Field Test with Helicopter Applications of Gardona Against Gypsy Moth in Pennsylvania

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ABSTRACT

In 1972, Gardona® WP was field tested against the gypsy moth, *Porthetria dispar* (L.), in Pennsylvania. Treatments of 1 gal/acre were applied by helicopter on six 100-acre plots, three at 1 pound AI/gal and three at 1.5 pound AI/gal. Check subplots of 0.1-acre were established throughout the area. Before and after spraying, evaluations were made by counting all visible egg masses and estimating defoliation to the nearest 10 percent. Kromekote cards and aluminum plates were used for qualitative and quantitative spray-deposit assessment. Leaves, soil, and water samples were collected at 1, 3, 6, 12, and 24 days after spraying for residue analysis. Analytical methods for determining residues are presented. Increases in both egg-mass density and percentage of defoliation were found after all treatments. Analysis showed no significant differences between the two doses nor between either of the two doses and the check. The kromekote cards and aluminum plates revealed minimal coverage (drops/cm^2) and deposits (gpa) at ground level. Data suggest that Gardona failed to affect the gypsy moth because (1) the study area had above-average precipitation, (2) the spray deposit and residue were highly variable, and (3) the residue dissipated rapidly from the leaves.

THE GYPSY MOTH, *Porthetria dispar* (L.), since its accidental introduction into the eastern United States, continues to spread and to defy a wide range of control actions. Increases in gypsy moth population to the west and south, resultant defoliation, and new spot infestations occur with increasing frequency.

Eradication and suppression of the gypsy moth, from 1890 to 1943, were attempted by applying creosote to egg masses, banding trees with burlap and sticky materials, spraying with lead arsenate *(Burgess 1930)*, and importing natural enemies from Europe and Japan *(Brown and Sheals 1944)*. From 1944 through 1957, DDT was used in control and eradication programs *(Nichols 1973)*. Use of the insecticide carbaryl (Sevin) as a substitute for DDT was begun in 1958 because of public concern over DDT residues.

Because carbaryl and trichlorofon (Dylox) are now used almost exclusively for gypsy moth control and containment programs, the need for other environmentally safe and nonpersistent insecticides became evident. Therefore the Animal Plant Health Inspection Service (APHIS) continued laboratory screening of named candidate toxicants against the gypsy moth in order to expand and create more chemical diversity in gypsy moth spray programs. Those toxicants that showed promise were to be field-tested by the Northeastern Forest Experiment Station's Forest Insect and Disease Laboratory to determine if the chemicals merited pilot-testing for operational feasibility by the Northeastern Area State and Private Forestry organization.

In early 1972, APHIS recommended that Gardona® (2-chloro-1-(2,4,5 trichlorophenyl) vinyl dimethyl phosphate) 75 percent WP be field-tested. Doane *(1964, 1966)* in earlier tests had shown Gardona[1] to be a promising toxicant against the gypsy moth when applied by backpack mistblower.

[1] Reg. U.S. Patent Office, by Shell Chemical Co., San Ramon, Cal. Mention of a proprietary product should not be taken as an endorsement by the U.S. Department of Agriculture, the Forest Service, or Rutgers University.

METHODS AND MATERIALS

Plot Layout

Six 100-acre plots were established in the Delaware State Forest near Marshall Creek, Pennsylvania. Five 0.1-acre (66X66-foot) subplots were randomly scattered in each 100-acre spray plot. All subplots were located at least 330 feet inside the plot boundary and were at least 198 feet apart. Fifteen 0.1-acre check subplots were scattered throughout the area, at least ¼ mile from any treated plot. Subplots were used to determine egg-mass densities and defoliation, and to serve as points of leaf and soil collections for residue analysis. Subplots were also used for sampling and determining the spray coverage and the amount of spray deposit reaching the forest floor under a closed canopy.

Plots were marked for spraying with bed sheets tied over 15- to 20-foot saplings nailed atop large corner trees. After spraying, all treated and check subplots were similarly marked with sheets to assist in identifying them on aerial photographs.

Treatments

Three plots were treated at the dose of 1 pound AI/gal and three plots at 1.5 pound AI/gal. No spray sticker was mixed with the formulation, but a measured quantity (0.1 percent w/v) of fluorescent tracer (Brilliant Sulfo Flavin FFA, General Analine and Film Corp.)[1] was included to aid in spray-deposit evaluation.

