Use of *Bacillus thuringiensis israelensis* and Methoprene to Control Asian Tiger Mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae), in Non-circulating Hydroponics Tanks

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Abstract. *Bacillus thuringiensis israelensis,* (Bti) and methoprene (isopropyl (2E-4E)-11-trimethyl-2,4-dodecadienoate) were tested for control of the Asian tiger mosquito, *Aedes albopictus* (Skuse) in non-circulating hydroponics tanks of lettuce. The results showed that Bti and methoprene formulations reduced mosquito larvae and pupae population for the duration of the lettuce crop (4 to 5 wk) compared to the non-treated control. Bti and methoprene treatments, however, caused reduced lettuce head weight and root growth. Leaf lettuce cultivars 'Red Sails' and 'Green Ice' were more tolerant to Bti treatment compared to 'Manoa'. Lowering the Bti (VectoBac G) application rate from 1.04 to 0.54g/m^2 increased head weight and root growth for 'Manoa' lettuce. The tank treatments presented in this study were not replicated due to the use of large, commercial-scale tanks and limited greenhouse space.

Introduction

In recent years, there has been great interest in stagnant water (non-circulating) hydroponic systems since it is cheaper to setup and operate and does not require electrical power compared to traditional circulating hydroponic systems (Kratky 1990). At present, there are several large commercial non-circulating hydroponic operations in Hawaii that have recently been established which are proving to be very successful. However, as non-circulating hydroponic operations expand and increase in number and size, growers are facing the problem of mosquitoes breeding in the stagnant water of the hydroponic tanks. The mosquitoes breeding within these hydroponic tanks pose no problem to crop production, but can be extremely irritating to farm workers.

The mosquito commonly found breeding in non-circulating hydroponic tanks in Hawaii is the Asian tiger mosquito, *Aedes albopictus* (Skuse). The *A. albopictus* life cycle starts with eggs hatching in water where they soon pass through the larval and pupal stages. The adults emerge with females searching out a blood meal to initiate egg production. Nutrient rich stagnant water, such as those used in non-circulating hydroponics, provide an ideal site for larval and pupal development. Furthermore upon adult emergence, the farm workers tending to hydroponic plants provide the blood meal that perpetuates the breeding of these mosquitoes.

Mosquito larvae and pupae can easily be controlled with today's chemical insecticides (Ali et al. 1995), however, none are cleared for use in hydroponics. In addition, the use of these insecticides is not an option for hydroponic growers in Hawaii since their produce is typically grown and sold under a "pesticide-free" label. This label precludes the application of traditional pesticides to all plant parts including the nutrient solution.

Bacillus thuringiensis israelensis, (Bti) (Ali et al. 1995; Becnel et al. 1996), and the insect growth hormone, methoprene (Ali et al. 1995; Basci et al. 1994; Becnel et al. 1996; Fargal et al. 1988; Sulaiman et al. 1994; and Toma et al. 1990), have been shown to be very effective in controlling *A. albopictus* in the laboratory and field and are not considered as

pesticides from the stand point of qualifying for the "pesticide-free" label. This study was conducted to investigate the effectiveness of Bti and methoprene to control *A. albopictus* larvae and pupae populations in non-circulating hydroponic tanks and their effect on lettuce, *Lactuca sativa* L., yield.

Materials and Methods

General methods. The Bti and methoprene formulations used in the experiments were Altosid (methoprene pellet, Sandoz Crop Protection Corporation, 1300 E. Touhy Ave., Des Plaines, IL), Mosquito Dunk (Bti floating donut, Summit Chemical Company, 7657 Canton Center Drive, Baltimore, MD.) and VectoBac G (*Bti* organic granules, Abbott Laboratory, Chemical and Agricultural Products Division, 14th Sheridan Road, North Chicago, IL). Altosid is a chemical growth regulator that disrupts the development of mosquito larvae and pupae preventing their development into adults. Altosid is formulated either as a liquid or a pellet, of which the pellet has longer residual activity. Since the recommended rate of application for the Altosid pellet formulation is between 210 and 280 g/ha, 280 g/ha (0.028 g/m2) was selected for testing in the hydroponic tanks. VectoBac G uses an organic vermiculite carrier to which *Bti* is impregnated. The recommended rate of application (note: commercial use on lettuce is not consistent with the pesticide label and therefore a violation of federal law if used commercially) for the VectoBac G formulation is 2.8 to 11.3 kg/ha. The 11.3 kg/ha rate (1.08 g/ m^2) was chosen for our experiment. The Mosquito Dunk is a donut-shaped *Bti* formulation designed to float. Its recommended dosage, which was used, is one dunk (15 g) / 9.29 m^2 of surface water (1.61 g/m2)

