SWATH WIDTH DETERMINATION FOR BEECOMIST®-APPLIED *BACILLUS THURINGIENSIS* (H-14) AGAINST *ANOPHELES QUADRIMACULATUS* LARVAE IN RICE FIELDS[1,2]

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ABSTRACT. Optimum flight path interval of Beecomist®-applied *Bacillus thuringiensis* var. *israelensis* (serotype H-14) (*Bti*) against *Anopheles quadrimaculatus* larvae was determined by assessing the effective swath width in rice fields. Droplet sensitive cards, laboratory-reared and naturally occurring populations of larvae were used to monitor aerial treatments 1 day posttreatment. Overlapping swaths were necessary to provide high levels of larval reduction. Based on tests where flight path intervals were 18.3, 36.6 and 73.2 m, optimal flight path interval was estimated to be approximately 67 m downwind of the extreme downwind flight path when flow rate was 1.44 liter/min.

INTRODUCTION

The Beecomist® spray head has proven effective for ultra low volume (ULV) application of *Bacillus thuringiensis* var. *israelensis* (serotype H-14) (*Bti*) to control mosquito larvae (Yates 1984, Sandoski et al. 1985) and malathion to control adult mosquitoes (Rupp and Sutherland 1976). This equipment represents an economically attractive alternative to conventional aerial application equipment in that undiluted materials can be applied. Heavy payloads of spray carrier are not required, permitting use of lighter, more economically operated aircraft. Currently, cost of conventional aerial application of *Bti* at label recommended rates exceeds cost of Beecomist-applied *Bti* at ULV rates (Anonymous 1983, Sandoski et al. 1985).

Application of materials via Beecomist spray head differs from high volume aerial application in that wind currents serve to distribute the material downwind of the flight path. Flight path thus is in the upwind portion of the swath, resulting in the asymmetrical deposition of material relative to flight path. Since wind currents are used to disperse the material in Beecomist applications, flight paths perpendicular to wind direction maximize carry of the material. Quartering wind directions do not allow for maximum carry of the material and may result in skips or reduced swath width.

Optimum flight path intervals in Beecomist applications depend on specific gravity, droplet size, altitude and wind. In turn these factors are affected by airspeed, sleeve rotation speed, flow rate and altitude. Other variables that must be considered are susceptibility of the target organism, canopy density and thermal activity. Effective swath width (region of material deposition) may in fact be greater or less than the arbitrary distance between flight paths. At relatively narrow flight path intervals, swaths may overlap with on-target dosage dependent upon number of overlaps from adjacent flight paths. When flight paths are too wide, skips in application of the control agent occur. Although previous studies have demonstrated the efficacy of Beecomist-applied *Bti*, optimum flight path interval has not been determined. To estimate optimum flight interval, this study assessed the effective swath width of Beecomist-applied *Bti* against *Anopheles quadrimaculatus* Say larvae in rice fields.

MATERIALS AND METHODS

GENERAL. Investigations were conducted in rice fields near Stuttgart, AR (Arkansas and Prairie counties) during the summers of 1984–85. Rice was in the vegetative stage during all tests. In tests in which effects of treatments were determined by monitoring naturally occurring larval populations, fields were selected on the basis of adequate numbers of *An. quadrimaculatus* larvae in 100 dip samples (near or greater than 0.5 larvae/dip) and uniformity of rice stand. In tests in which effects of treatments were monitored using laboratory-reared larvae exposed in floating cages, field selection was based solely upon uniformity of rice stand. Plots within the same field were separated by a minimum of 150 m. Teknar® AC [1500 AA units/mg (*Aedes aegypti*) Linn.] was the source of *Bti* in all tests.

Applications were made using a Piper Pawnee 260 with a Beecomist Model 360A spray head mounted on the spray boom of the starboard wing approximately 0.7 m from the outer edge of the wing and 4.3 m from the

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center of the aircraft as previously described by Sandoski et al. (1985). Flight speed was 161 km/hr at an altitude of approximately 15 m. Flight path was perpendicular to the wind with the Beecomist spray head on the upwind wing of the aircraft. Sleeve revolutions/min of the spray head in absence of *Bti* flow were monitored with a stroboscope and determined to be approximately 11000 revolutions/min.

