A CHIRONOMID (DIPTERA: CHIRONOMIDAE) MIDGE POPULATION STUDY AND LABORATORY EVALUATION OF LARVICIDES AGAINST MIDGES INHABITING THE LAGOON OF VENICE, ITALY[1]

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ABSTRACT. Chironomid larval densities in the saltwater lagoon of Venice, Italy, were assessed in the spring of 1984. Four organophosphates; chlorpyrifos, temephos, fenthion and fenitrothion, and three pyrethroids; cypermethrin, permethrin and deltamethrin, were tested in the laboratory against field-collected larvae. Three industrial formulations of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) were also tested as midge larvicides.

Only *Chironomus salinarius* occurred in the benthic samples taken from different sections of the lagoon. The densities of this species ranged from 0 to 38,976 larvae/m^2. The highest mean larval density of 15,673/m^2 was encountered in a section of the lagoon adjacent to Venice airport and receiving large quantities of raw sewage. The lowest mean density (< 1 larva/m^2) existed in another area of the lagoon receiving discharge from chemical industry.

Cypermethrin and permethrin were 21-233X more active against the larvae than the four organophosphates. Chlorpyrifos was the most active organophosphate. Formulations of *B.t.i.* were economically ineffective against the larvae.

INTRODUCTION

Massive swarms of chironomid midges (Diptera: Chironomidae) emanating from lentic and lotic habitats situated amid urbanized areas are a serious nuisance to man. In many parts of the world, occasional or regular control efforts are directed against the midge larvae or adults to reduce their populations. A description of the nature of nuisance and economic problems caused by midges and a review of chironomid control are previously available (Ali 1980). The association of human allergic reactions, such as asthma and rhinitis, with seasonal midge emergence has also been reported (Cranston et al. 1981), and it is believed that chironomids are potentially a worldwide cause of allergy (Cranston et al. 1983).

Most available literature on chironomid control deals predominantly with freshwater species of midges although chironomids are also a problem in some saltwater habitats (Ali and Majori 1984). In one saltwater situation, in and around the lagoon and waterways of Venice, northern Italy, annoyance and economic loss (mostly tourism) due to chironomid swarms have greatly increased in the past 3–4 years (M. Tanda Dall'asta, personal communication). In this historically important tourist area of the world, there is concern for the potential of airplanes skidding over periodic massive accumulations of dead midges on the runways. Also, there is a severe nuisance of adult swarms to passengers and crews of cargo vessels and other boats.

Reported here is a study conducted in the spring of 1984 to assess chironomid larval densities in the lagoon of Venice. Laboratory tests were made on susceptibility of the midge larvae to several organophosphorus and pyrethroid insecticides and to three industrial formulations of the biocide, *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*), during 1983 and 1984.

MATERIALS AND METHODS

STUDY AREA. The lagoon is located along the Mediterranean (Adriatic) Sea in the southeast portion of northern Italy at approximately 45° 26′ N latitutde and 12° 35′ E longitude. Separating the lagoon from the sea are four narrow strips of land (see Fig. 1). The lagoon is approximately 48 km long and 14 km wide at its widest point and covers 55,000 ha. It receives water primarily from the Adriatic Sea through three major openings which range from 0.5 to 0.8 km in width. A small river, Dese, and a few small channels (Orellino, Brenta, and others) bring very small amounts of freshwater to the lagoon from surrounding land areas. Water depth in the lagoon ranges from a few cm to almost 1.5 m; the overall depth may increase daily up to one meter or more depending upon the tidal flux. The lagoon contains an extensive network of 3–15 m deep canals and channels periodically dredged to maintain the water depth. They are lined with thousands of

