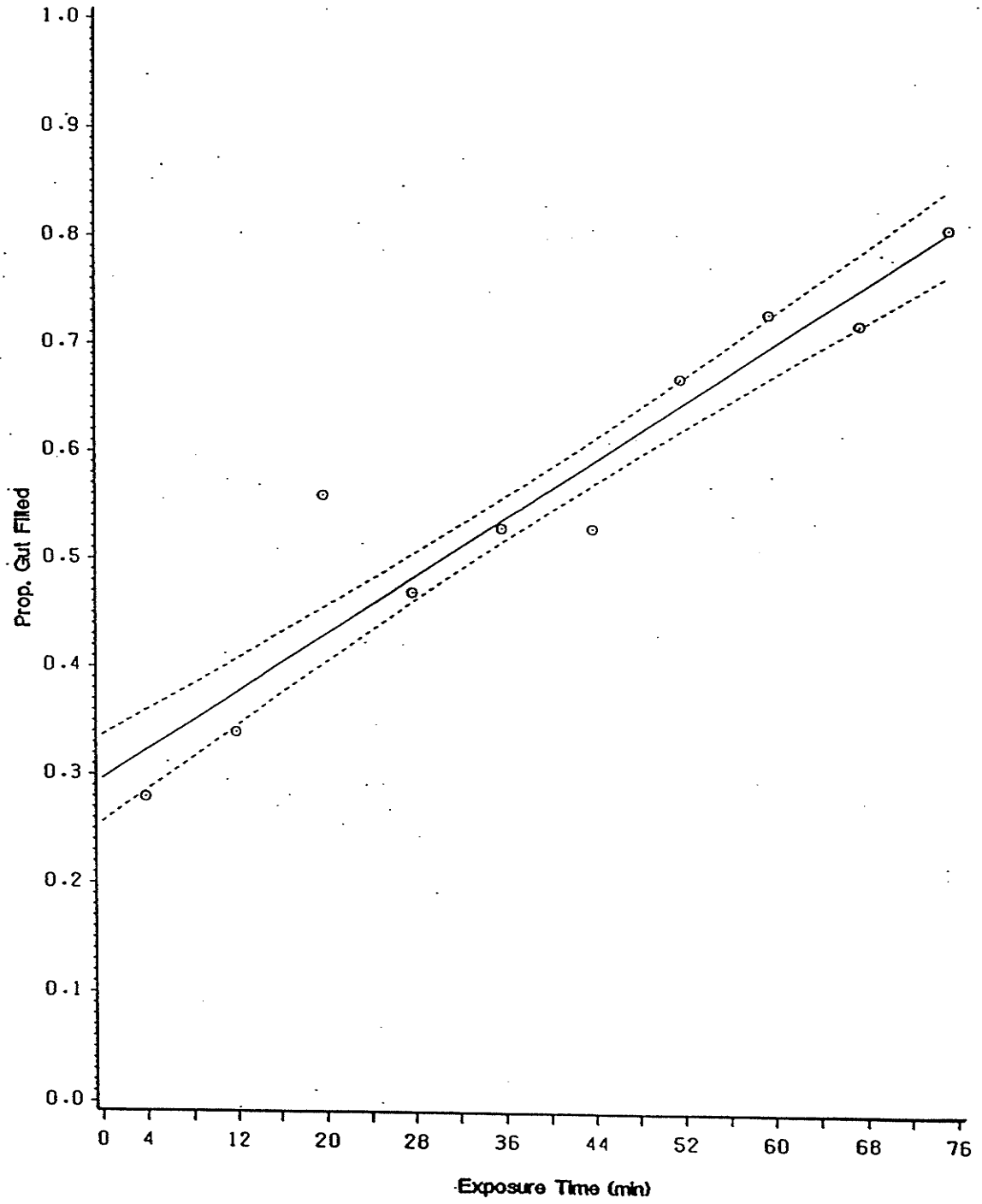

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Prop. Gut Filled
0
4
12
20
28
36
44
52
60
68
76
Exposure Time (min)

Figure 11. The mean proportion gut filled in S. vittatum larvae versus time at 35 ppm suspended solids. Trials 1 and 2 pooled. Circles = mean proportion gut filled at each exposure time. The solid line = the regression line and the dotted lines = 95% confidence limits for the mean predicted values.

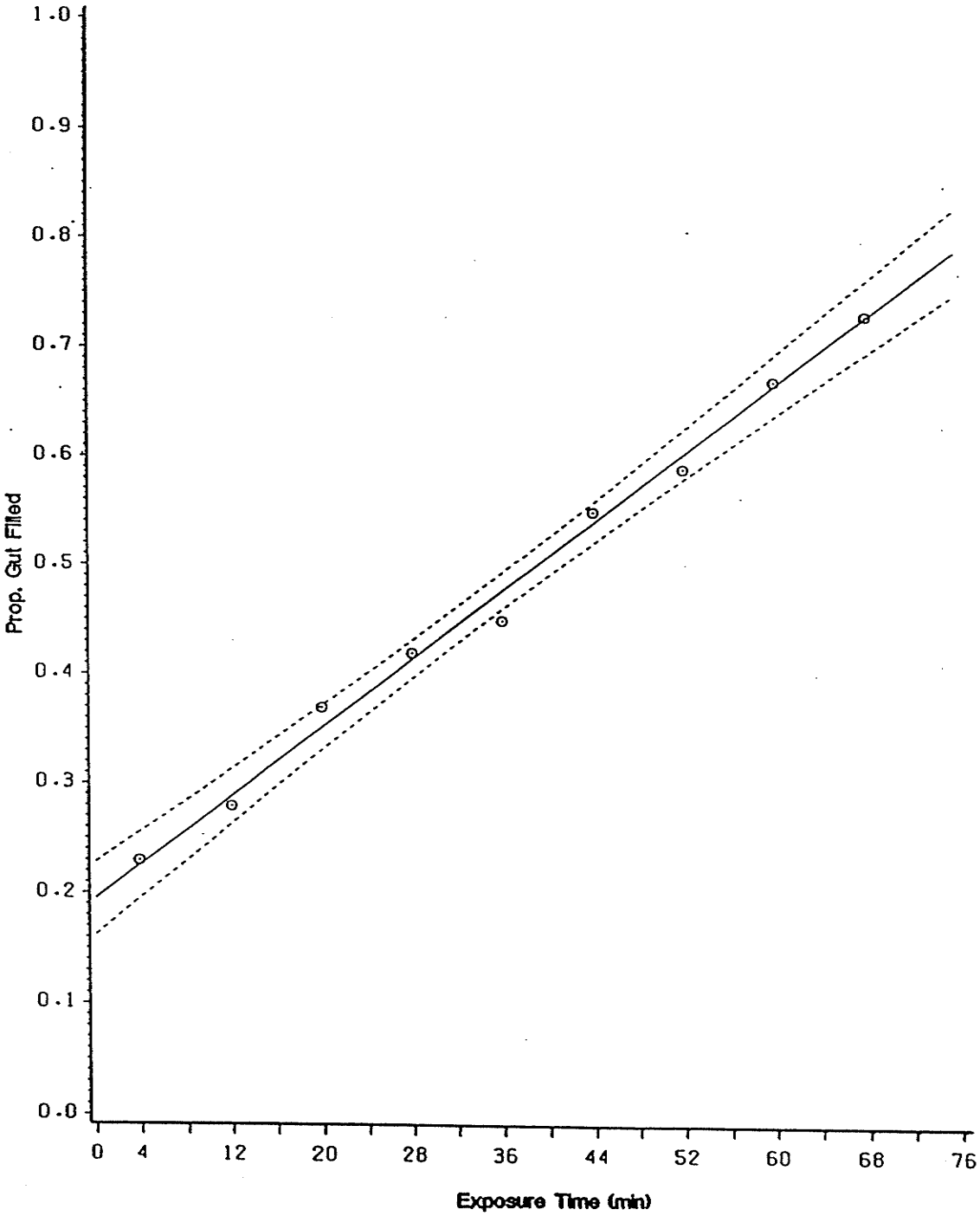
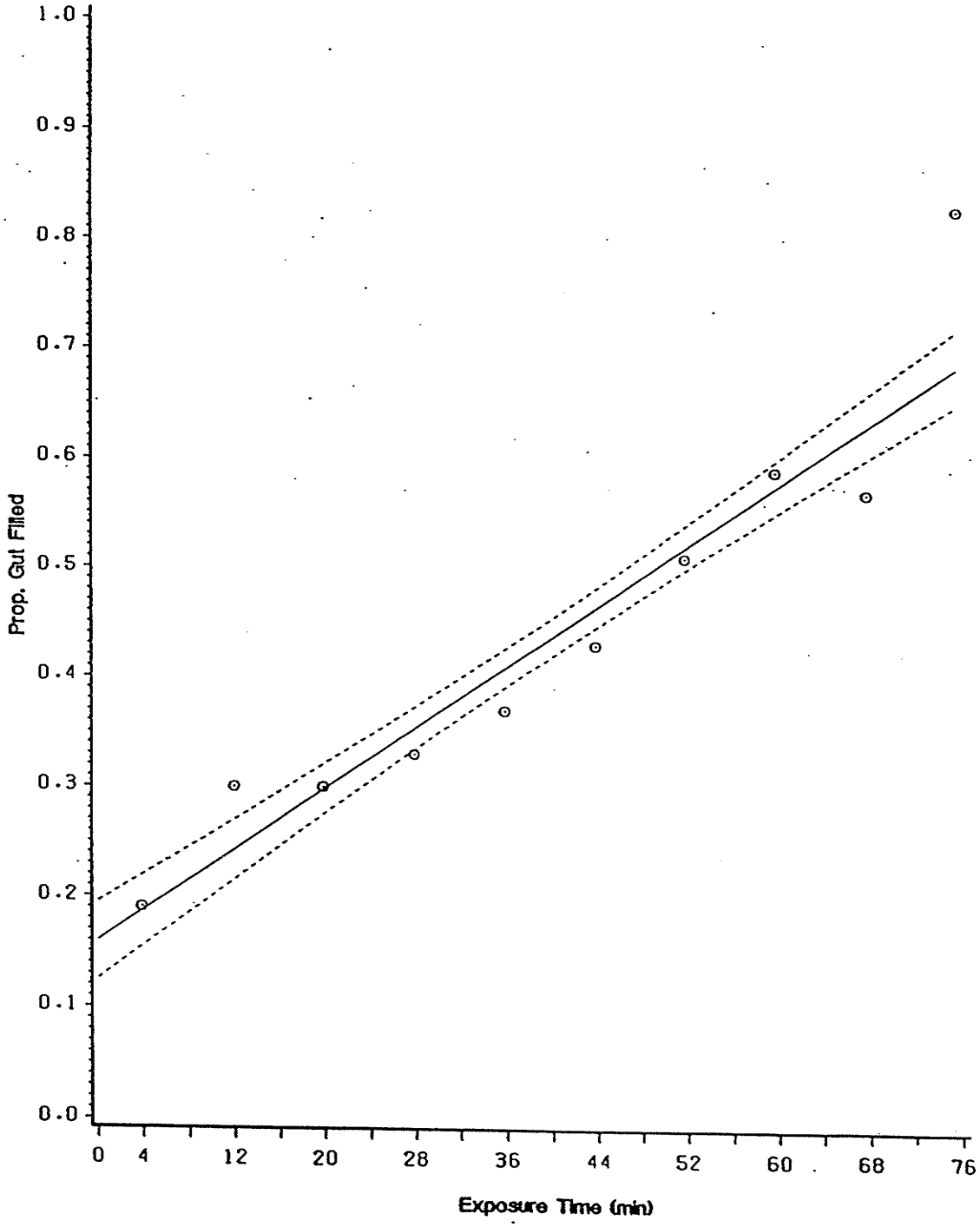