Four plots were sprayed on 2 June and two plots on 3 June, using a Bell 47G-2 helicopter equipped with 30 hollow-cone disk-type teejet nozzles having D4 tips and No. 25 cores on a 30-foot boom. Boom pressure was about 48 psi, and the flow rate was 11.5 gal/min. The helicopter was flown 35 feet above the canopy at 60 mph, was calibrated at 1 gal/acre, and had a swath width of 95 to 100 feet. The nozzle orifices where directed downward at 90° to the thrust line of the helicopter.

This equipment combination was chosen to produce an unconfirmed spray atomization of 250 to 300 μ in volume median diameter (vmd). (Volume median diameter is the drop diameter that divides the spray volume in half; 50 percent of the spray volume is in drops of larger diameter and 50 percent in drops of smaller diameter.)

By 2 June, leaves were at least 50 percent expanded, and most gypsy moth larvae were in their second stadium. At each spraying, wind speeds were 3 mph or less. On 2 June, 4.5 hours after spraying, 0.28 inch of rain fell in the area. Also on 3 June, 8 hours after spraying, 0.08 inch of rain fell. Rain fell on 26 days, totaling 8.85 inches for June, which was 5.02 inches above normal.

Egg-Mass Counts

Before and after spraying, estimates were made of egg-mass densities by counting all visible egg masses in each 0.1-acre subplot, using binoculars when necessary. No attempt was made to count egg masses under rocks and debris. The mean number of egg masses in the five subplots per treatment plot was used to estimate number of egg masses per acre.

Defoliation Estimates

All trees of the white and red oak groups having a dbh of 2 inches or greater were number-tagged in each 0.1-acre subplot. The average number of preferred host trees in the treated and check subplots was 22, range 3 to 34. Defoliation estimates were made in increments of 10 percent on all numbered trees. Before-spraying foliage estimates were begun the first day of spraying (2 June) and were completed by 5 June. After-spraying foliage estimates were begun on 26 July and were completed on 1 August. The same individual made both before and after foliage estimates to minimize subjective error.

Spray-Deposit Assessment

Kromekote cards and aluminum plates were placed in each 0.1-acre subplot in all treated plots for qualitative and quantitative spray-deposit assessment. The forest canopy

in all subplots was judged to be the closed type. The kromekote cards, eight per subplot, were placed along two sides of each subplot border in the direction of the flight path for determining the number of spray drops reaching the forest floor, thus providing an index of deposit coverage. Four aluminum plates were placed in each subplot to sample spray deposit reaching the forest floor for the quantitative determinations of deposit in terms of gallons per acre (gpa). Cards and plates were supported by wire card holders *(Maksymiuk 1959)*. Cards and plates were collected for assessment immediately after spraying.

The spray drops on the kromekote cards were counted under a dissecting microscope with a UV illuminator. The quality of spray deposit was determined by taking the mean number of drops from 40 cards for each treatment combination, expressed as number of drops/cm^2.

The quantity of spray deposit was determined by washing the aluminum plates with 10 ml of 95-percent ethanol. The concentration of fluorescent Brilliant Sulfo Flavin FFA dye was determined with a spectrofluorometer. The deposit removed from the aluminum plates was converted to gpa from the actual concentration of dye in the formulation.

Sampling and Preparation for Residue Analysis

Available analytical methods were combined and modified for more efficient chemical assay of residues *(Shell 1967, 1970; Beroza and Bowman 1966)*.

Leaf and soil samples were collected from each treatment subplot and from 6 of the 15 check subplots on 3, 5, 8, 14, and 26 June. Separate leaf samples were taken from both upper and lower sections of the same tree crown. Each sample was a composite from the four tree quadrants. Water was sampled at nine locations in and adjacent to the various plots. Soil samples were taken at random points within the subplots.

Leaf samples were frozen, chopped in a Hobart food chopper, and refrozen to await analysis. Soil samples were frozen for storage, then thawed and screened to remove leaves, roots, and stones before extraction. Water samples were refrigerated until analyzed.

Extraction from Leaves

For each assay, a 100-g sample of chopped frozen leaves was placed in a mason jar, 400 ml of 1:3 hexane-isopropanol solution was added, and the mixture was blended for 3 minutes on a high-speed blender. The sample was filtered directly into a 500-ml graduated cylinder through a plug of glass wool in a powder funnel.

