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Pilot Control Project Using Trichlorfon and *Bacillus thuringiensis* against Forest Tent Caterpillar in Southwest Alabama

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PILOT CONTROL PROJECT USING TRICHLORFEN
AND BACILLUS THURINGIENSIS AGAINST
FOREST TENT CATERPILLAR IN SOUTHWEST ALABAMA

By

Lawrence P. Abrahamson*
Senior Research Associate
State University of New York
College of Environmental Science and Forestry
Syracuse, N.Y. 13210

James D. Harper
Insect Pathologist
Department of Zoology-Entomology
Auburn University Agricultural Experiment Station
Auburn University, Ala. 36849

Ira I. Ragenovich*
USDA Forest Service
Entomologist
Southwestern Region
Albuquerque, N.M. 87102

James R. Hyland
Entomologist
Alabama Forestry Commission
Montgomery, Ala. 36104

*At the time of this project, Abrahamson's position was Pesticide Specialist, Southeastern Area, State and Private Forestry, Atlanta, Ga., and Ragenovich's position was Entomologist, Southeastern Area, State and Private Forestry, Pineville, La.

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PILOT CONTROL PROJECT USING
TRICHLORFON AND BACILLUS THURINGIENSIS
AGAINST FOREST TENT CATERPILLAR
IN SOUTHWEST ALABAMA

SUMMARY

Registered rates of trichlorfon and Bacillus thuringiensis were applied aerially to forest tent caterpillar (Malacosoma disstria Hübner) infested water tupelo ponds in Alabama in March and April, 1975. Data were collected to determine the effectiveness of these insecticides in preventing defoliation on large scale plots, and to determine their impact on selected nontarget organisms in a swamp situation.

Aerial application of trichlorfon provided rapid and nearly complete collapse of the forest tent caterpillar population in a 900-acre (364 ha) pond. Timing and application parameters were near optimum for this treatment. Treatment efficacy was evident from heavy larval mortality, cessation of defoliation, and an increase in canopy foliage which was measurable within a week following application. Foliage loss in the untreated control block reached nearly 95 percent in this same period. Population reduction was sufficient to prevent occurrence of damaging populations in 1976.

B. thuringiensis provided excellent kill when applied against late instar larvae. However, aerial application of B. thuringiensis over 1500 acres (607 ha) of tupelo was delayed 5 days past the planned application date because of inclement weather. This delay, coupled with poor coverage, resulted in inadequate protection of the forest canopy. The succeeding generation was heavy enough to completely defoliate the canopy in 1976.

Studies on the impact of both insecticides on nontarget organisms showed no acute toxicity to birds following treatment, nor to turkeys which were force fed trichlorfon-killed tent caterpillar larvae. No differences attributable to the treatments were detected in zooplankton, aquatic macroinvertebrate, or phytoplankton populations within the plots.

In the forest canopy, significant numbers of nontarget terrestrial invertebrates were apparently

killed following treatment with trichlorfon. Treatment with B. thuringiensis resulted in much lower levels of mortality of nontarget organisms which, during the first 7 to 10 days following treatment, were equal to or less than that measured in the untreated control plot. Subacute levels of trichlorfon residues were detected in tissue analyses of birds, snakes, mammals, and snails 3 and 26 days after treatments.

INTRODUCTION

The forest tent caterpillar, Malacosoma disstria Hübner, has been epidemic in swamp lands consisting of water tupelo, Nyssa aquatica L., and blackgum (swamp tupelo), Nyssa sylvatica var. biflora (Walt.) Sarg., in southern Louisiana and Alabama for many years (Morris, 1965). Tupelo and blackgum have been partially to completely defoliated by the forest tent caterpillar on an average of 500,000 acres (202,350 ha) per year in Louisiana and 50,000 acres (20,235 ha) per year in Alabama during the last 20 years. An unusually heavy infestation in Alabama in the late 1950's and early 1960's prompted local landowners to support the work of Federal and State entomologists in determining methods for controlling the pest. Several chemicals were tested for efficacy in 1960-61. DDT was found to be effective, but was considered potentially dangerous to the extensive wildlife habitat involved. Further tests concerning minimal rates and environmentally safe insecticides were planned but were never conducted (Morris and Orr, 1962).

Further interest in developing insecticides for control of the tent caterpillar was shown in 1968. Two insecticides, trichlorfon and Bacillus thuringiensis, were found to be highly effective in small plot tests (Abrahamson and Harper, 1973; Abrahamson and Morris, 1973; Harper and Abrahamson, 1979) while presenting low environmental hazard. Both were subsequently registered for aerial application against this pest.

In 1969, Abrahamson (unpublished data) initiated a 5-year impact study to determine the effects of tent caterpillar defoliation on incremental growth of water tupelo. The study was located in the Mobile-Tensaw River Basin near Mobile, Ala. Results of this study have indicated that water tupelo, which is repeatedly defoliated

by forest tent caterpillar, will sustain an average incremental growth loss of approximately 45 percent. These results suggest the desirability of population management to reduce growth losses.

In the fall of 1974, a cooperative pilot project was established by the USDA Forest Service, the Alabama Forestry Commission, Auburn University, and Scott and International paper companies to investigate the effectiveness and environmental impact of trichlorfon and B. thuringiensis when applied on an operational basis. The treatments were applied and the major portion of the information for the impact studies were collected during the spring of 1975. Monitoring of forest tent caterpillar populations was continued for 2 years after treatment, until the summer of 1977, to assess possible long-term population suppression.

The objectives of this pilot project were: (1) to determine if the registered rates of trichlorfon and B. thuringiensis would significantly prevent forest tent caterpillar defoliation on large scale plots and (2) to determine the environmental impact of trichlorfon and B. thuringiensis on selected nontarget organisms in the Mobile-Tensaw River Basin.

METHODS

Plot Selection

The criteria for plot selection included: (1) a similar past history of defoliation by the forest tent caterpillar based on the amount of defoliation over the past 10 years; (2) the presence of natural or manmade boundaries (rivers, lakes, levees, powerlines, etc.) to minimize reinfestation possibilities from adjacent untreated areas; (3) approximately the same established population levels based on egg mass counts made during plot selection; and (4) ease of access.

Initial field work began in mid-February, 1975. Branch samples were shot from trees, using .22 magnum rifles and hollow point bullets, to determine potential larval populations. Estimates were based on the number of egg masses per 4-foot branch tip. Normally, 2.5 egg masses per branch sample means that complete defoliation can be expected (Abrahamson and Harper, 1973). Three areas of approximately 1,000 acres (404.7 ha) and

one area of approximately 500 acres (202 ha), all having sufficient forest tent caterpillar populations based on egg mass count, were delineated. Compass lines were flagged to mark boundary lines when natural or obvious boundaries, such as rivers or powerlines, could not be used.

The corners of all plots were marked for pilot orientation by placing nylon parachute panels in tree tops using the method described by Abrahamson and Harper (1973).

An arrow attached to a nylon fishing line was shot over two trees on the corners to be marked. Nylon parachute cord was tied to the fishing line and pulled over the trees. The parachute panel was then attached to the cord, pulled up (figure 1), positioned between the tops of the trees, and tied securely in a position that would be visible from the spotter and spray planes.

A 1,000-acre (404.7 ha) plot received no treatment and served as the control or check plot. A 900-acre (364 ha) plot was treated with trichlorfon (Dylox 4)⁵ and is hereafter referred to as the Dylox plot. A 1,000-acre (404.7 ha) plot was treated with B. thuringiensis (Dipel WP)⁶ and is hereafter referred to as the Dipel I plot. A 500-acre (364 ha) plot was treated with two applications of B. thuringiensis about a week apart and will be referred to as the Dipel II plot.

The Dylox plot (figure 2) was located in a bend of the Tensaw River near Stockton, Ala., in Baldwin County. It was surrounded on three sides by the Tensaw River. The east branch of the "T" in Mifflin Lake provided access to the west side of the Dylox spray plot. This area had an average egg mass count of 3.81 per branch sample.

The Dipel I plot (figure 2) was located between Middle River on the east and Bayou Zeast on the west. The old power line served as the south boundary and the new power line served as the north boundary, with access on all sides. This area had an average egg mass count of 6.79 per branch sample.