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Prop. Gut Filled
0
4
12
20
28
36
44
52
60
68
76
Exposure Time (min)

Figure 12. The mean proportion gut filled in S. vittatum larvae versus time at 55 ppm suspended solids. Trials 1 and 2 pooled. Circles = mean proportion gut filled at each exposure time. The solid line = the regression line and the dotted lines = 95% confidence limits for the mean predicted values.


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Prop. Gut Filled
0
4
12
20
28
36
44
52
60
68
76
Exposure Time (min)

DISCUSSION

Observations

Black fly larvae do not feed continuously. At any one time there is a proportion of the larvae that are not feeding. Chance (1977) reported that larvae stop feeding for various reasons: to clean their fans, to graze on the substrate, to move to a different site of attachment, to fight neighboring larvae, or apparently to rest.

The larvae stop feeding and close their cephalic fans whenever they are disturbed by any movement of the substrate to which they are attached. Consequently any inadvertent jarring of the bioassay beakers causes the larvae to stop feeding for a few minutes. In fact attaching the rubber tubing used to flush the beakers causes a vibration sufficient to cause the larvae to stop feeding momentarily. The current generated by flushing the bioassay beakers also interrupts larval feeding. This limitation in the protocol of the bioassay machine contributed to the variability observed in the results of the bioassay and feeding studies. This source of error could be reduced by making design changes to the bioassay machine. Attaching all tubing permanently to the beakers would reduce accidental disturbance of the bioassay beakers. However there is no

obvious way to reduce the disturbance caused by flushing the beakers. The impact of disturbances such as these on the feeding behavior of black fly larvae has not been addressed in the literature.

The larvae also stop filtering if the concentration of suspended solids changes suddenly. This necessitated a second acclimation period, of at least four hours, after the addition of bentonite. Three different second acclimation periods 3, 4, and 13 hours, were tried using 25 ppm suspended solids. Larvae ingested at a higher rate after the 4 hr acclimation period than after the 3 hr period. Apparently the larvae became accustomed to the sediment load between 3 and 4 hours.

The black fly larvae were able to remove a noticeable amount of the suspended solid load from the water overnight. Black fly larvae remove significant quantities of particulate matter from streams (Ladle et al 1972, Reisen 1974). In the present experiments there was only 250 ml of water in the beakers, hence it is not surprising that the larvae removed a noticeable amount of suspended solids overnight. In addition particulate matter ingested by the larvae was not made available again to the larvae after it was excreted. Larvae excreted compact circular fecal pellets, which sank to the bottom of the bioassay beakers and were not resuspended at the current velocity used in these experiments. Similar observations have also been made by Ladle and Griffiths (1980). These observations explain why

ingestion rate at the 13 hr acclimation period was not significantly different from that at the 3 hr acclimation period. Over the 13 hrs the larvae had removed much of the sediment from the water. At the end of the 13 hr acclimation period there were little or no suspended particles in the water. As a result the larvae were no longer accustomed to a high sediment load and when the suspended solids were reintroduced after the flush the larvae reduced or stopped feeding.

The current velocity of 8.7 cm/sec was far below that commonly reported in streams and rivers in the field (Hynes 1970). However, it is important to note that in the present study the velocity was estimated within 1 cm of the larval attachment surfaces. Velocity measurements in the field are not commonly done this close to the substrate. In flowing water, the velocity of the water decreases rapidly with increasing proximity to the substrate. Close to the substrate, in what is referred to as the boundary layer the velocity is near zero. The thickness of the boundary layer is controversial but Chance and Craig (1986) have shown that a black fly larva's body is in the boundary layer when the larvae is filtering. Nowell and Jumars (1984), reviewed this subject. In fact the present study's experimental conditions of 8.7 cm/sec are probably very similar to conditions experienced by black fly larvae in the field.

There were significant differences in current velocity

among the bioassay beakers. This was caused by the plastic bottles that were suspended inside the bioassay beakers. The bottles tended to wobble from side to side slightly when they were rotated, some bottles wobbling more than others. This design problem must be corrected in the future if the machine and protocol of Lacey et al. (1982a) is to be useful.

A different population of S. vittatum larvae was used in the preliminary studies from that used for the bioassay and feeding studies. However, preliminary studies and the bioassay and feeding studies were not compared to each other in the analysis, so potential variation due to different populations is unimportant.

Laboratory colonies of black fly larvae are not readily available. While successful colonization of blackflies has been achieved (Simmons and Edman 1982), no laboratory colonies were available for this study. Field collected larvae were used. This problem was further compounded by the fact that S. vittatum is composed of two sibling species, IIIL-1 and IS-7. These two siblings are sympatric in Manitoba (Rothfels and Featherston 1981), as a result it is impossible to insure that all larvae used in this study were of one sibling or the other. Attaining a laboratory colony of blackflies should be a high research priority.

Not all the data in the feeding studies have been presented. Two feeding trials were run at 45 ppm suspended solids. In both these trials few or none of the larvae

ingested the charcoal marker. As a result analysis was impossible in these trials. It is difficult to explain this phenomenon. Ordinary dechlorinated tap water was used in all the experiments. Dechlorination was achieved by allowing the water to sit overnight in open pails. Dr. K.W. Stewart of the University of Manitoba, Zoology Department pointed out that the Winnipeg water system is extremely variable, especially in chlorine content (personal communication). Algal blooms in the fall make it necessary to increase the chlorine levels in the city water supply. Often the chlorine is bound to organic matter in the water. Sudden die off of algae combined with continued application of high levels of chlorine often leads to periods of unusually high chlorine concentrations in tap water. These high levels cause sublethal effects in fish at the University of Manitoba (Dr. K.W. Stewart personal communication). Periods of high chlorine levels can range from several hours to several days in length. The trials at 45 ppm suspended solids were run at the time of year when this is a problem. It is possible that during the time these trials were run, the chlorine levels in the tap water were unusually high. Its also possible that due to the high level of organically bound chlorine the method of dechlorination used in this study was inadequate at this time. These high chlorine levels may have stressed the larvae which caused cessation of feeding.

Effects of Temperature, B. t. H-14 Exposure Time, and Concentration of Suspended Particles on Efficacy of B. t. H-14 against S. vittatum Larvae

Influence of Temperature on Efficacy of B. t. H-14

The efficacy of B. t. H-14 is greater at higher temperatures (Lacey et al. 1978, Lacey and Federici 1979, Molloy et al. 1981). However Undeen and Colbo (1980) reported high mortality of larvae of four genera of black fly larvae at temperatures of 3-5 C after treatment with B. t. H-14 (100,000 viable cells/ml). It is likely that similar mortalities would have resulted at a lower concentration of B. t. H-14 had the temperature been greater. The results from this study support the former findings.

Ingestion rate varies directly with temperature (Lacey and Mulla 1979, Ladle et al. 1972, Mulla and Lacey 1976). These findings and those of the present study do not reflect the reduced toxicity of B. t. H-14 at lower temperatures but rather reduced intake of toxicant. It is also possible that enzyme activity in the larval gut is reduced at lower temperatures. This would also cause a reduced rate of toxin activation.

