

AEROSOL APPLICATION OF INSECT PATHOGENS

L. A. FALCON · ANN SORENSEN N. B. AKESSON

Results from these initial studies into insect pathogen application demonstrated the promising potential for effective dispersal of insect pathogens with ground aerosol rigs.

The cost of application of insect pathogens has become one of the major obstacles in putting them on a competitive level with chemical pesticides. Because many insect pathogens have a very short effective residual life, applications at high dosages must be repeated at close and frequent intervals. This short effective residual life is an inherent characteristic, as most insect pathogens are not mobile and cannot escape from unfavorable situations. Thus, sunlight, temperature, amount of moisture, and the pH of the immediate environment may be critical. Placed on an actively growing plant, leaves, stems, and fruit may soon grow away from the pathogens, exposing untreated areas or diluting existing deposits. In the case of bacteria and viruses, there is no fuming or contact action on the host, and they must be ingested in order to destroy the host.

Timing critical

Because an adequate titer of pathogens is required, timing of application becomes critical. Given the necessity for frequent applications with insect pathogens and the resulting costs, broad-spectrum hard chemical pesticides continue to be favored by the farmer or pest control operator despite the long-range dangers of insect resistance, possible pest resurgences, and environmental pollution. This research has been directed toward reducing application costs and has led to the delineation of what are

BOLLS PER ACRE AND QUANTITY DAMAGED BY
BOLLWORM IN FOGGER-APPLIED VIRON/H STUDY
FIELD AT PALLA RANCH, KERN COUNTY, 1972

	Total bolls*	Bolls damaged by bollworm** counted Oct. 6	
	no./acre	no./acre	%
No Predators	346,000 a	66,000 a	19.39 a†
No Predators + Viron/H	328,000 a	26,000 b	8.13 b
Natural Predation + Viron/H	314,000 a	17,000 bc	5.43 b
Natural Predation only	319,000 a	9,000 c	2.83 b

* From about 29,000 plants per acre

** Includes only bolls ½ inch or longer

† Means followed by the same letter do not differ significantly by Duncan's multiple range test at the 1% level.

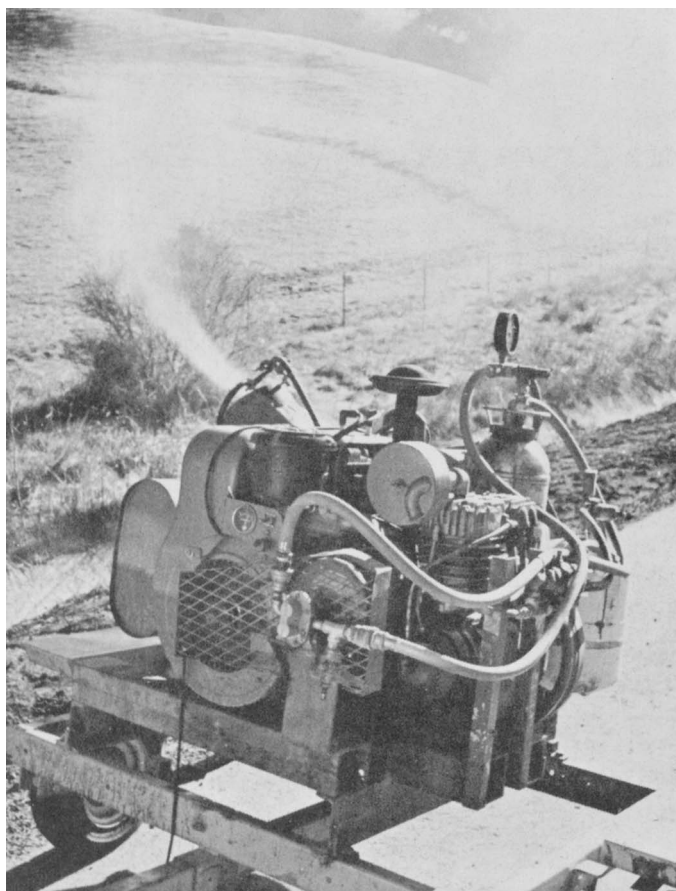
believed to be the main problems and possible solutions in practical field usage of insect pathogens.

