Control of chironomid midge larvae in wastewater stabilisation ponds: comparison of five compounds

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Abstract
Chironomid midge larvae are a valuable component of wastewater stabilisation pond (WSP) ecology. However, in high numbers, adult midge swarms can be a nuisance to near-by urban areas. Improving WSP treatment by incorporating aerobic or maturation ponds or by the addition of pre-treatment to reduce organic loading also increases the availability of aerobic sediment (midge larva habitat) in the pond system and the potential for midge nuisance problems. The efficacy of Maldison, an organophosphate traditionally used to control midge larvae in New Zealand WSPs, was compared to Bacillus thuringiensis var. israelensis (Bti), Methoprene, Pyriproxyfen and Diflubenzuron which are all more specific to insects and have fewer adverse environmental effects. Initial laboratory trials established the concentration of each compound required to achieve 95% control of the midge population. During 21-day small-scale trials within the WSP, Bti, Diflubenzuron and Maldison reduced live larvae numbers substantially (80–89%) compared to controls and adult midge emergence was markedly reduced by all compounds (72–96%). Large-scale trials with Bti (Vectobac® WGI powder (1000 mg/L) only caused a slight reduction in midge larvae numbers compared to controls and had little effect on adult emergence, however, Methoprene (Prolink XRG granules) (50 mgAI/L) reduced midge adult emergence by ~ 80% over 25 days and has been used successfully to control several midge nuisance outbreaks.

Keywords Chironomid; control; larvae; midge; methoprene; nuisance; wastewater stabilisation ponds

Introduction
Chironomid midge larvae are commonly found in aerobic bottom sludges of wastewater stabilisation ponds (WSPs) (Cranston, 1995) and are recognised as a valuable component of WSP ecology (Pinder, 1995; Tokeshi, 1995), contributing to treatment by consuming algae, bacteria and detritus (reducing pond TSS and nutrient levels), which are then completely removed from the system when the adult midge emerges from the pond. Midge adults swarm around pond margins at dawn and dusk, where they mate, and females lay eggs back into the pond (Tokeshi, 1995). When present in high numbers, and blown by the wind or attracted by light to near-by urban areas, swarms of adult midges can cause considerable nuisance (Ali, 1996). Common nuisance problems include invasion of buildings, disruption of outdoor activities and accumulation beneath exterior lighting.

Nuisance problems typically occur with newly commissioned WSP systems where aerobic pond sediments provide ideal midge larva habitat. The nuisance is usually only short-term as the midge larvae population declines when anaerobic sludge accumulates on the pond bottom; restricting the available midge larva habitat to the pond edges. However, pond sediments of established WSPs can temporarily become aerobic (due to favourable environmental conditions or reduced organic loading) and cause temporal midge nuisance problems (Spiller, 1964). Thus, a potential downside of improving the design of WSPs to provide higher levels of treatment (e.g. by incorporation of aerobic or maturation ponds) is the increase of available aerobic sediment (midge larva habitat) in the pond system and the higher susceptibility of near-by urban areas to midge nuisance.
problems. Likewise, installation of a mechanical treatment plant ahead of a WSP system and the consequent improvement in pond water quality can result in a similar increase in midge larvae habitat and nuisance from adult swarms (e.g., Broza et al. 2000).

This paper compares the efficacy of Maldison (a compound that has traditionally been used to control midge larvae in New Zealand (NZ) WSPs) to four alternative compounds (\textit{Bacillus thuringiensis} var. \textit{israelensis} (Bti), Methoprene, Pyriproxyfen and Diflubenzuron) that are more specific to insects and have fewer adverse environmental effects. When comparing the efficacy of several types of control agent it is important to understand their different modes of action and the relevant ‘endpoint’ for control.

Maldison is a fast acting, broad-spectrum organophosphate insecticide that is effective upon external contact or after ingestion. Maldison causes disruption of the central nervous system, but in insects it becomes a much stronger inhibitor when it is metabolised to malaoxon (Extoxnet, 2000). Endpoints: larvae mortality, reduced pupation and reduced adult emergence. \textit{Bacillus thuringiensis} var. \textit{israelensis} (Bti) is a naturally occurring soil bacterium that contains a proteinaceous toxin which is activated by enzymes in the intestine of the larvae. The protoxin disintegrates the intestine causing death within a few hours up to a few weeks depending on the dose ingested and chironomid species (Liber et al. 1998). Endpoints: larvae mortality, reduced pupation and reduced adult emergence.

