DROPLET SIZE AND SPRAY VOLUME EFFECTS ON COTTON CANOPY PENETRATION AND THIRD INSTAR *HELIOTHIS VIRESCENS* (Lepidoptera: Noctuidae) MORTALITY

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Abstract

Research was initiated to identify effects of droplet size and volumetric application rate on insect mortality and insecticide deposition when applied to cotton for control of *Heliothis virescens* (tobacco budworm). Karate (lambda cyhalothrin) 2.08 SC insecticide was applied to mature cotton in three distinct droplet sizes and three volumetric application rates. Third instar budworm mortality occurring in leaf-disk bioassays was highly correlated with insecticide deposits (μg/leaf disk). Mortality occurring at the upper canopy level was negatively correlated with volumetric application rate. At the upper canopy level, droplet size was positively correlated with deposits, and small droplets deposited significantly less insecticide than the medium and large droplets. Droplet size did not significantly affect larval mortality. Results from this study do not support a recommendation to either increase the volumetric application rate or decrease droplet size in order to improve insect control within a cotton canopy.

Introduction

Cotton insect control recommendations for insecticides typically include insecticide rate and volumetric application rate (VAR). Other parameters that may be governed by the applicator are generally ignored in recommendations because of lack of definitive research. Smith and Luttrell (1996) reviewed the literature concerning relationships between insect control and application variables and stated that 'the literature is essentially void of sound scientific data that relate physical properties of spray deposits to insecticide efficacy'. Those physical properties that can be manipulated by the applicator include dosage (amount of pesticide applied per unit area), droplet size (usually expressed as a volume median diameter), deposit density (related to droplet size and VAR), and pesticide concentration (related to VAR and dosage). Unfortunately, most pesticide efficacy research reports do not identify factors related specifically to droplet size and VAR, thus compounding the results and making it impossible to separate the value of the two factors in analyzing insect mortality and pesticide efficacy data.

The effect of droplet size on pesticide deposition and efficacy may be an important factor in row-crop pest control where coverage is a critical issue. There is some evidence that penetration into the cotton plant canopy is facilitated by smaller droplet size. Sumner et al. (unpublished research report) compared several sprayers with different volumetric application rates and droplet sizes and concluded that smaller droplet sizes tended to drift to the underside of cotton leaves and that larger droplet sizes were found deposited on the upper side of leaves. Similarly, aphid control with sprays of a contact insecticide applied at 3 gal/acre was found to be equal to or better than control by sprays applied at 5 or 10 gal/acre with dosage held constant but applied with different sized nozzles (Reed, unpublished research results). In that study, it was assumed that small droplets from the smaller nozzles provided better coverage under the leaves and deeper in the crop canopy than larger droplets from the larger nozzles. Smith and Luttrell (1987) reported that mortality of budworm larvae resulting from vegetable oil sprays of permethrin increased with decreasing droplet size, but droplet size of permethrin sprays in water did not affect mortality. Atypically large droplets applied with ULV sprayers have been avoided by heliothine larvae under some conditions, (Polles, 1968), a fact which adds yet another parameter to an already complex puzzle. These studies indicate that development of a recommendation for optimum droplet size would be of value to pesticide applicators; however, not all research in this realm has resulted in the same conclusion. Coates (1996) reported that white fly control and insecticide deposition was similar for applications made by electrostatic sprayers and a controlled droplet applicator, as compared to deposits by hydraulic nozzles. His conclusion indicated that variation in deposit was too great to separate the differences in either deposit or insect mortality between nozzle types.

Volumetric application rate is also a factor that may be manipulated by the applicator. When good coverage is important, as in herbicide application, a high VAR is usually recommended. It is also often recommended for insecticide applications, but in the case of insect control, little documentation exists to verify the utility of high VAR recommendations. The primary target of insecticide applications in cotton is the plant surface because it has been determined that insecticide residue is responsible for a high percentage of heliothine mortality on cotton (Wofford, 1985; MacQuillan et al., 1976). When dosage remains constant, concentration of the insecticide spray solution is inversely proportional to the VAR. Thus, when high volumetric application rates are used to help obtain or improve good coverage of the cotton plant, the concentration of insecticide in each droplet is reduced, resulting in yet another factor to be considered in recommendations for tobacco budworm control in cotton. Wofford et al. (1987) considered this factor in a laboratory study with leaf disks sprayed at different distances from the nozzle and reported that bioassay mortality of budworm larvae resulting from water carried permethrin increased with increased VAR.

