SOME ASPECTS OF FLY CONTROL ON DAIRY FARMS

I. EVALUATION OF SOME INSECTICIDE FORMULATIONS AS FOGS IN MILKING PARLORS

II. RESIDUAL EFFECTS OF DERMAL APPLICATIONS OF MALATHION TO DAIRY CATTLE

Thesis for the Degree of M. S.

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Arthur Louis Wells

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SOME ASPECTS OF FLY CONTROL ON DAIRY FARMS I. EVALUATION OF SOME INSECTICIDE FORMULATIONS AS FOGS IN MILKING PARLORS II. RESIDUAL EFFECTS OF DERMAL APPLICATIONS OF MALATHION TO DAIRY CATTLE

By

Arthur Louis Wells

AN ABSTRACT

Submitted to the College of Science and Arts of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Entomology

1957

Approved _____

ABSTRACT

A study was made in 1956 on the Michigan State University campus to evaluate the effectiveness of eight insecticides against house flies and stable flies and to determine some of the physiological effects of malathion on dairy cows.

Experimental oil sprays of Crag F-21, CFR-Mal, Crag LSO-30, Crag LSO-31, Tabutrex, 1 percent and 5 percent malathion, and 7.3 percent Korlan (Dow ET-14) were dispersed as fogs into a milking parlor. Cages of laboratory-reared house flies and stable flies were exposed to fogs dispersed in one-half and two minutes, with additional exposures of one, three, and five minutes. The knockdown resulting from exposure to the fogs was recorded, after which the flies were taken to a laboratory where the mortality was noted two hours later.

The results indicated that the stable flies were more susceptible than were the house flies to the fogs. Only the malathion and Korlan were effective against the house flies. The LSO-30 exhibited a knockdown of stable flies when they were exposed to the fogs over three minutes. All of the materials except Tabutrex showed high toxicity to stable flies.

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To study the physiological effects of malathion, groups of four Holstein cows were treated dermally with 0.5 percent and 1 percent malathion emulsions daily, 1 percent emulsion every third day, and 4 percent dust daily. Blood and milk samples were obtained from the cows periodically to determine the effect on cholinesterase activity and the amount of malathion residue in the milk. Daily fly counts were made on the cows to study the effects of the malathion on the fly populations. Milk and butterfat production records and weekly weight records were kept to determine if they were affected by the malathion.

The results indicated that there was a wide variation in the susceptibility of the cows to fly attacks. Although the emulsion treatments tended to lower the fly populations on the cows, the difference could not be proven statistically. The cows receiving the dust treatments appeared to attract more flies than the untreated cows. The 1 percent applications of malathion inhibited the cholinesterase activity in the cows for three months after the last applications. The 0.5 percent emulsion and dust applications affected the cholinesterase activity for a shorter period than the 1 percent treatments.

Malathion was excreted in the milk in small amounts for a week following the applications of the 1 percent emulsions. The 0.5 percent emulsion and dust resulted in the excretion of malathion in

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the milk for three days after the final treatments. The malathion treatments did not affect the milk or butterfat production or the weights of the cows. No external evidence of toxicity to the cows was observed during the project by the cooperating veterinarian.

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INTRODUCTION

Each year livestock farmers are confronted with the problem of flies on their livestock and around their barns. This is especially apparent to dairymen who wish to keep their cattle and milking areas free from flies. The enforcement of the Federal Food, Drug and Cosmetic Act, as amended, has put limitations on the use of insecticides in these situations to avoid possible contamination of meat and dairy products. A dairy farmer must take these limitations into consideration while planning a fly-control program on his farm.

In the past, dairymen have relied on the use of space sprays and the direct application of oil-base sprays to their cattle for the control of troublesome flies. Several new methods for the application of fly toxicants have been developed in recent years. Among these are the use of residual-type sprays, back-rubbers, treadle sprayers, and aerosol machines. These methods have been advanced partially by the changes in dairy farming practices. The use of pen-type barns and milking parlors is one of these changes. The application of fly toxicants as fogs in the milking parlors is one of the most recent developments for fly control in these locations.

Many synthetic insecticides have been developed in the past fifteen years to combat flies and other insects. Among these have been DDT and other organic insecticides. Since some species of flies have acquired resistance to certain of these compounds, other chemicals have been developed to replace them in fly-control programs. Before new materials can be put into use commercially they must be tested for toxicity to flies and their possible physiological effects on warm-blooded vertebrates.

The main objectives in this study were: (1) to study and compare the effect on flies of eight new insecticides when they are used as fogs in a milking parlor; (2) to study some of the physiological effects of dermal applications of malathion on dairy cows, with special emphasis on milk contamination and cholinesterase inhibition; and (3) to study the effects of these applications on the fly populations on the cows.

LITERATURE REVIEW

The Use of Insecticides as Fogs and Space Sprays

Many insecticides have been tested and compared for their effect on flies when applied as fogs or space sprays. The method proposed by Peet and Grady (1928), or modifications thereof, have been used in the initial tests of many of these insecticides. A modification of this method was used by Atkeson, Smith, Borgmann, and Fryer (1944) in testing sixteen materials as space sprays against caged house flies. They stated that "the results indicate the importance of a lethal dosage at the time of knockdown and indicate that percentage of kill is a better measure of the toxicity of a livestock spray than is the percentage of knockdown."