In order to duplicate typical hydroponic farming conditions, 4 tanks measuring 2.44 m long, 1.22 m wide by 19 cm deep were constructed with 1.90 cm thick exterior grade 2.44 x 1.22 m plywood. The sides were constructed with 5.10 x 20.30 cm lumber. The inner surface of the tank was lined with 3 layers of 6 mil thick black polyethylene sheeting. A 2.44 m long, 1.22 m wide by 1.30 cm thick plywood sheet was used for the tank cover and also served as the support for the forestry tubes (3.80 cm diameter x 20.30 cm long plastic tubes) in which the lettuce seedlings were planted. To support the forestry tubes the plywood cover was drilled with 3.80 cm diameter holes spaced 22.90 x 22.90 cm on center (60 holes per tank). Half of the holes were used for planting and the remaining holes were left open to allow mosquitoes to freely lay eggs within the hydroponic tank. The upper surface of the plywood was painted white to reduce surface temperature.

Lettuce seedlings of cultivars 'Manoa', 'Red Sails', and 'Green Ice' were prepared by first sowing seeds 0.25 cm deep into seedling trays filled with a standard potting mix (Promix BX, Premier Horticulture Inc., Red Hill, PA). After 2 wk, the lettuce seedlings were transplanted into foresty tubes containing a 1:1:1 mixture of peat (ProMix BX): horticultural perlite (medium grade): vermiculite (no. 2 grade). The seedlings were grown under greenhouse conditions for approx. 2 wk before transplanting.

The nutrient solution was prepared by adding 227 liters of water into each hydroponic tank, as described by Kratky 1993, which submerged the bottom of the forestry tube 2.50 cm. The following nutrients were added to each tank; 72 g of 4-18-38 hydroponic mix (Chem-gro, Hydro-gardens, Inc., Colorado Springs, CO 80932), 72 g $CaNO_3$, 15 g KNO_3, and 43 g $MgSO_4$. No additional fertilizers were added to the tanks. Electrical conductivity (1.5±0.5 milli-siemens, *mS*) and pH (6.5±1.0) of the nutrient solution was maintained throughout the growing period.

Mosquito larvae and pupae populations within each tank were estimated by visually counting live mosquito larvae and pupae within a 0.75 m^2 area of the 3 m^2 tank and then multiplying the count by 4. The entire tank could not be counted since movement of water or distur-

Table 1. Effect of Mosquito Dunk (1.61 g/m^2) and Altosid (0.028 g/m^2) treatments on mean head and root weight (g), and root length (cm) ± SEM of 'Manoa' lettuce when grown in non-circulating hydroponic culture.

Treatment [x]	Head wt (g)	Root wt (g)	Root Length (cm)
Control	109.0 ± 8.5	6.4 ± 0.9	10.1 ± 1.0
Mosquito Dunk	106.6 ± 6.1	3.9 ± 0.5	5.9 ± 0.4
Altosid	103.6 ± 8.0	2.3 ± 0.4	5.8 ± 0.7

[x] N=40 for each treatment.

bance of the lettuce plants might result in lower yields (Kratky, 1993). Lettuce heads were harvested and weighed when "market size" was obtained. Root growth measurements and/or ratings were also recorded at time of harvest.

The experimental tanks were built to duplicate tanks that are currently used in commercial hydroponic lettuce farms in Hawaii. Due to the size of these tanks and the limited greenhouse space available for this study, treatments were not replicated. However, the experimental hydroponic tanks should closely reflect plant growth and mosquito control in commercial operations.