Methoprene, Pyriproxyfen and Diflubenzuron are insect growth regulators (IGRs) that are specific to insects and tend to sorb to organic matter thereby targeting benthic organisms (including chironomids). An advantage of IGRs is that the treated larvae are not killed immediately and remain in the aquatic food chain (Ali et al., 1993). Methoprene and Pyriproxyfen are both juvenile hormone analogues (JHAs). Methoprene interferes with the insect’s life cycle preventing metamorphosis of larvae to adults but is not toxic to pupae. Therefore adults will still emerge from pupae present at the time of application, but larvae will pupate then fail to emerge (Extoxnet, 2000). Endpoint: reduced adult emergence. Pyriproxyfen interferes with larval development of pupae by disrupting the sequence of gene activation. Therefore adults will emerge from pupae present at the time of application but larvae will fail to pupate. Endpoints: reduced pupation and reduced adult emergence. Diflubenzuron is a chitin synthesis inhibitor (CSI) that prevents exoskeleton hardening after moulting and is effective through contact or ingestion (Extoxnet, 2000). Therefore midge larvae are most susceptible prior to moulting. Endpoints: larvae mortality, reduced pupation and reduced adult emergence.

Experiments were conducted at the North Shore WWTP, Auckland, NZ, where there was concern that an upgrade of the mechanical treatment plant upstream of the two-pond WSP system and consequent reduction in the organic load would increase the available habitat (aerobic sediment) for chironomid midge larvae in the ponds and the potential for midge nuisance problems.

\section*{Methods}

\subsection*{Small-scale field trials}

The results of initial laboratory trials (96-hour acute static tests for the fast acting toxins Maldison and Bti, and 10-day chronic static tests for the IGRs Methoprene, Diflubenzuron, and Pyriproxyfen using midge larvae (\textit{Chironomus zealandicus}) cultured from egg masses collected from the North Shore WSPs) were analysed and compared with literature values to derive the following concentrations of each compound for use in field trials: 30 $\mu$g active ingredient (AI)/L Maldison (Malathion 50EC); 1000 $\mu$g/L Bti (Vecto-bac®WG, 3000 international toxic units (ITU)/mg); 50 $\mu$gAI/L Methoprene (Prolink); 10 $\mu$gAI/L Pyriproxyfen (Admiral 10EC); and 50 $\mu$gAI/L Diflubenzuron (Dimilin
Unfortunately, Pyriproxyfen which has been found to provide better control of midges than both Methoprene and Diflubenzuron (Ali et al., 1999) could not be tested further as it was not currently approved for water-related applications in NZ.

Small-scale trials were conducted in enclosures at a site in Pond 1, where high numbers of midge larvae had routinely been recorded in the pond sediment. The enclosures (0.785 m², 471 L) were made from 1.6 m sections of 1 m diameter fibreglass pipe that were driven into the pond substrate. Triplicate enclosures for each treatment and 6 control enclosures were randomly positioned at 0.6 m water depth along a line parallel to the pond edge. The trials of each compound were conducted at two concentrations: (1) the concentration recommended from the laboratory trials, and (2) 10× that concentration, to account for factors such as insecticide degradation and sediment adsorption which may decrease toxicity in the pond environment. Maldison (a liquid emulsion) was directly applied and mixed into the enclosure water. Bti and Diflubenzuron (both powders) were applied by premixing in water taken from the enclosure then mixing the suspension into the enclosure water. Methoprene pellets (42.5 gAI/kg) were evenly scattered over the water surface and sank to the bottom of the enclosure.

The enclosure water was aerated with bubble diffusers to ensure aerobic conditions (DO > 3 g m⁻³). Numbers of live larvae and pupae and emerging adults were monitored in the enclosures before and over 21 days after application of the control compounds. Larvae and pupae numbers were counted from a sieved (500 μm mesh) sediment sample taken from each enclosure using an Eckman grab sampler (15 cm × 15 cm). Adult emergence numbers over a 24 h period were monitored using an emergence trap in each enclosure that caught the adults on adhesive paper. Statistically significant differences between treatments were detected using a one-way ANOVA (p < 0.05) with post hoc analysis of least significant difference using DataDesk® software.