During application, *Bti* flow was initiated at least 50 m prior to flight over the plot and was discontinued a minimum of 50 m beyond the plot. Time of application and wind velocity varied among tests. At time of application, wind speed was determined at 1.5 m above ground using a battery-powered hand-held anemometer.

In some tests droplet sensitive cards (Kromekote® Cards, Forshaw Chemical Co., Charlotte NC) were attached horizontally to wooden stakes placed in rice fields such that site disturbance was minimized. Cards (10 × 10 cm) were located at rice canopy level and/or below canopy level 5 cm above the water surface. Location of cards above and below the canopy allowed a relative assessment of impingement on vegetation. Cards were examined 45 min posttreatment for the presence or absence of *Bti* droplets.

In tests in which *An. quadrimaculatus* larvae were held in floating cages, larvae were reared from a native population using the techniques described by Dame et al. (1978) and Bailey et al. (1980) with modifications as described by D. L. Kline (personal communication 1985). Second and third instar larvae were placed in floating cages (10 larvae/cage) to monitor treatment efficacy. Two types of cages were used: (1) open, and (2) closed. The open cages allowed for interchange of water between the cage and the rice field. Lids were not used on either cage type. Both types of cages were constructed from 12 × 16 cm plastic cylindrical containers. Open cages had a 7 cm diam opening in the bottom and four 3.5 × 5 cm openings spaced equidistantly around the circumference. Openings were covered with 35 mesh nylon screen. Four table tennis balls affixed to equidistantly spaced 3 cm diam openings on the container side served to float the cage so that the lower half of each cage (both types) and the screened openings on the sides of the open cages were below the water surface. Larvae were placed in cages prior to treatment and mortality was monitored 24 hr posttreatment. Cages used as checks were located in the rice field upwind of the treatment zone. Larval reduction data for cages were corrected for check mortality using Abbott's formula (Abbott 1925, transformed (arcsine) and subjected to analysis of variance. Means of larval mortality for cage type, transect, and distance were separated using Duncan's Multiple Range Test ($P = 0.05$) (Duncan 1955). An 80% reduction in larvae was arbitrarily established as the standard for effective control.

In tests where treatment efficacy was assessed on the basis of percentage reduction of naturally occurring populations of *An. quadrimaculatus* larvae, larval density was estimated immediately following treatment and 1 day posttreatment. Estimation of pretreatment larval populations was made immediately following treatment so as to accurately align the plot and flight direction parallel and perpendicular to wind direction, respectively. "Pretreatment" sampling was concluded within 2 hr of treatment as time available for sampling was limited by activity of *Bti*, Lacey and Lacey (1981) noting onset of *Culex quinquefasciatus* Say larval mortality at 2 hr when administered an LC_{100} concentration. Four sampling transects were established parallel to wind direction immediately posttreatment and extended from 0–18.3 m upwind to 73–146 m downwind of flight path. Transects were equidistantly spaced from adjoining transects and sides of the plots. Sampling stations spaced 0.8–24.4 m apart were established along each transect for a total of 116 samples/transect. Samples were made using 450 ml dippers fitted with wooden handles. Dips were made at the surface of the water and in close association with vegetation to assure maximum encounter of *An. quadrimaculatus* larvae. Number of larvae collected was recorded for each sampling station. Number of samples per station varied among tests. Percentage reduction in *An. quadrimaculatus* larvae 1 day posttreatment was based upon sample totals for intervals of each transect (four stations/interval). Percentage reductions were transformed (arcsine) and subjected to analysis of variance. Means of samplers and sampling intervals were separated using Duncan's Multiple Range Test ($P = 0.05$) (Duncan 1955).