Morrison et al. (1950) used an aerosol generator to apply insecticides as fogs in a dairy barn for house fly and horn fly control. Space sprays over the backs of the cows were also used in their fly-control program. Fisher (1955) reported the use of fogs as a method for applying quick-knockdown sprays in dairy barns. He used a portable air compressor attached to an Insectojet nozzle. The nozzle was suspended from the ceiling of a stanchion-type dairy barn and the sprays were dispersed as fogs over the backs of the

cows before milking. He obtained effective control of insects in the barn and on the cattle with a piperonyl butoxide-pyrethrin spray applied by this method regularly as needed.

The Direct Application of Insecticides to Cattle

Effect on flies. The first reports available for the effect of repellents on dairy cattle were presented by Graybill (1914). He stated that up to that time mostly oils were used on cattle to protect them from horn flies and stable flies. Pyrethrum powder had been used on cattle and was found to have a repellent effect on flies for about a day. He indicated that light-colored cows were bothered less by flies than were those with dark markings.

One of the more recent studies on the protection of cattle from horn flies was made by Laake et al. (1950). They applied two quarts of aqueous spray to each cow in several dairy herds. They found that a 0.5 percent methoxychlor suspension protected the cows for seventeen days against horn flies, a 0.5 percent toxaphene emulsion gave eighteen days' protection, and a 0.025 percent lindane emulsion gave fourteen days' protection. A synergized pyrethrin emulsion gave approximately six days' protection from the horn flies.

Physiological effects of fly sprays on cows. The effects of flies and fly sprays on milk production were first studied in the early 1900's, but the first controlled experiments were conducted by Freeborn et al. (1925). They found that the milk production of cows confined in heavy infestations of stable flies for a month was 9 percent below that of noninfested cows. When the cows were sprayed daily with a nontoxic oil spray with the flies present, the cattle with stable flies lost 21 percent in milk production while those with horn flies lost 13 percent. When sprayed with a pyrethrum oil spray the cows with stable flies lost 12.4 percent of the normal milk flow. As a result of further tests (Freeborn et al., 1928), they stated that "the loss to dairy production caused by flies is sometimes greatly overestimated and that often greater damage to the cows results from the spray than from the flies." This conclusion was drawn after discovering that daily applications of an oil spray increased the body temperatures of the cows as much as 3° F., increased the respiration rate 40 percent over the controls, and decreased the milk production over 20 percent.

Shaw and Atkeson (1943) indicated that there was no significant difference between the production of cows sprayed with oil-base sprays and unsprayed cows. Bruce and Decker (1947) reported that herds treated with DDT or Rhothane maintained milk production

better than herds treated with repellent-type sprays. They also noticed an inverse correlation between changes in milk production and fly abundance. Granett and Hansens (1956), after applying 10 percent methoxychlor and Crag F-21 concentrate sprays diluted with water, found a significant difference for two days between the horn fly and stable fly populations on treated and untreated cows. They also found a significant increase over the checks in milk production on the second day from both treatments, and from the Crag F-21 treated cows on the third day. They indicated that the cows varied in their susceptibility to fly attacks. Morrison et al. (1950), as a result of a study of fly control on dairy cattle, indicated that Jersey cows were significantly less attractive than Holsteins to flies and that "there was considerable variation of horn fly populations on the Holsteins with the flies seemingly showing preference for the cows on which black color predominated."

Wilson et al. (1933) reported that when oil sprays were applied directly to cattle the skin was affected and the body temperatures were raised slightly. Regan and Freeborn (1936), in further studies on the physiological effects of fly sprays on dairy cattle, found that the bodily effects of oil sprays were greater when they were applied during temperatures exceeding 80° F. The temperature increase apparently was caused by the lack of evaporation of

water from the skin. They also reported that emulsions of pyrethrum and pine oil with a small amount of petroleum oil was as efficient and was less detrimental to cows than petroleum sprays. In the same report they stated that the loss of milk production due to infestations of house flies and horn flies was negligible when compared to stable flies.

Hyperkeratosis in calves has been caused by topical applications of mineral seal oil (Hoekstra et al., 1954a). This same condition was not produced when pyrethrin-sulfoxide emulsions or suspensions were applied to calves for the same length of time. The addition of lindane, methoxychlor, and thiocyanate compounds to mineral seal oil did not increase the severity of the skin condition (Hoekstra et al., 1954b). The oil also caused a depression of the vitamin A concentration in the blood plasma. The effects were greater on young calves than on older ones. The materials were applied at double the manufacturer's recommended safe levels of usage.

Melvin (1932) reported that oil sprays at the rate of 1.6 ounces per cow caused body temperatures to rise and respiratory rates to increase. The increase in body temperatures was greater when air temperatures exceeded 85° F. When the cows were exposed to the sun, the dark-colored animals had higher temperatures

than did those which were light-colored. Milk-producing cows were affected by these conditions more than were heifers. After exposing cows and heifers to house flies and stable flies, he found that large populations of house flies did not affect the body temperatures of the cattle, but that two hundred or more stable flies per animal caused a measurable rise in body temperatures and respiratory rates of both heifers and producing cows.