Influence of Suspended Particles on Efficacy of B. t. H-14

Concentration of suspended solids influences the rate at which black fly larvae feed (Chance 1977, Elouard and

Elsen 1977, Fredeen 1964, Glotzel 1973, Kurtak 1978, Wotton 1978). At higher concentrations of particulate matter, feeding is inhibited (Gaugler and Molloy 1980). These authors demonstrated an inhibition of filter feeding behavior at 10 ppm with certain particles. At 50 ppm all food and several types of inert particles, including chalk caused an inhibition of filter feeding behavior. This inhibition resulted in reduced feeding, but not an absolute cessation of ingestion. Gaugler and Molloy (1980) demonstrated that this feeding inhibition can effect the efficacy of B. t. H-14. When feeding inhibition was induced by a high concentration of suspended solids before treatment with B. t. H-14, the efficacy of the bacterial formulation was reduced to insignificant levels. Larvae stopped feeding and therefore did not ingest the toxicant. Conversely when feeding inhibition was induced after exposure to B. t. H-14, mortality increased by as much as 90 % of the LD-50 level. This was attributed to an increased gut retention time which resulted from feeding inhibition. Under these conditions more of the toxicant was digested. Gaugler and Molloy point out that the effects of adding high concentrations of suspended solids and B. t. H-14 simultaneously were not determined.

In the preliminary studies of the influence of suspended solids on B. t. H-14 efficacy the suspended particulates were introduced during the acclimation period

and were maintained until after the B. t. H-14 exposure period. As a result, inhibition of feeding occurred which reduced the efficacy of the endotoxin. However if the suspended solids had been present after exposure to the bacterium as well, the drop in efficacy would not have been as great. The larvae would have continued to have a reduced feeding rate. As a result, while the larvae may have ingested a small amount of toxin, the longer gut retention time may have allowed a greater proportion of toxin to be activated. This problem was addressed in the later bioassay studies when suspended solids were present throughout the trials.

The limited loss of efficacy that occurred at 10 ppm suspended solids was not significantly different from 90% mortality without bentonite. However the almost total loss in efficacy occurring at the other two concentrations, 55 and 505 ppm, agrees well with the results of Gaugler and Molloy (1980).

Influence of B. t. H-14 Exposure Time on Efficacy of B. t. H-14

Frommer et al. (1980) showed that susceptibility of S. vittatum larvae to B. t. H-14 increased as exposure time increased up to a maximum of 60 min. The results of the present study do not agree well with Frommer's findings. The 30 min exposure period was significantly more effective than the 15 min exposure in only two conditions: at 12 C and 10 ppm suspended solids. Mortality after the 15 and 30

min B. t. H-14 exposure periods was not significantly different at 22 C and 5 C because of the larval feeding behavior at these temperatures. At 22 C, when feeding rate is maximal, most of the larvae ingested a lethal dose of the endotoxin before 15 min. At 5 C larvae feed more slowly and most failed to ingest a lethal dose even after 30 min.

Mortality after the two B. t. H-14 exposure times was not significantly different at 55 ppm and 505 ppm because of the high degree of feeding inhibition. As a result less toxicant was ingested even at 30 min exposures.

Toxicity Studies

As sediment load increases, LC-50 and LC-90 values of B. t. H-14 increase (Figure 8 and 9). Thus as sediment load increases efficacy of B. t. H-14 deceases.

The lower (LC-10) and upper (LC-90) limits of dose mortality curves show more variability than do the middle of the curve (LC-50). This makes estimates of LC-50 values more precise than LC-90 values. Therefore LC-50 values are used to test whether there is a significant linear regression of toxicity of B. t. H-14 on suspended solid loads.

There is a major problem when using B. t. H-14 for black fly control in rivers with high sediment loads. Control of black fly larvae is generally aimed to achieve ninety percent mortality of the larvae. At suspended solid loads of 55 ppm these results indicate approximately 35 ppm

of B. t. H-14 would be needed to kill 90% of the larvae. This value is far greater than that needed for other pesticides (Sebastien 1986), which would make B. t. H-14 more costly to use than other pesticides. The 95% fiducial limits of the LC-90 values also vary widely. This would cause results of different field trials under these same conditions to be highly variable. Fredeen (1964) reported that the suspended solid concentration of the Saskatchewan River, where control of black fly larvae is needed, annually averages as much as 400 ppm and is sometimes as high as 3000 ppm. It is unlikely that B. t. H-14 applications under these conditions will be successful.

Efficacy studies such as the present study should be carried out on target species of blackflies including S. arcticum and S. luggeri. These species may react differently to high levels of suspended solids than does S. vittatum.

Feeding Studies

Other authors have attempted ingestion rate studies similar to the present study. Mulla and Lacey (1976) introduced dye particles into streams to mark the larval guts. Larvae were then removed after various exposure times, fixed in alcohol, and the progress of the dye plug measured. These authors also noted that at times some larvae did not ingest the dye particles. They attributed this to non-uniform distribution of dye suspension in the

running water or to absence of feeding. However, Mulla and Lacey (1976) did not indicate what proportion of the larvae did not ingest the dye particles. Mulla and Lacey (1976) did not attempt to measure greater than 100% gut clearance. Clearance was deemed to have occurred once all the larvae in the sample were free of dye. They did not differentiate between larvae that had voided the gut marker and ones that had not ingested it. As a result actual gut clearance rates may have been longer than these authors realized.

Lacey and Lacey (1983) found that, in a similar study of S. fulvinotum Cerqueira and Mello, the only individuals which failed to ingest the marker were pharate pupae. This is remarkable because the time the larvae were exposed to the dye (gut marker) was only 5-10 sec. S. fulvinotum may filter feed continuously. S. fulvinotum larvae spent considerably more time with their fans open than was reported by Kurtak (1973) and Craig and Chance (1982) for S. vittatum. Also S. fulvinotum was only occasionally seen to flick its fans rapidly as has been reported for S. vittatum (Kurtak 1973, Craig and Chance 1982). Craig and Chance (1982) hypothesized that larvae with less frequent mouthpart movements may filter more efficiently than those which clean their fans more often because the latter have their fans adducted (not exposed to food) for a greater period of time. Lacey and Lacey (1983) suggested that the greater feeding efficiency of S. fulvinotum is an adaptation to the

comparatively low seston levels of the waters that they inhabit (0.37 to 2.01 ppm).

Kurtak (1978) measured the time needed for black fly larvae to fill their guts with dye. He did not notice whether some larvae failed to feed for periods of time. Fredeen (1964) also measured the time needed to fill larval black fly guts. He noticed that some larvae feed slower than others but attributed it to local differences in current velocity. Wotton (1978) and Ladle et al (1972) also conducted ingestion rate studies on black fly larvae. In common with the present study they used charcoal as a marker. However, these authors made no reference to larvae not ingesting the gut marker.

Feeding studies reported here show that ingestion rate remains constant as sediment load increases within the sediment loads tested, except at 25 ppm. At 25 ppm suspended solids ingestion rate was significantly greater than at all other sediment loads. It is possible S. vittatum has an optimum ingestion rate that occurs around 25 ppm suspended solids. However, the ingestion rate of the trial using the 3 hour second acclimation period at 25 ppm suspended solids was not significantly different from all the other sediment loads. It is doubtful that one hour of acclimation could make that much difference in the ingestion rate. Since the ingestion rate at 25 ppm suspended solids was not replicated, as were all the other sediment loads,

its results should be considered with caution. However, if some unknown factor were influencing the ingestion rate of the larvae in this trial (25 ppm 4 hr 2nd acclimation period) the factor would be expected to reduce ingestion rate rather than enhance it, assuming the the conditions within the bioassay beakers did not stress the larvae to begin with.

The results of the ingestion rate studies agree with those in the literature. Gaugler and Molloy (1980) found that suspended solids at levels of 50 ppm caused inhibition of filter feeding behavior. However, the feeding behavior of black fly larvae consists of three components, filter feeding, ingestion, and digestion (Hart and Latta 1986). Filter feeding is the filtering of particulate matter out of the water with the fans. The rate depends on the proportion of time the fans are fully extended and actively filtering, as well as other factors such as current velocity and suspended solid load. The second component of feeding behavior is the ingestion rate, namely the rate at which particles are introduced into the gut from the fans. The third component is digestion which was not examined in this study. Gut clearance experiments in this study measured ingestion rate only. Gaugler and Molloy (1980) measured filter feeding rate only. They found that larvae actively filtered particles from the water a smaller proportion of the time at higher sediment loads as compared to low sediment loads. They pointed out that inhibition resulted

in reduced feeding, but not an absolute cessation of ingestion. Within the range of suspended particle loads tested in the present study (10-55 ppm, except 25 ppm) black fly larvae ingestion rate remained relatively constant as suspended solid loads increased. Gaugler and Molloy (1980) pointed out that what changes is the filtering rate.