The solution was poured into a 500-ml separatory funnel containing 50 ml of distilled water and shaken. The wash was repeated six times, using 100 ml of distilled water for each wash. The wash water was discarded. The emulsion formed was broken by stirring with a glass rod. The hexane extract was stored in glass bottles in the refrigerator over 5 g of sodium sulfate. In the case of very low residue values (<0.10 ppm), 50 ml of the solution were concentrated to 10 ml by using a flash evaporator with water bath at 40°C.

Extraction from Soil

Each 40 g of frozen and sifted soil sample was placed in a 500-ml Erlenmeyer flask and slurried with 25 ml of distilled water. To the flask 200 ml of acetonitrile were added, and the flask was shaken for 1 hour on a Burrell wrist-action shaker. The solution was filtered through fluted Whatman No. 1 filter paper into a 100-ml graduated cylinder, and 75 ml were poured into a 250-ml separatory funnel containing 100 ml of distilled water. Gardona was extracted by using three aliquots hexane: 60 ml, 40 ml, and 40 ml respectively. Each time, the acetonitrile-water layer was removed into the beaker, and the hexane was run into a 250-ml Erlenmeyer flask. The acetonitrile-water was returned to the separatory funnel for the next extraction. When the extractions were completed, the hexane solution was dried over 15 to 20 g sodium sulfate for at least 15 minutes. The extract was decanted into a clean Erlenmeyer

flask and condensed to 1 ml, using a concentrating tube and flash evaporator with water bath at 40°C.

Extraction from Water

An 800-ml water sample was saturated with sodium chloride (about 290 g) in a 1-liter Erlenmeyer flask. The saturated solution was poured into a separatory funnel and shaken for 3 minutes with 800 ml hexane. The hexane layer was removed to a 1-liter flask and dried over 50 g sodium sulfate. The extraction was repeated with the same 800-ml water sample. The extracts were combined and condensed to 1 ml by flash evaporation with water bath at 40°C.

Gas Chromatography Measurements

Instrument: Micro Tek 220; detector: flame photometric; inlet temp: 220°C; column temp: 220°C; detector temp: 215°C; column: 36 X ¼ inch O.D. aluminum; packing: 5 percent OV 101 on acid washed chromosorb W; nitrogen: 100 ml/min; hydrogen: 100; oxygen: 25; injection: 5 μl.

RESULTS AND DISCUSSION

Increases in egg-mass densities were found after every treatment combination (table 1). Generally, those plots having the lowest initial egg-mass density had the highest percentage increase. Analysis (ANOVA) showed no significant difference in egg-mass counts between the two levels of treatment; nor was there any significant difference between any treatment combination and the check.

All treatment combinations showed increases in average percentage of defoliation. Those treatment combinations having higher before-spraying egg-mass densities generally had greater after-spraying defoliation. Although there is numerical evidence of some foliage protection, there was no significant difference in after-spraying defoliation between the two doses and no significant difference between either of the two doses and the check.

Quantitative and qualitative data obtained from the aluminum plates and kromekote cards show tremendous variation in both gpa and number of spray drops/cm² at ground level (table 2). The overall range of deposit on aluminum plates was 0.0001 to 0.066 gpa. The mean gpa deposit for 1.0 pound AI/gal was 0.00507 and for 1.5 pound AI/gal was 0.01592.

No kromekote card in any subplot had a droplet density range of 15 or more drops/cm². Few cards fell in the medium range of 5 to 15 drops/cm². More than 90 percent of all cards were in the light range—five or fewer drops/cm². The mean number of drops/cm² for 1.0 and 1.5 pound AI/gal was 2.41 and 1.75 respectively.