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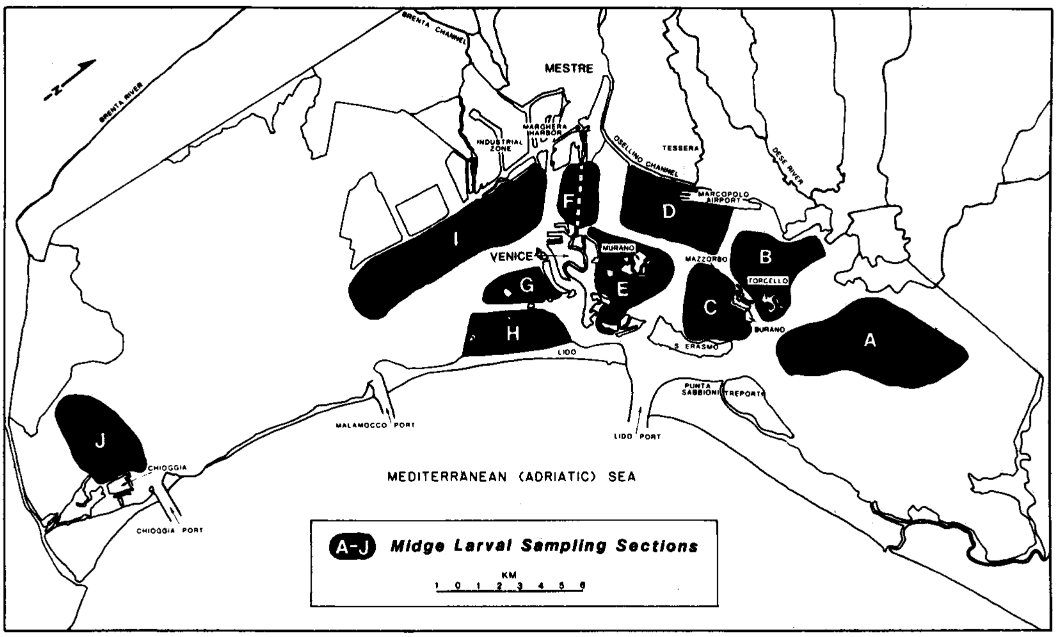

Fig. 1. The lagoon of Venice, Italy, showing the main islands, surrounding land areas, and the midge larval sampling sections A to J in the lagoon.

permanent wooden markers to facilitate the movement of cargo and passenger vessels and other boats.

The city of Venice is located on an island of approximately 600 ha in the middle of the lagoon and is connected to the mainland city of Mestre by a 4 km long bridge. The Venice airport is located on the mainland ca. 6 km north of Venice, and the harbor (Marghera Harbor) is situated adjacent to an industrial area near Mestre (Fig. 1). There are a number of other densely populated islands in the lagoon such as Murano, Burano, Torcello, Mazzorbo and S. Erasmo which range from < 25 to ca. 200 ha. Several other large and small cities and towns are located on the peripheral land areas; the strips of land partitioning the lagoon and the sea also support a number of towns. The northeast and the western portions of the lagoon are very shallow and are predominated by exposed marshland under low water conditions. The water is turbid in most areas but in the shallow sections the bottom is visible. The pH ranges from 8 to 11 and salinity from 15 to 38 g NaCl/liter. The lagoon receives large amounts of partially depurated industrial discharge along several km of the mainland mostly near Marghera harbor. There are numerous sources of domestic raw sewage in the lagoon as the cities of Venice and Lido, and other inhabited islands do not have any functional sewage treatment facilities. The lagoon also receives large quantities of raw sewage from the city of Mestre. This sewage is carried by the Osellino channel and continously emptied into the lagoon near the Venice airport. Thus, the nutrient loading in the lagoon is heavy. The details of physico-chemical and biological parameters in the lagoon were previously described (Anonymous 1977, 1984). The sediment composition of the lagoon bottom varies spatially. Generally, the areas of high input of organic pollutants have soft and thick deposits of decomposing organic matter and mud, while those away from such inputs have sand bottom covered with bivalves and gastropods. Growth of the algae, *Ulva rigida* C. A. and *Enteromorpha* spp., is widespread covering large areas of the lagoon bottom in patches.