Mosquito Dunk and Altosid treatments on Manoa lettuce. 'Manoa' lettuce was grown as described above. 'Manoa' was selected since it is one of the most widely grown lettuce cultivars among non-circulating hydroponic growers in Hawaii. 'Manoa' lettuce seeds were started by sowing seeds into seedling trays on 2 July 1996 and transplanted into forestry tubes 16 July 1996. Forty transplanted seedlings were used for each treatment. Three tanks were prepared as above and treated with either Mosquito Dunk, Altosid or left non-treated (control) with application rates as described above. Salinity and pH readings and mosquito larvae and pupae counts were recorded weekly. Lettuce head weight and root length measurements (length and weight of roots protruding from the forestry tubes) were recorded on 15 August 1996.

VectoBac G and Mosquito Dunk treatments on lettuce. Lettuce cultivars 'Manoa', Red Sails', and 'Green Ice' were seeded 23 August 1996. Ten transplants from each cultivar were planted into separate rows within each tank. The tanks were prepared as described above and treated with VectoBac G, Mosquito Dunk, or left non-treated (control). The plants were 10-days-old at time of transplanting. All lettuce cultivars were harvested 1 October 1996. Data on numbers of mosquito larvae and pupae, lettuce head weight, and root measurements were collected as described above at the time of harvest.

VectoBac G application rate treatment on 'Manoa' lettuce. To observe the influence of application rate on 'Manoa' lettuce yield, plants were grown as described above and treated with 3 rates of VectoBac G. The application rates were 0.54 g /m^2 (0.5 x), 1.08 g/m^2 (1.0 x), and a 0 g rate which served as the control. The lettuce seeds were sown 20 Sept 1996, transplanted 1 Oct 1996 and harvested 12 Nov 1996. Data on numbers of mosquito larvae and pupae, lettuce head weight, and root measurements were collected as described above.

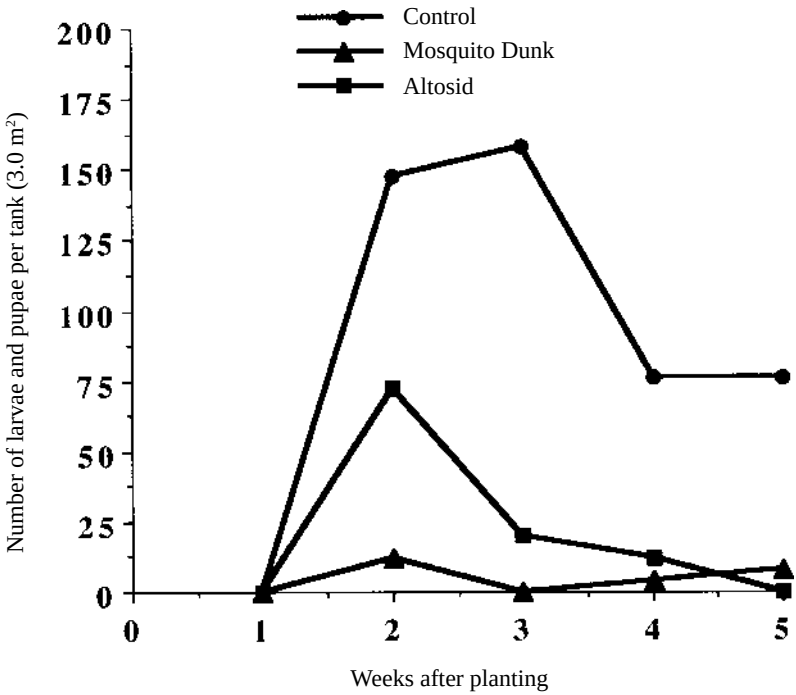

Figure 1. Effect of Mosquito Dunk and Altosid treatment on *A. albopictus* larvae and pupae in non-circulating hydroponic tanks.

Results and Discussion

Mosquito Dunk and Altosid treatment on Manoa lettuce. There were no differences in average lettuce head weight between the control, Mosquito Dunk, and Altosid treatments (Table 1). Weight and length of roots, however, were significantly lighter and shorter compared to the control. The Altosid treated plants had 64% reduction in root weight and 43% reduction in root length. Plants treated with Mosquito Dunk had 39% reduction in root weight and 42% reduction in root length. The reduced root weight and length may be a result of the petroleum-based ingredients used in the formulation of pellets and floating dunks. Plants grown in non-circulating hydroponic culture are very sensitive to these chemicals as they often leave an oily film on the surface of the nutrient solution affecting gas exchange that may in turn inhibit root growth. Circulating hydroponic systems do not display such sensitivity since these films tend to disperse with moving water.