⁵Agricultural Chemicals Division, Mobay Chemical Corp., Kansas City, Mo.

⁶Abbott Laboratories, North Chicago, Ill.

Figure 1.—Hoisting a nylon parachute panel to mark the corners of the spray plots. (photo by James D. Harper)

Figure 4.—Three-hundred gallon (1,135.6 L) mixing tanks, recirculating apparatus, and pumps used in mixing the B. thuringiensis formulation.

Figure 3.—Typical subplot with three sample trees in the forest tent caterpillar pilot control project. Nets were part of a non-target organism study conducted in conjunction with the project (see Appendix IV). (Photo by James D. Harper)

Figure 2.—Location of the forest tent caterpillar project plots in the Mobile-Tensaw River Basin, Ala., 1975. Arrows through plots indicate the location of the subplots.

The Dipel II plot (figure 2) was located next to the Dipel I plot to facilitate the application of Dipel. It was bounded by Bayou Zeast on the east and south, the Mobile River on the west, and the new powerline on the north, with access on all sides. This area had an average egg mass count of 5.50 per branch sample.

The control plot (figure 2) was located about 1 mile north of the Dipel plots and 2 miles west of the Dylox plot, to minimize the possibility of water contamination and drift from the sprayed plots. It was located between the Middle River on the west, Bottle Creek on the east, and Alligator Lake and Dominic Creek on the north. This plot had an average egg mass count of 5.01 per branch sample.

Subplot Selection

A diagonal line was marked with flagging tape through each of the four plots (figure 2). Twenty subplots were located at equal intervals along each line. The first and last subplots were located at least 500 feet (155 m) from the plot boundary to avoid any edge effect. Three dominant and/or co-dominant water tupelo or blackgum trees in each subplot were selected and individually marked and identified with tree marking paint, providing 60 sample trees per plot (figure 3).

Determination of Optimal Spray Date

Beginning in early March, egg masses were taken from tree tops approximately twice a week to determine the initial hatch date and to monitor subsequent larval development. Treatment of the plots was begun when the tupelo leaf expansion was one-half completed, and the insects were at least in the second instar of development.

Aerial Application

Materials and formulations used in the project were as follows:

* Trichlorfon: .75 pound (680 g) a.i. per acre, applied at the rate of 24 ounces (680 g) of trichlorfon per acre + Rhodamine B Extra Base.⁷

⁷Rhodamine B Extra Base (General Analine Film Corp.) was formulated in tetrahydro-furfuryl alcohol. Both dyes were added at the rate of 3.87 g per gallon at 0.1 percent (weight per volume) of final spray.

* B. thuringiensis: One application at the rate of 3.6 BIU per acre⁸ (.5 pound in 1 gallon of water per acre [560.4 g per 9.36 L per ha]) + Rhodamine B Extra F⁷ + 8 ounces of Chevron Spray Sticker per 100 gallons (.06 L per 100 L) of water.

* B. thuringiensis: One application as above, and a second application 7 days later, that was similar, but did not contain Rhodamine B Extra F dye.

* Control: No treatment.

Rhodamine B Extra Base and Rhodamine B Extra F were added to respective formulations to allow analysis of spray swath width and droplet density on Kromekote cards prior to operational application, as well as on foliage taken from plots following application.

Trichlorfon was pumped directly into the spray tank of the aircraft from the shipping containers. The dye was added and agitated with the airplane's recirculation systems. Dipel WP was mixed with water, dye, and spray sticker in two 300-gallon (1,135.6 L) mixing tanks equipped with agitators and recirculation apparatus (figure 4). It was then pumped directly into the aircraft spray tank.

Local meteorological data were obtained through the National Weather service located at the Mobile Municipal Airport. One-day forecasts and 5-day extended forecasts were used to aid in predicting optimal spray conditions. Portable meteorological instruments were set up at the loading site to monitor wind speed and direction, temperature, and humidity. The loading site was located at Bay Minette Municipal Airport, approximately 10 miles east of the spray plots.

Swath width and droplet spectrum were determined by placing Kromekote cards at intervals on open ground near the airstrip and spraying them with complete formulations at an operational spray height, air-speed, and spray pressure.

Trichlorfon and initial B. thuringiensis applications were made with a twin-engine fixed-wing aircraft (DC-3) with a 500-gallon (1,892.65 L) carrying capacity. Nozzles used for the trichlor-

⁸BIU = Billion International Units of potency.

ton treatment were Spraying Systems diaphragm check valves with Teejet flat fan 8010 spray tips. Twelve nozzles were used, six along the trailing edge of each wing concentrated aft of the engine area. The nozzles were directed straight down. The plane speed was 150 miles (241 km) per hour, the spray pressure was 50 pounds per square inch (psi) (3.5 kg/cm^2), and the expected droplet volume mean diameter (VMD) was 150 microns. The trichlorfon application was made on March 26, 1975, from 5:50 p.m. to 6:45 p.m. Table 1a gives meteorological data at the airport during the trichlorfon application period.

Nozzles used for the B. thuringiensis treatment were the same type and size used in the trichlorfon treatment. Sixty nozzles were initially evenly spaced along the boom, 30 on each side of the fuselage. Through calibration tests, it was determined that the pressure and flow were reduced on the outboard part of the boom, probably due to the small 1-inch boom. As a result, eight more nozzles were added—four to each side, concentrated behind the engine area. The nozzles were directed down and back at about a 30° angle to the line of flight. The aircraft speed was 150 mph (241 km), the spray pressure was 40 psi (2.8 kg/cm^2), and the expected VMD was 250 microns. Application was made on March 31, 1975, from 7:20 a.m. to 8:50 a.m. Table 1b gives meteorological data at the airport during the B. thuringiensis application period.

During both treatments, an observer was present in a chase plane to direct the spray plane to the treatment plots and monitor the application. Radio communication was maintained between the chase plane and the spray plane, airport, and ground flight-line marking crews. Swath widths were marked by helium-filled balloons raised above the tree tops from boats on the river.

Within 16 hours following treatment, foliage samples were collected by shooting branches from the tops of dominant trees on the subplots in each pond. Foliage samples were sent to the Pacific Northwest Forest Experiment Station, Corvallis, Oreg., where droplet spectrum and deposit assessment were completed.

The second application of B. thuringiensis on the Dipel II plot was made on April 7. Two Piper Pawnee aircraft with 150-gallon (567.8 L) capacities, fitted with 10 Roto-spin nozzles and cali-

brated to deliver 1 gallon per acre (9.4 L/ha), were used for this application.

Efficacy Evaluation

An estimate of treatment effect was based on defoliation before treatment, and defoliation and refoliation after treatment. Defoliation and refoliation on treated and control plots were estimated on individual sample trees from the ground, and on an overall plot basis by using low level aerial photographs. The percentage of defoliation on individual subplot trees was ocularly estimated from the ground within 4 days before spraying and at weekly intervals after spraying. The same individual made all estimates throughout the project to reduce variation. On each sampling date, data were recorded as a percent of defoliation per tree.

Aerial Estimates: At the time of approximate peak defoliation, 35 mm infrared aerial photographs were taken of the plots to determine the extent of defoliation and refoliation of the plots. About 1 month after application, 9 x 9-inch (22.8 x 22.8 cm) infrared aerial photographs of the plots were taken using a Wild RC-10 aerial camera. Evaluation of these photographs indicated the overall effectiveness of the sprays in reducing or stopping tent caterpillar defoliation and clearly showed any skips that occurred during aerial application.

Insect counts were not taken as a measure of treatment efficacy. Variations between larval samples were found to be as great within treatments as between them. Sampling methods to overcome this problem have not been developed.

Population Recovery

Egg mass counts were made in each of the study areas in the year following treatment, and in three of the four areas 2 years after treatment. In addition, visual estimates of the degree of defoliation from aerial and ground observations of each of the study plots were made in both years. These data allowed determination of the length of time and rate of recovery of the populations following suppression by each treatment.

Nontarget Organism Monitoring

Effects of trichlorfon and B. thuringiensis on mammals, birds, fish, crustaceans, insects and

Figure 5.—
Infrared aerial photograph, taken 5 weeks after treatment with trichlorfon, showing streaking and protection of plot. Blue streaks are defoliated areas not protected by insecticide.