Larval sampling. Many shallow areas (< 30 cm) of the lagoon supporting dense growths of algae were inaccessible by boat for midge larval sampling. Also, it was impossible to collect larval samples from all areas of the lagoon in the available time because of the very large size of the habitat. However, 10 large sections of the lagoon were selected for sampling and are marked as A to J on Fig. 1. The selection of these sections mostly depended upon their proximity to midge complaint areas, or their situation in relation to significant sewage input or industrial wastewater discharge into the lagoon. The sampling sections ranged from ca. 5 to 35 km^2. Prior to collecting samples, an approximate grid on each section was created by utilizing the channel markers and other visual structures in the area. Thus, depending upon the size of a section, 10 to 55 sampling sites, each at an interval of 0.5–1.0 km in a grid, were established in each section. At each site, one mud sample was collected with a modified Petersen dredge (ca. 22 x 13 x 13cm). Each mud sample was carefully washed through a 315 μm (pore size) net, and the residue of each sample was examined in the laboratory to count and identify chironomid larvae. At each sampling site water temperature and depth were recorded. At some locations dissolved oxygen near the lagoon bottom was also measured.

In sections E and G, a number of channels (ca. 3 m) existed. In these sections, 10 benthic samples were randomly taken from the channels and 10 from the nearby shallow areas (0.7–1m) to compare the density of midge larvae at the two levels of water depth.

LARVAL BIOASSAYS. Four organophosphorus insecticides; chlorpyrifos, temephos, fenthion and fenitrothion, and three pyrethroids; cypermethrin, permethrin and deltamethrin were tested in the laboratory against a mixture of 3rd and 4th instar larvae collected from the lagoon. Technical grade material of each insecticide was utilized to prepare a 1% stock solution in acetone, and serial dilutions in acetone were made as needed. The required amount of a toxicant was added to a disposable paper cup containing 10 larvae in 100 ml of water and 5 g of sterilized sand. Each test consisted of 5–6 triplicate concentrations of a test material plus three untreated checks maintained in the laboratory. Each chemical was tested on three different occasions. The larval mortality was checked after 24 hr and was corrected for mortality in the checks (Abbott 1925). The corrected mortality at different concentrations of a compound was subjected to log-probit regression analysis.

The larval bioassays were also conducted with three industrial formulations of *B.t.i.* These formulations were: Bactimos® (WP, 3500 *Aedes aegypti* [AA] International Units [IU]/mg), Vectobac® (WP, 2000 AA IU/mg) and Teknar™ (FC, 1500 AA IU/mg). The procedures for testing the biocide were previously described (Ali et al. 1981).

RESULTS AND DISCUSSION

Larvae of *Chironomus salinarius* Kieffer were the only chironomids occurring in the samples taken during the period of this investigation. Other chironomid species, *Halocladius* spp. and *Thalassomya* sp., may occasionally occur in negligible numbers in some areas of the lagoon (U. Ferrarese, personal observations). It is evident from Table 1 that *C. salinarius* was present in all sampled sections of the lagoon. However, the larval density varied considerably within a section and between sections. The overall mean larval density in the sampled areas was 5,127/m² and ranged from 0 to 38,976/m² (Table 1). The highest mean density (15,673 larvae/m²) was encountered in section D adjacent to Venice airport and the lowest (< 1 larva/m²) in section I receiving the industrial discharge. The mean density of larvae in a 2–3 km wide area around Venice and Murano was 1437 (section E) and 1983 larvae/m² (section G). The larval infestation in section D was more dense and widespread than in the other sections as indicated by the lowest coefficient of variation (CV) (57%) of larval density among the sections sampled. The overall CV of larval density in the areas sampled amounted to 155% and ranged from 57 to 486% in different sampling sections, thus indicating a wide range of spatial variability of distribution of *C. salinarius* in the lagoon.

A comparison of larval density of *C. salinarius* in 3 m deep channels and in shallow areas (0.7–1.0 m) of the lagoon is shown in Table 2. It is obvious that the channels supported a significantly lower number of midge larvae than the surrounding shallow areas.

Table 3 presents the relative larvicidal activity of organophosphates; chlorpyrifos, temephos, fenthion and fenitrothion, and pyrethroids; cypermethrin, permethrin and deltamethrin against 3rd and 4th instar *C. salinarius*. Among the organophosphates, chlorpyrifos was the

Table 1. Larval density and distribution of *Chironomus salinarius* in different areas of the lagoon of Venice, Italy (April–May, 1984).