There was great variation in residue among subplots within a given treatment combination (table 3). For example, residues 1 day after treatment ranged from

Table 1.—Mean estimates, before- and after-spraying, of egg masses/acre and defoliation, by plot and treatment

Treatment (lb AI/acre)	Average egg masses/acre			Average defoliation		
	Before	After	Net increase	Before	After	Net increase
	No.	*No.*	*No.*	*Pct.*	*Pct.*	*Pct.*
1.0	320	2,812	2,492	6	19	13
1.0	368	2,070	1,702	8	18	10
1.0	1,976	3,758	1,782	7	29	22
Mean	956	2,935	1,979	7	22	15
1.5	732	2,586	1,854	16	25	9
1.5	844	4,276	3,432	10	27	17
1.5	3,244	5,114	1,870	8	32	24
Mean	1,607	3,992	2,385	11	28	17
None	1,241	5,541	4,300	8	34	26

Table 2.—Quantitative and qualitative spray-deposit assessment of Gardona 75 percent WP applied by helicopter

Treatment (lb AI/acre)	Aluminum plate, deposit		Kromekote cards, drops/cm²	
	Average	Range	Average	Range
	gpa	*gpa*	*No.*	*No.*
1.0	0.00092	0.0007 - 0.0013	3.13	1.59 - 6.09
1.0	.00842	.0004 - .0251	2.27	.59 - 4.44
1.0	.00587	.0007 - .0250	1.82	.50 - 3.50
Mean	.00507	—	2.41	—
1.5	.02784	.0069 - .0573	3.03	2.00 - 5.19
1.5	.00135	.0001 - .0073	.70	.31 - 1.00
1.5	.01857	.0031 - .0660	1.51	.97 - 2.34
Mean	.01592	—	1.75	—

Table 3.—Mean Gardona residues on leaves, in soil, and in water, assayed by gas chromatography
[Data pooled to plots and treatments; Rutgers University, 1972]

Treatment (lb AI/acre)	Leaves (days after treatment)					Soil (days after treatment)					Water (days after treatment)		
	1	3	6	12	24	1	3	6	12	24	1	3	6
	ppm	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppb*	*ppb*	*ppb*
1.0	12.35	2.85	1.50	0.45	0.08	0.27	0.16	0.14	0.36	0.16	—	—	—
1.0	14.19	3.06	1.52	.76	.08	.05	.26	.28	.28	.14	0.4	6.3	0.2
1.0	18.94	1.06	1.40	.65	.03	.12	.14	.25	.13	.07	2.8	1.1	.4
Mean	15.16[1]	2.32	1.47	.62	.06	.15[2]	.20	.22	.26	.12	1.6[3]	3.7	.3
1.5	25.70	3.78	2.30	.80	.11	.23	1.52	.78	.37	.46	—	—	—
1.5	17.00	4.15	2.30	.86	.05	.18	.31	.34	.20	.15	3.1	.8	.1
1.5	24.30	2.78	1.98	.80	.05	.43	1.57	.28	.27	.23	1.8	1.2	.2
Mean	22.33	3.57	2.19	.82	.07	.28	1.13	.47	.28	.28	2.5	1.0	.2
None	.11[4]	.13	.03	.04	.0	.02[5]	.04	.01	.07	.0	.1[6]	.2	.1

[1] Mean based on 30 assays; [2] 15 assays; [3] 2 assays; [4] 12 assays; [5] 6 assays; [6] 3 assays.

0.09 to 100.7 ppm on leaves. At 3 days after treatment the range was not nearly as great: 0.09 to 14.7 ppm. Residues on leaves continued to dissipate rapidly, and at 24 days after treatment 10 of the 30 subplots had no detectable residues.

Although the leaf assay means of the two doses at 1, 3, and 6 days after treatment showed a difference of about 50 percent, corresponding to the difference in pound AI/gal applied, and in the expected direction, the variation in residue was so large that an analysis to determine differences between the two doses could not be established. Furthermore, in laboratory tests conducted by APHIS, second-instar gypsy moth larvae were fed an artificial diet fortified with levels of Gardona ranging from 63 to 1,000 ppm; and at 63, 167, and 200 ppm, which correspond somewhat to ppm residues detected on leaves, larval mortality was <10 percent after 72 hours *(McLane 1973)*.

Residues in soil did not exceed 2.9 ppm for any sample at any time. For water, the highest level detected was 6.3 ppb; and by 6 days after treatment, residues had dissipated to <0.4 ppb.

The failure of Gardona to either protect foliage or to reduce egg-mass density may have resulted from one or any combination of the following: (1) above-average rainfall in the study area (June 8.85 inches, July 5.06 inches); (2) insufficient and highly variable spray deposit, demonstrated by data from the qualitative and quantitative spray-deposit assessment; and (3) rapid dissipation of residues from leaves, confirmed by the chemical assay.

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