Figure 6.—
Infrared aerial photograph, taken 4 weeks after treatment with Dipel, showing streaking caused by alternating strips of protected and unprotected forest canopy.

Figure 8.—
Aerial photograph of the control plot in the forest tent caterpillar pilot project in the Mobile-Tensaw River Basin shows complete defoliation 5 weeks after insecticides were applied to treatment plots. (Photo by James D. Harper)

Figure 7.—Estimated average mean defoliation by forest tent caterpillar and recovery rates of plots treated with trichlorfon and *B. thuringiensis* in the Mobile-Tensaw River Basin, Ala., 1975. The dates of the treatment applications are indicated by triangles. The standard deviation for each mean is given in parenthesis.

Foliage loss in the control plot (figure 8) was essentially complete 8 days following the initial B. thuringiensis application (13 days following the trichlorfon application). The rapidity with which foliage was lost in the absence of treatment is well illustrated in figure 7.

Loss of foliage in the control plot averaged about 7 percent per day from March 24 through April 1. Each day of delay in application resulted in the corresponding increased degree of damage. This delay, coupled with poor coverage as discussed later, combined to cause less than satisfactory economic control with the single application of B. thuringiensis and marginally acceptable control with the dual application. However, the defoliation patterns resulting from both of these treatments would have been quite acceptable had they been initiated on the same populations 5 days earlier. Protection of all plots, trichlorfon included, would have benefitted from treatment on or around March 23.

Spray Deposit and Distribution Assessment

Spray deposit analysis of foliage indicated that actual droplet VMD was larger than expected for trichlorfon—175 microns instead of 150 microns. The nozzles should have been rotated slightly forward to achieve the desired VMD.

Spray deposits were found on all but three subplots, with considerable variance between subplots (0–4.62 droplets per square centimeter). None of the subplots actually occurred in an unprotected, streaked area, but three subplots which did not have droplets on the foliage samples were located near the edges of these streaks in the forest canopy (figure 5). Other subplot samples had relatively low droplet density counts, yet did not coincide with canopy streaks or unprotected crown areas.

The method of droplet assessment used was unable to detect 35 micron or smaller droplets (Neisess, 1974). Since such droplets of contact insecticides were undoubtedly extremely toxic, their presence could explain the protection level achieved in subplots where droplet densities were relatively low. This method of determining spray coverage and droplet density for trichlorfon was therefore only useful as a gross indicator of spray distribution. Fine droplets which drifted outward from the detectable swath edges may account for

a wider effective swath width than would be expected from the Kromekote card analysis of swath width.

The spray deposit for B. thuringiensis showed that the actual droplet VMD was 241 microns, which was close to the 250 VMD expected. A great deal of variance in the number of drops on foliage occurred (0–16.8 droplets per square centimeter).

Subplots with very low droplet densities were found to correspond with the locations of the streaks seen in figure 6, as well as with the highest foliage loss estimates. Thorough coverage and precise location of flight lines would appear to be much more critical for control with B. thuringiensis than for trichlorfon. While small droplets are effective for contact insecticides, larger droplets have been shown to increase persistence and efficacy of B. thuringiensis applications (Thompson et al., 1977). Small droplets spreading out from the edges of the swath or drifting downwind would not produce the effect of similar-sized droplets of the contact insecticide.

Population Recovery

Table 2 shows tent caterpillar egg mass numbers over 3 years of the project. Numbers in all plots dropped following treatment and increased again in the three measured plots 2 years later, although not to the initial population levels.

In the Dylox plot, the drop in numbers of egg masses 1 year after treatment can be attributed to the effective suppression of the forest tent caterpillar. The residual population was too low to produce sufficient progeny to completely defoliate the stand. Immigration by moths apparently did not occur, at least in large numbers. That gravid moths do not normally fly far from their point of pupal eclosion is supported by the fact that egg mass counts the following year were much higher on the western side of the Dylox plot—where no natural boundary separated the untreated and treated populations—than on the eastern side of the plot where the Tensaw River provided a 200 to 300-foot (61 to 91.4 m) natural barrier to immigration. Two years later, the average egg mass number per sample unit had increased to almost 2, and numbers were more uniformly distributed across the plot. Although defoliation progressed

Table 2.--Mean egg mass counts and final defoliation estimates for forest tent caterpillars over the 3 years of pilot control study in the Mobile-Tensaw River Basin.

Treatment	1975		1976		1977	
	Mean egg mass count*	Mean percent defoliation†	Mean egg mass count	Mean percent defoliation	Mean egg mass count	Mean percent defoliation
Dylox	3.81 ± 1.66	19	1.35 ± 1.55	30	1.88 ± 1.74	99+
Dipel I	6.74 ± 3.96	77	3.23 ± 2.72	99+	---	99+
Dipel II	5.50 ± 3.53	44	2.45 ± 1.74	85	3.17 ± 3.25	99+
Control	5.01 ± 3.40	99+	1.55 ± 3.15	30	3.13 ± 2.42	99+

*Mean number of egg masses per 4-foot (122 cm) branch tip.

†Percent defoliation in first week of May at end of caterpillar feeding period.

slowly, the pond canopy was completely defoliated by the end of the larval development period.

Assessment of egg mass numbers in the Dipel plots for the 2 years subsequent to treatment indicated that satisfactory suppression of the post-treatment generation was not achieved in either the Dipel I or the Dipel II plots. Both had significant reductions in average egg mass counts the following year—63 percent in the Dipel I plot and 59 percent in the Dipel II plot. However, the reduced levels were sufficiently high to produce larval populations capable of completely defoliating the respective areas.

It seems probable that the failure to achieve complete coverage on these plots resulted in larger and healthier residual populations than would have occurred in the absence of treatment. Defoliation in the control plot was progressing at a rate similar to that in the Dipel I plot prior to treatment (figure 7). Total loss of foliage in the control plot, about 3 weeks before completion of larval development, resulted in starvation and subsequent reduction in population size and egg mass quality. Only 30 percent defoliation occurred on the control plot in 1976. This pattern did not occur in the Dipel plots. Following complete utilization of foliage in the unprotected strips, larvae were able to move into the edges of adjacent, previously protected foliage, and complete development rather than starve. This resulted in residual populations of larvae which completed development normally and produced enough eggs and subsequent larvae to defoliate the stands in 1976.

In 1977, both Dipel I and Dipel II plots, as well as the control plot, had elevated populations and were completely defoliated 2 to 3 weeks before the normal pupation period. Although populations of forest tent caterpillars collapsed in high population areas in the Mobile-Tensaw River Basin, there were sufficient areas of low population to sustain epidemic conditions in the basin. Because this chronic epidemic condition has existed for decades, it appears that population collapses will not solve the forest tent caterpillar problem in the Mobile-Tensaw River Basin. If the cost-benefit becomes favorable, management of the insect populations will be necessary.

On the basis of the work reported here, trichlorfon could be used effectively to reduce forest tent caterpillar populations for at least 2

years. While B. thuringiensis was demonstrated by this and previous studies to control forest tent caterpillars, the effectiveness would be improved by more timely applications and better control over application parameters.

Nontarget Organism Impact Studies

Although both trichlorfon and B. thuringiensis are registered for use against forest tent caterpillars, they had never been applied in a swamp situation. Because the Mobile-Tensaw River Basin is a swamp situation, with a fragile aquatic environment, it was felt that the impact of these insecticides on nontarget organisms should be monitored. Consequently, five impact studies were conducted by State, Federal, or university regulatory or research personnel, as part of their regular duties or under grants or contracts from Scott and International paper companies and the USDA Forest Service. The appendices to this publication present summaries of the purpose, methods, and results of each of these studies.

RECOMMENDATIONS

Based on the results of the project, the following recommendations can be made concerning the treatment of forest tent caterpillar infestation in the Mobile-Tensaw River Basin.

Pesticide

1. Under conditions of application and testing, trichlorfon performed adequately and could be used for up to 2 years of protection in areas of at least 1,000 acres (404.7 ha).