TEST 1. A 0.73 ha (73.2 × 100 m) plot was established in a 13 ha rice field. At treatment, rice was approximately 0.3–0.4 m in height. Water depth was 5 cm with 6 cm of rainfall occurring 8 hr posttreatment. The efficacy of Beecomist-applied *Bti* from 6.7–106.7 m downwind of flight path was assessed (Fig. 1). *Bti* was applied in one flight path 6.7 m upwind of the plot. Based upon flight path interval used in LFD_{50} and LFD_{90} estimation (18.3 m) as described by Sandoski et al. (1985), the system was pressurized at 124.2 kPs and *Bti* was applied at the flow rate corresponding to 0.11

liter/ha (0.54 liter/min). Application was made at 0900 with winds 12.8–16.1 km/hr.

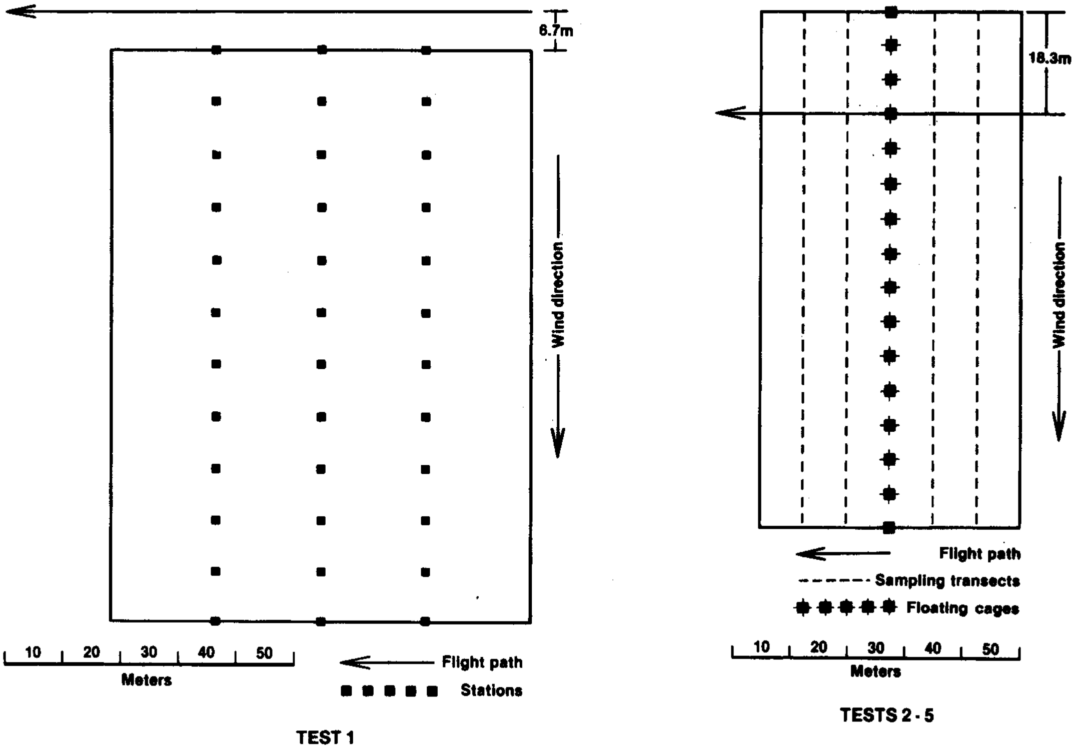
Three 100 m transects were established 18.3 m apart and parallel to wind direction. Each transect consisted of 12 stations spaced 9.1 m apart. Located at each station were two droplet sensitive cards (one located 5 cm above the water surface and one located directly above the canopy) and two floating cages, one open and one closed. Open cages were utilized to approximate the effect of the application on native larval populations, since *Bti* deposited in open floating cages was subject to dilution with ambient rice field water. Closed floating cages were used to determine if mortality at a given location was due to direct impact of *Bti* in the floating cage or movement of the material in the water. The objective was to determine if effective swath width resulted from direct impact of the *Bti* or movement subsequent to application. Six cages were used as checks (three open and three closed).

TEST 2. A 0.42 ha plot (45.7 × 91.3) was located in a 48 ha rice field. Rice was 0.7–0.9 m in height. Water depth was less than 5 cm. Efficacy of Beecomist-applied *Bti* from 18.3 m upwind to 73.2 m downwind of flight path was assessed (Fig. 1). Area upwind of flight path was monitored since region of deposition was inapparent in Test 1.