**Large-scale field trials**

Methoprene and Bti were tested individually and in combination at two areas in Pond 1 (that typically had high numbers of midge larvae) to determine how factors such as dilution and dispersion in the pond water or adsorption to the pond sediment influenced performance, and to test the ease of applying each compound. Area SH on the north-eastern side of Pond 1 stretched 160 m along the shore (at a depth of 0.5 m) and extended 30 m into the pond to a 2 m depth. Area SI on the southern side of Pond 1 stretched 80 m along the shore (at a depth of 0.25 m) and extended 20 m into the pond to a 2 m depth. Both trial areas were divided into four sites (Control, Methoprene, Bti/Methoprene and Bti) each 40 m × 30 m in Area 1 and 20 m × 20 m in Area 2, and separated to reduce water movement using polythene curtains extending to just below the water surface, that were weighted on the pond bottom and held in place with stakes.

Methoprene granules (15 gAI/kg) were used to achieve a more even distribution than the larger, higher strength pellets (42.5 gAI/kg) used in the small-scale trial. The granules were applied using a rotary dispenser (10 m swath) fitted to the front of a flat-bottomed boat at a rate of 32 kg total ingredient (TI)/ha to give a concentration of 50 μgAI/L in the water column (assuming a 1 m water depth). Bti (wettable powder) was mixed with 200 L of pond water in a plastic drum and the continuously mixed suspension was applied to the trial sites by pumping through a boom manifold (with 16 hoses that reached down to the pond sediment surface) that was fitted to the front of the flat-bottomed boat. An application rate of 10 kg TI/ha was used to give a concentration of 1000 μg/L in the water column (assuming a 1 m water depth). A hand-held boom manifold (2 m swath with 4 hoses), was used for inshore areas. Both compounds were re-applied on day 20.
Midge larvae were enumerated from two sieved (500 μm mesh) sediment samples that were collected using a 0.023 m² Eckman grab sampler from four monitoring stations within each trial site. Following the first application, sediment samples were randomly collected at pond depths between 0.25 m and 1 m, however, following the second application, all sediment samples were collected at a uniform pond depth of 0.75 m to control for a possible depth effect. Pupae were not enumerated because previously their numbers were highly variable. Sediment samples were collected on days 3, 5, 10, 13 and 17 after the first application of the compounds and on days 24, 27, 31 and 34 following the second application. Numbers of adults emerging from the trial sites were monitored using emergence traps placed within each monitoring station (water depths: 0.25–1 m after the first application; 0.75 m (above larvae sampling sites) following the second application). Emergence traps were deployed for 24 h periods, once prior to the first application of the compounds (to assess the initial populations); on days 3, 5, 10, 13 and 17 after the first application; and on days 24, 25, 26, 27, 31, 32 and 33 following the second application.

Results and discussion
Small-scale field trials
Small-scale field trials were conducted in enclosures within the WSP to determine the efficacy of each compound to control a sample of the midge population at 1 × and 10 × the concentration recommended by the laboratory tests. The average percentage change in live larvae and pupae (per m²) and adult emergence (per emergence trap) for each of the compounds compared to controls are given with 95% confidence limits in Table 1. Despite a gradual increase in the midge population within the control enclosures, all four compounds (Maldison, Bti, Methoprene and Diflubenzuron) were effective at reducing the midge population by the end of the 21-day experimental period.

Compared to controls, treatment with Maldison reduced live larvae numbers by 80% and 92% by day 11 at the 30 μgAI/L and 300 μgAI/L concentrations, respectively, and pupae by 100%. By day 14 numbers of emerging adults were significantly reduced by 88% and 83%, respectively. The 30–300 μgAI/L concentration used in this experiment is much lower than the 22,000 μgAI/L that was routinely used at the Mangere WSP, NZ to achieve 100% larvae mortality (Spiller, 1964), but falls within the ranges suggested by Ali (1981b) for effective control (4–56 μgAI/L) and for control of a Maldison-sensitive species in a 1 m deep pond (25–50 μgAI/L).