2. Bacillus thuringiensis has potential as an effective insecticide. However, timing of application is very critical, and must be adhered to if this material is to be used effectively in an operational manner.

Carefully controlled, small-area applications of B. thuringiensis by a small aircraft may be used for cosmetic or nuisance control around loading, recreation, or other sensitive areas. Previous studies have shown that these controlled, small-area applications are possible, although multiyear control will not necessarily be achieved.

3. Additional studies to more clearly define the impact of B. thuringiensis and trichlorfon on nontarget aquatic invertebrates in an aquatic environment, such as the swamp situation, may be desirable.

Spray Applications

1. A more visible and accurate swath width marking system should be used to achieve adequate coverage and reduce skips in application. This is more critical with formulations having a larger droplet size than it is with those having a small droplet size, where the spray disperses more readily.

2. When treating large areas, use of large aircraft, such as the DC-3, is practical. Material can be applied much faster than with small aircraft having lower payload capacities and narrower swath widths. This can be critical in areas where weather patterns change quickly.

3. Treatment of large areas would be more cost effective in terms of multiyear protection than small acreages. Treatment of smaller areas would have no multiyear protection effects, especially in areas adjacent to an infestation.

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APPENDIX I

Effects of Force-fed Trichlorfon-killed Forest Tent Caterpillars on Turkeys

Conducted by:

M.K. Causey

Department of Zoology-Entomology

Auburn University

Auburn Ala.

The bottomland forest habitat of north Mobile and Baldwin Counties, Ala., is an area noted for high population densities of various forest wildlife species. One of the most highly prized game species is the eastern wild turkey (Meleagris gallopavo). Wild turkeys are known to consume quantities of insects when foraging, and it was of some concern to wildlife biologists that tent caterpillars killed by the aerial application of trichlorfon might be consumed in harmful quantities by wild turkeys within the area. This test was conducted to observe the effect of trichlorfon-killed forest tent caterpillars in the diet of penned turkeys.

Methods: Large numbers of dead forest tent caterpillar larvae were collected from the test site, and untreated larvae were collected from outside the treatment area. Larvae were placed in plastic bags and transported in ice chests to Auburn University where they were frozen. Twelve adult domestic White Holland turkeys (six males and six females) were separated into treatment and con-

trol groups composed of three males and three females each. Individual weights of the birds were recorded.

The penned turkeys were maintained on a diet of Purina turkey starter. In addition, daily for 6 consecutive days, each control turkey was forcefed 50 grams of untreated larvae, and each treatment turkey was force-fed 50 grams of trichlorfon-killed larvae. Both groups of turkeys were observed daily. After 6 days, the force-feeding was discontinued and the turkeys were observed for a 2-week period. At the end of the 3-week period, all turkeys were weighed again.

Results: There were no visible differences in the appearance or behavior of the treatment and control birds during the force-feeding period or the subsequent 2-week period. All turkeys appeared alert and healthy throughout the entire period. When the turkeys were weighed at the end of the test, every bird had gained or maintained body weight.

The daily consumption of 1.76 ounces (50 g) of trichlorfon-killed forest tent caterpillar larvae for 6 consecutive days did not appear to affect the health of the turkeys used in the experiment.

APPENDIX II

Ground Assessment of Acute Toxicity of Trichlorfon to Fish and Wildlife

Conducted by:
L. Johnson and J.R. Davis
Game and Fish Division
Alabama Department of Conservation and Natural Resources

This assessment was made to provide information on the immediate effects on wildlife and fish of trichlorfon, when applied on a large scale basis in a swamp situation.

Methods

The assessment was based strictly on the observations of biologists who moved through the treated area in boats. Most observations concerned the bird and fish populations. Notes were

taken on the number of species of birds and on the actions of individual birds. Observations were made before treatment application, and again from 14 to 20 hours following treatment application.

Results

Based on field observations, there were no noticeable differences in the number of species of birds or in the number of individual birds before and after treatment. No distressed birds were observed, nor were any harmful effects to the fish population noted.

The late afternoon application of trichlorfon was probably desirable from the standpoint of reducing the exposure of birds to treated larvae.

APPENDIX III

Monitoring of Fish-Food Micro- and Macroorganism Production

Conducted by:
J.W. Lawrence
Department of Fisheries and Allied Aquaculture
Auburn University
Auburn, Ala.

The primary objectives of the fish-food micro- and macroorganism production study were: (1) to monitor the zooplankton populations, prior to, and following aerial applications of trichlorfon and *B. thuringiensis* and (2) to determine the relative abundance of macroinvertebrate fish-food organisms within treated and nontreated areas. A secondary study included an evaluation of phytoplankton populations within treated and control areas.

The Aquatic Habitat

For the greater part of the year, the forested swamp regions of the Mobile-Tensaw River Basin are covered with water less than 2 feet (61 cm) deep. Connecting channels and canals within the swamp are 4 feet (122 cm) or more in depth. These areas normally support a varied population of fish-food organisms as well as a population of fish.

During the study period, the rivers were in varying stages of flooding, and the waters covering the forested areas ranged from 4 to 8 feet (122 to 244 cm) in depth. This degree of flooding also subjected all of the study areas to a rather high rate of water current. The water level was higher at the end of the study than it had been when the study began.

All sampling was done in the Dylox, Dipel I, and the control plots.

Part I.—Study of Zooplankton Populations in Treated and Untreated Areas

In this study, the terms "zooplankters" and "net plankters" are considered as synonymous because the microorganisms were collected by straining relatively large volumes of water through a plankton net. These organisms, which are large enough to be seen by the naked eye but still so small that they have to be identified under low magnification, constitute the main animal diet of small fish and larger invertebrates which inhabit the aquatic habitat. Since zooplankters form a vital part of the food web of fishes, their susceptibility to large scale pesticide applications is of concern. The 1975 treatments to large forested areas within the Mobile Delta offered an excellent opportunity to collect information on the effects of trichlorfon and B. thuringiensis to resident populations of zooplankton in this area.

Methods

The collection of zoo- or net plankters was accomplished by pumping 10.6 gallons (40 L) of surface water through a standard Wisconsin 80-mesh plankton net. Four samples from various sites within each area were taken in March and again in April. Sampling locations were normally near the edges of the tree plots and were located where access by boat was possible at each sampling date. The collected samples of organisms were washed from the plankton net into 100-ml wide-mouthed plastic bottles, placed in ice chests, and returned to the laboratory. An aliquot of each sample was placed in a Sedgewick-Rafter counting cell, and the numbers of the various groups of organisms per liter of river water were determined. The dominant organisms in each major group were identified.

Results

Pretreatment sampling for zooplankton was accomplished on March 5. Post-treatment sampling for zooplankton was accomplished on April 1 in the control and trichlorfon treatment areas, and on April 8 in the control and B. thuringiensis treated areas. Table 3 gives the analyses of the samples collected from all areas according to the average number of organisms in each major zooplankton group per sample on each sampling date.

The dominant zooplankton found in the various samples were the following: Alona sp., Alona quadrangularis, Ascomorpha sp., Bosmina longirostris, Ceriodaphnia pulchella, Chironomid larvae, Chydorus sp., Chydorus sphaericus, Copepod nauplii, Cyclopoid, Cyclops sp., Filinia sp., Killickottia bostoniensis, Keratella cochlearis, K. valga, Platytas patulus, Polyarthra sp., Trichocerca sp.

According to the data, no differences in zooplankton populations could be attributed to either trichlorfon or B. thuringiensis treatments. The increase in numbers from March to April was believed due to natural population increases that typically occur in early spring. Because of spring flooding, water flow in all study areas varied considerably between sampling dates, making it difficult to assess the causes for changes in zooplankton densities.

Part II.—Study of the Macroinvertebrate Populations

Organisms that live in the river swamps endure all conditions that exist throughout their life cycle. Therefore, the organisms that are found at a specific location reflect the conditions or minimal water quality that existed in their lifetime.

Because organisms vary considerably in their tolerance to various conditions of water quality, different organisms become indicators of conditions that have existed in the swamp. In general, a large diversity of aquatic organisms indicates good water quality, while a lack of diversity—even though large numbers of individuals of a few species may be present—indicates limiting conditions to which only certain organisms are tolerant.