Treatment consisted of *Bti* applied in a single flight path over the plot 18.3 m downwind of the extreme upwind side of the plot. Flow rate was increased to 1.44 liter/min to insure a zone of high larval reduction, flow rate corresponding to 0.29 liter/ha. Wind speed at time of application (0700) was 4.8–8.0 km/hr.

Open floating cages were placed in the field immediately posttreatment at 6.1 m intervals in a line bisecting the plot 7.6 m from adjacent sampling transects. A total of 16 cages were installed from 18.3 m upwind to 73.2 m downwind of the flight path. Six open cages were used as checks. Naturally occurring larval populations were also monitored with sampling transects spaced at 7.6 m intervals. Sampling stations were established at 0.8 m intervals along each transect. One dip sample was taken at each station.

TESTS 3–5. Three 0.35 ha plots (38.1 × 91.3 m) were established; two in a 32 ha rice field (Tests 3 and 4) and one in a 64 ha rice field (Test 5). Rice in both fields was 0.7–0.9 m in height. Water depth was less than 5 cm in Tests 3 and 4, and 18 cm in Test 5. No addition of water occurred during the tests. Due to



TEST 1

TESTS 2-5

Fig. 1. Test format showing wind direction, flight path, sampling transects, and location of stations and floating cages in Tests 1–5.

problems associated with larvae in floating cages (predators, time constraints and inconsistency of results with previous field observations), floating cages were eliminated subsequent to Test 2. Treatments, application, flow rate, sampling scheme and sampling transects were as described for Test 2. Wind speed at time of application (0700–0800) was 4.8–8.0, 9.7–12.9, and 4.8–8.0 km/hr, for Tests 3, 4, and 5, respectively.

Tests 6–8. Results of Tests 1–5 indicated that overlapping swaths were necessary for adequate control of *An. quadrimaculatus* larvae. Test design for Tests 6–8 was modified to include three passes of the aircraft over the plot at varying intervals. Two plots [1.84 ha (91.5 × 200.9 m) and 2.17 ha (91.5 × 237.5 m)] were located in a 16.2 ha field (Tests 6 and 7, respectively) and a 2.84 ha plot (91.5 × 310.7 m) was located in a 32.4 ha field (Test 8). At treatment, rice in all tests was approximately 0.5–0.7 m in height and water depth was less than 5 cm. No addition of water occurred during the tests. Efficacy of Beecomist-applied *Bti* was assessed upwind, between flight paths and to 146 m downwind of the furthermost downwind flight path (Fig. 2).

Treatments consisted of *Bti* applied in swaths of 18.3 m in Test 6, 36.6 m in Test 7 and 73.2 m in Test 8. The extreme upwind flight path of each test was 18.3 m from the upwind side of each plot. Applications were made with specifications and equipment consistent with Tests 2– 5. Time of application was 0700–0845 with winds 4.8–8.0 km/hr in Tests 6 and 7, 1.6–4.8 km/hr in Test 8.

Four transects extended from 18.3 m upwind of the extreme upwind flight path to 146 m downwind from the furthermost downwind flight path. Fifty-eight sampling stations were established along each transect. Two dip samples were taken at each sampling station. Three stations were spaced 6.1 m apart in the 18.3 m zone upwind of the extreme upwind swath. The first station was located on the extreme upwind edge of each plot. Each of the three swaths in each plot was sampled at stations located directly beneath the flight paths with two stations equidistantly spaced between adjacent flight paths.