Hart and Latta (1986) conducted a study in which they measured both the filter feeding rate and the ingestion rate. These authors measured flick rate (the frequency with which larvae open and close their cephalic fans) and used this to measure filter feeding rate. They reported that ingestion rate rose asymptotically with increasing food availability, leveling off at a concentration of about 100 ppm. Flick rate rose with increasing food concentration even after the ingestion rate had leveled off. The number of particles ingested per flick failed to increase with increasing food concentration. Hart and Lata's (1986) study was different from Gaugler and Molloy's (1980) and the present study in two ways. Firstly, Hart and Latta (1986) used an overwintering population of Prosimulium rather than S. vittatum, and they ran their tests at about 6 C. Secondly, they used a suspended particle (pollen) that is extremely nutritious. Feeding rates of insects and other animals are affected by the quality of their foods (Browne 1975, Cammen 1980). Hart and Latta (1986) argued that the use of pollen would be more representative of field

conditions. However, suspended solids in lotic waters are usually composed largely of inert non-nutritious material (Wotton 1978). The suspended solids used by Gaugler and Molloy (1980) and the present study are probably more representative of river suspended solids than is pollen. Hart and Latta (1986) showed that the black fly larvae they used had a constant ingestion rate only at suspended solid loads greater than 100 ppm. Simulium vittatum larvae ingestion rate remained constant, in the present study, at suspended solid levels of 10-55 ppm, except 25 ppm. The greater nutrient value offered by pollen may stimulate black fly larvae to increase their ingestion rate. Gaugler and Molloy (1980) found that filter feeding was inhibited at suspended solid levels above 50 ppm. Hart and Latta (1986) found no evidence of filter feeding inhibition even at levels over 100 ppm. Again this may be explained by the different nutrient value of the suspended solids used, and/or the different species and temperature used by Hart and Latta.

It is possible that black fly larvae require a consistent ingestion rate over a range of suspended solid loads. Black fly larvae have a very low assimilation rate (Wotton 1978). As a result they must process a large amount of material to obtain adequate nutrients. However, too rapid an ingestion rate may not allow enough time to digest fully the small amount of nutrients present in most river conditions.

At low suspended solid loads black fly larvae must have their fans extended and filtering a large proportion of the time to attain the appropriate ingestion rate and thus suitable nutrition. At high suspended solid loads the larvae may trap a greater amount of suspended solids on the fans per unit time. If ingestion rate stays constant, the larvae would simply reduce the time spent filtering.

This theory would adequately explain all the findings in the present study. In the preliminary and bioassay studies increasing sediment loads drastically reduced efficacy of B. t. H-14. If larvae were filtering at a much reduced rate in the presence of B. t. H-14 and high suspended solids then they would trap a smaller amount of the protoxin in their fans, and would ingest less of it.

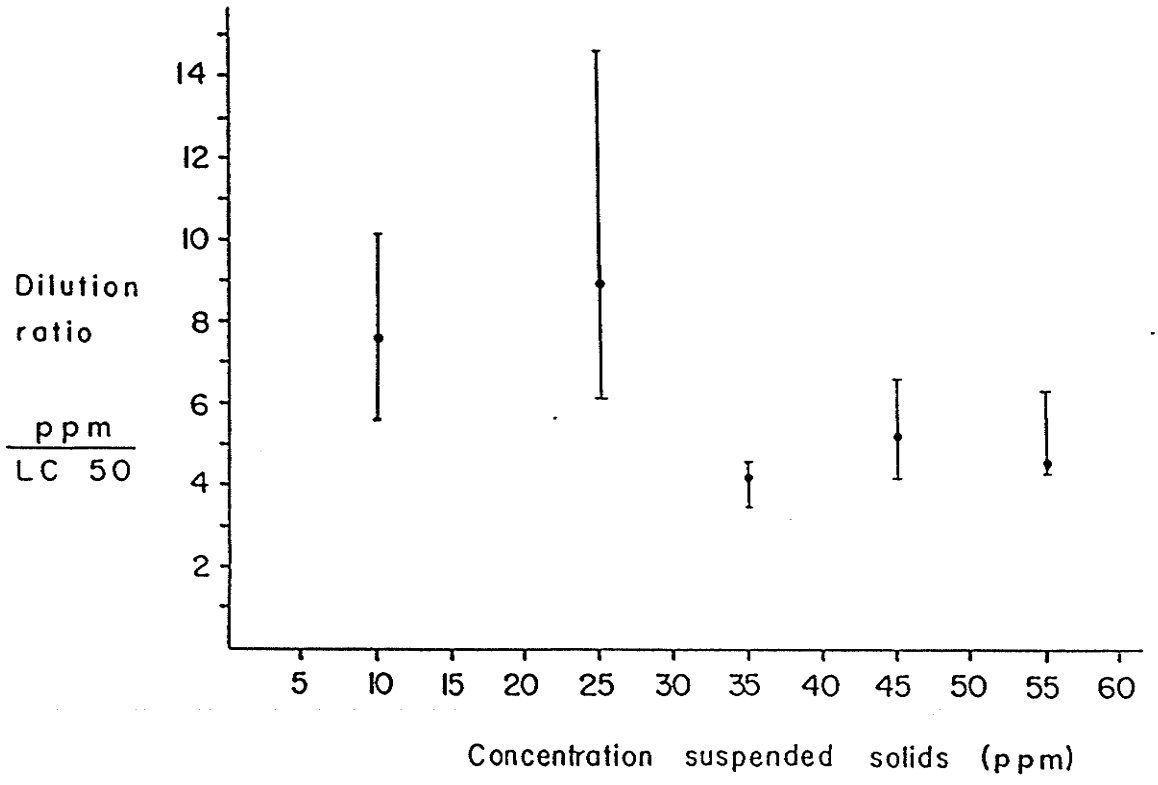
It may be argued that this drop in efficacy of B. t. H-14 at high sediment loads was caused by the "competition" of suspended particles with the B. t. H-14 particles. Black fly larvae filter feed indiscriminately with respect to the type of particles found in the water. Contents of larval guts generally reflect the contents of the water (Kurtak 1979, Wotton 1977, Thompson 1987). If simple competition were at work here, increasing the quantity of suspended solids would necessitate a corresponding increase in the number of B. t. H-14 particles to insure the larvae ingested the same minimum amount of toxin that is needed to be lethal. If this was in fact what was happening the ratio of the

suspended solid load over the LC-50 would remain constant as the suspended solid load increased. For example at 10 ppm suspended solids, the LC-50 is 1.32 ppm B. t. H-14. The ratio is 7.58. Increase the suspended solid load by 10 and you would also have to increase the B. t. H-14 concentration by 10 to get similar mortality. The actual suspended solid/LC-50 ratios are given in Figure 13 along with their 95 % confidence limits. The slope of the regression line of dilution ratio on concentration suspended solids is not significantly different from zero ($P \geq .05$). Therefore the results of the LC-50 determinations can be explained by simple competition alone.

While the results of the present study are adequately explained by the theory that filter feeding rate changes while ingestion rate stays constant (over the range of conditions tested), some of Gaugler and Molloy's (1980) results are not. These authors found that efficacy of B. t. H-14 could be increased 90% by the addition of high levels of a particular suspended solid (Tetra-min) after B. t. H-14 exposure. They attributed this to feeding inhibition (decrease in filtering rate) which led to increased gut retention time and increased digestion of the toxin. These authors did not actually measure ingestion rate (gut retention time). While filter feeding rate was reduced under the conditions described above, ingestion rate was constant in the present study.

It is interesting that Gaugler and Molloy's (1980)

Figure 13. The calculated suspended solid load/LC-50 ratio of B. t. H-14 against S. vittatum larvae versus concentration suspended solids. Confidence limits (95 %) are indicated.


Dilution ratio
ppm
LC 50
14
12
10
8
6
4
2
5
10
15
20
25
30
35
40
45
50
55
60
Concentration suspended solids (ppm)

results, increased efficacy of B. t. H-14 following feeding inhibition resulting from exposure to high sediment loads, varied according to the nutrient value of the suspended particle used. Tetra-min was more inhibitory than was chalk. In fact the addition of chalk after B. t. H-14 exposure did not cause a significant increase in mortality. Generally in Gaugler and Molloy's (1980) results the more nutritious a suspended solid appears the more successful the particle was at inhibiting feeding in black fly larvae. These authors reported that Tetra-min, rabbit chow, and dog food are better inhibitors than are clay, chalk, and charcoal.

It would obviously be advantageous for the larvae to be able to decrease ingestion rate (increase gut retention time) in the presence of a suspended solid with a high nutrient value, allowing greater digestion and absorption of the available nutrients. In the presence of a suspended solid with little nutrient value it may be better to decrease gut retention time (increase ingestion rate) in order to increase the quantity of material ingested. This strategy would require a digestive system that could digest all or most of the available nutrients in the gut during the time in the gut.

In the present study the nutrient level of the suspended solid load decreased (50% Tetra-min at 10 ppm) as suspended solid loads increased (9% Tetra-min at 55 ppm).

According to the above reasoning, gut retention times would be expected to be longer at the lower (more nutritious) suspended solid loads. However, there were no statistically significant differences in gut retention times between suspended solid loads of, 10, 35, or 55. In fact retention times tend to be longer in the presence of less nutritious but more numerous suspended solids.

The opposite theory to the one above is that black fly larvae have a rapid ingestion rate (short gut retention time) in the presence of a nutritious suspended solid. In this case the larvae would be trying to assimilate as much as possible. In the presence of a poorly nutritious suspended solid the larvae would decrease the ingestion rate (long gut retention time) to insure that all the available nutrients are digested and absorbed. For this hypothesis to be correct efficiency of digestion must be extremely limited, allowing only a small proportion of the available nutrients to be digested per unit time. Hart and Latta's (1986) work seems to support this theory. Ingestion rate increased with the increasing concentration of a very nutritious suspended solid up to 100 ppm, and then leveled off. The authors indicated this was because the limit of the larval filtering efficiency had been reached.

As mentioned above, it is known that the feeding rates of insects and other animals are influenced by the quality of their food (Browne 1975, Cammen 1980). Unfortunately there has been no systematic study of the effect (if any) of

nutritional value of suspended solids on rates of ingestion by black fly larvae.

