Evaluation of various control agents against mosquito larvae in rice paddies in Taiwan

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ABSTRACT: A field test was conducted in rice paddies adjacent to Wufeng, Taichung County in Central Taiwan to evaluate the efficacy of control agents against mosquito larvae. The agents included *Bacillus thuringienesis israelensis* (Bti), two *Lagenidium giganteum* products (*Lg* product A and T), and temephos. The major mosquito species found in the rice paddies were *Culex tritaeniorhynchus/vishnui* and *Anopheles sinensis*. Compared to controls, a 7-day treatment with Bti or *Lg* products A and T caused overall reductions in the number of immatures (larvae and pupae) of *Cx. tritaeniorhynchus/vishnui* of 77.5%, 49.7%, and 21.9%, respectively, whereas temephos caused an increase of 66.9%. The overall reductions in *An. sinensis* were 85.4%, 8.6%, 44.6%, and 92.1%, respectively. There was no significant reduction in the number of mosquito larvae following 42 days of treatment with these agents. In summary, 1-week treatments with both biological control agents produced moderate overall reductions in mosquito larvae in rice paddies. The insecticide temephos, on the other hand, was very effective at suppressing the larvae of A*n. sinensis* but significantly increased the number of *Cx. tritaeniorhynchus/vishnui* larvae in temephos-treated plots. ***Journal of Vector Ecology*** **30 (1): 126-132. 2005.**

Keyword Index: Culex tritaeniorhynchus, *Anopheles sinensis*, control agent, rice paddies.

INTRODUCTION

Culex tritaeniorhynchus Giles and *Anopheles sinensis* Wiedemann are the main mosquito species in rice paddies in Taiwan. The former species (Rosen et al. 1989) along with other vectors, *Cx. vishnui* Theobald (Cates and Detels 1969) and *Cx. fuscocephala* Theobald (Wang et al. 1962), are the major vectors of Japanese encephalitis in Taiwan. To prevent the spread of infection in humans, vaccination of children became a national annual campaign in Taiwan in 1968, and only sporadic human cases have been reported each year since (19 cases in 2002 and 25 cases in 2003) (Center for Disease Control/Taiwan, 2004). However, a rather high infection rate (over 50%) has often been found in pigs across the island each summer since the beginning of the vaccination program.

In addition, since 1980, the adult mosquito population of *Cx. tritaeniorhynchus* has increased faster than that of *Cx. vishnui* (Rosen et al. 1989, Lin and Lu 1995). The past larval pool collections of the two species showed that the ratio of *Cx. tritaeniorhynchus* to *Cx. vishnui* was 1:20 between 1970 and 1972 in Taoyuan (Mitchell and Chen 1974), but in northern Taiwan, this ratio was 4.5:1 from 1980 to 1983 (Rosen et al. 1989) and 1004:1 from 1991 to 1993 (unpublished data, CDC/Taiwan).

In addition to the vaccination of children, insecticides have been used to help control mosquito populations. As part of our routine disease control practice, adulticides are sprayed in residential areas immediately after a human case of Japanese encephalitis is identified. Larvicides are applied the following year in the breeding sites nearby to control the immatures. Unfortunately, *Cx. tritaeniorhynchus* is reported to be highly resistant to some organophosphorus insecticides; for example, larvae resistant to chlorpyrifos and temephos have been reported in Sri Lanka (Karunaratne et al. 1998) and in Japan (Takahashi and Yasutomi 1987, Yasutomi and Takahashi, 1987), respectively. No such resistance of *Cx. tritaeniorhynchus* has been reported in Taiwan. In addition to insecticides, some biological control agents, such as *Bacillus thuringiensis* ssp. *israelensis* de Barjac (Bti), *Lagenidium giganteum* Couch (*Lg*), and insect growth regulators have been used to kill mosquito larvae in various habitats.

The objective of this study was to evaluate the efficacy of three agents against mosquito larvae in Taiwan's rice paddies as part of the effort to control Japanese encephalitis vectors. We also briefly discuss the effects of rice paddy water quality on the performance of the biological agents.