Sampling sections[a]	Mean larval density/m² in lagoon bottom mud	Range	CV (%)	n
A	1,729	0–18,720	242	20
B	4,652	40–18,384	105	16
C	1,536	0– 7,392	150	12
D	15,673	432–38,976	57	55
E	1,437	0–10,368	234	15
F	970	288– 2,592	69	10
G	1,983	48– 7,776	109	16
H	1,335	0–10,080	168	36
I	<1	0– 1	486	24
J	156	0– 896	178	11

[a] The location of each sampling section in the lagoon and the approximate area of the lagoon covered by each section are shown in Fig. 1. Water temperature at the sampling sites ranged from 13 to 22°C, water depth 0.5 to 1.8 m, and the dissolved oxygen measurements taken at a few sites ranged from <2 to 9 ppm during the study period.

Table 2. Larval density of *Chironomus salinarius* in shallow areas[a] and deep channels[a] in the lagoon of Venice, Italy (May 1984).

Area (depth)	No. larvae/sample in 10 samples										Mean[b]
Shallow (0.7 – 1 m)	77	103	16	17	26	92	81	18	32	62	52.4
Deep (ca. 3 m)	0	0	0	1	2	1	0	0	0	1	0.5

[a] Samples collected from sections E and G around Venice and Murano (see Fig. 1).
[b] Difference between the means is significant at the 0.01 level of probability (Student's *t*-test).

most active (LC_{90} = 0.0071 ppm) and fenitrothion was the least active with LC_{90} = 0.07 ppm. Chlorpyrifos was 4X, 6X and 10X more active than temephos, fenthion and fenitrothion, respectively. Temephos showed approximately 2X and 3X better activity than fenthion and fenitrothion, respectively. Among the pyrethroids, both cypermethrin and permethrin were highly toxic to the larvae with similar LC_{90} values of 0.00034 and 0.0003 ppm, respectively. Deltamethrin was ca. 13–14X less active than cypermethrin and permethrin. A comparison of activity of the three pyrethroids and the four organosphosphates against the test larvae indicates that cypermethrin and permethrin were 21–233X more active than the organophosphates, and even the least active pyrethroid, deltamethrin, was 2X more active than the most active organophosphate, chlorpyrifos.

The activity of *B.t.i.* formulations, Bactimos, Vectobac and Teknar, are summarized in Table 4. Considering the relative potencies (no. of AA IU/mg) of the three formulations, Vectobac showed slightly better activity against *C. salinarius* larvae than Bactimos; Teknar was the least active. Overall, the three formulations of *B.t.i.* were economically ineffective against *C. salinarius* larvae. Bactimos and Vectobac, respectively, were 101X and 135X less active against the larvae than the least active organophosphate fenitrothion, and that even in double the exposure time (48 hr) than the 24 hr exposure period used for the evaluation of organophosphates.

This study indicated that *C. salinarius* prevails

Table 3. Comparative susceptibility of *Chironomus salinarius*[a] to organophosphate and pyrethroid insecticides in the laborabory.

	24-hr lethal concentration (ppm)				
Insecticide	LC_{50}	C. L.[b]	LC_{90}	C. L.[b]	r^2
		Organophosphates			
Chlorpyrifos	0.00099	0.00083 –0.0012	0.0071	0.0052 –0.0099	0.99
Temephos	0.0040	0.0034 –0.0046	0.027	0.019 –0.35	0.99
Fenthion	0.0078	0.0064 –0.0092	0.046	0.035 –0.057	0.98
Fenitrothion	0.012	0.0093 –0.0147	0.070	0.046 –0.094	0.99
		Pyrethroids			
Cypermethrin	0.000065	0.000056–0.000074	0.00034	0.00025–0.00043	0.85
Permethrin	0.000073	0.000063–0.000083	0.00030	0.00023–0.00037	0.89
Deltamethrin	0.00071	0.00058 –0.00084	0.0043	0.0027 –0.0059	0.78

[a] Late 3rd and early 4th instar field-collected larvae from the lagoon of Venice, Italy, April–May, 1984.
[b] 95% confidence limits.