In small streams and shallow swamps that can

Table 3.--Numbers of zooplankton per liter obtained from pre- and post-spray sampling of water in water tupelo plots treated with insecticide for control of the forest tent caterpillar.*

Zooplankton	Dylox plot		Dipel I plot		Control plot		
	March 5	April 1	March 5	April 8	March 5	April 1	April 8
Rotifers	11.8	85.4	15.4	70.4	34.5	107.8	43.1
Copepods	5.7	16.7	11.3	15.0	18.2	26.9	24.2
Adults	-	0.9	-	5.5	1.9	4.7	8.7
Nauplii	5.7	15.8	11.3	9.6	16.3	22.1	15.4
Cladocera	4.4	3.8	6.2	13.2	6.7	3.8	8.7
Totals	21.9	105.9	32.9	98.6	59.4	138.6	76.0

*All figures are averages of four samples, except for the April 1 and April 8 samples from the control plot, which are averages of two samples, and the two Dipel plot samples, which are averages of three samples.

be surveyed by wading, the sampling may be accomplished by hand-picking the organisms from rocks, sticks, leaf masses, etc. In water bodies too deep to employ this method, it is common practice to use substrate material that can be placed in the habitat, recovered after a given period of exposure, and examined for colonization by organisms. This technique is somewhat selective, but it is standardized and allows better comparisons between locations and over periods of time. The latter method was used in this study.

Methods

Macroinvertebrate organisms were collected with a modified Dendy plate sampler (Hester and Dendy, 1962). The total attachment area of the plate samplers was 0.5 square foot (465 sq. cm). Four samplers were placed at each of four sites within each plot, providing a total of 16 samplers per plot. Each plate sampler was suspended 5 to 6 feet (152 to 183 cm) below the water surface. A line attached to the sampler was tied to an overhanging limb or tree trunk, and an anchor line and anchor were suspended below, to maintain the plate at a fixed position in the water column. An effort was made to locate each sampler in an area of water 8 feet (244 cm) deep or more, to allow for water level fluctuations during the sampling period.

When retrieving a sampler, the suspending line was slowly lifted until the sampler was just under the surface of the water, at which point the line from the sampler to its anchor was cut. A 1-gallon (3.8 L) container was lowered beneath the sampler, and the sampler and its contents were gently removed from the water. The sampler and its container were stored in an ice chest and returned to the laboratory. In the laboratory, the container and its contents were kept aerated until the materials on the plates could be removed, separated, counted and preserved for further identification.

Results

Because of the delay in treatment of the B. thuringiensis area, samplers were collected on separate dates. To compensate for these unequal exposure periods, one-half of the plates (two sites) within the control area were collected on each date.

Table 4 gives the average number of organisms in each group per square foot of sampler area. The diversity of organisms was about the same in both treatment and control areas. Little evidence exists to indicate any detrimental effects on aquatic macroinvertebrates in the trichlorfon-treated area. The data from the B. thuringiensis-treated area may indicate that a reduction in larval forms of aquatic insects, particularly chironomids, occurred. However, the high numbers of chironomids recovered in the control area were small forms, 95 percent of which were collected on plates positioned at only one site within the control area. More intensive sampling of the control area may have provided a more accurate estimate. The delay before the collection of the second group of control samples could have allowed time for chironomid egg deposition and hatching and could account for the large increase in numbers.

In summary, there were considerable differences in numbers of macroinvertebrate organisms present on the samplers collected on the separate dates. From the numbers obtained on trichlorfon-control areas on April 1, it can only be surmised that the differences could well be attributed to sampling error. Likewise, based upon changes that occurred between numbers that were collected on April 8, it would appear that some degree of sampling error was present. The extent of this error is unknown, and further sampling under similar conditions must be done before the effects of B. thuringiensis upon larval forms of aquatic insects can be evaluated.

Part III.—Study of the Phytoplankton Populations

The productivity of zooplankton and macroinvertebrates within aquatic habitats depends partly on the supply of microscopic plants or phytoplankton. Therefore, to assess the natural productivity or abundance of micro- and macroscopic fauna within an area, some measure must be made of phytoplankton abundance. The most widespread method of determining phytoplankton abundance is to count the numbers of individuals per milliliter of water. Such counts not only indicate the level of productivity, but they also may reflect the source of the nutrient supply.

Table 4.--Average number of organisms recovered per square foot of sampler area from trichlorfon and B. thuringiensis control areas in the Mobile-Tensaw river basin.

Groups of Organisms	Average recovery per square foot			
	Trichlorfon	Control	<u>B. thuringiensis</u>	Control
Stalked protozoa	147.0	6.0	25.0	180.0
Hydra	1.8	87.2	13.2	12.0
Turbellaria	0.4	2.4	12.6	12.0
Rotifers	6.4	10.8	48.1	102.0
Nematodes	11.8	7.2	18.6	48.0
Oligochaeta	9.2	11.2	35.1	60.0
Snails	2.2	9.6	0.5	1.0
Clams	0.2	0	0	0
Water mites	9.2	0	1.8	6.0
Cladocera	10.8	114.0	167.1	24.0
Ostracod	2.8	1.2	1.2	48.0
Copepod	3.0	9.2	13.0	90.0
Isopod	2.8	2.4	2.7	0
Amphipod	1.2	7.6	2.1	1.0
Water boatman	1.6	2.8	0	0
Mayfly	0	0	0	30.0
Chironomid	5.6	7.2	106.7	846.0
Ceratopogonid	0	0.4	0	0
Coleoptera	0	0	0.1	0
Totals	216.0	279.2	447.8	1,460.0

In a study to evaluate the influence of an insecticide application upon the zooplankton and macroinvertebrates within the aquatic habitat of a forested area, the abundance of the food supply for these organisms must be known. Sampling of the treatment and control areas for phytoplankton abundance was accomplished on March 5, April 1, and April 8.

Methods

Water samples for phytoplankton counts and identification were collected by pumping 16.9 ounces (500 ml) of water into a flat-bottomed, 1-liter (1 quart), widemouthed plastic container. Samples were treated with a killing-preserving solution, stored, and returned to the laboratory where the suspended matter was allowed to settle for 48 hours or more. The excess water was then carefully removed by a siphon until 1.9 ounces (50 ml) remained in the container. The settled material in the bottom was carefully resuspended, and a .03 ounce (1 ml) aliquot was withdrawn and placed in a Sedgewick-Rafter counting cell. The various groups of organisms were identified and counted by using a microscope.

Results

Table 5 gives data on the numbers of different groups of phytoplankton per milliliter which were collected from the trichlorfon, control, and B. thuringiensis treatment areas on March 5. The dominant phytoplankton taxa found were: Actinastrum sp., Ankistrodesmus falcatus, A. nanoselene, Closterium spp., Coelastrum sp., Cosmarium sp., Crucigenia spp., Eudorim sp., Golenkinia sp., Gomphosphaeria sp., Melosira sp., M. granulata, Navicula sp., Oscillatoria sp., Pandorina sp., Phacus sp., Planktosphaeria sp., Scenedesmus spp., S. acuminatus, S. quadricauda, Synedra sp., Tabellaria sp., and other pennate diatoms, Tetrastrum sp., T. heterocanthum, Trachelomonas sp.

Reduction in phytoplankton numbers occurred in all areas checked. The degree of reduction was very similar in each of the three study plots, indicating that factors other than the treatments caused the decreases.

Flooding, which occurred during the entire sampling period, probably interfered with normal development of phytoplankton populations.

Normally, the numbers of phytoplankton would be expected to increase rather than decrease from early to late spring.

Literature Cited

- Hester, F. E., and J. S. Dendy.
1962. A multiple-plate sampler for aquatic macroinvertebrates. Trans. Amer. Fisheries Soc. 91:420-421.

APPENDIX IV

Impact of Trichlorfon and Bacillus thuringiensis on Nontarget Terrestrial Invertebrates

Conducted by:

J.D. Harper and H.B. Cunningham
Department of Zoology-Entomology
Alabama Agricultural Experiment Station
Auburn, Ala.

The impact of trichlorfon and B. thuringiensis on nontarget invertebrates, when applied in forest habitats, has not been well documented. Trichlorfon is generally known to be a relatively broad spectrum insecticide while B. thuringiensis is relatively specific to certain lepidopterous larvae.