Altosid and Mosquito Dunk treated tanks had lower numbers of larvae and pupae compared to the nontreated control for the duration of the lettuce crop (Fig. 1). While the Mosquito Dunk treated tanks did have some larvae after 3 wk, the larvae were small and no pupae were observed. Both larvae and pupae were observed in the Altosid treated tank one wk following treatment. Although larvae and pupae were seen in the Altosid treated tank, they probably did not develop into adults. Altosid is a juvenile hormone that prevents the

Table 2. Effect of Mosquito Dunk (1.61 g/m^2) and VectoBac G (1.08 g/m^2) treatment on mean head and root wt (g), and root length (cm) ± SEM of 3 lettuce cultivars grown in non-circulating hydroponic tanks.

Cultivar	Treatment[x]	Head wt (g)	Root wt (g)	Root length (cm)
'Manoa'	Control	120.7 ± 15.7	8.5 ± 1.3	7.7 ± 0.6
	Mosquito Dunk	81.0 ± 7.6	3.2 ± 1.2	4.6 ± 0.5
	VectoBac G	69.3 ± 17.2	3.9 ± 0.8	6.4 ± 1.1
'Red Sails'	Control	105.9 ± 13.4	5.6 ± 1.3	7.4 ± 0.8
	Mosquito Dunk	52.1 ± 13.3	3.0 ± 1.2	3.4 ± 0.9
	VectoBac G	124.9 ± 13.1	8.6 ± 1.6	8.2 ± 1.0
'Green Ice'	Control	144.4 ± 11.0	12.6 ± 1.3	9.3 ± 0.6
	Mosquito Dunk	76.8 ± 14.6	3.9 ± 1.4	5.0 ± 1.2
	VectoBac G	114.6 ± 19.b	5.6 ± 1.6	6.9 ± 1.2

[x] N=10 for each treatment.

development of larvae and pupae into adults. Hence, 2 wk following treatment, most of the individuals observed within the Altosid treated tanks were either unusually large larvae or pupae. After 5 wk, no larvae or pupae were observed in the Altosid treated tank, 8 larvae and pupae were observed in the Mosquito Dunk treated tank compared with 76 larvae and pupae for the control tank.

VectoBac G and Mosquito Dunk treatments on lettuce. The results of head and root weight and root length for all cultivars treated with Mosquito Dunk and VectoBac G are presented in Table 2. 'Red Sails' had head weights that were not significantly different from the controls after treatment with VectoBac G, but 'Green Ice' had a lower head weight. 'Manoa' had a 42.6 % reduction in head weight. There was a significant reduction of root weight and length for 'Green Ice' using VectoBac G. Compared to the control, 'Manoa' root weight and length was significantly lowered by 54.1% and 16.9%, respectively.

The Mosquito Dunk affected the head weights of all 3 cultivars. Head weight was reduced by as much as 50.8 %. Similiar significant reductions in root weight and length were also observed.

Apparently, Mosquito Dunk and VectoBac G greatly reduced root growth in 'Manoa' lettuce. The reduced root growth may have been directly related to the lighter head weights observed for 'Manoa' lettuce. 'Green Ice' and 'Red Sails' had similiar reductions in head weight and root growth when treated with Mosquito Dunk.

There were no mosquito larvae and pupae developing within the tanks during the cropping period. The absence of larvae and pupae may have been due to the hot and dry weather during this period.

VectoBac G application rate treatment on 'Manoa' lettuce. 'Manoa' lettuce treated with 0.5x rate of VectoBac G had similiar head weight and root length compared to the control (Table 3). Root weight for 0.5x VectoBac treated plants, however, were 39.0% lighter than the control. Plants treated with VectoBac G at 1.0 x rate displayed 69.0% decreased

Table 3. Effect of VectoBac G application rate on the mean head and root wt (g), and root length (cm) ± SEM of 'Manoa' lettuce grown in non-circulating hydroponic tank culture.