Table 4. Comparative susceptibility of *Chironomus salinarius*[a] to three industrial formulations of *Bacillus thuringiensis* var. *israelensis* in the laboratory.

		48-hr lethal concentration (ppm)				
Formulations		LC_{50}	C. L.[b]	LC_{90}	C. L.[b]	r^2
Bactimos	WP	4.46	4.18– 4.74	7.07	6.42– 7.72	0.99
Vectobac	WP	5.40	5.06– 5.74	9.46	8.37–10.55	0.99
Teknar	FC	14.63	13.09–16.17	38.26	31.40–45.12	0.98

[a] Late 3rd and early 4th instar field-collected larvae from the lagoon of Venice, Italy, September–October 1983.
[b] 95% confidence limits.

in large numbers in the lagoon. Among the different sections of the lagoon sampled, the area of section D near Venice airport supported the highest numbers of *C. salinarius* larvae, while section I adjacent to the industrial zone and Marghera harbor, contained almost no larvae. The large populations of *C. salinarius* near the airport may be due to high levels of organic pollution because large quantities of raw sewage collected from Mestre is continously discharged into that area of the lagoon. The lagoon areas surrounding Venice, Murano, Burano, and other islands also receive raw sewage from several hundred domestic and business sources. The sewage input from these sources increases considerably during the spring and summer periods due to the influx of thousands of tourists in the area. It is likely that the midge populations also increase in the lagoon areas receiving higher levels of organic pollutants during the tourist season. The lack of midge larvae in section I may be due to some chemical pollutants as the area of section I annually receives 25 mt of heavy metals, 246 mt of fluorides, 9996 mt of organic chloride solvents, 919 mt of mineral oil and 175 mt of chlorine (Anonymous 1977). A list of organic and chemical pollutants and their quantitative distribution in the lagoon were also given in a recent publication (Anonymous 1984).

Previously, no data on the quantitative composition of chironomid populations in the lagoon of Venice were available although some qualitative (taxonomic) work on the midge fauna of Venice and some other northeastern areas of Italy was reported by Marcuzzi (1947, 1948, 1949). Ferrarese (1982) had provided a brief history of chironomid research in Italy. Recently, Ali and Majori (1984) studied the midge problem in two saltwater lakes of Orbetello, central Italy; the lakes were occupied by *C. salinarius* with mean larval density in each lake exceeding 4,000/m^2 and ranging from $<$ 100 to $>$25,000/m^2. The larval densities of *C. salinarius* encountered in the lagoon of Venice were similar or higher than in the Orbetello lakes. Midge densities exceeding 4000 larvae/m^2 were also reported to exist in a variety of man-made and natural freshwater habitats in the U.S.A. producing chironomids at nuisance levels and requiring management (Ali and Mulla 1976, Mulla et al. 1976).

The larval susceptibility of *C. salinarius* populations in the lagoon to chlorpyrifos, temephos, and fenthion is compatible with the previous study of Ali and Majori (1984) on these insecticides against *C. salinarius* in the Orbetello lakes. Also, the activity of cypermethrin and permethrin was comparable with some previous studies (e.g., Ali 1981, Ali et al. 1978) on a number of experimental pyrethroids against freshwater species of pestiferous chironomids of California and Florida. Although the pyrethroids are far superior in activity against chironomids than the organophosphates, the pyrethroids at mosquito and midge larvicidal rates have a much lower index of safety to nontarget invertebrates and fish (Mulla et al. 1978a, 1978b). To reduce *C. salinarius* in the Venice area, chlorpyrifos and temephos, as larvicides, could be used at the time of acute need. The insecticidal treatments should be made only in some parts of the lagoon which support heavy populations of the pest. Such partial area treatments will perhaps facilitate rapid recolonization and population restoration of the reduced nontarget organisms from untreated areas. The deep channels in the lagoon would not require any chemical treatment.

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