Atkeson, Borgmann, Smith, and Shaw (1944) also found that some base oils, after continued application to cows, caused a skin reaction. They further reported that the best index of the effect on the skin was the unsulfonatable residue content. In most cases oils containing 92.5 percent or more unsulfonatable residues were the least harmful. Saybolt viscosity of an oil was not a reliable index of skin reaction from direct applications.

Residues in milk. Soon after the extensive use of organic insecticides began in the 1940's people became concerned about food contamination by insecticide residues. Since then, extensive analytical studies have been made on residues, especially in milk from cows which have been treated for parasites or fed insecticide-treated forage. Milk and other dairy products are known to acquire

odors or off-flavors very easily from the cows' diet or metabolic activity, and the presence of insecticides is no exception.

Knipling (1950) listed three ways in which dairy products may become contaminated with insecticides: (1) ingestion of residues on forage fed to cows; (2) absorption from treatment for parasites; and (3) ingestion from treatment of barns. Carter et al. (1949) also included such methods of contamination as careless use of milking equipment, mechanical transfer of the materials during hand milking, or ingestion of residues from licking treated animals.

Some of the first research on insecticide residues in milk was done by Howell et al (1947). They found that a Jersey cow excreted a maximum of 20 p.p.m. DDT in her milk for 126 days after forty-five daily applications of a 5 percent suspension spray. A Holstein which had received the same treatment excreted a maximum of 18 p.p.m. DDT for 21 days after the first positive analysis. Another Jersey which had been sprayed with 5 percent DDT daily for 20 days first excreted DDT six days after the first application. She excreted a maximum of 33.6 p.p.m. on the twentieth day and continued until the end of her lactation period 119 days later.

Furman and Hoskins (1948), after spraying cows with a 0.5 percent benzenehexachloride suspension without preventing the animals from licking themselves, found as high as 5.5 p.p.m. of the

gamma isomer equivalent in the milk the first day after treatment as determined by an analysis of the butterfat. The residue was practically gone on the eighth day. When self-licking was prevented, the cows excreted 3.2 p.p.m. gamma equivalent in the butterfat, and it was practically gone within a week. Knipling (1950) reported that lindane appeared in amounts up to 2 p.p.m. in the milk the first day after the application of a 0.1 percent spray to dairy cows.

Carter and Mann (1949) reported that a cow sprayed to the point of saturation with a 0.5 percent DDT suspension excreted as high as 3.0 p.p.m. DDT in the milk for a period of approximately five weeks. Later, Carter et al. (1949), after extensive experiments with direct applications of 0.5 percent suspensions to Jersey cows, found that DDT, TDE, and possibly chlordane were eliminated in the milk. They had no conclusive evidence that toxaphene and methoxychlor were eliminated, since the amounts of organic chlorine found in the milk were so small. They further found that pasteurization did not decompose the DDT content in milk samples.

Research workers at the United States Department of Agriculture laboratories at Kerrville, Texas, have done extensive research on insecticide residues in milk. Claborn et al. (1950) reported that the direct applications of 0.5 percent DDT and TDE emulsions caused about the same residue in milk, and that two quarts of the

sprays per cow caused more contamination than did one quart. The residue in the milk increased when a supplemental residual spray was applied to the barn.

Claborn and Wells (1952) reported that dieldrin, methoxychlor, and Dilan have also been excreted in the milk following one application of 0.5 percent sprays to Jersey cows. Methoxychlor was found to be excreted in lesser amounts than were the other materials, and emulsions were found to be absorbed faster than were suspensions. In other tests at Kerrville (Claborn, 1956), eight chlorinated hydrocarbon insecticides and malathion were compared for milk contamination following similar dermal applications. Dieldrin was found to cause the greatest contamination, followed in decreasing order by DDT, TDE, Dilan, toxaphene, Strobane, methoxychlor, Perthane, and malathion.

Claborn et al. (1956) reported the results of experiments on the residual effects of malathion in milk after dermal applications to cows. After spraying Jersey cows with two quarts of 0.5 and 1 percent emulsions and suspensions, they found that the cows excreted malathion ranging from 0.08 to 0.36 p.p.m. in 4 percent fat-corrected milk in all samples of milk taken five hours after treatment. Traces were found in samples taken one day later, but samples taken after three and seven days were free of any residues.

The higher concentrations caused larger residues than did the lower concentrations, and the suspensions caused more residues than did the same concentrations applied as emulsions.

Residues in meat. Many of the organic insecticides which have been used for fly control have been found to contaminate the meat of treated cattle. Claborn (1956) has compiled reports that residues of DDT, TDE, methoxychlor, dieldrin, heptachlor, chlordane, "Gamma chlordane," Strobane, and toxaphene have been found in the fat of cattle which had been sprayed with the insecticides. Claborn et al. (1956) found no malathion residues in the fat of Hereford cattle which had been sprayed with 0.5 percent suspensions or emulsions sixteen times at weekly intervals.