CONCLUSION

This study has shown that efficacy of B. t. H-14 is reduced at lower temperatures and that the reduced efficacy is due to reduced feeding rates at lower temperatures. Work needs to be done to partition the effects of temperature into components of filtration rates and ingestion rates.

This study has also shown, in general, that longer B. t. H-14 exposure times are more efficacious than short ones. However, there are some conditions under which this does not apply: at high suspended solid loads which inhibit feeding of S. vittatum, at water temperatures either too cold for the larvae to ingest a lethal dose of the toxin even at prolonged exposures, and at water temperatures that are warm enough to allow larvae to ingest a lethal dose even under short exposure periods.

Thirdly this study shows that increased suspended solid loads decrease the efficacy of B. t. H-14. However, the mechanism causing this effect is still unclear. In the past it was believed that a simple decrease in feeding was responsible for the reduced efficacy of B. t. H-14. This study combined with the earlier work cited suggests that feeding behavior of black fly larvae consists of two components, filter feeding rate and ingestion rate.

More is needed to ascertain the effects of: nutritional value of suspended particles, efficiency of digestion, concentration of suspended particles, and how these three factors interact on the overall feeding behavior of black fly larvae.

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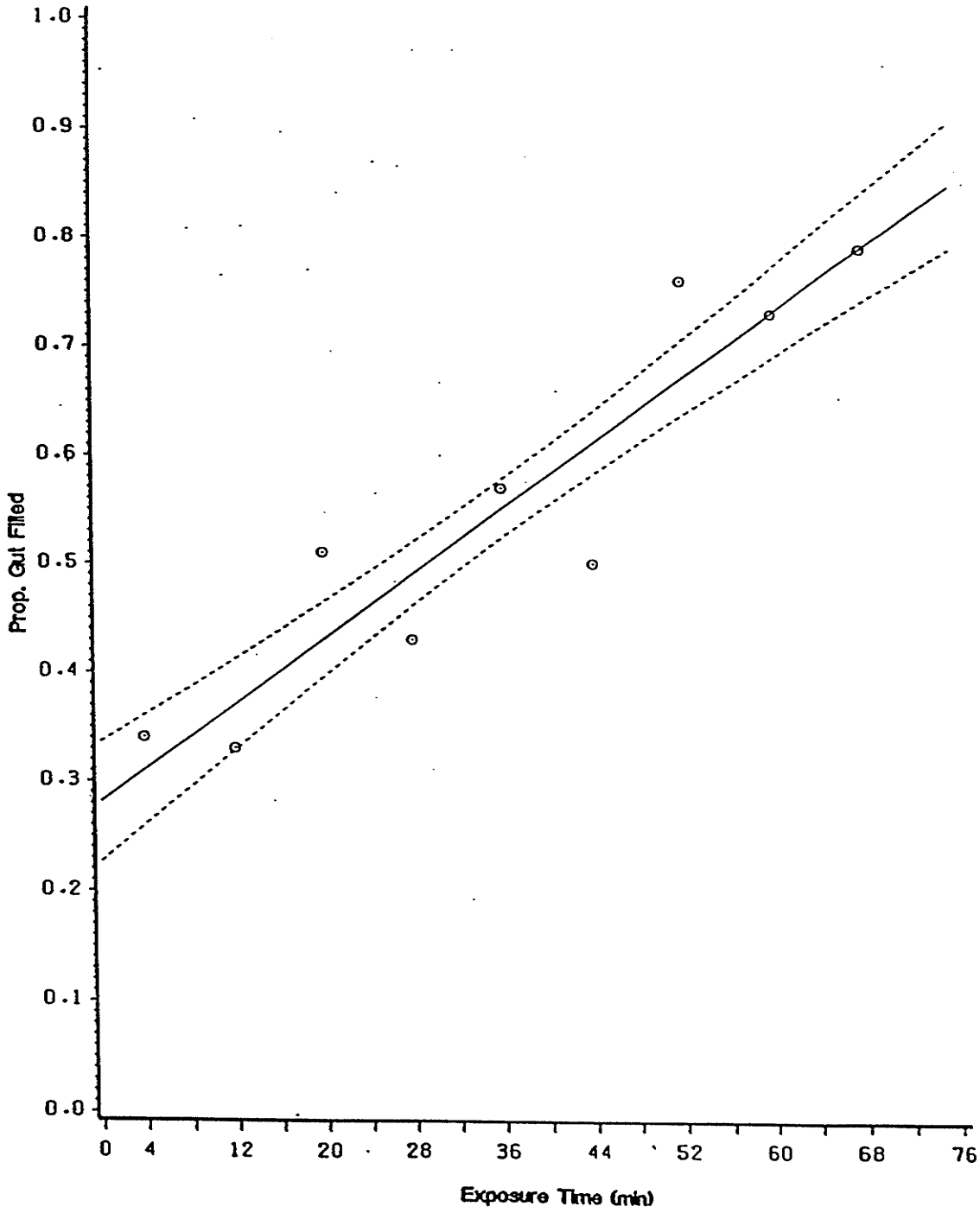
APPENDIX 1

Figure 14. The proportion gut filled of S. vittatum larvae after 4-76 min exposure to 10 ppm suspended solids. Replicate number 1. Circles represent mean proportion gut filled at each exposure period. Solid line = regression line and the dotted lines = 95 % confidence limits for the mean predicted values.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Exposure Time (min)

Figure 15. The proportion gut filled of S. vittatum larvae after 4-76 min exposure to 10 ppm suspended solids. Replicate number 2. Circles represent mean proportion gut filled at each exposure period. Solid line = regression line and the dotted lines = 95 % confidence limits for the mean predicted values.

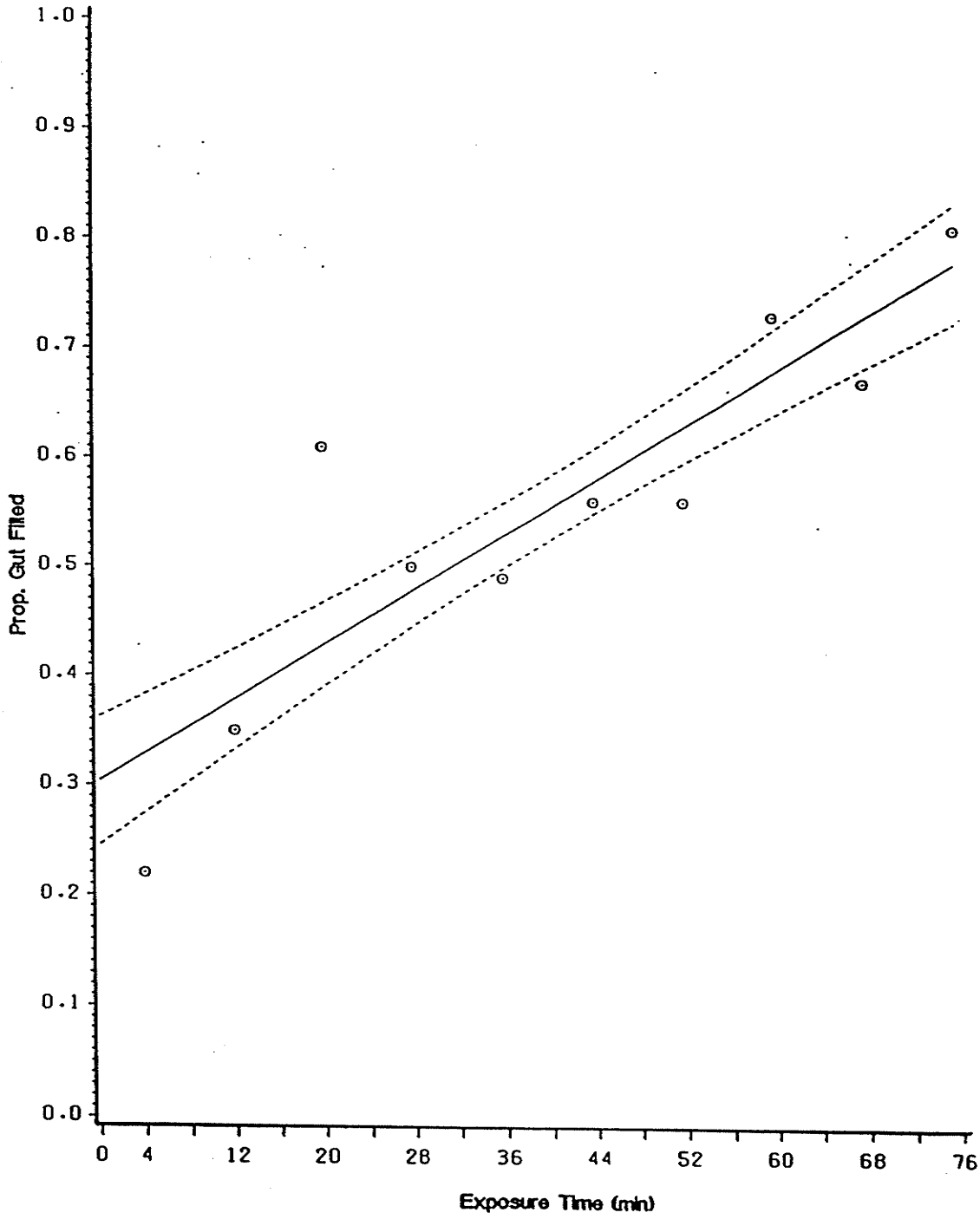
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Figure 16. The proportion gut filled of S. vittatum larvae after 4-76 min exposure to 25 ppm suspended solids. Replicate number 1 (4 hr second acclimation period). Circles represent mean proportion gut filled at each exposure period. Solid line = regression line and the dotted lines = 95 % confidence limits for the mean predicted values.