MATERIALS AND METHODS

A field test was conducted in rice paddies adjacent to Wufeng, Taichung County in Central Taiwan from April 9 to May 21, 1999. A randomized complete block design with four blocks and five treatments was used. The plot sizes ranged from 118.9 to 193.9 m^2. The field trials commenced on the 55th day after the planting of rice (February 13, 1999). An irrigation ditch provided water with one gate opening to each plot. Water was allowed to flow into the plots and was stopped by closing the gate when the level in the plots reached the level in the irrigation ditch.

The larvicides tested included two non-commercial products of *L. giganteum*: *Lg* product A (AgraQuest, Inc., Davis, CA) and *Lg* product T (Taiwan Sugar Research Institute, Tainan, Taiwan), which are provided as emulsifiable concentrates. We also tested two commercial products: *B. thuringiensis* (Bti; Vectobac G, 200 International Toxic Units/mg, Abbott Laboratories, North Chicago, IL) and temephos (Abate 1-SG, American Cyanamid, Wayne, NJ). The *Lg* products, Bti, and temephos were tested at 30 oz/acre, 1 g/m^2, and 1 ppm, respectively, for the first application. The *Lg* products were first diluted with tap water and sprayed onto the paddy plot using a 1-gallon sprayer equipped with lance and nozzle (GLORIA-Compression Sprayer Type no. 141T; H. Schulte-Frankenfeld GmbH & Co., Wadersloh, Germany). One plot served as the control and received no treatment. A 14-cm-diameter dipper was used to collect mosquito larvae just prior to the spraying and 2, 4, 7, 14, 20, 28, 35, and 42 days after the first spraying. The test was terminated before the rice harvest on day 42. In each plot, immatures were sampled systematically at 30 sites that were 2 to 3 m apart. Five dips were taken at each site with a total of 150 evenly spaced dips per plot. Except for *Cx. tritaeniorhynchus* and *Cx. vishnui*, which cannot be distinguished by visual examination, the species of collected larvae and pupae were identified in the field.

Data, except those collected before spraying, were analyzed by randomized complete block design with one repeated measurement (time). A single comparison of control and treatments was also conducted separately to determine the pair-wise differences. The dip counts from the treatment plots were compared to dip counts in the controls to assess the reduction in larval number. Due to the low density of mosquito larvae found in the test paddies, 270 *Cx. tritaeniorhynchus/vishnui* larvae collected from nearby rice paddies were released into each treated plot 1 to 2 days before spraying. A second spraying was conducted on the 20th day after the first application based on the poor results obtained at that time. The application rates for temephos and *Lg* products were also increased to 1.5 ppm and 60 oz/acre, although Bti was applied at the same rate.

The water temperature was recorded at the sites for the entire study period. On each day that mosquito larvae were sampled, we also measured the pH, conductivity, and concentration of NO_2-N, NO_3-N, NH_3-N, S_2, Cl^-, Mg-$CaCO_3$, and Ca-$CaCO_3$ for each of the treatments using a handheld multi-parameter instrument (Multi340i; Wissenschaftlich-Technische-Werkstätten company, Weilheim, Germany).

RESULTS

Mosquito species

At least three mosquito species were found in the rice paddies during the survey periods: *Cx. tritaeniorhynchus/vishnui*, *An. sinensis*, and *Cx. fuscanus* Wiedemann. The compositions of the two major species by time for each treatment are presented in Figure 1. The percentages of Cx. *tritaeniorhynchus/vishnui* collected in the control, *Lg* product, and Bti-treated plots varied from 27.2% to 69.0%, whereas those in the temephos-treated plots were 42.3% on day 0 and increased to between 66.4 and 98.8% thereafter. It was obvious that the number of *Cx. tritaeniorhynchus/vishnui* found in the temephos-treated plot was much higher than the number of *An. sinensis* in the same plot as well as the number of both *Cx. tritaeniorhynchus/vishnui* and *An. sinensis* in the other plots.