Sampling stations were concentrated in the zone downwind of the furthermost downwind flight path to determine the range of larval reduction corresponding to the effective swath width, i.e., that area downwind of the furthermost downwind flight path where adequate reduction of larval populations was achieved. Stations were located at 3 m intervals along

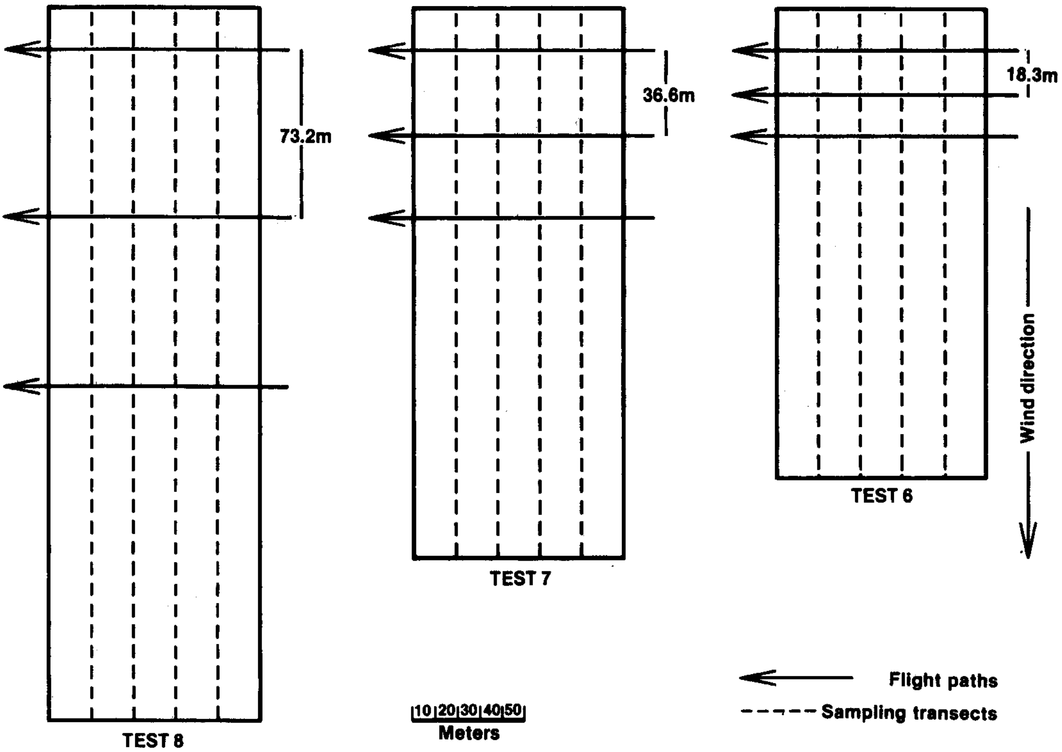


Fig. 2. Test format showing wind direction, flight path and sampling transects in Tests 6–8.

each transect in this zone. Stations were grouped at 12.2 m intervals (four stations/interval) for analysis of reduction in number of larvae/dip. Stations located between swaths and upwind of the furthermost upwind swath were not included in the statistical analysis. In addition to samplers and sampling intervals, flight path intervals were separated using Duncan's Multiple Range Test ($P = 0.05$). For each flight path interval, sampling intervals (four stations/interval) were plotted versus mean percentage reduction. A minimum of 80% reduction in number of larvae/dip was arbitrarily established as the standard for determination of optimum flight path interval and effective swath width was determined by extrapolation of the distance downwind of the furthermost downwind flight path where > 80% reduction in number of larvae/dip was achieved.

RESULTS AND DISCUSSION

Two approaches existed for estimation of optimum flight path interval. One approach was to estimate this distance based upon an estimated lethal field dosage (LDF_{90}) or some value representing a minimal dosage whereby overkill was avoided. Another approach was to disregard overkill and utilize a high flow rate in an attempt to increase downwind carry of the material. Since flight path interval is an arbitrary value, a constant flow rate was monitored over a range of flight path intervals.

In Test 1, Beecomist-applied *Bti* was monitored from 6.7 to 106.7 m downwind of flight path with droplet sensitive cards and floating cages containing *An. quadrimaculatus* larvae. Posttreatment observation of droplet sensitive cards revealed that *Bti* deposits were detected at all station intervals, from 6.7–106.7 m downwind of the flight path. Twenty-eight of the 36 cards (77.8%) placed 5 cm above the water surface were marked with *Bti* (Table 1). Cards placed above the canopy folded due to wind and were omitted.