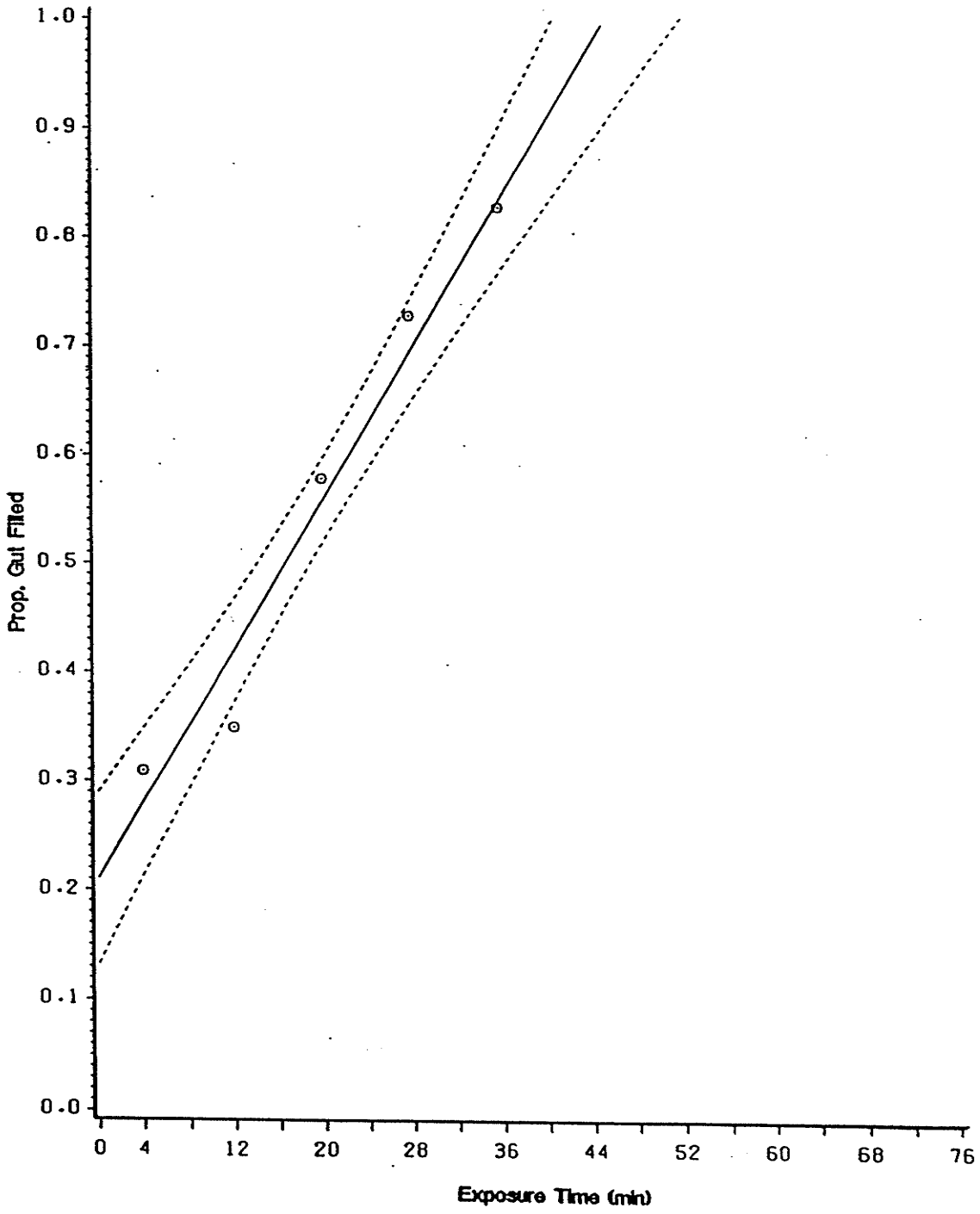
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Figure 17. The proportion gut filled of S. vittatum larvae after 4-76 min exposure to 25 ppm suspended solids. Replicate number 2 (3 hr second acclimation period). Circles represent mean proportion gut filled at each exposure period. Solid line = regression line and the dotted lines = 95 % confidence limits for the mean predicted values.

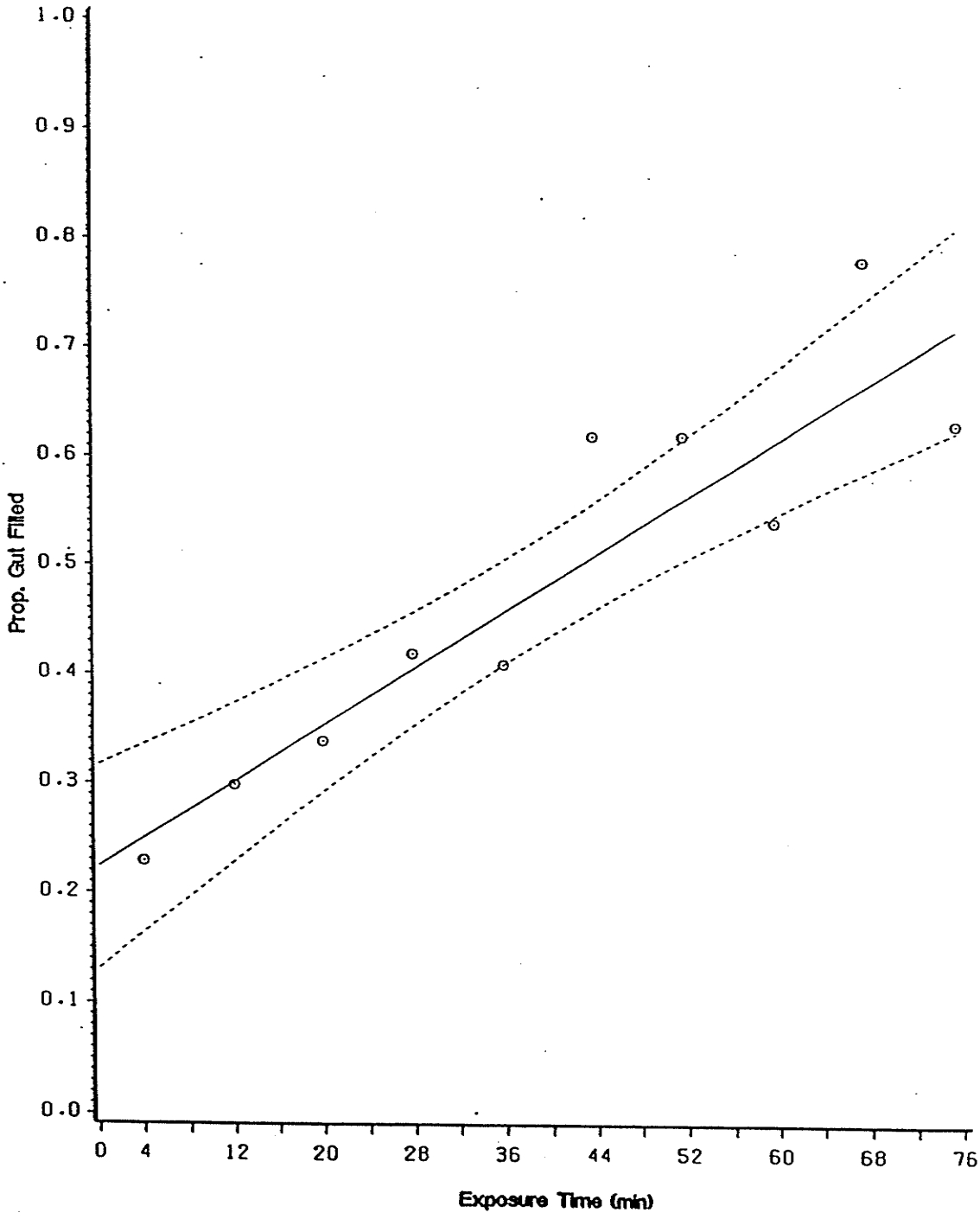
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Figure 18. The proportion gut filled of S. vittatum larvae after 4-76 min exposure to 25 ppm suspended solids. Replicate number 3 (13 hr second acclimation period). Circles represent mean proportion gut filled at each exposure period. Solid line = regression line and the dotted lines = 95 % confidence limits for the mean predicted values.