Larvicidal effects

During a 7-day period, significant differences were noted among different treatments or times for *Cx. tritaeniorhynchus/vishnui* (F=3.62, $P<0.05$; F=10.21, $P<0.01$) and *An. sinensis* (F=7.63, $P<0.01$; F=4.45, $P<0.01$). There was no interaction between treatments and time for either species. The total number of *Culex* (21 larvae per 150 dippers) in temephos-treated plots within the 7-day period was significantly higher than in plots treated with *Lg product* A (6 larvae per 150 dippers) or Bti (3 larvae per 150 dippers) but not in the control (13 larvae per 150 dippers) or *Lg* product T-treated plots (10 larvae per 150 dippers) (Table 1). There were no significant differences between the other treatments. For single comparison between control and treatments, no significant difference was detected. Within 7 days, Bti and *Lg* products A and T caused overall reductions in the number of Cx. tritaeniorhynchus/vishinui immatures of 77.5%, 49.7%, and 21.9%, respectively. In contrast, temephos caused the number of immatures to increase by 66.9%.

As for *An. sinensis*, the total number of immatures in the control (14 larvae per 150 dippers) was significantly higher than the numbers in plots treated with Bti (2 larvae per 150 dippers) or temephos (1 larva per 150 dippers) but not *Lg* products T (8 larvae per 150 dippers) or A (13 larvae per 150 dippers)(Table 1). For single comparison between control and each treatment, the numbers of *An. sinensis* immatures in the control was significantly larger than the numbers in plots treated with temephos (F=17.98, $P<0.01$) or Bti (F=15.45, $P<0.01$) but not much different from the numbers in plots treated with either *Lg* product A or T (F=4.20 and 0.15, respectively; $P>0.05$). Compared to controls, the overall reductions in the numbers of *An. sinensis* immatures in plots treated with temephos, Bti, and *Lg* products T and A compared were 92.1%, 85.4%, 44.6%, and 8.6%, respectively.

Significant differences from days 2 to 7 were found for both species. The numbers of immatures of *Cx. tritaeniorhynchus/vishnui* dramatically dropped in Bti-treated plots on days 2 and 4 and then rebounded on day 7 (Figure 2). Similar decreases were also found in the numbers of *An. sinensis* immatures in Bti and temephos-treated plots (Figure 3).

In the 42-day long trial where two applications of product were added, significant differences were detected among the different treatments (F=9.54, $P<0.01$) for *Culex* group and among the different blocks (F=42.41, $P<0.01$), treatments (F=4.08, $P<0.05$), and times (F=17.19, $P<0.01$) for *An.*

Table 1. Effects of 7-day treatments with temephos, *Bti*, and *Lg* products A and T on immatures of *Culex tritaeniorhynchus/vishnui* and *Anopheles sinensis* in rice paddies.

Treatment**	*Cx. tritaeniorhynchus/vishnui*		*An. sinensis*	
	Total no. per 150 dippers*	Percent Reduction	Total no. per 150 dippers*	Percent Reduction
Temephos				
Small larvae	7.42	-8.6	0.83	87.5
Large larvae	10.75	-111.5	0.08	98.7
Pupae	2.83	-325.0	0.17	71.4
Total immatures	21.00 a	-66.9	1.08 c	92.1
Control				
Small larvae	6.83	-----	6.50	-----
Large larvae	5.08	-----	3.92	-----
Pupae	0.67	-----	0.58	-----
Total immatures	12.58 ab	-----	13.67 a	-----
Lg Product T				
Small larvae	3.92	42.7	3.08	52.6
Large larvae	4.67	8.2	3.92	40.5
Pupae	1.25	-87.5	0.58	0.0
Total immatures	9.83 ab	21.9	7.58 ab	44.6
Lg Product A				
Small larvae	3.08	54.9	4.08	37.2
Large larvae	2.83	44.3	7.67	-16.5
Pupae	0.42	37.5	0.75	-28.6
Total immatures	6.33 b	49.7	12.50 a	8.6
Bti				
Small larvae	1.50	78.0	1.00	84.6
Large larvae	1.33	73.8	0.83	87.3
Pupae	0.00	100.0	0.17	71.4
Total immatures	2.83 b	77.5	2.00 bc	85.4

*Total immatures within each species followed by the same letter were not significantly different (α=0.05) according to the Least-Significant-Difference method.
**n=12 for each treatment.