To determine this impact, an assessment of the numbers and types of organisms removed from tree canopies treated with each agent was considered desirable. This information could be of importance in understanding or predicting whether serious shifts in ecosystem balances might be expected following use of either agent. This project was designed to determine major groups of organisms and the relative numbers of each group affected by each control agent.

Methods

A specially designed collecting trap (Cunningham and Harper, 1977) was used to determine the numbers of tent caterpillars and nontarget arthropods falling from treated canopies in each of the four treatment areas (figure 3). Three of these traps—each with 10.76 square feet (1 m²) of collecting area—were hung beneath the tree canopy in each subplot on the sample lines established for defoliation assessment in the pilot study. This system provided 60 traps per treatment area.

Table 5.--Numbers of phytoplankton per milliliter obtained from pre- and post-spray sampling of water in water tupelo plots receiving insecticide treatment for control of the forest tent caterpillar.

Phytoplankton groups	Trichlorfon plot		B. thuringiensis plot		Control plot	
	March 5	April 1	March 5	April 8	March 5	April 1
				April 8		April 8
Diatoms	697.5	62.3	104.3	47.9	163.3	30.8
Pennate	652.0	27.5	79.4	29.2	128.5	29.2
Centric	45.5	26.6	24.9	18.7	34.8	1.6
Green	147.9	15.5	369.1	42.7	417.2	21.9
Filamentons	1.9	0.6	4.4	3.7	10.1	1.0
Coccoid	128.5	14.9	286.1	39.0	200.6	20.8
Flagellates	17.6	0	75.8	0	87.4	0
Blue-green	11.8	9.1	16.6	0.5	12.0	1.7
Filamentons	0	7.5	5.3	0	8.5	1.7
Coccoid	11.8	1.6	11.3	0.5	3.3	0
Other pigmented flagellates	129.5	0	70.6	0	115.2	0
Totals	986.7	87.0	560.6	91.0	707.7	54.4
						147.4

Traps were hung before the application of Insecticides. Each was emptied at varying intervals following treatment--normally 6 to 8 days--over a period of about 30 days. All material in each trap was transferred to alcohol vials and returned to the laboratory for processing.

With the help of stereoscopic microscopes, all nontarget organisms were separated from the tent caterpillars, frass, and organic debris, and placed in alcohol vials for later identification. Total numbers of organisms were determined for all sampling periods. Identifications of the materials to families were completed for only the first two collections in each treatment. In later collections, identifications were made to the family level for all insects, when possible, or to orders when insufficient identifying characters were present on immature or damaged specimens.

Comparisons between numbers of various groups, as well as total numbers of groups and specific groups present, were made to determine whether the treatments caused more or less mortality to occur in nontarget species in the treated areas than in the untreated area. By examining the numbers of organisms trapped over time in each area, it was possible to determine whether initial numbers decreased significantly over the sampling period. All data were converted to "numbers captured per hectare per hour" to make comparisons between treatments. This was necessary because some sampling periods differed in length.

Results

Large numbers of arthropods were collected in the hoop traps in all treatment areas, as well as in the untreated check plot, throughout the sampling period (figure 9). Definite patterns were seen in the collections within each treatment area. In the check plot, catches decreased gradually from March 25 through April 11 and then rapidly increased (over threefold) between April 12 to 17. Initial captures in the Dylox plot were over three times those in the untreated plot. Fallout dropped rapidly through April 10, and then began to increase slightly. Both Dipel plots had gradually increasing trap catches from March 26 through August 8-9. In the following week, the catches in both of these plots, as in the untreated plot, increased rapidly.

Materials collected during the first 7 to 9 days of

sampling, from all treatments combined, were sorted into 112 different taxa or categories, principally families of insects (table 6). Numbers of different taxa found in the Dylox, Dipel I, Dipel II, and untreated plots were 91, 39, 48 and 67, respectively. The last figure shows the large amount of natural fallout which occurs even in the absence of treatment.

Most taxa were collected in very low numbers. However, both total numbers and diversity of organisms collected were significantly higher in the Dylox plot than in the other three plots. Twenty-nine taxa were found in significantly higher numbers in the Dylox traps than in all other plots, and 37 were higher than in the untreated plot only. Dipel I and II plots had zero and three taxa, respectively, which were present in significantly higher numbers than the untreated plot. The untreated plot had two taxa which were present at significantly higher levels than were found in the Dylox plot, three that were higher than in the Dipel I plot, and three that were higher than the Dipel II plot.

Taxa which appeared in high numbers in the trichlorfon traps (25 or more per hectare per hour) were spiders, mites, psocopterans, thysanopterans, hemipteran nymphs, sisyrids, scolytids, scaptiids, staphylinids, unidentified beetles (larvae), unidentified lepidopteran larvae, cecidomyiids, chironomid adults, empids, scatopsids, sciarids, unidentified adult dipterans, eulophids, formicids, and pteromalids (table 6).

In the Dipel I plot, only chironomid adults, scatopsids, unidentified adult dipterans, and formicids were found at these levels. In the Dipel II plot, spiders, mites, psocopterans, chironomid adults, scatopsids, sciarids and ants each surpassed 25 per hectare per hour in the traps.

Untreated plot traps collected at this level (25 or more hectare per hour): spiders, psocopterans, thysanopterans, staphylinids, chironomid adults, scatopsids, sciarids, unidentified larval dipterans, and formicids. Adult chironomids and other adult Diptera undoubtedly were collected by being accidentally caught while flying. However, the significantly higher number of chironomids captured in the Dylox plots probably indicates some knockdown due to treatment.

Total trap catches per time unit during the first 7 to 9 collecting days were significantly higher in

Figure 9.--Collecting periods, fallout trap catches (numbers of organisms per hectare per hour), and treatment application dates (vertical arrows) for impact study on nontarget canopy-dwelling organisms in a pilot insecticide study on forest tent caterpillars in the Mobile-Tensaw River Basin, 1975.

Table 6.--Arthropods collected from tree canopies in fallout traps, following treatment with insecticides for control of forest tent caterpillars in water tupelo stands in southwest Alabama. All figures are numbers collected per hour per hectare during the trapping period.*†

Taxa	Dylox	Dipel I	Dipel II	Untreated
Snails	2.7a	0.0a	1.0a	1.6a
Diplopoda	0.0a	1.3a	0.0a	0.0a
Spiders	338.2a	24.2b	93.6b	66.9b
Pseudoscorpions	3.4a	0.0b	1.0ab	0.0b
Mites	38.5a	22.9ab	55.2a	10.6b
Collembola				
Poduridae	1.4a	0.0a	0.0a	0.0a
Isotomidae	0.0a	1.3a	0.0a	0.0a
Entomobryidae	9.6a	3.8a	7.3a	13.1a
Unidentified	0.0a	0.0a	1.0a	0.8a
Ephemeroptera				
Unidentified	1.4b	0.0b	8.3a	0.0b
Odonata				
Coenagrionidae	12.3a	1.3b	7.3ab	1.6b
Corduliidae	1.4a	0.0a	0.0a	0.0a
Orthoptera				
Blattoidea	3.4a	1.3a	2.1a	0.8a
Tetrigidae	2.7a	5.1a	5.2a	1.6a
Unidentified	8.9a	0.0b	0.0b	0.0b
Isoptera				
Unidentified	0.7a	0.0a	0.0a	0.0a
Embioptera				
Teratembidae	0.0a	0.0a	1.0a	0.0a
Unidentified	13.7a	0.0b	0.0b	0.0b

*Trapping periods were March 25–April 2 for Dylox, March 26–April 1 for Dipel I, March 26–April 2 for Dipel II, and March 26–April 3 for the untreated check plot.

†Values within a given taxon followed by the same letter do not differ significantly (0.05) according to Duncan's New Multiple Range Test.