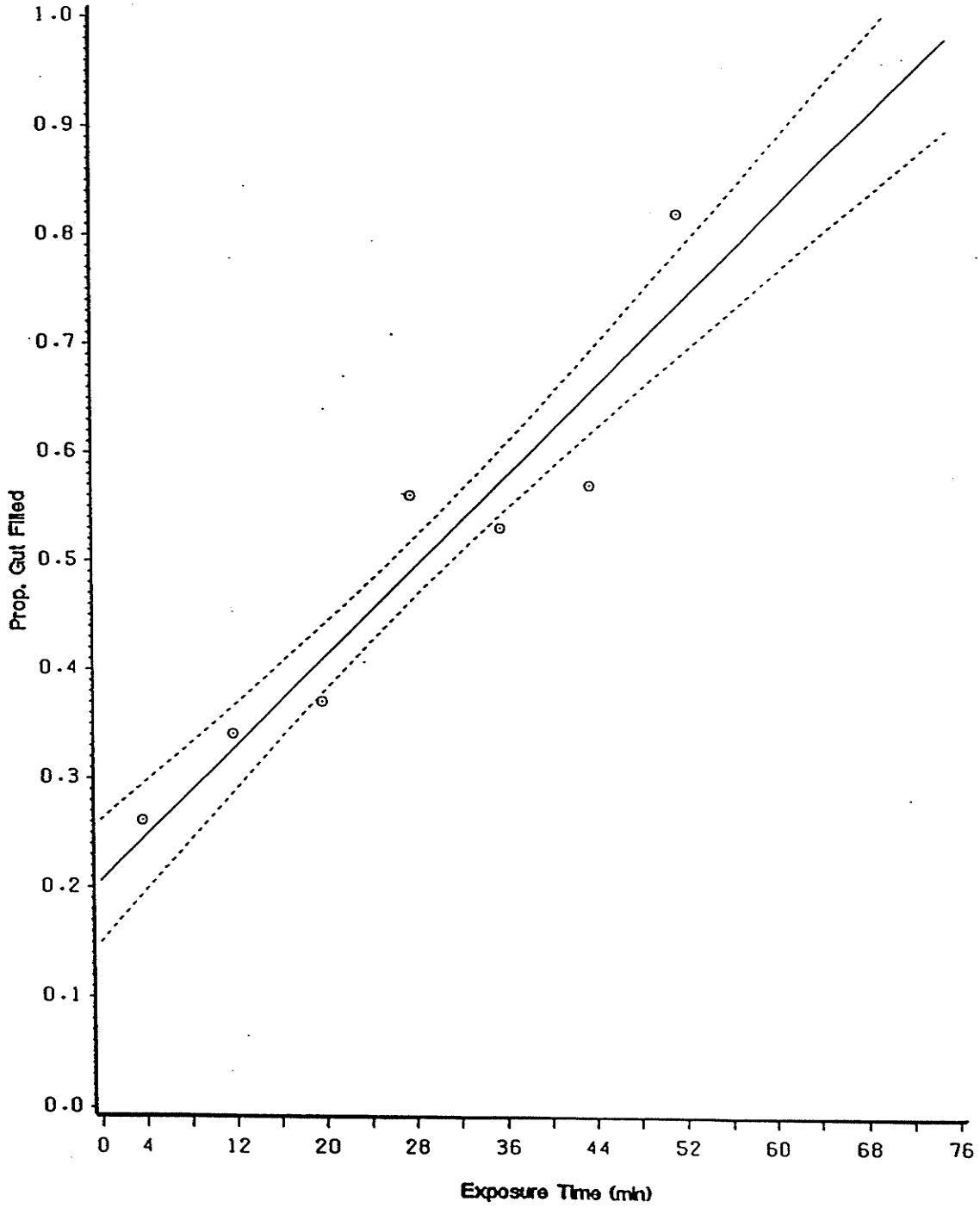
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Figure 19. The proportion gut filled of S. vittatum larvae after 4-76 min exposure to 35 ppm suspended solids. Replicate number 1. Circles represent mean proportion gut filled at each exposure period. Solid line = regression line and the dotted lines = 95 % confidence limits for the mean predicted values.

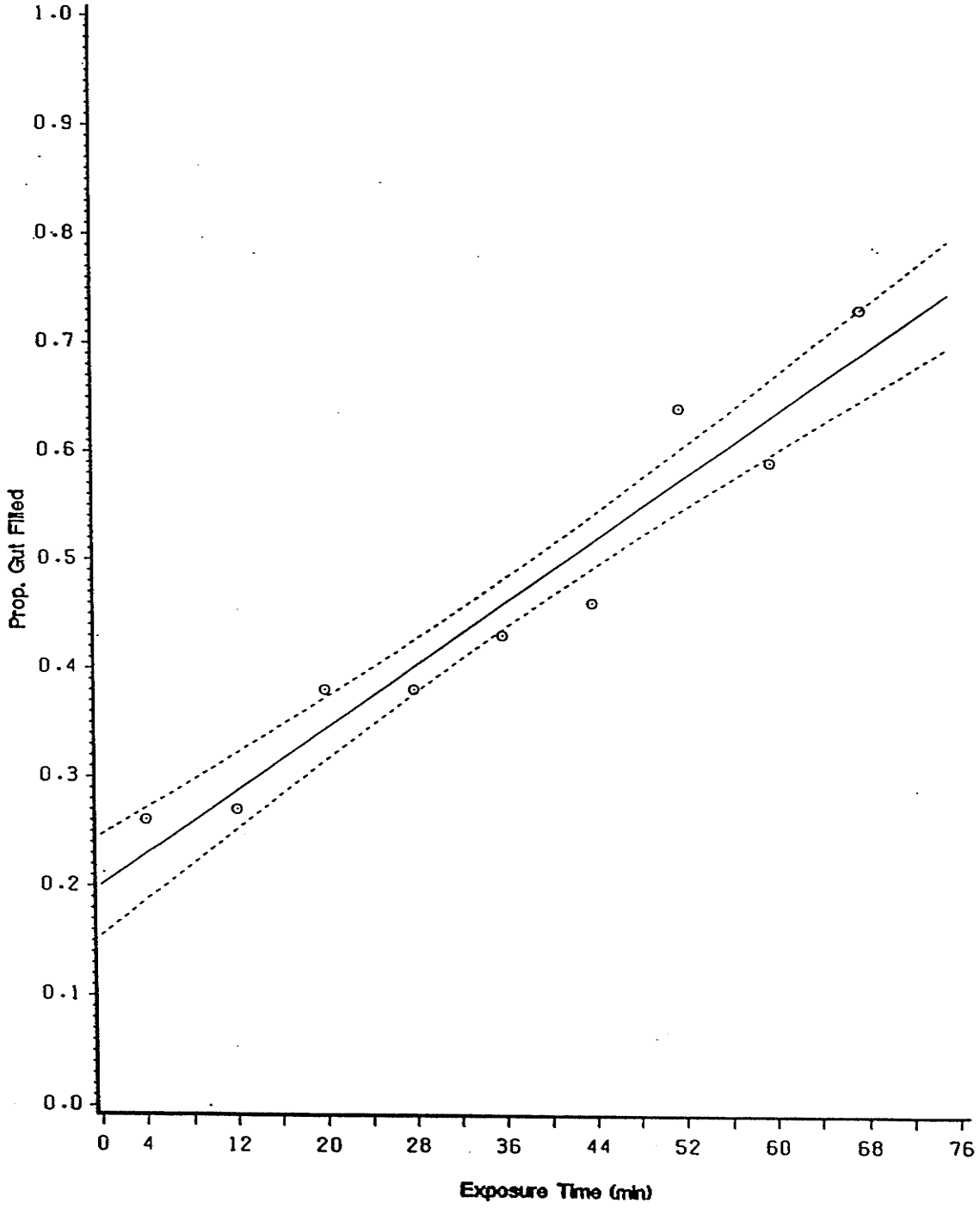
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Figure 20. The proportion gut filled of S. vittatum larvae after 4-76 min exposure to 35 ppm suspended solids. Replicate number 2. Circles represent mean proportion gut filled at each exposure period. Solid line = regression line and the dotted lines = 95 % confidence limits for the mean predicted values.