The fate of malathion after dermal application. March et al. (1956) have traced the fate of dermally applied malathion in calves with the use of radioactive phosphorus in the malathion molecule. Two 200-pound Jersey calves were sprayed with one pint of a 0.5 percent emulsion spray twice at two-week intervals, and the calves were sacrificed one and two weeks after the second application. The various tissues and organs, including the bones, hide, and brain, were analyzed for malathion and radioactive phosphorus by chemical assay and radioassay. Urine samples collected after the second spraying

were analyzed for malathion and water-soluble metabolites. The urine analyses indicated that the greatest absorption and elimination of malathion took place within 24 hours after application. The data indicated that between 96 and 99 percent of the malathion eliminated was in the form of water-soluble metabolites. The residues were found as metabolites and degradation products throughout the animals, but only the hide contained unchanged malathion. The highest levels of radioactivity were found in the bones, hide, liver, thymus, pancreas, and tongue. This indicated that the phosphorus from the degraded compound was used in the normal phosphorus metabolism of the animals. No detectable malathion (less than 0.2 p.p.m.) was found in the various cuts of meat by chemical assay.

Cholinesterase inhibition by malathion. Hamblin et al. (1956) have summarized the results of three experiments on the effects of dermally applied malathion on the cholinesterase activity of cattle. These studies have been made by the American Cyanamid Company, manufacturer of malathion, and the United States Department of Agriculture.

In one experiment a 1.25 percent malathion emulsion spray containing 2.5 percent sucrose was applied weekly over a seven-week period to the point of run-off to five dairy cows and four

1-week-old calves. Blood samples taken just prior to each application and at 24 and 72 hours after spraying showed no indication of erythrocyte cholinesterase inhibition in the cows. The cholinesterase activity of the calves remained at approximately 80 percent of the pretreatment level although due to the lack of sufficient data this level of activity could not be attributed definitely to the malathion.

Twelve Hereford cattle were used in one experiment for studying cholinesterase activity. Four of the cattle were treated with a 0.5 percent malathion emulsion spray, four were treated with a 0.5 percent suspension, and four were left untreated as controls. The sprays were applied to the point of run-off at weekly intervals for a total of sixteen applications. The erythrocyte cholinesterase activity was determined during the week prior to the first spraying and weekly thereafter up to three months following the last application. The cholinesterase activity of all the treated animals was depressed after one treatment. The cholinesterase activity of the blood samples from the animals treated with the emulsion was depressed steadily after five consecutive treatments. It remained at this level of activity until the last application. The cholinesterase activity began regeneration after this last

treatment and continued for about three months before it was comparable with the level of activity of the controls.

Application of the malathion suspension treatments caused a sharp depression after two treatments and continued downward to the minimum activity after nine treatments had been applied. It remained at this level of activity until the posttreatment regeneration began. The period of partial or complete recovery was about three months. The cholinesterase activity of the cattle receiving the suspension was depressed to a greater extent than of those being treated with the emulsion. No gross evidence of toxicity was observed in any of the animals.

Four Jersey cows were treated twice, one week apart, in another experiment. Two of the cows received, to the point of run-off, a 1 percent emulsion spray, and the other two received the same amount of a 0.5 percent suspension spray. No significant depression of cholinesterase activity was found from analyses made one hour before and 24 hours after each treatment and one week after the last treatment.

PROCEDURE

The Effects of Insecticide Fogs on Flies

Eight insecticides were tested as fogs for their relative toxicity to flies. These formulations, listed in Table I, were composed of both commercial and experimental fly toxicants. All of the materials except the Crag F-21 and Crag CFR-Mal were formulated as oil-base sprays. These two materials were concentrate sprays which were used as formulated in this project. All of the Crag formulations were based on butoxypolypropylene glycol (Crag Fly Repellent). Tabutrex, which is a repellent-type material, was included to compare its action on caged flies with known toxicants. The malathion and Korlan are both phosphate insecticides.

The insecticides were dispersed as fogs into a closed milking parlor by the use of a four-opening Insectojet unit.[1] The unit was installed in the milking parlor so that the nozzles were one foot below the ceiling approximately in the center of the room. It was connected by pipe and hose to a one-quarter horsepower electric

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[1] Insectojet 4-2 (Spraying Systems Company, Bellwood, Ill.).

TABLE I

INSECTICIDE FORMULATIONS TESTED FOR THEIR TOXICITY TO FLIES WHEN DISPERSED AS FOGS IN A MILKING PARLOR

Insecticide	Percentage Composition
Crag F-21 Emulsifiable Concentrate[a]	
Butoxypolypropylene glycol	49.84
Methoxychlor, technical	5.00
Methylated napthalenes	40.50
Petroleum distillate	0.55
Inert mixed alkyl aryl poly ether alcohols	4.11
	100.00
Crag CFR-Mal Livestock Spray (Concentrate)[a]	
Butoxypolypropylene glycol	50.00
Malathion, technical 95%	5.26
Solvent and carrier	44.74
	100.00
Crag LSO-30 Livestock Spray[a]	
Butoxypolypropylene glycol	8.60
Pyrethrins	0.03
Piperonyl butoxide, technical	0.25
Methoxychlor, technical	1.01
Petroleum distillate	90.11
	100.00
Crag LSO-31 Livestock Spray[a]	
Butoxypolypropylene glycol	6.20
B-butoxy-B'-thiocycanodiethyl ether	0.60
Methoxychlor, technical	1.00
Petroleum distillate	92.20
	100.00
Tabutrex Insect Repellent Oil Base Spray[b]	
Tabutrex	2.00
Petroleum distillate	98.00
	100.00

[a]Furnished by Union Carbide and Carbon Company, New York, N.Y.