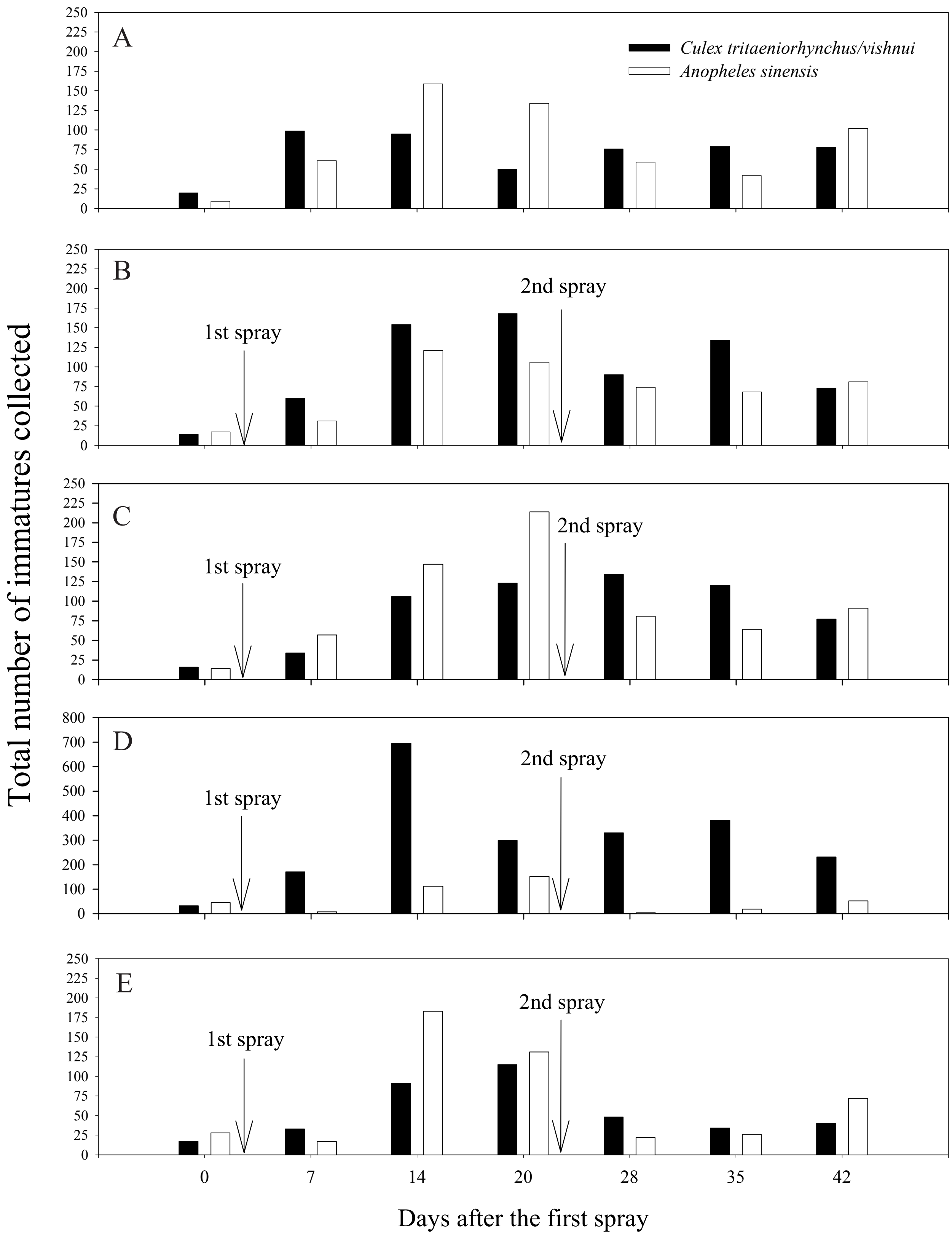

Figure 1. Mosquito control in rice paddies in Taiwan. Shown are the total number of immatures in control plots (A), and plots treated with *Lg* A (B), *Lg* product T (C), temephos (D), or Bti (E).