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Because of the questions concerning the optimum droplet size and VAR for improving control within a cotton canopy, a test was designed to relate these factors to pesticide deposition and tobacco budworm (*Heliothis virescens*) mortality. The deposits and insect control at the top of the cotton plants in the test was of secondary importance. This and related research is designed to provide recommendations for the best possible use of insecticides for cotton pest control.

Materials and Methods

Suregrow 501 cotton variety was planted in 96.5 cm (38 in.) row-spacing on May 15 at the Black Belt Branch Experiment Station, Mississippi Agricultural and Forestry Experiment Station, Brooksville, MS. At the time of the applications on 21 and 28 August, cotton averaged 24 nodes and terminal height was approximately 4.5 ft (1.37 m) ± 12 in (0.3 m).

Nine nozzle/pressure/ground-speed configurations were chosen to provide three droplet sizes and three application rates within a range of grower acceptability (Table 1). The VMD (Volume Median Diameter) of a Karate 2.08 SC solution (dosage was 0.01 lb(AI)/acre) at three volumetric application rates was determined by replicated evaluations using a Malvern laser droplet analyzer (Model 2600Lc, Malvern Instruments Ltd., Spring Lane South, Malvern, Worchester, England). The nine treatments developed from five nozzle/pressure combinations delivered the following flow rates and standard deviations (SD) expressed in ml per minute: TX-4 at 40 psi, 257 (10.3); TX-6 at 54 psi, 415 (10.5); D2-23 at 22 psi, 258 (6.2); 8001 at 30 psi, 333 (5.13); D4-25 at 17 psi, 713 (18.0). Ground speeds were calculated using these flow rates in order to deliver 6, 12 or 18 gallons per acre (GPA).

Applications of insecticide were made with a high-clearance plot spray tractor using a compressed-air powered spray system. The boom was 6 ft (1.82 m) above the ground and approximately 16 in (40 cm.) above the cotton terminals. Nine nozzles were spaced at 19 in (48.3 cm) along the boom with one nozzle placed directly over each row and one between the rows allowing full coverage of four rows. Spray pressure was determined from a pressure gauge (0-60 psi) mounted on the boom. Plot size was four, 38 inch (0.96 m) rows by 50 ft (15.3 m). Tractor speed was set using an ultrasonic speed sensor and speedometer (Micro-Trak Systems, Inc., P.O. Box 3699, Mankato, MN). Because the ultrasonic sensor was influenced by the tall foliage, the speed was set in a bare turn row located beside the plots prior to spraying each treatment. Engine speed was constant throughout the application of any one treatment. Wind speed was negligible on 28 Aug, but was estimated to be 1-5 mph (1.61-8 km/h) on 21 Aug, blowing westerly in the same direction as the rows.

The statistical design was randomized complete block (five replications) with each VAR/droplet size combination considered as a treatment. The treatments were applied in sequence of nozzle type, speed and pressure to avoid continual changing of nozzles, speed and pressure settings. Because of the time it required to complete spraying and sampling, the test could not be completed in a single day as originally planned. Plots designated to be sprayed with the TX-4, D2-23 and 8001 nozzles at the 6 and 12 GPA rate were treated on 21 Aug, except that only 2 replicates using the 8001 nozzle at 12 GPA were sprayed. The remainder of the plots was treated on 28 Aug. Two additional plots located outside the replicated area were treated for the mid-sized droplet and 12 GPA on 28 Aug. to verify that the deposit and larval mortality results did not differ from that of 21 Aug. In addition, two untreated plots located outside the replicated area were sampled on 21 Aug. to provide results from untreated control plots on each day. Temperature and humidity at the beginning of the trials on 21 Aug at 2:00 p.m. were 92° F and 65% RH. Temperature and humidity at the start of the trial on 28 Aug were 91° F and 41% RH. The soil was dry and there was no dew on the plants during the trial.

Evaluation of the spray application involved both bioassay using third instar tobacco budworm (*Heliothis virescens* (Fabricius)) and gas chromatograph (GC) assay of insecticide deposits. Samples for GC analysis were collected immediately after the spray had dried on the cotton by collecting leaf discs from each of two levels by use of a leaf disc cutter. The upper level leaves were the first fully open leaves about six inches down from the terminals of the plants that would have an optimum chance for spray deposition. The mid-canopy level was composed of leaves with petioles originating from the main stem at approximately one half of the plant height down from the terminal, a position that would be difficult for spray to reach. Ten leaf disks (38.1 mm (1.5 in.) diameter) were taken from ten randomly chosen upper leaves in each plot and placed in a clean 100 ml glass container with a screw top containing a Teflon liner. These were immediately placed in an insulated container containing ice. A 10-leaf disk sample was similarly collected from the lower canopy level. The disks in each container were then submerged in 30 ml of hexane and washed for one min, after which the leaf disks were counted and removed and the rinsate was sealed and placed on ice in an insulated container. These were then held in a freezer at 0° F over the weekend and delivered to the GC laboratory on the following Monday morning. GC samples were analyzed at the Analytical Support and Food Safety Laboratory, Mississippi State University, Mississippi State, MS.