[b]Furnished by Consolidated Research and Testing Laboratories, Chicago, Ill.

TABLE I (Continued)

Insecticide	Percentage Composition
Malathion 1% Oil Base Spray[c]	
Malathion, premium grade	1.00
Solvent	5.00
Deobase	94.00
	100.00
Malathion 5% Oil Base Spray[c]	
Malathion	5.00
Piperonyl butoxide	0.50
Pyrethrins	0.05
Solvent and carrier	94.45
	100.00
Korlan (Dow ET-14) Oil Base Spray[d]	
Korlan	7.30
Solvent and carrier	92.70
	100.00

[c]Furnished by American Cyanamid Company, New York, N.Y.

[d]Furnished by Dow Chemical Company, Midland, Mich.

air compressor[1] in an adjoining room. The compressor maintained a constant pressure of 30 pounds per square inch at the nozzles. The insecticides were siphoned from a plastic jar which was attached to the fogging unit.

The insecticide formulations varied in viscosity; therefore, each material was calibrated for its rate of emission from the unit. The materials were found to be emitted faster when the jar was full than when it was nearly empty. To compensate for this difference, the jar was filled to the same depth with each material before the initiation of a test.

The milking parlor in which the comparison tests were made had a volume of 3,250 cubic feet into which the fog diffused. Existing insecticide vapors were eliminated from the room by forced ventilation as much as possible before each material was tested. The milking parlor was located in one of the loose-housing dairy barns on the Michigan State University campus. The barns were about two miles from the laboratory where the flies were reared.

The house fly, Musca domestica L., and stable fly, Stomoxys calcitrans (L.), were used as test species in this project. The specimens that were exposed to the fogs were reared from artificial

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[1] Industrayer (The Tanglefoot Company, Grand Rapids, Mich.).

media in a controlled-temperature cabinet. The original culture of house flies was obtained from collections in the vicinity of the dairy barns on the Michigan State University campus. The stables flies were from a laboratory culture.

The house flies were fed a milk-sugar solution and allowed to deposit eggs on media in the breeding cages. The eggs and small larvae were placed in a controlled-temperature cabinet to complete their development. After emergence the adult flies were transferred to holding cages and fed for at least 24 hours before being exposed to the insecticides.

The stable flies were reared similarly except for their food. They were fed warm citrated bovine blood from saturated cellulose cotton. The blood was obtained fresh weekly from a local slaughter-house. The flies deposited eggs on the dried blood in the feeding dishes, after which the eggs were washed off with distilled water and transferred to the larval media.

An hour before each test was conducted the flies to be used were placed in a walk-in freezer at 0° F. for a few seconds to inactivate them while they were counted and transferred to the test cages. The counting was done in a room at 50° F., after which they were brought back to normal room temperature. The test cages were made of 18-mesh galvanized screen rolled into cylinders

5-1/2 inches long and 2 inches in diameter and held with rubber bands. The ends were covered with cheesecloth and secured with rubber bands. Twenty-five flies, disregarding the sex, were put into each cage. Twenty-seven cages of each species were used to test each insecticide. All of the cages were marked with a wax pencil for identification before exposing them to the fog. New cages were used for each test.

The cages of flies were then taken to the milking parlor for the exposure tests. They were suspended from the ceiling of the room on three wires. Each was located approximately five feet from the nozzle unit in different corners of the room. Four loops were formed in each wire to suspend the cages 18, 24, 30, and 36 inches from the ceiling.

Twelve cages were placed in the wire loops, and the doors and windows were closed to prevent any air currents or any of the fog from escaping. The air compressor was run for one-half minute, three cages were removed from the fog immediately, and the number of paralyzed flies in each was recorded. The flies were considered paralyzed if they were unable to walk around or climb up the sides of the cages. After noting the knockdown, they were taken out of the barn away from any traces of the fog. Three additional cages were removed at one, three, and five minutes after

the compressor had been turned off. After recording the knockdown in each, they were also taken out of the barn while the test for that material was being completed.

The milking parlor was cleared of all insecticide vapors by forced ventilation before the second part of the test was undertaken. This prevented the flies from being exposed to an unknown amount of insecticide. After ventilating the room, twelve more cages of the same species of flies were placed in the wire loops and the compressor was run for two minutes. At the end of this period, and one, three, and five minutes later, the cages were removed and treated as described above.

Three of the twenty-seven cages of flies that were taken to the barn were not exposed to the fog. The mortality in these cages due to handling was recorded before taking them back to the laboratory. These data were used in analyzing the effects of the insecticides on the flies. The entire fogging process was repeated for each insecticide on both house flies and stable flies.

After the flies had been exposed to the insecticide fogs they were taken back to the laboratory where they were kept apart to prevent intercontamination. The mortality of flies in each cage was checked and recorded again two hours after exposure. In pretesting experiments little difference was found between the mortality of

stable flies that were fed warm beef blood after exposure to the fog and those which were unfed. Therefore, the flies used in these tests were not fed after exposure.