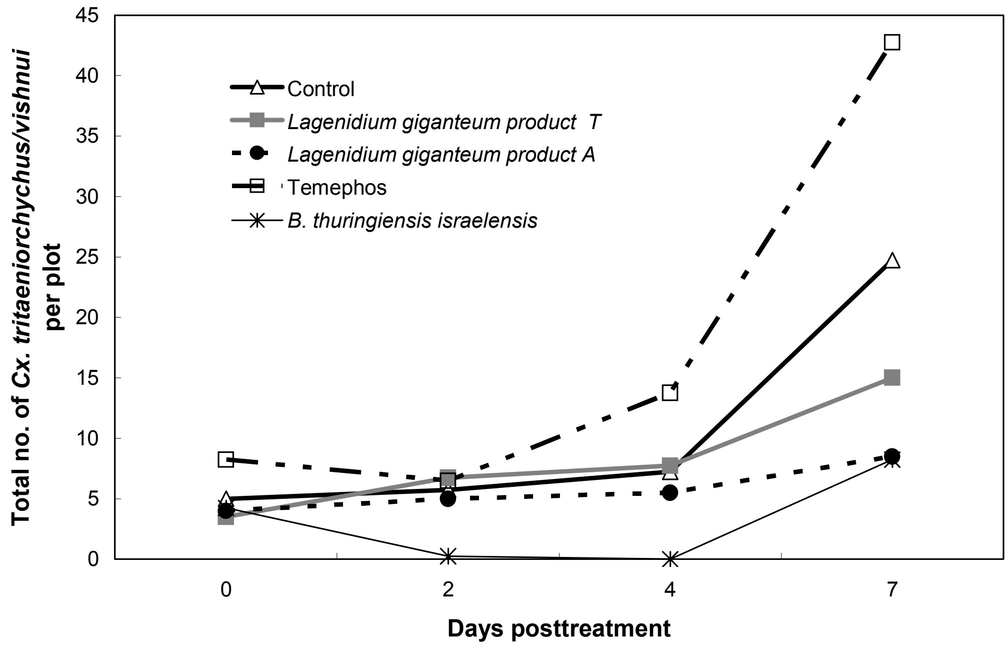

Figure 2. Efficacy of control agents on *Cx. tritaeniorhynchus*/*vishnui* mosquitoes in rice paddies in Taiwan. Shown are the effects of *Lg* products A and T, Bti, and temephos on the average total number of cx. *tritaeniorhynchus*/*vishnui* per plot.

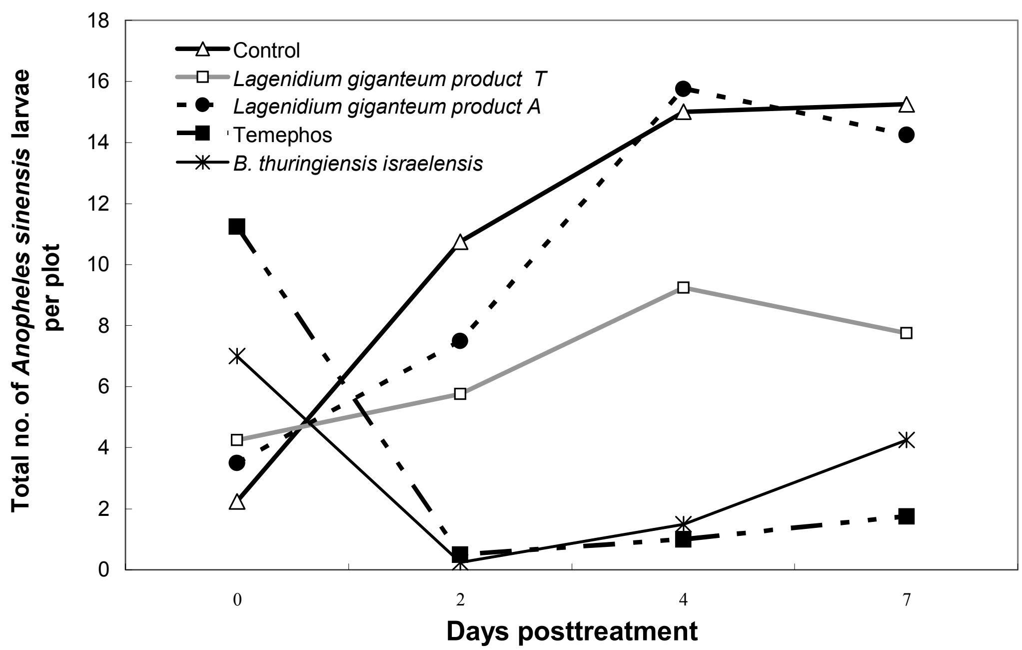

Figure 3. Efficacy of control agents on *An. sinensis* mosquitoes in rice paddies in Taiwan. Shown are the effects of *Lg* products A and T, Bti, and temephos on the average total number of *An. sinensis* per plot.