Budworm bioassay samples were made by separately collecting 20, 50 mm (1.97 in.) diameter leaf disks from randomly chosen leaves from each canopy level and placing them in a 7.6 cm (3 in) diameter petri dish. The Petri dishes were immediately placed in an insulated container that

contained ice. Leaf disks for bioassays were transported with the GC samples to the laboratory where disks were immediately placed individually in 50 mm (1.97 in.) diameter petri dishes with two, 50 mm diameter filter papers moistened with five drops of water dispensed with a disposable Teflon pipette. A single, third instar tobacco budworm reared on commercial insect diet was placed on the upper surface of the leaf disk in each dish. The dish was then closed with a lid and placed in an insulated container without ice at approximately 75° F and held for evaluation of mortality at 24h ± 1h. At 24 h, based on the approximate time of sampling in the field, the larvae were evaluated for mortality. Those larvae not responding to probing by a pencil point were considered dead.

Samples of each of the 6, 12 and 18 GPA spray solutions were collected at the field in 100 ml glass vials with Teflon lined caps. These were placed in insulated containers with ice and transported to the laboratory. In order to quantify deposits, 1.27 μg of active ingredient from each solution was micro-applicated on 3 replications of 10 leaf discs to provide a standard for calculating actual field deposits based on GC results. The average recovery of these samples was 61.6% of the amount applied with the micro-applicator. Mean corrected deposit results are shown in Table 2. Data were corrected for the 61.6% recovery rate prior to statistical analyses.

Results

There were no statistical differences in insect mortality or deposits between the same treatments applied on the two different days, and all data were subsequently analyzed as a single data set. The correlation between insecticide deposit in micrograms per disk and percent mortality was determined by determining the best fitting regression line (Figure 1) (Statgraphics Plus Statistical Package, nonlinear regression module, Manugistics, Inc., Rockville, MD). Three data points were determined to be outside the 95% confidence interval and were subsequently excluded from all analyses. The best fitting equation was percent mortality=73*deposit^0.389, and the adjusted R^2 value was 74.4. The R^2value indicates that 74.4% of the variation in the budworm mortality data was explained by the equation.

Statistical analyses were completed using data from plots receiving insecticide only since mortality of larvae from the untreated control was negligible and GC recovery of insecticide from leaf disks from control plots was essentially below the detection level. The analysis of variance summary (Table 3) indicates that significant differences (p=0.05) in larval mortality occurred between sampling levels and within volumetric application rates. The analysis of variance summary for insecticide deposits in μg per disk (Table 4) indicates that significant differences occurred in sampling level, droplet size, and volumetric application rate. The only significant interaction was the sampling level*VAR interaction related to larval mortality. Correlation coefficients indicate that mortality decreased as VAR increased, and that the deposited insecticide is positively correlated with insect mortality (Table 5).

Within the upper sampling level, the 18 GPA rate resulted in significantly less mortality than the 6 GPA rate (LSMEAN; P=0.0001) OR 12 GPA RATE (LSMEAN; P=0.0001). However, small droplets resulted in significantly lower insecticide deposits than did the medium (LSMEAN; P=0.0087) or large droplets (LSMEAN; P=0.0083). The deposits resulting from medium and large droplets did not differ. Droplet size caused no differences in percent larval mortality at the upper sampling level. Volumetric application rates did not affect the amount of insecticide deposit in the upper sampling level. The 18 GPA rate resulted in significantly lower mortality than did the other two volumetric rates (LSMEAN; P=0.0001). The linear regression of mortality on VAR for the upper canopy level was significant (R^2=0.46; P=0.0001), and the negative slope indicated an inverse relationship between mortality and VAR (Fig. 2). The significant (R^2=0.14; P=0.0104) regression of deposit on VAR for the upper level had a slight positive slope (Fig. 3).