The approach taken toward the application and assessment of insect pathogens is similar to that used for chemical insecticides. This research has been twofold: (1) to develop application methods to effectively disseminate insect pathogens at a minimum cost—this was accomplished by using cold aerosol generator systems which deliver fine aerosols (drop size range 10 to 40µm volume mean diameter (VMD), and coarse aerosols (drop size range 40 to 100µm VMD); (2) to develop accurate means of assessing coverage and efficacy of pathogens applied in this manner. To this end both physical and biological methods have been developed.

On August 15, 1969, a preliminary

run was conducted at Skagg's Island in California with a Micro-gen fogger generator equipped with four Belvoir nozzles. A nuclear polyhedrosis virus (NPV) (Viron/H 690) was applied at a rate of 35 to 50 oz per minute with green cotton bolls or cups of synthetic diet exposed at varying distances downwind. These were subsequently fed to first instar bollworm larvae (*Heliothis zea*). Two carriers were tried: (1) refined cottonseed oil and (2) skim milk (dried powder 10gm per 100 cc water). The cottonseed oil proved a more effective carrier, with 70 to 100% mortality recorded for bollworm larvae at distances out to 500 ft.

Following the demonstration of the potentiality of an aerosol system for pathogen distribution, a series of tests were conducted in 1970 and 1971 with commercially available aerosol ground rigs using tracer dyes to assess coverage. In the summer of 1970, experiments with cold aerosol applications were carried out jointly with staff from the Department of Agricultural Engineering, University of California, Davis. Two machines were tested: (1) a cold aerosol generator with a Belvoir nozzle capable of atomization in the range of 10 to 40µm VMD, and (2) an aerosol machine which had been developed for mosquito control and which produced a droplet range of 30 to 90µm VMD. The latter machine was equipped with a Calblower



Prototype cold fogger generator used in 1972-73 studies for aerosol application of insect pathogens.

and two Spraying Systems twin fluid nozzles using air at 40-50 psi at approximately 2 cfm each.

Both NPV (in refined cottonseed oil) and *Bacillus thuringiensis* (Bt) (in 50% refined cottonseed oil, 35% water, 15% diesel fuel) were applied in combination with fluorescent dye as a marker. It was found that by applying the pathogens under temperature inversion conditions and low wind velocities very wide swath coverage was obtained by both rigs. Measurable deposits of dye particles were recovered on mylar sheets one mile downwind, the extent of sampling. Although the use of fluorescent particles proved adequate in assessing spray coverage, difficulties in formulation and the possibility of detrimental effects on the pathogens severely limited the use of this method in further studies.

Fluorescent labeling

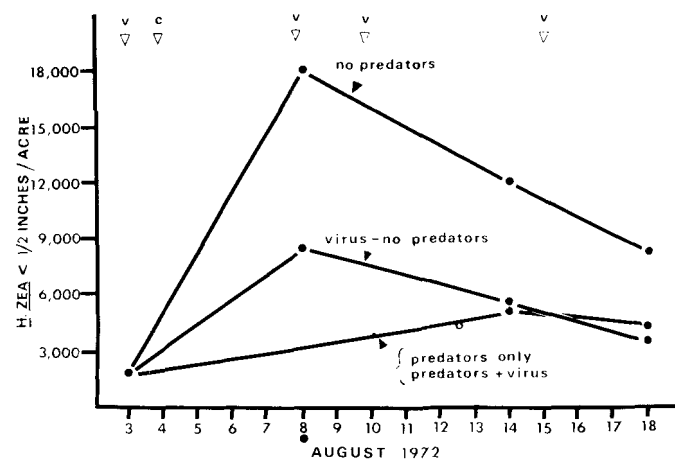
Another approach, a fluorescent antibody labeling technique, was modified for virus detection purposes and tested in August of 1971 with adequate results. While the recovery of tagged insect pathogens downwind from the fogger gave no information as to the effectiveness of the treatment, the experimental data collected during this period provided the

information used in the development of a prototype cold fogger generator built expressly for the dissemination of insect pathogens.