Table 6.--Continued

Taxa	Dylox	Dipel I	Dipel II	Untreated
Psocoptera				
Psocidae	1.4a	0.0a	0.0a	0.0a
Peripsocidae	68.8a	6.4b	0.0b	18.8b
Liposcelidae	0.7a	0.0a	0.0a	0.0a
Pseudocaeciliidae	4.1a	0.0a	0.0a	1.6a
Unidentified	122.4a	12.5c	72.8b	36.8bc
Thysanoptera				
Phlaeothripidae	2.8a	1.3a	10.4a	6.5ab
Unidentified	112.1a	24.1b	13.5b	34.3b
Hemiptera				
Anthocoridae	2.1a	0.0a	0.0a	0.0a
Corixidae	0.0a	0.0a	1.0a	0.0a
Enicocephalidae	4.1a	0.0b	1.0ab	0.0b
Hydrometridae	0.0a	1.3a	0.0a	0.0a
Miridae	8.9a	0.0b	0.0b	0.0b
Pentatomidae	11.0a	0.0b	0.0b	0.0b
Saldidae	0.0a	0.0a	0.0a	1.6a
Unidentified nymphs	65.3a	5.1b	8.3b	8.2b
Homoptera				
Aleyrodidae	0.7a	0.0a	0.0a	0.0a
Cicadellidae	4.8a	2.6a	1.0a	5.7a
Eriosomatidae	2.8a	0.0a	0.0a	0.0a
Unidentified nymphs	7.5a	3.8a	9.4a	13.0a
Neuroptera				
Chrysopidae	6.9a	2.6ab	1.0ab	0.0b
Corydalidae	1.4a	1.3a	2.1a	1.6a
Mantispidae	1.4a	0.0a	0.0a	0.0a
Sisyridae	33.7a	0.0b	1.0b	1.6b

Table 6.--Continued

Taxa	Dylox	Dipel I	Dipel II	Untreated
Coleoptera				
Anobiidae	1.4a	0.0a	0.0a	0.0a
Anthicidae	0.0a	1.3a	0.0a	3.3a
Carabidae	8.3a	0.0b	1.0b	4.9ab
Chrysomelidae	2.7a	0.0a	0.0a	4.1a
Ciidae	0.0a	0.0a	0.0a	1.6a
Coccinellidae	0.0a	0.0a	0.0a	1.6a
Corylophidae	0.0a	0.0a	0.0a	1.6a
Cucujidae	0.0a	0.0a	0.0a	3.3a
Curculionidae	5.5a	0.0b	2.1ab	0.0b
Elaterridae	1.4a	0.0a	0.0a	0.8a
Erotylidae	0.0a	0.0a	0.0a	1.6a
Halplidae	1.4a	0.0a	0.0a	0.0a
Heteroceridae	0.0b	0.0b	0.0b	3.3a
Histeridae	2.7a	0.0a	0.0a	3.3a
Hydrophilidae	0.0a	0.0a	0.0a	1.6a
Lampyridae	0.0a	1.3a	0.0a	0.0a
Lathridiidae	18.6a	0.0b	2.1b	2.4b
Noteridae	1.4a	0.0a	0.0a	0.0a
Phalacridae	2.8a	0.0a	0.0a	0.0a
Platypodidae	2.1a	0.0a	0.0a	1.6a
Scarabaeidae	1.4a	0.0a	0.0a	0.0a
Scolytidae	25.4a	2.5b	5.2b	9.0b
Scraptiidae	35.8a	0.0b	0.0b	0.8b
Scydmaenidae	1.4a	0.0a	0.0a	0.0a
Staphylinidae	134.1a	6.4b	8.3b	156.1a
Unidentified	59.8a	7.7b	13.5b	8.2b
Trichoptera				
Hydropsycidae	1.4a	0.0a	0.0a	1.6a
Leptoceridae	11.7a	3.8a	6.2ab	1.6b
Unidentified	1.4a	0.0a	0.0a	0.0a
Lepidoptera				
Geometridae	4.1a	0.0a	0.0a	0.8a
Microlepidoptera	12.4a	0.0b	5.2b	0.0b
Unidentified larvae	51.5a	2.6b	4.2b	4.1b

Table 6.--Continued

Taxa	Dylox	Dipel I	Dipel II	Untreated
Diptera				
Cecidomyiidae	109.3a	5.1b	21.8b	24.5b
Ceratopogonidae	2.1b	2.5b	0.0b	11.4a
Chironomidae	1,476.2a	595.3b	860.5b	718.2b
Chloropidae	0.0a	1.3a	3.1a	1.6a
Culicidae	0.7a	0.0a	1.0a	0.0a
Dolichopodidae	2.0a	0.0a	0.0a	0.0a
Empididae	30.9a	0.0b	0.0b	0.0b
Mycetophilidae	2.1a	0.0a	0.0a	0.0a
Phoridae	3.4a	1.3a	1.0a	3.3a
Psychodidae	5.5a	0.0a	0.0a	4.9a
Sarcophagidae	0.0a	0.0a	0.0a	0.8a
Scatopsidae	55.7ab	66.0ab	106.2a	26.9b
Sciaridae	55.7a	19.1b	46.8ab	36.8ab
Sciomyzidae	4.1a	0.0b	0.0b	0.0b
Syrphidae	1.4a	0.0a	0.0a	0.0a
Tipulidae	4.1a	0.0a	0.0a	0.0a
Unidentified larvae	3.4a	0.0a	0.0a	76.8a
Unidentified adults	77.0a	27.9b	13.5b	21.2b
Hymenoptera				
Aphidiidae	24.1a	0.0b	0.0b	3.3b
Apidae	1.4a	0.0a	1.0a	0.0a
Bethylidae	2.1a	0.0a	0.0a	1.6a
Braconidae	11.7a	0.0b	4.2b	0.0b
Ceraphronidae	17.2a	0.0b	1.0b	1.6b
Chalcidoidea	10.3a	0.0b	0.0b	2.4b
Diapriidae	0.0a	0.0a	1.0a	0.0a
Encyrtidae	11.0a	1.3b	0.0b	0.8b
Eulophidae	33.7a	1.3b	7.2b	3.3b
Eupelmidae	0.0a	0.0a	0.0a	0.8a
Eurytomidae	3.4a	0.0a	0.0a	1.6a
Formicidae	373.3a	67.2b	119.7b	187.9b
Halictidae	0.7a	0.0a	0.0a	0.0a
Ichneumonidae	0.7a	0.0a	0.0a	0.0a
Mymaridae	4.1a	0.0a	0.0a	4.1a
Platygastridae	2.8a	1.3a	6.2a	6.5a
Pteromalidae	28.2a	1.3b	3.1b	4.1b
Scelionidae	4.8a	0.0a	0.0a	1.6a
Sphecidae	0.0a	0.0a	0.0a	1.6a
Unidentified	4.1a	2.6a	0.0a	0.8a
Insecta				
(Unidentified)	1.4a	0.0a	2.1a	0.0a
Totals	3,658.6a	942.3c	1,553.1b	1,593.1b

the Dylox plot than any other area (table 6). Dipel I plot fallout was significantly lower than Dipel II and untreated check plot fallout. Dipel II plot fallout was not significantly different from the check plot fallout.

The general trends from data in figure 9 and table 6 indicate the following:

1. The trichlorfon treatment caused a rapid knockdown of organisms from the canopy. The numbers remaining were so low that natural fallout during the second week post-spray was much lower than that recorded in the untreated area.
2. Both B. thuringiensis areas showed some increase in fallout following treatment. This was possibly related to treatment, because the untreated plot catches decreased slightly during this same period. However, in neither Dipel nor untreated plots were specific taxa associated with these increases or decreases. The shifts were spread over the entire spectrum of taxa, indicating that the changes in numbers were probably normal, rather than being treatment induced.
3. All plots showed an increase in fallout in mid-April. This increase was highest in the plot with the greatest number of active larvae (the untreated plot) and lowest in the plot with the fewest larvae (Dylox plot). The Dipel plots, which had intermediate levels of control, showed intermediate increases. These responses could be due, at least in part, to increased activity of forest tent caterpillar larvae. As seen in figure 5, foliage in the untreated plot was completely removed by April 10 to 12. The same was true of the unprotected areas of the Dipel-treated plots. Increased larval wandering occurred at this time (personal observations), and disturbance of other arthropods, resulting in release or dislodging, undoubtedly increased as a result. The relatively small increase in capture numbers in the Dylox plot, where tent caterpillars were nearly eliminated (except for a few strips of canopy), would support this hypothesis.