The Direct Application of Malathion to Dairy Cows

Description of the cows used. The Hostein cows used in studying the residual effects of malathion when applied directly to cattle were part of the experimental dairy herd of Michigan State University. They were used primarily for serological and nutritional research. Complete records of their feed rations and milk and butterfat production, as well as weekly weights, were kept. All of the producing cows were on a twice-daily milking schedule. The cows were turned out about three hours daily into a loafing yard for exercise; otherwise their activities were controlled in a stanchion-type barn.

The twenty cows used in this study were selected from the herd and divided into groups of four cows each. They were divided so there would be no more than one cow in any group which was on another research project at the same time. Three groups of four cows each were used at the start of the project and two more groups of four were added later. These five groups were used for four treatments and a control.

The ages, weights, and stages of pregnancy and lactation periods of the cows used in this study are presented in Table II. Their ages ranged from two and one-half to ten years, with an average of approximately five and one-half years. All of the cows had freshened at least once before the start of the project. They were at different stages of pregnancy and lactation periods at the time of this study. Although the lactation periods of some of the cows ended during the summer, all were in production during the period of treatment. Their weights and milk production were thus affected by these conditions. Their weights on September 1, 1956, ranged from 890 to 1,250 pounds, with an average of 1,088 pounds.

Treatments. The malathion was applied externally to the cows as 0.5 and 1 percent emulsion sprays and as a 4 percent dust. They were applied to the back and sides of the cows from the shoulders to the rump, including the legs, tail, and udder. The materials were not applied to the neck where the veni-punctures were made to obtain blood samples for the cholinesterase study. There were no other insecticides used in the barn during the course of the project.

All of the treatments were applied to the cows about 8:30 A.M., after the morning milking period and after the fly populations

TABLE II

AGES AND STAGES OF LACTATION PERIODS OF HOLSTEIN COWS USED IN MALATHION TOXICITY STUDY

Cow No.	Date of Birth	Last Calving Date	Last Breeding Date	Weight (9/1/56) in lbs.
	One Percent Malathion Emulsion Daily			
A-78	5/27/48	2/7/56	6/12/56	1114
A-95	5/17/51	12/4/55	2/23/56	1232
A-98	10/26/52	12/29/55	5/23/56	1062
K-129	9/4/49	2/12/56	11/5/56	1070
	One Percent Malathion Emulsion Every Third Day			
568	1/7/53	2/20/56	5/23/56	1080
A-94	6/14/51	10/17/55	2/17/56	1250
K-139	9/27/50	4/7/56	11/2/56	1058
K-303	7/8/52	12/12/55	4/6/56	1066
	One-half Percent Malathion Emulsion Daily			
A-67	7/7/46	12/8/55	4/7/56	1026
A-77	5/25/48	5/12/56	8/10/56	1160
A-80	7/30/48	7/4/56	10/1/56	1210
218	12/1/52	3/23/56	7/25/56	1000
	Four Percent Malathion Dust Daily			
T-3	2/2/50	6/16/56	10/4/56	1054
T-4	2/2/50	4/24/56	11/5/56	1100
A-103	2/17/53	7/11/56	11/2/56	1010
K-131	11/19/49	8/17/56	open	1140
	Untreated			
T-17	2/1/51	3/3/56	6/5/56	1080
A-110	10/15/53	3/18/56	8/10/56	1000
K-239	11/11/53	1/18/56	11/2/56	890
K-213	6/5/51	1/23/56	4/4/56	1148

were counted for that day. The number of treatments which each group received depended upon the amount of enzyme inhibition in their blood. While the project was being organized it was decided to discontinue treating the cows if the cholinesterase activity decreased 50 percent from the pretreatment level. This point was established for the health of the cows since the accumulative effect of malathion in this type of application was uncertain.

A 57 percent malathion (5 lb. actual malathion per gal.) emulsifiable liquid (premium grade) furnished by the American Cyanamid Company was used to prepare the sprays. The sprays were applied to the point of run-off with a one-gallon compressed air sprayer. This was found to use approximately one quart of the spray. This amount of the 1 percent spray contained nine grams of actual malathion. One group of four cows received this treatment daily from July 30 through August 6, 1956, and another group received the same treatment every third day from July 30 through September 19. Another group of four cows was treated in the same manner from August 15 through September 19 with a 0.5 percent spray. One quart of this emulsion contained 4.5 gm. of actual malathion.

A 4 percent malathion dust was used to treat one group of four cows. It was applied daily from August 28 through September

19, 1956, with a hand garden duster[1] at the rate of 1.25 ounces (1.4 gm. of actual malathion) per cow.

Determination of fly control. The effectiveness of the insecticide treatments on the fly populations was determined by keeping a daily record of the number of flies on the cows. The fly populations on the cows were composed mostly of house flies, Musca domestica L. Although stable flies, Stomoxys calcitrans (L.), were numerous in the barn at times, they were never present on the cows in numbers comparable to those of the house flies. The daily fly populations were determined by counting the flies on the right side of the cows from the shoulders back to the tail and down to the knees. The counts were made on the treated cows before the insecticides were applied. The counts were made on the control animals at the same time.