Mortality of *An. quadrimaculatus* larvae in cages was low in Test 1. Corrected mortality averaged only 10.0% and ranged from 0 to 57%. Check mortality averaged 10.0 and 13.3%, respectively, for the open and closed floating cages. No significant difference in larval mortality among distances was revealed 24 hr posttreatment. Closed floating cages exhibited significantly (PR > F = 0.0009) higher levels of larval mortality (19%) than did open cages (3.7%). Higher mortality in closed cages may have been due to lack of circulation and subsequent dilution of *Bti* with ambient water. Significantly greater (PR > F= 0.0078) larval mortality was exhibited in the center transect (21.9%) than in the outside transects (6.8 and 4.5%). No significant differences were detected between the two outside transects.

Although larval mortality was obtained at the farthest stations (107.6 m downwind), *Bti* dosage was insufficient to provide adequate levels of control over any part of the plot. Based upon flight path interval utilized by Sandoski et al. (1985), flow rate corresponded to the LFD_{74} value. These results were inconsistent with previous studies (Yates 1984 and Sandoski et al. 1985) and field observations in which adequate control was obtained where *Bti* droplets were detected. Previous studies in which high levels of *An. quadrimaculatus* larval reduction were obtained with Beecomist-applied *Bti* (Sandoski et al. 1985) were conducted with wind speeds of 4–8 km/hr. Wind speed at time of application was 12.8–16.1 km/hr. Deposition of *Bti* may have been inconsistent with previous studies. In the test format for Test 1, placement of larvae and droplet sensitive cards required approximately 2.5 hr. Prediction of wind direction prior to plot layout was required. Investment of time and manpower precluded postponement of the test due to high wind speed.

Test format was subsequently modified to eliminate prediction of wind direction, allowing for postponement or cancellation of tests. Droplet sensitive cards were eliminated be-

Table 1. Application of *Bacillus thuringiensis* var. *israelensis* at varied rates via Beecomist® spray head against caged and naturally occurring populations of *Anopheles quadrimaculatus* larvae in rice fields.

Test number	Wind speed (km/hr)	Flow rate (liter/min)	% of droplet sensitive cards marked by *Bti* (below canopy)	Mean % mortality of larvae in floating cages[1]		Pretreatment mean ± S.E. of larvae/dip	No. of swaths and flight path (m)	Range and mean of larval reduction
				open cages	closed cages			
1	12.8–16.1	0.54	77.8	3.7B	19A	—	1	0–57.0 (10.0)
2	4.8–8.0	1.44	—	51.8[2]	—	0.36±0.03	1	0–98.0 (18.6)

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[1] Means of % mortality in Test 1 not followed by the same letter are significantly different (P=0.05); Duncan's multiple range test.

[2] Seven of 16 floating cages contained predators and were excluded from mean % mortality.

cause of dew associated with early morning applications. Open floating cages and naturally occurring populations of larvae were subsequently used to better estimate effective swath width of Beecomist-applied *Bti* against natural populations of *An. quadrimaculatus*.

In Test 2 and subsequent tests, flow rate was increased approximately 3X of that in Test 1. No significant difference was revealed for sampling intervals in Tests 2–4. In Test 2, reduction in number of larvae/dip averaged 18.6% (Table 1) and exceeded 80% at one sampling interval 10.2 m downwind of flight path). Results from floating cages showed no trend in mortality related to flight path location. Seven of the 16 cages contained predators at 24 hr posttreatment and were excluded from the results. The remaining nine cages averaged 51.8% corrected mortality. Check mortality averaged 10%. Due to consumption of larvae by predators, use of floating cages and laboratory-reared larvae was subsequently discontinued. Remaining tests were based solely upon monitoring of naturally occurring populations of larvae.

Reduction of larval populations was low across all distances in Tests 3–5 (Table 2). No significant differences were revealed among distances in any of the three tests. Mean reductions in number of larvae/dip were 42.0, 15.7 and 36.4% for Tests 3, 4, and 5, respectively. Range of reductions in number of larvae/dip were 3.2–85.4, 0–76.0, and 3.1–79.0% for Tests 3, 4 and 5, respectively. In Test 3, reduction in larvae exceeded 80% at two sampling intervals; 41.9 and 67.3 m downwind of the flight path.