Bti significantly reduced live larvae numbers by 93% by day 4 at the 10,000 μg/L concentration compared to controls and by day 11, larval numbers were reduced by 61% and 89% and pupae numbers by 100% and 100% at the 1000 μg/L and 10,000 μg/L concentrations respectively. By day 14, numbers of emerging adults were significantly reduced by 75% at the 10,000 μg/L concentration compared to controls and by day 21, adult emergence was reduced by 72% and 96% at the 1000 μg/L and 10,000 μg/L concentrations respectively. Previous field studies have shown that Bti is effective for midge larvae control e.g. at 1600 μg/L (1200 ITU) (Charbonneau et al. 1994), 88% larvae reduction at 2500 μg/L (1000 ITU) (Ali, 1981a) and 71% larvae reduction at 9000 μg/L (200 ITU) (Liber et al., 1998). Typical reapplication rates are 2–4 weeks (Ali, 1981a; Liber et al., 1998). Therefore, Bti would appear to be an effective replacement for Maldison.

As expected, Methoprene did not significantly reduce larvae numbers compared to controls, but by day 11, pupae numbers were significantly reduced by 100% at both concentrations, and by day 14 adult emergence was significantly reduced by 93% and 98% at the 50 μgAI/L and 500 μgAI/L concentrations, respectively. These results are in agreement with Ali (1991) who found that 22 μgAI/L (for a 1 m depth lake; 0.22 kgAI/ha) reduced adult emergence by 64–98% for 7 weeks.
Table 1 Results of small-scale field trials. Mean % change relative to controls in live midge larvae, pupae and emerging adults in enclosures treated with Maldison, Bti, Methoprene, and Diflubenzuron relative to the control (no treatment) over 21 days.

<table>
<thead>
<tr>
<th>Treatment (AI) \ Day</th>
<th>Live larvae</th>
<th>Live pupae</th>
<th>Adult emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Maldison 30 μg/L</td>
<td>-54</td>
<td>-80*</td>
<td>-33</td>
</tr>
<tr>
<td>Maldison 300 μg/L</td>
<td>-70</td>
<td>-92*</td>
<td>-96*</td>
</tr>
<tr>
<td>Bti 1000 μg/L</td>
<td>-41</td>
<td>-61*</td>
<td>-83*</td>
</tr>
<tr>
<td>Bti 10 000 μg/L</td>
<td>-93*</td>
<td>-89*</td>
<td>-100*</td>
</tr>
<tr>
<td>Methoprene 50 μg/L</td>
<td>13</td>
<td>-17</td>
<td>-33</td>
</tr>
<tr>
<td>Methoprene 500 μg/L</td>
<td>42</td>
<td>-23</td>
<td>-31</td>
</tr>
<tr>
<td>Diflubenzuron 50 μg/L</td>
<td>116*</td>
<td>-7</td>
<td>-89*</td>
</tr>
<tr>
<td>Diflubenzuron 500 μg/L</td>
<td>15</td>
<td>-25</td>
<td>-85*</td>
</tr>
</tbody>
</table>

(negative % implies a decrease, positive % implies an increase, *statistically significant, \( P < 0.05 \))
Diflubenzuron performed more quickly and was slightly more effective than Methoprene; significantly reducing live larvae numbers by 89% and 85% compared to controls by day 21 at the 50 μgAI/L and 500 μgAI/L concentrations, respectively. By day 11, numbers of pupae were reduced by 100% at both concentrations and adult emergence was reduced by 95% at the 50 μgAI/L concentration. By day 21 numbers of emerging adults were significantly reduced by 96% and 95% at the 50 μgAI/L and 500 μgAI/L concentrations, respectively. Previous studies have demonstrated similar control of adult emergence by Diflubenzuron at concentrations of: 0.028–0.056 kgAI/ha for 24 d (Ali and Lord, 1980) and 30 μgAI/L. (Liber et al., 1998). Our results also corroborate studies that have found Diflubenzuron to be more effective at controlling midges than JHAs such as Methoprene (Ali and Lord, 1980). However, for exposure periods greater than 21 days, the slow release pellet and granule formulations of Methoprene could be more effective than Diflubenzuron powder, the only formulation presently available in NZ. Diflubenzuron was not tested further because of concern over possible effects on non-target organisms at the marine outfall.