Treatment[x]	Head wt. (g)	Root wt. (g)	Root length (cm)
Control	65.5 ± 12.6	4.1 ± 0.8	4.1 ± 0.5
VectoBac G (0.54 g/m^2)	72.7 ± 5.9	2.5 ± 0.5	4.4 ± 0.5
VectoBac G (1.08 g/m^2)	20.3 ± 4.2	0.3 ± 0.1	0.6 ± 0.3

[x] N=40 for each treatment.

head weight, 92.7% reduction in root weight and 85.4% root length compared to the control.

Larvae and pupae number were consistently high for the last 3 wk in the control tank with as much as 72 larvae and pupae at the fourth wk (Fig. 2). Tanks treated at the 0.5x and 1.0x rate had no larvae and pupae for the entire cropping period, except for the fourth wk in the 0.5x treated tank where only 4 small larvae were observed.

The recommended rate for VectoBac G application is 2.79 to 11.29 kg/ha. The highest rate of application (11.29 kg/ha or 1.08 g/m^2) was used for these experiments. Reduced yield and inhibited root growth was observed at this rate. At the 0.5x application rate 0.54 g/m^2, yields similiar to the control were obtained, however, a 40% reduction in root weight was observed. Mosquito larvae and pupae were adequately reduced at the 0.5x rate, although 100% control was not observed. Thus, a split-application of the 0.5x rate (2 applications of 0.25x administered 2 wk apart) may have a longer lasting control and be safer for the plants compared to a one-time 0.5x application.

In conclusion, Bti and methoprene formulations tested were very effective in controlling *A. albopictus* mosquito larvae and pupae within the nutrient solution of noncirculating hydroponic tanks. These formulations, however, reduced head weight and root growth of lettuce. Of the 3 lettuce cultivars tested, 'Manoa' lettuce is the most susceptible to yield and root growth reduction. However, lowering the application rate of VectoBac G from 1.0x to 0.5x on 'Manoa' lettuce resulted in the production of heads similiar to that of the control. While this rate of application did not control the mosquito larvae for the full 4 wk cropping period, the larvae numbers recorded at the 4 wk period was very low. Split-applications at lower dosages are currently being investigated.

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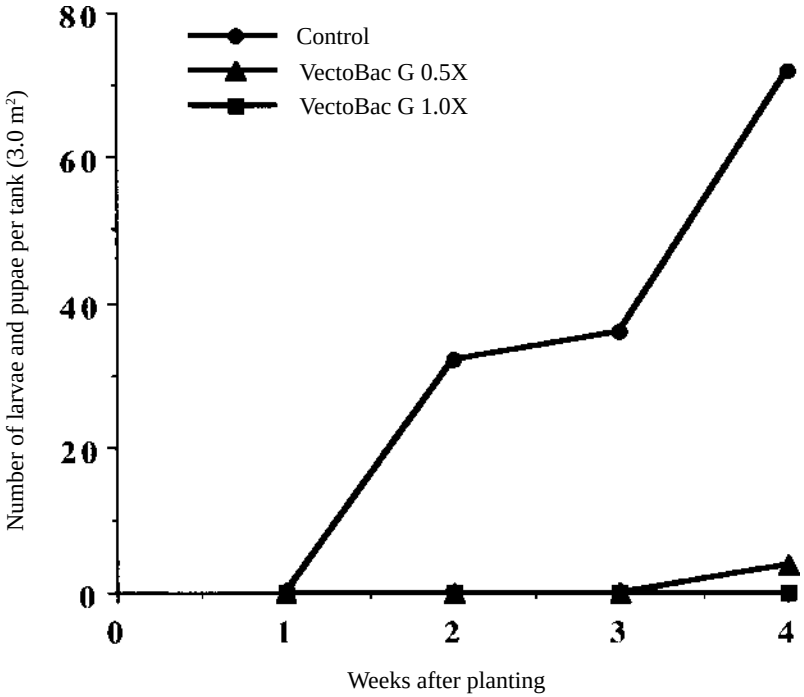

Figure 2. Effect of VectoBac G application rates on *A. albopictus* larvae and pupae in non-circulating hydroponic tanks.

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