The mid-plant sampling level resulted in only one significant effect: that of VAR on deposited insecticide. The 18 GPA rate resulted in significantly less insecticide deposit than the 6 (LSMEAN; P=0.0001) and 12 GPA (LSMEAN; P=0.0075) rates, and the 12 GPA rate resulted in significantly less recovery than the 6 GPA rate (LSMEANS, P=0.0196). Figure 4 indicates that the negative slope for the regression of deposit on VAR for the mid-plant sampling level was significant (R^2=0.38; P=0.0001).

Summary

Droplet size had no effect on budworm mortality at either canopy level. Deposits increased slightly with higher VAR at the upper canopy level and decreased slightly with higher VAR at the mid-plant canopy level. Mortality was negatively correlated with VAR at the upper canopy level. This indicates that the concentration of insecticide in each droplet (compounded in this study with VAR) may be a factor affecting larval mortality since insecticide concentration is inversely proportional to the VAR. Although some differences in insect mortality in this study were statistically significant, numerical differences between treatments were slight. However, the data indicate that lower volumetric application rates are as effective as the higher rates. If this holds true in commercial insecticide applications targeting indigenous budworm populations, considerable application time and cost can be saved by reducing VAR.

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Table 1. Nozzle, speed and pressure combinations used to obtain three volumetric application rates with 3 distinct droplet sizes. Volume Median diameter and the percent of the spray volume occurring in droplets <= 105 µm is listed parenthetically following the nozzle designation.

VAR	Nozzle	VMD (μm), % Drops<105μm	Speed (MPH)	Pressure (PSI)
6 GPA	TX4	121 μm, 38%	3.5	40
	D2-23	207 μm, 16%	3.6	22
	8001	284 μm, 8%	4.6	30
12 GPA	TX4	121 μm, 38%	1.8	54
	D2-23	207 μm, 16%	1.8	22
	8001	284 μm, 8%	2.3	17
18 GPA	TX6	123 μm, 38%	1.9	54
	D2-23	207 μm, 16%	1.2	22
	D4-25	302 μm, 6%	3.3	17

Table 2. Percent recovery (and standard deviation) of Karate 2.08 SC spray solution on leaf disks from two canopy levels, three volumetric application rates, and three droplet sizes. Data have been adjusted to account for the 61.4% recovery of known amounts applied to leaf discs with a micropipette.

Droplet Size	**Upper level**	**Mid-plant**
	6 GPA	
SMALL (121-123 μm)	68 (18.4)	34 (11.7)
MEDIUM (207μm)	109 (21.1)	44 (22.5)
LARGE (284-302 μm)	97 (11.6)	49 (12.9)
	12GPA	
SMALL (121-123 μm)	74 (25.0)	32 (8.4)
MEDIUM (207μm)	97 (21.4)	37 (15.0)
LARGE (284-302 μm)	95 (18.4)	27 (9.4)
	18GPA	
SMALL (121-123 μm)	83 (33.6)	15 (4.9)
MEDIUM (207μm)	81 (16.4)	19 (13.3)
LARGE (284-302 μm)	99 (28.6)	21 (8.7)

Table 3. Analysis of variance (Type III SS) summary of larval percent mortality for all data from upper and mid canopy level combined. D=Drop size; L=Level; R=Replicate; VAR=Volumetric Application Rate.

Source	DF	SS	Mean Square	F Value	Pr > F
R	4	453.86	113.46	0.64	0.6392
L	1	20890.28	20890.28	116.97	0.0001*
R*L	4	55.99	13.99	0.08	0.9886
VAR	2	4348.70	2174.35	12.17	0.0001*
D	2	960.43	480.21	2.69	0.0758
VAR*D	4	1204.50	301.12	1.69	0.1643
VAR*L	2	1317.67	658.83	3.69	0.0305*
D*L	2	453.49	226.74	1.27	0.2880
VAR*D*L	4	400.47	100.11	0.56	0.6921

Table 4. Analysis of variance summary (Type III SS) deposits (μg/disk) for all data from upper and mid canopy level combined. D=Drop size; L=Level; R=Replicate; VAR=Volumetric Application Rate.

Source	**DF**	**SS**	**Mean Square**	**F Value**	**Pr > F**
R	4	0.38	0.09	1.95	0.1123
L	1	11.63	11.63	239.36	0.0001*
R*L	4	0.45	0.11	2.33	0.0654
VAR	2	0.47	0.23	4.86	0.0109*
D	2	0.55	0.27	5.75	0.0051*
VAR*D	4	0.28	0.07	1.45	0.2267
VAR*L	2	0.25	0.12	2.60	0.0819
D*L	2	0.18	0.09	1.87	0.1621
VAR*D*L	4	0.16	0.04	0.83	0.5096

Table 5. Correlation between volumetric application rate (VAR), droplet size, percent mortality and deposit (μg/disk) of Karate for all data from both sampling levels combined. Marked (*) correlations are significant at p<0.05 (Pearson's correlation coefficient. Data exclude results of the untreated check and outliers.