Each of the treatment plots in the test was unreplicated. Differences in numbers of organisms taken in traps in the four areas could be due, in part, to relatively higher or lower numbers in the four populations and/or to variation in

species composition. However, the four test areas were of very similar habitat types, and extreme differences in canopy-dwelling arthropod populations would seem unlikely.

Summary

A considerable number and diversity of insects fall from these water tupelo canopies naturally. Interactions among species, particularly among high levels of tent caterpillars and other arthropods sharing the same branches, probably account for much of the natural fallout.

The initial high numbers collected in the Dylox plots, followed by the depression to the lowest level collected in any sampling period, indicate that this treatment removed significant numbers of nontarget organisms from the forest canopy.

The two Dipel-treated areas showed steadily increasing trap captures over the test period. Some of the increase was possibly due to the treatment. However, no particular group or groups of organisms contributed to this increase. Rather, the level of fallout appeared to increase slightly for most organisms and may have been an indirect response to altered tent caterpillar activity rather than a direct response to the treatment.

Literature Cited

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APPENDIX V

Analysis of Residues and Pathological Effects of Trichlorfon and Bacillus thuringiensis on Selected Small Mammals, Birds, Reptiles, and Mollusks

Conducted by:
William L. Thomas
U.S. Fish and Wildlife Service
Atlanta, Ga.

Contact of insecticides with members of the wildlife complex represents a direct effect of

treatment. An indirect effect may result when the controlling agents are assimilated into the food chain during the normal feeding processes of nontarget organisms. An example would be when birds feed on tent caterpillars that have come into contact with or ingested some of the insecticide. This study was conducted to determine the residue levels and pathological effects of trichlorfon and B. thuringiensis on wildlife within the treatment areas.

Methods

Pretreatment collections (control) were taken from the two study areas and adjacent habitat 1 to 9 days before the application of the pesticides. A total of 24 species of birds, 4 species of reptiles, and 3 species of mammals was collected. Visual observations were made of the relative abundance and feeding activity of the various birds to see if any noticeable changes occurred after the treatments.

Post-treatment collections were begun 1 hour after the trichlorfon was applied and continued the next day. Collections were made again 26 days later. In the B. thuringiensis area, collections were made on the 2 days following application.

In both treatment areas, birds were the principal specimens collected. Mammals were extremely scarce because of the flooded situation. Birds were taken both from the interior of the study area and along the forest edge.

Pre- and post-treatment specimens were taken by shotgun, .22 caliber rifle, mouse traps baited with a peanut butter-oatmeal mixture, and hand capture (snakes). Most of the specimens were obtained in early morning or late afternoon; however, collections were made throughout the day. Upon collection, the specimens were immediately identified, wrapped in aluminum foil and frozen on dry ice. The frozen specimens were then sent to Auburn University for storage until laboratory analysis could be performed.

Gas chromatograph tissue analyses, according to Devine (1949), were done on specimens collected in the Dylox plot to determine the presence of trichlorfon residue.

Results

Specimens collected in the Dipel plot were examined for gross organ pathologies attributable to B. thuringiensis. Reliable gross observations concerning the presence or absence of pesticide-induced lesions could not be made because of the advanced autolysis present in all post-treatment specimens. Deterioration was a result of a failure in the freezing equipment in which the specimens were stored.

Sixty birds were collected before the aerial application of trichlorfon. All specimens were negative when checked for trichlorfon residues. In some cases a number of birds had to be used in one composite to have sufficient tissue for analysis. Both the pre- and post-treatment collections were too few in number to allow for statistical analysis.

Trichlorfon residues were found in birds, mammals, snakes, and snails collected within the treatment area. Of those bird samples collected within 3 days after treatment, 33 of 34 samples were positive at the 0.01 ppm or higher level for trichlorfon residues (table 7). This represents 78 of 79 birds collected, as well as 20 of 21 species. The single negative avian species was an American egret which feeds more on vertebrates than on insects.

Of the birds collected 26 days after treatment, 10 of 15 bird samples had detectable trichlorfon residues. This represents 9 of 14 species or 20 out of 31 birds which were collected (table 7).

The single mammal (a gray squirrel), both composites of snails, and the two species of aquatic snakes collected within 3 days after treatment contained detectable levels of trichlorfon. No snails, reptiles, or mammals were collected in the 26-day post-treatment collection.

It was surprising that residues were still present in the birds collected 26 days after application. Chemagro Corporation⁹ reported that trichlorfon in an outdoor pond (pH 7.0 and 29°C

⁹Anonymous. 1971. Dylox—The effects on the environment. Chemagro Corp., Kansas City, Mo. Unpublished.

Table 7.--Average residue levels detected in bird specimens collected from treatment plots following application of trichlorfon for control of forest tent caterpillars in water tupelo stands in southwest Alabama.*

Species	Parts per million*		
	Before Treatment	1 to 3 days after Treatment	26 days after Treatment
Parula warbler	.000 (5)	.129 (17)	-
Myrtle warbler	.000 (5)	.251 (6)	-
Prothonotary warbler	.000 (1)	.180 (5)	-
Hooded warbler	-	-	.000
Yellow-throated warbler	.000 (2)	1.600 (4)	-
White-eyed viro	.000 (2)	.070 (3)	.000
Blue-gray gnatcatcher	-	1.353 (3)	-
Tufted titmouse	.000 (3)	.097 (5)	-
White-throated sparrow	.000 (8)	.105 (10)	-
Swamp sparrow	.000 (3)	.090 (2)	-
Field sparrow	-	.072 (1)	-
Cardinal	.000 (4)	.047 (6)	-
Brown thrasher	.000 (2)	-	-
Gray-cheeked thrush	.000 (1)	.048 (1)	-
Wood thrush	-	-	Trace (2)
Hermit thrush	-	.024 (1)	-
Carolina chickadee	.000 (5)	.820 (2)	-
Great crested fly catcher	.000 (1)	-	Trace (1)
Rufous-sided towhee	.000 (6)	.041 (1)	-
Carolina wren	-	.068 (3)	-
American gold finch	-	-	.103 (2)
Indigo bunting	-	-	.000 (5)
Yellow-billed cuckoo	-	-	.133 (1)
Red-bellied woodpecker	.005 (5)	.047 (1)	-
Downy woodpecker	.000 (5)	.213 (3)	-
Hairy woodpecker	-	.094 (2)	-
Yellow-bellied sapsucker	-	.021 (1)	-
Red-winged blackbird	.000 (1)	-	-
Common grackle	.000 (1)	-	-
Purple grackle	-	-	.029 (1)
Common crow	.000 (1)	-	-
Eastern kingbird	-	-	.000 (1)
Cat bird	-	-	.101 (1)
Yellow-crowned night heron	.000 (2)	-	-
Barred owl	.000 (2)	-	-
Red-shouldered hawk	.000 (1)	-	-
Common egret	-	.000 (1)	-

*Number in parenthesis indicates numbers of specimens collected. Dash indicates none of that species collected.

average temperature), with exposure to sunlight and wind, showed a half-life of only 0.3 days. In mammalian studies, Robbins, Hopkins, and Eddy (1956) reported that trichlorfon (Bayer L13/59) and/or metabolites were very rapidly excreted through the urine. Using radioactive labeled Bayer L13/59, peak levels were detected in Hereford cow urine 2.5 hours after ingestion.

Birds observed during and after the spray application did not display any noticeable behavioral changes, although the presence of trichlorfon residues indicated that organo-phosphate poisoning had occurred. Based upon the numbers of insects in the gizzards and forestomachs of collected specimens, feeding ability of the birds did not seem to be seriously impaired.

Birds, mammals, snakes, and snails contained subacute levels of trichlorfon residues in their tissue. Some reversible physiological damage could be expected within the animals. However, based upon the reversible effects of organo-phosphate poisoning and the normally short life of trichlorfon, it was concluded that this pesticide would not have a significant detrimental effect on wildlife.

Additional negative impacts on the avian population would result from a decrease in insect populations in the treatment area. The insect populations of the southern flood plains are an important food supply for migrating birds.

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