sinensis. No significant interactions between treatments and time for either species were noted. At the beginning of the trial, population densities of both species were low and gradually increased to a peak after 14 or 21 days (Figure 1). Thereafter, there was a decrease in the numbers collected, especially for *An. sinensis* in the temephos-treated plot. For single comparison between control and each treatment, the number of immatures in the control was significantly smaller than the numbers of *Culex* immatures in temephos-treated plots (F=24.70, $P<0.01$) but significantly larger than the number of *An. sinensis* immatures in temephos-treated plots (F=6.82, $P<0.05$). Also, there was no difference with Bti or *Lg* products A or T for the *Culex* group (F=0.13, 0.38, and 0.13, respectively; $P>0.05$) or for *An. sinensis* (F=1.71, 0.88, and 1.43, respectively; $P>0.05$). Overall, after 42 days of treatment, no marked reduction was observed in the number of mosquito larvae with any of the treatments (up to 24.3% and 38.0% in the *Culex* group and *An. sinensis*, respectively).

Physio-chemical conditions

The water temperature at the study sites varied between 21°C and 30°C, which was suitable for fungal growth. The water in the tested plots was characteristically hard ($[Mg^{2+}]$ = 0.84 ± 0.12 mg/L and $[Ca^{+2}]$ = 0.58 ± 0.08 mg/L), weakly acidic (pH = 6.33 ± 0.03), and with moderate levels of organics and high concentrations of NO_3-N (1.94 ± 0.07 mg/L) and NH3-N (0.64 ± 0.18 mg/L). In addition, the ionic content was very high (conductivity = 284.60 ± 9.08 µS/cm).

DISCUSSION

In the present study, the biological control agents, *B. thuringiensis israelensis* and *L. giganteum*, were found to be effective for 1 week at reducing the number of mosquito immatures in rice paddies in Taiwan. Temephos, on the other hand, was very effective at controlling immatures of A*n. sinensis* but significantly increased the number of *Cx. tritaeniorhynchus*/*vishnui* larvae. The 42-day trial showed that there was no long-term effect, which might be due to the poor water quality at the study sites, the short duration of efficacy of certain agents, and the introduction of new egg rafts laid by females from nearby untreated rice paddies.

The use of a secondary treatment at 20 days in our study was based on the duration of efficacy of *L. giganteum*. *Lg* products effectively reduce the number of mosquito larvae for the first 5 days and last for 20 days (Hallmon et al. 2000, Guzman and Axtell 1987). However, the duration of efficacy is much shorter for Bti and temephos. Generally, the duration of efficacy in the commercial Bti is not greater than 7 days (Mulla et al. 1993, Su and Mulla 1999) and 14 days for the temephos in streambed pools (Shililu et al. 2003). Reapplications of Bti and temephos within less than 14 days are thus required to obtain longer durations of efficacy, which explains why Bti and temephos did not have long-term efficacy in our study.

In our study, we found a significantly higher number of *Cx. tritaeniorhynchus*/*vishnui* in temephos-treated plots than in the control plots. This result may be due to resistance to temephos, which has been reported in previous laboratory studies in Japan (Takahashi and Yasutomi 1987, Yasutomi and Takahashi, 1987). In those studies, the resistance ratio was more than 125,000:1, and the insensitivity of acetylcholinesterase to the compounds was identified as the major factor leading to resistance. Further studies should be performed to determine whether this is also a problem in Taiwan.

Water quality and temperature affect the infection of mosquito larvae by *L. giganteum*. Several other studies have indicated that *L. giganteum* have low tolerance to certain environmental conditions, including organic water pollution, salinity, and water hardness (Jaronski and Axtell 1982, Merriam and Axtell 1982, Kramer 1990, Guzman & Axtell 1987). Because our study site was part of a pig farm, the water was characteristicly hard and high in nitrogen, which probably explains the low efficacy of *L. giganteum*, especially in the long-term trial.

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