Large-scale field trials
Based on the small-scale trial results, large-scale field trials were conducted at two sites (SH and SI) within Pond 1 to determine the efficacy of Bti and Methoprene alone, and in combination, to control the midge population. The midge larvae population was highly variable at the control sites during the experiment (2,600–22,000/m² at Area SH, and 3,800–11,400/m² at Area SI (Figure 1), reflecting the inherent variability in the WSP midge population as cohorts of larvae overlap one another.

We expected Bti to greatly influence larvae numbers, however, only a slight reduction was observed compared to controls. A detectable reduction in live midge larvae numbers was not recorded at Area SH until day 24 (4 days after the second application) with numbers reduced from 17,000/m² (day 19) to 3,300/m², while numbers at the control sites increased from 7,800/m² to 21,500/m² over the same period (Figure 1). Larvae numbers at Area SI were no different to controls throughout the trial period (Figure 1).

Bti only had a slight effect on adult emergence at both trial areas; with numbers at Area SH varying between 2–26/trap (control 5–36/trap) and those at Area SI varying between 2–15/trap (control 1–26/trap) (Figure 2). Field trials previously conducted at Mangere WSP, NZ using Bti (Vectobac 12AS) were also found to be ineffective (pers comm. Sam Tan, Mangere WWTP). Poor control by Bti in field trials has been attributed to dilution and dispersion of the Bti suspension in the water column (Ali, 1981a; Liber et al., 1998) and this probably occurred in the Mangere trial where the Bti was applied to the water surface. Charbonneau et al. (1994) found that larvae instar stage was important, with second and third instars more susceptible to Bti than the fourth-instar larvae, but this is an unlikely factor in the present study that was conducted over 35 days. Thus, dispersion of the Bti into the water column above the sediment or within the sediment itself is the most plausible explanation. We had hoped to use a concentrated Bti briquette in the large-scale trial, which could have alleviated this problem, but it was not approved for use in NZ at the time.

Compared to the control sites, Methoprene also had little control on live midge larvae numbers which varied between 3,400–10,400/m² at Area SH and 4,700–11,200/m² at Area SI (Figure 1). This result was expected as larvae exposed to Methoprene do not die immediately. The effect of Methoprene on adult emergence was clearly evident at both trial areas, with the numbers decreasing after both applications. On average, Methoprene reduced adult emergence by 80% compared to controls at both trial areas (Figure 2). Methoprene appeared to have greatest effect on adult emergence 8–10 days following
Figure 1  Live larval numbers at sites in large-scale field trial areas (SH and SI) treated with Bti, Methoprene, or Bti/Methoprene combined (all white symbols) compared to control sites (black symbols) over 35 days.

Figure 2  Adult emergence at large-scale field trial areas (SH and SI) treated with Bti, Methoprene, and Bti/Methoprene combined (all white symbols) compared to control sites (black symbols) over 34 days.
application, which was probably the time when affected larvae would have emerged. When Bti and Methoprene were applied in combination, the contribution of Bti was difficult to discern, while Bti/Methoprene control of adult emergence was similar to that at the Methoprene only sites (Figures 1 and 2). There appeared to be little additive benefit of using Bti and Methoprene together. Control of adult emergence by Methoprene started to decline 17 days after application suggesting a two-week reapplication period for the Prolink XRG granule formulation used.

Conclusions
Methoprene (50\,\mu\text{g} AI/L, concentrated, slow release granule) provided effective midge control in the large-scale WSP trials. Definitive control was not observed with Bti (1000\,\mu\text{g}/L, wettable powder); however, the efficacy of Bti should be re-evaluated if a concentrated, slow-release formulation becomes available in NZ. Both Diflubenzuron and Pyriproxyfen showed promise for midge control in laboratory and small-scale field trials and should also be considered for re-evaluation if concentrated, slow-release formulations are registered in NZ. This study highlights that laboratory studies and small-scale field trials must be validated at large-scale before final conclusions are drawn on the efficacy of the control. The difficulty of interpreting field data due to natural variation of the WSP midge population was a feature of these trials.

Following this research, Methoprene was adopted as the midge control compound for the North Shore WSPs. During the 2003/2004 NZ summer Methoprene was applied to the WSPs on two occasions following complaints of midge nuisance by residents of urban areas close to the pond system. On both occasions the WSP midge population was successfully controlled, and no further nuisance complaints were received.

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References