Based upon the high levels of larval reduction exhibited by Beecomist-applied *Bti* at flow rates lower than used in Tests 2–5 (Sandoski et al. 1985), overlapping swaths were critical to achieving high levels of larval reduction. Thus in Tests 6–8, three passes of the aircraft were made over the plot to take advantage of overlap from adjacent swaths. Since flow rate was maintained at 1.44 liter/min dosage decreased as flight path interval increased. Flight path intervals were 18.3, 36.6 and 73.2 m for Tests 6, 7 and 8, respectively. In these tests displacement of *Bti* downwind was determined and arbitrarily established as that point downwind from the extreme upwind flight path where $>$ 80% reduction in number of larvae/dip was first achieved. Posttreatment reductions in larvae/dip indicated displacement of *Bti* approximately 10 m downwind in Test 6 (18.3 m flight paths) and 35 m downwind in Test 7 (36.6 m flight paths). This level of reduction was never achieved in Test 8 (73.2 m flight paths). Overall reduction means for *An. quadrimaculatus* larvae were 87.1, 84.9 and 16.2% for the 36.6, 18.3 and 73.2 m flight path intervals, respectively (Table 2). The former flight path intervals did not differ significantly but both were significantly different from the mean of the 73.2 m flight path intervals. A significant interaction between flight path interval and sampling interval was also indicated ($P > F = 0.0223$). As flight path interval and sampling interval increased, reduction in larval population decreased.

Reductions in number of larvae/dip exceeded 80% where flight path intervals were 18.3 and 36.6 m, respectively. The increase in flight path interval from 36.6 to 73.2 m decreased dosage and may have negated the effect of overlap from adjacent swaths, thus reducing the effect of the treatment. Based upon this level of reduction in number of larvae/dip, optimum flight path interval was estimated.

The 18.3 and 36.6 m flight path intervals maintained $>$ 80% reduction in number of larvae/dip for up to 67 m downwind of the extreme downwind flight path (Fig. 3). Optimal flight path interval was estimated to be approximately 67 m. The 73.2 m flight path interval never exceeded 80% reduction in

Table 2. Application of *Bacillus thuringiensis* var. *israelensis* at 1.44 liter/min via Beecomist® spray head against naturally occurring populations of *Anopheles quadrimaculatus* larvae in rice fields.

Test number	Wind speed (km/hr)	Pretreatment mean ± S.E. of larvae/dip	No. of swaths and flight path interval (m)	Range and mean of[1] larval reduction (%)
3	4.8– 8.0	0.65 ± 0.05	1	3.2– 85.4 (42.0)
4	9.7–12.9	0.55 ± 0.04	1	0.0– 76.0 (15.7)
5	4.8– 8.0	0.84 ± 0.05	1	3.1– 79.0 (36.4)
6	4.8– 8.0	0.67 ± 0.05	3 (18.3)	2.0–100.0 (84.9) A
7	4.8– 8.0	0.46 ± 0.03	3 (36.6)	16.0–100.0 (87.1) A
8	1.6– 4.8	0.78 ± 0.06	3 (73.2)	0.0– 80.0 (16.2) B

[1] Means of Tests 6, 7, and 8 not followed by the same letter are significantly different (P = 0.05); Duncan's multiple range test.