U.C. prototype

During the winter of 1971-1972 the Department of Agricultural Engineering at the University of California, Davis, assembled a prototype cold fogger generator to be used with internal and external mix type nozzles (see photo). Preliminary runs were made with the prototype fogger in July of 1972. A series of 5 spray tests were conducted on alfalfa. The purpose of these studies was threefold: (1) calibrate the fogger and establish optimum machine settings; (2) test carriers for commercial Bt formulations (Dipel wettable powder); and (3) develop assay and bioassay procedures to monitor spore coverage. Maximum coverage was achieved at machine settings of 100 psi air pressure and 10 to 15 psi fluid pressure. Crude cottonseed oil proved to be a more effective carrier than a 40% sucrose solution, based both on increased mortalities in the bioassays with first instar larvae of cabbage looper (*Trichoplusia ni*) and on a greater field recovery of viable spores. Use of a spore assay technique developed by D. E. Pin-

GRAPH 1. EFFECT OF VIRON/H ON BOLLWORM LARVAE—showing where insect predators had been eliminated by application of dimethoate on August 4, fogger applications of Viron/H significantly reduced the number of bollworm larvae. Viron/H had no additive effect on the level of predation in the control plot where dimethoate was not applied.



nock provided an accurate method of assessing coverage of Bt on cotton as well as on alfalfa and sugar beets. Within 24 hours after samples were taken, an accurate count of the number of viable spores per unit area of leaf surface was available. In addition, samples from the assayed leaves were also fed to first instar larvae of cabbage looper, bollworm, and beet armyworm (*Spodoptera exigua*) to measure biological activity of the spore deposits. This technique, coupled with a series of pre- and post-treatment field population counts and observations on field-collected worms, provided useful information on both coverage and efficacy of each treatment.

Calibration studies

Following the calibration studies on alfalfa, Bt (Thuricide) was applied to cotton on the J. G. Boswell Co. Ranch, Tulare Lake Basin, Corcoran, California. Comparisons showed a similar pattern of coverage for cotton, with a maximum of 300 viable spores/mm² of leaf surface recovered at 125 ft from the fogger. Distances sampled followed a geometric progression of either 100, 200, 400, 800 ft, etc. or 125, 250, 500, 1000 ft, etc. The same pattern of spore distribution occurred with each run, although the exact number of spores recovered varied. At approximately 250 ft a sharp drop-off in spore deposits occurred. Viable spores were recovered out to 1600 ft, which was the extent of the sampling. Upon analysis of the data it was decided that the standard commercial Bt formulations tested were too dilute for application by this method. Specially prepared highly concentrated formulations were needed to achieve maximum coverage with the fogger equipment.

A practical demonstration of insect control potential was carried out on cotton against bollworm during the latter part of the season at the Palla Ranch in Kern County. Initially, dimethoate, a chemical insecticide was applied to a five acre area to eliminate insect predators. As anticipated, this resulted in a serious outbreak of bollworm. Four fogger applications of Viron/H in crude cottonseed oil were applied to one half of the dimethoate-treated area over a two-week period in August. The Viron/H treatments resulted in a significant reduction of the bollworm larval population and consequently in the quantity of bollworm-damaged bolls (graph 1, table 1).

Additional data were obtained at the Vignello Ranch in Kern County. Here bolls, squares, and leaf discs were bio-assayed after Viron/H applications to assess total plant coverage. Leaf discs showed the greatest mortality (virus deaths in first instar bollworm larvae). Squares and bolls were difficult to work with because of reduced chance of recovering larvae, but the results showed that these fruiting parts retained enough virus to kill the larvae.

Modifications

In 1973, modifications in the fogger system enabled us to increase pressure to 120 psi air and 70 psi fluid. The first series of tests were carried out in July against cabbage looper and beet armyworm at the J. G. Boswell Ranch in Corcoran. Four side-by-side plots of 80 acres each were used. One plot was treated aerially with Thuricide 16B (2 quarts in 6 quarts of water). A second plot was treated with the fogger using Thuricide 16B without dilution. This formulation of Bt is a highly concentrated experimental preparation. In a third plot, NPV of cabbage looper was applied in crude cottonseed oil. Suppression of the cabbage looper larval populations as compared with that in the check plot resulted from each treatment. Preliminary analysis indicated an almost equivalent control based on field count data for both aerial and fogger applications at distances sampled.