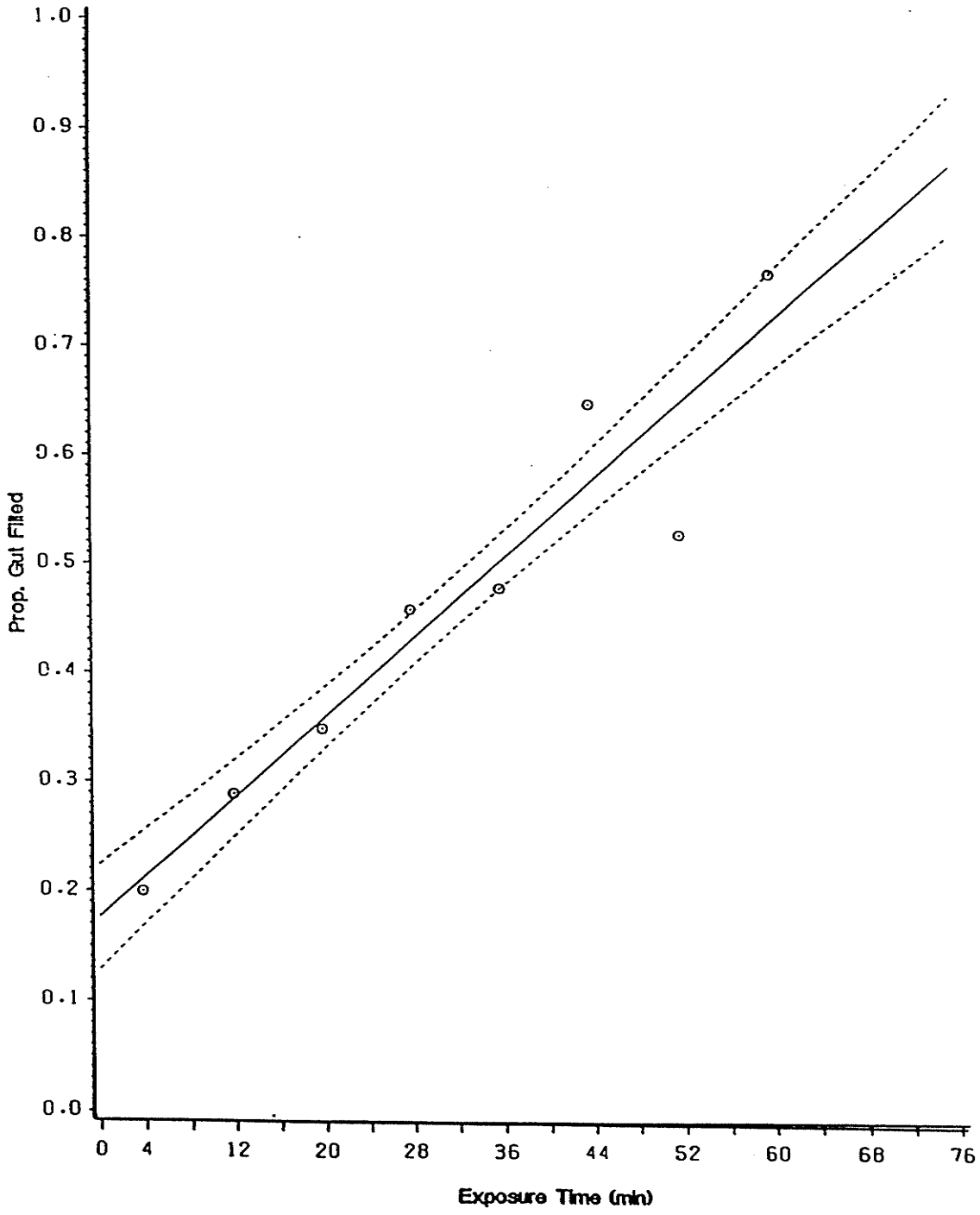
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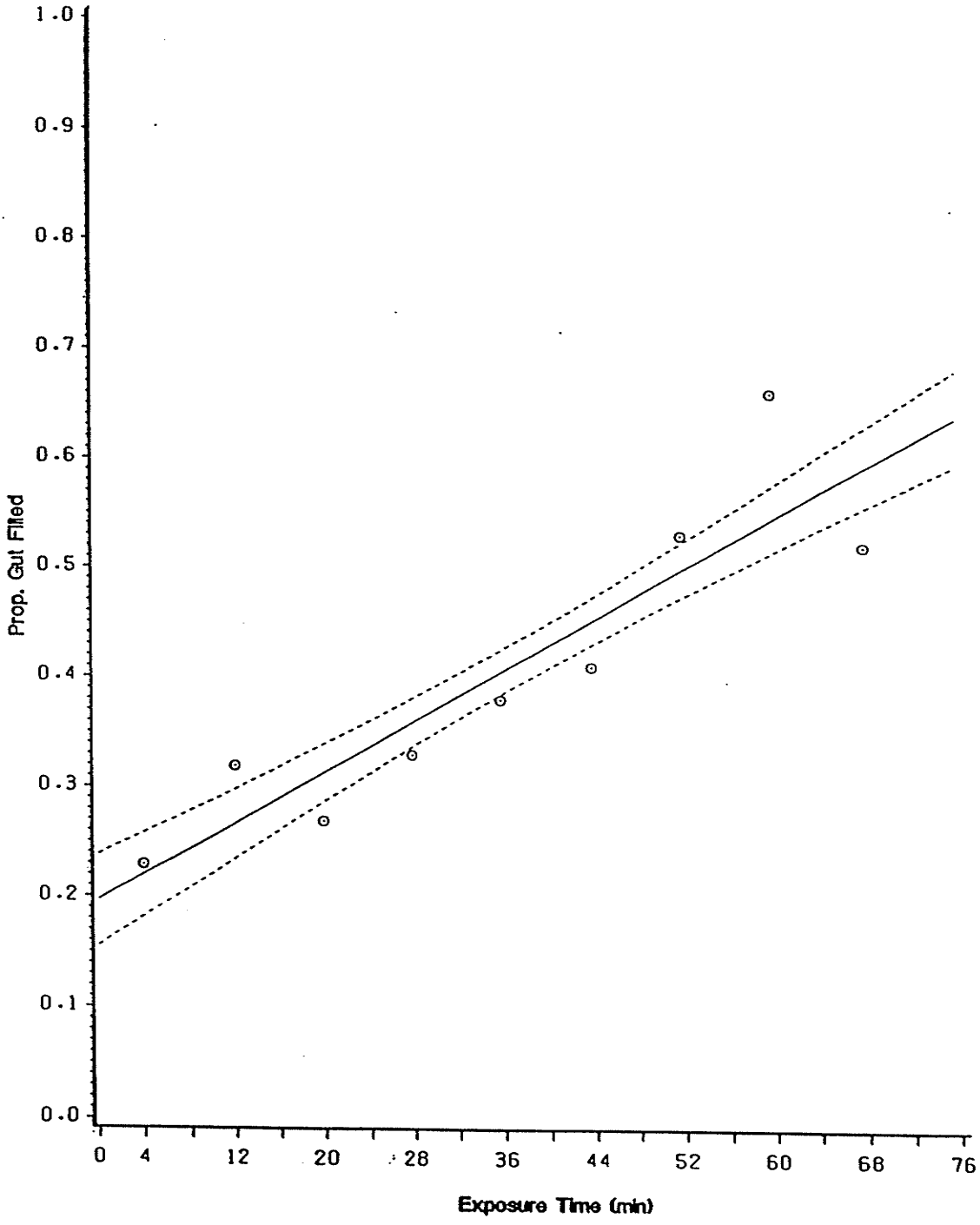
Figure 21. The proportion gut filled of S. vittatum larvae after 4-76 min exposure to 55 ppm suspended solids. Replicate number 1. Circles represent mean proportion gut filled at each exposure period. Solid line = regression line and the dotted lines = 95 % confidence limits for the mean predicted values.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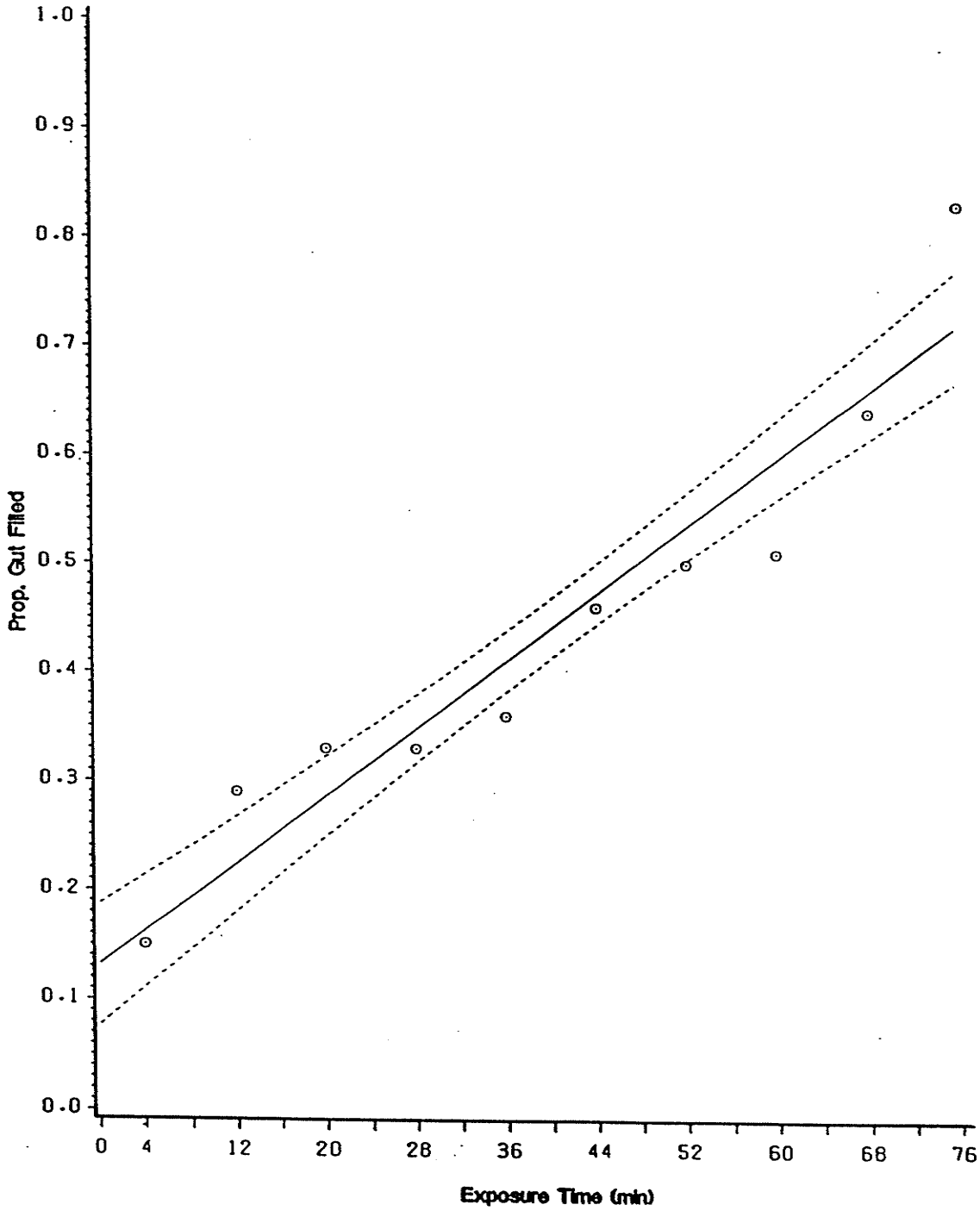
Exposure Time (min)

Figure 22. The proportion gut filled of S. vittatum larvae after 4-76 min exposure to 55 ppm suspended solids. Replicate number 2. Circles represent mean proportion gut filled at each exposure period. Solid line = regression line and the dotted lines = 95 % confidence limits for the mean predicted values.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