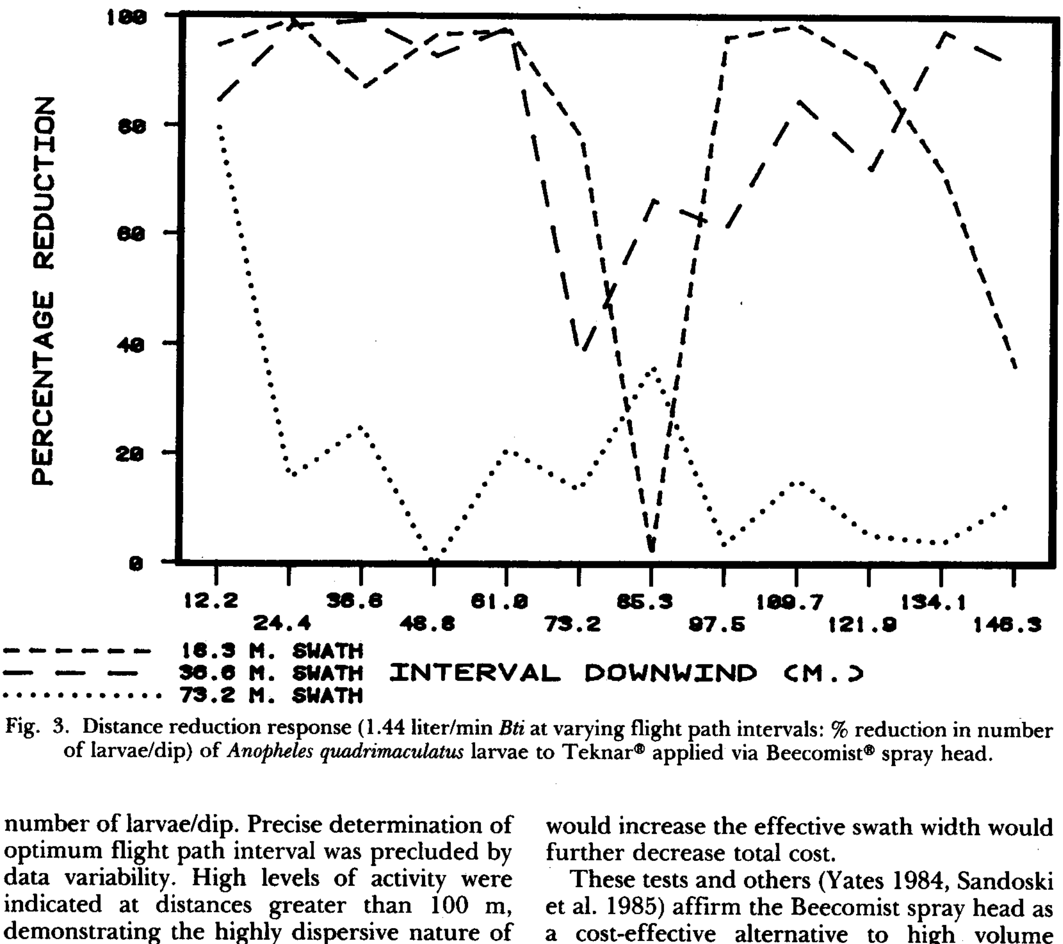

Fig. 3. Distance reduction response (1.44 liter/min *Bti* at varying flight path intervals: % reduction in number of larvae/dip) of *Anopheles quadrimaculatus* larvae to Teknar® applied via Beecomist® spray head.

number of larvae/dip. Precise determination of optimum flight path interval was precluded by data variability. High levels of activity were indicated at distances greater than 100 m, demonstrating the highly dispersive nature of Beecomist-applied *Bti*.

The estimate of optimum flight path interval (67 m) based upon tests 6 and 7 is open to question as flight path interval in Test 8 was 73.2 m and reduction in number of larvae/dip never exceeded 80%. Based on these results, further investigations should include tests in which large rice fields with naturally occurring populations of *An. quadrimaculatus* larvae are treated with Beecomist-applied *Bti* at 67 m flight path intervals and flow rates of 1.44 liter/min and lower. Effect of wind speeds greater than 8 km/hr should be investigated as effects on swath width area not conclusive. Data from Tables 1 and 2 suggest that wind speed in excess of 8 km/hr results in irregular deposition of material and reduced larval control. Interaction of those factors affecting Beecomist applications must be determined. Data presented here are unique for the aircraft and equipment involved.

Increases in flow rate accompanied by increases in flight path interval must be considered as cost of application currently exceeds cost of material. Any tactic which would increase the effective swath width would further decrease total cost.

These tests and others (Yates 1984, Sandoski et al. 1985) affirm the Beecomist spray head as a cost-effective alternative to high volume aerial application of *Bti* on large areas. As effective swath width, optimum flight path interval and flow rate are more precisely determined, cost of treating large areas of rice for larval mosquito control will be further decreased.

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