Comparisons of the 1973 results with those obtained in 1972 showed that the spore concentration on leaves was considerably higher in 1973 (graph 2). To assess NPV efficacy leaf damage estimates were made following treatment. The plant growth measurement method for leaf damage estimation was used. On each of five plants per meter row of

cotton, five flag leaves beginning with the first leaf over $\frac{1}{2}$ inch in length were examined for percent damage—four one-meter row samples represented the equivalent of 1/1000th of an acre. Leaf damage was sharply reduced in the treated plots (from 43% damage in the check plot to 0 to 10% damage in the treated areas).

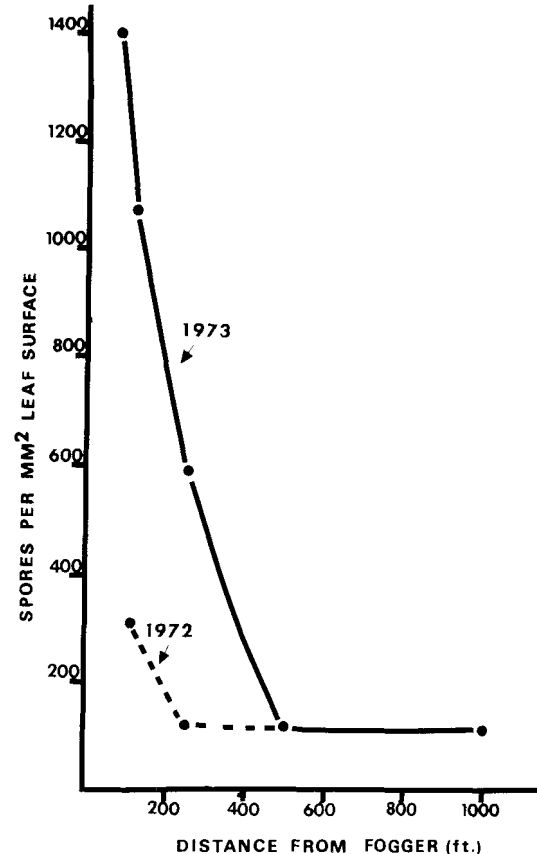
Larvae feeding

Experimental data has shown that following ingestion of Bt or NPV, larvae will cease feeding 2 to 3 days before they die. The data would seem to confirm the presence of non-feeding, yet visible, larvae. For this reason, field counts cannot be relied upon to give a totally accurate picture of the amount of damage occurring after treatment with these insect pathogens. In August a single application of cabbage looper NPV was applied to control an outbreak of cabbage loopers at the Logoluso Farms in Madera, Fresno County. The single fogger application successfully induced an epizootic which completely decimated the population.

Later in the season, application equipment was again compared at the Shubin Ranch near Kerman. Conventional application by air and fogger applications of Thuricide 16B were compared in adjacent cotton fields (20 acres each treatment). Comparisons of horizontal coverage showed a rather even distribution of spores over the tops of the cotton plants for the conventional aerial application and an expected drop-off with distance in spore recovery from the fogger application. Vertical assessment showed that conventional aerial application results in spore deposits concentrated on the top portions of the plant with greatly reduced coverage as lower levels are sampled. The fogger, in comparison, covered the entire plant evenly, reaching even the lowest leaves on the plant (graph 3).

Louis A. Falcon is Associate Insect Pathologist, and Ann Sorensen is Research Assistant, Department of Entomological Sciences, University of California, Berkeley. Norman B. Akesson is Professor, Department of Agricultural Engineering, University of California, Davis. The Micro-gen fogger generator was loaned by the U. S. Navy Biological Research Laboratory, Oakland, California; the Dipel wettable powder was provided by Abbott Laboratories; and the Thuricide was provided by International Minerals & Chemicals Co.

GRAPH 2. A COMPARISON OF SPORE RECOVERY DATA OBTAINED AFTER FOGGER APPLICATIONS OF Bt—shows (1) the downwind pattern of spore distribution provided by the fogger equipment and (2) the higher deposits obtained in 1973 as a result of increasing flow rate and using a highly concentrated formulation of Bt (Thuricide 16B).



GRAPH 3. VERTICAL ASSESSMENT OF Bt SPORE DISTRIBUTION—showing top to bottom coverage on cotton (1) under the spray swath of an airplane and (2) 250 ft. downwind from the fogger.