The amount of black markings on the cows varied between individuals; therefore, outline drawings of the markings on all of the cows were made. These were made to determine if flies were attracted to cows with certain markings more than others. No record was kept of the location of the flies on the color markings while making the daily counts.

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[1] Pestmaster Garden Duster (D. B. Smith and Co., Utica, N.Y.).

Meteorological data. The temperature and climatic conditions for the local area during the testing period were obtained from the East Lansing office of the United States Weather Bureau. These data were obtained to study the association of climatic factors and the fly populations on the cows.

Blood sampling for cholinesterase study. The blood samples for the determination of the cholinesterase activity were taken at various intervals for durations depending upon the amount of enzyme inhibition and rate of regeneration. Two samples of blood were taken from all of the cows before a treatment was applied. This was done to determine the normal level of enzyme activity. Samples were taken from the cows receiving the 1 percent spray daily the day after the first application and then at three-day intervals for three samples. They were then sampled at weekly intervals for seven weeks, after which they were taken at two-week intervals for a month with a final sample three weeks later. The data for this group were completed on November 12, 1956, ninety-eight days after the final application of insecticide.

The cows receiving the 1 percent spray every third day were sampled at intervals corresponding with the 1 percent daily group, with the exception of the last sample. This sample was

omitted since the regeneration of the cholinesterase activity was not followed back to the initial level in this group.

The group being treated with the 0.5 percent spray was sampled twice before the first treatment to determine the normal level of enzyme activity. They were sampled again after one application and then at weekly intervals for six weeks, followed by two samples at two-week intervals. The sampling of this group was not continued until the cholinesterase activity returned to normal.

The cows receiving the dust treatment were sampled twice before the first application and then again the following day. Weekly samples were then taken for four weeks, followed by two samples at two-week intervals. Since the enzyme activity was not depressed to a great extent during the project, the sampling was terminated at that time.

Samples of blood from the untreated cows were taken at intervals throughout the duration of the project to compare their cholinesterase activity with that of the treated cows. The exact days of sampling are given in the data presented here.

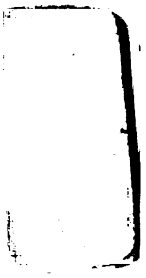
The blood samples were taken after the morning milking period of each sampling day. The cows were first sponged with soap and warm water in the area of the jugular vein where the veni-punctures were made. About 3 ml. of blood from each cow

were collected into graduated heparinized tubes for each cholinesterase analysis. The samples were gently agitated for even dispersion of the anticoagulant and transported in crushed ice to the laboratory.

The cholinesterase analyses were determined by Miss Florence Hermenze in a laboratory provided by the Agricultural Chemistry Department of Michigan State University. The electrometric method of Michel (1949) was used in making the analyses. After centrifuging the blood samples to separate the plasma from the erythrocytes, the determinations were made on the washed erythrocytes only. As a rule, the initial tests were analyzed on the day of blood sampling and duplicate tests determined the following day. The average of the two tests on each blood sample are given as the final result.

Analysis of residue in milk. Milk samples for making the residue analyses were taken at intervals during the testing period. The duration for which these were obtained depended upon the treatment applied to the cattle. It was assumed that the malathion would not remain without hydrolyzing in their systems for more than ten days after the last treatment; therefore, sampling of the milk was discontinued at that time.

One sample of milk was taken from each of the cows before treatment. They were then sampled at or before the third milking



after the first treatment was applied. The milk from the cows treated with the 1 percent spray daily was sampled three times during the eight days of treatment. It was then sampled at the third, ninth, and fifteenth milkings after the last treatment was applied. Samples of milk from the cows receiving the 1 percent spray every third day were taken twelve times during the period of treatment, and then at the third and seventeenth milking after the last application.

Milk from the group receiving the 0.5 percent spray daily was sampled six times during the 36 days of treatment, first at three-day intervals and then weekly. It was sampled again at the third and seventeenth milkings after the last treatment. Five samples were taken from the group receiving the dust during the 23 days of treatment. The milk was also sampled at the third and seventeenth milkings after the last application was made. Milk from the group of untreated cows was obtained fifteen times during the course of the project to determine if they had acquired malathion in their systems.

The first two groups of samples were taken during the morning milking period, but, because of inconvenience, the remaining samples were taken during the evening milking. Before putting the milking machine on the cows, their udders were washed and the

first milk was examined in a strip-cup for the presence of any inflammation of the udder. After each cow in the herd was milked, the milk was weighed and sampled for butterfat tests. A liter sample of milk was then taken and poured into glass jars for the residue analyses.

The samples were then taken to a laboratory and transferred to plastic sacks which in turn were put into paper sacks for reinforcement and ease in handling. They were then placed in a walk-in type freezer at approximately 0° F., where they were frozen. They remained frozen until the residue analyses were made by Dr. Zenobius Stelmach. The colorimetric method developed by the American Cyanamid Company was used for making the residue analyses. This method of analysis is accurate to 0.02 p.p.m. of malathion in the whole-milk sample.