	VAR	Drop Size	% Mortality	Deposit
VAR	1			
Drop Size	-0.05	1		
% Mortality	-0.28 *	-0.08	1	
Deposit	-0.18	0.14	0.71 *	1

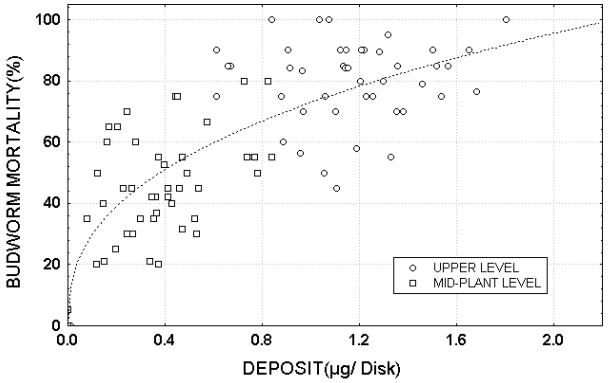

Figure 1. Scatterplot of mortality (%) of third instar tobacco budworm larvae versus deposit (μg/disk) for all droplet sizes, volumetric application rates and sampling levels combined. The nonlinear regression line (y=73*x^0.389) indicates a significant, positive relationship between larval mortality and deposit. R2=74.4.

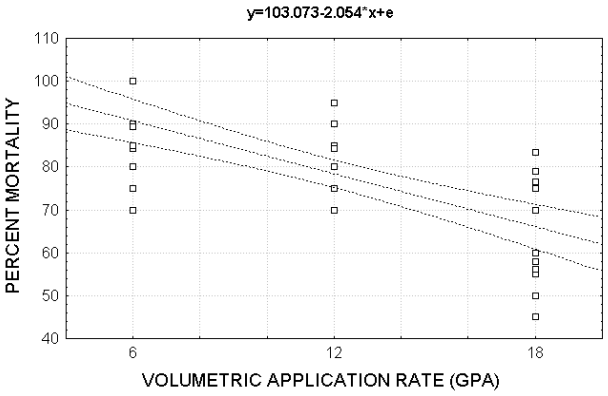

Figure 2. Percent larval mortality related to volumetric application rate in the upper sampling level (linear regression and 95% confidence interval (CI). R^2=0.46, significant at P=0.0001.

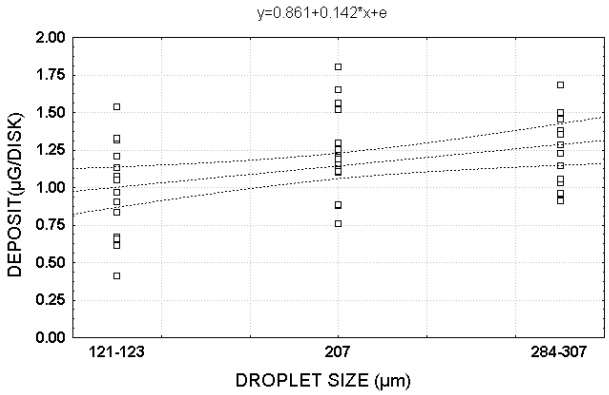

Figure 3. Deposit related to droplet size for upper sampling level (linear regression and 95% CI). R2=0.14, significant at p=0.0104.

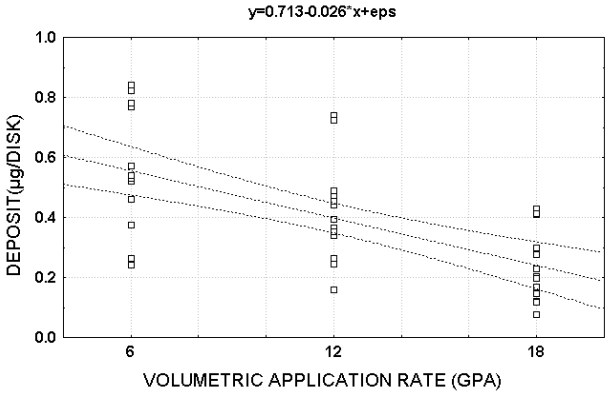

Figure 4. Deposit related to volumetric application rate at the mid-plant sampling level (linear regression and 95% CI). R^2=0.38, significant at P=0.0001.