The first milk samples were analyzed on August 2, 1956, and the last ones were completed on December 26, 1956. All of the samples were not analyzed immediately after they were taken, but were determined at different intervals after the actual sampling. This was due to the amount of time required to process the samples in making the analyses.

PRESENTATION OF DATA

The Effects of Insecticide Fogs on Flies

Mortality from exposure to fogs. The knockdown and mortality of flies resulting from exposure to the insecticide fogs are presented in Tables III through VI. The data obtained from exposing house flies to the fogs dispersed in one-half minute and two minutes are given in Tables III and IV, respectively. The amounts of each material that were emitted from the nozzle-unit into the milking parlor during the periods in which the air compressor was operating are given in part A of each table, along with the knockdown resulting from this fog. The mortality resulting from this exposure is given in part B of each table. Corresponding data for the stable flies are presented in Tables V and VI. The mortality in the untreated cages of house flies and stable flies ranged from 0 to 5 percent at the milking parlor. The mortality two hours later ranged from 0 to 13 percent. These losses apparently were due to the handling of the cages. All of the toxicity data from the exposed cages of flies are corrected with these losses in the untreated cages by the use of Abbott's formula (Abbott, 1925).

TABLE III

CORRECTED[a] KNOCKDOWN AND MORTALITY OF HOUSE FLIES RESULTING FROM EXPOSURE TO VARIOUS INSECTICIDE FOGS DISPERSED INTO A MILKING PARLOR FOR ONE-HALF MINUTE

Insecticide	Amount Emitted in 1/2 Minute (ml.)	Additional Exposure to Fog after Its Introduction			
		None	1 Min.	3 Min.	5 Min.
Part A. Percent knockdown[b] (data obtained immediately after removal of flies from fog)					
Crag F-21	3.5	0	0	0	0
CFR-Mal	5.0	0	0	0	0
Crag LSO-30	27.0	0	0	0	0
Crag LSO-31	28.0	0	0	0	1.3
Tabutrex	20.0	1.3	2.7	0	0
Malathion 1%	31.0	0	0	0	0
Malathion 5%	24.0	0	0	0	0
Korlan (Dow ET-14) 7.3%	22.5	0	0	0	0
Part B. Percent mortality[b] (data obtained two hours after removal of flies from fog)					
Crag F-21		0	0	0	0
CFR-Mal		0	1.3	0	0
Crag LSO-30		0	0	0	0
Crag LSO-31		1.3	1.3	0	1.3
Tabutrex		1.3	2.7	0	0
Malathion 1%		0	0	0	0
Malathion 5%		38.7	22.7	21.3	17.3
Korlan (Dow ET-14) 7.3%		98.7	97.3	98.7	98.7

[a] All toxicity data corrected by mortality in untreated cages of flies.

[b] Data based on 75 flies (three cages of 25 flies each).

TABLE IV

CORRECTED[a] KNOCKDOWN AND MORTALITY OF HOUSE FLIES RESULTING FROM EXPOSURE TO VARIOUS INSECTICIDE FOGS DISPERSED INTO A MILKING PARLOR FOR TWO MINUTES

Insecticide	Amount Emitted in 2 Minutes (ml.)	Additional Exposure to Fog after Its Introduction			
		None	1 Min.	3 Min.	5 Min.
Part A. Percent knockdown[b] (data obtained immediately after removal of flies from fog)					
Crag F-21	14.0	5.3	5.3	0	0
CFR-Mal	20.0	1.3	0	0	0
Crag LSO-30	108.0	1.3	1.3	0	4.0
Crag LSO-31	112.0	0	0	0	0
Tabutrex	80.0	0	0	0	0
Malathion 1%	124.0	0	0	0	0
Malathion 5%	96.0	0	0	0	0
Korlan (Dow ET-14) 7.3%	90.0	2.7	0	0	0
Part B. Percent mortality[b] (data obtained two hours after removal of flies from fog)					
Crag F-21		4.0	2.7	0	4.0
CFR-Mal		1.3	0	6.7	1.3
Crag LSO-30		1.3	1.3	0	6.7
Crag LSO-31		1.3	1.3	1.3	0
Tabutrex		0	0	1.3	0
Malathion 1%		14.7	2.7	6.7	2.7
Malathion 5%		73.3	76.0	74.7	86.7
Korlan (Dow ET-14) 7.3%		98.7	98.7	98.7	98.7

[a]All toxicity data corrected by mortality in untreated cages of flies.

[b]Data based on 75 flies (three cages of 25 flies each).

TABLE V

CORRECTED[a] KNOCKDOWN AND MORTALITY OF STABLE FLIES RESULTING FROM EXPOSURE TO VARIOUS INSECTICIDE FOGS DISPERSED INTO A MILKING PARLOR FOR ONE-HALF MINUTE

Insecticide	Amount Emitted in 1/2 Minute (ml.)	Additional Exposure to Fog after Its Introduction			
		None	1 Min.	3 Min.	5 Min.
Part A. Percent knockdown[b] (data obtained immediately after removal of flies from fog)					
Crag F-21	3.5	0	0	0	0
CFR-Mal	5.0	0	0	0	0
Crag LSO-30	27.0	0	0	9.3	52.0
Crag LSO-31	28.0	0	0	0	0
Tabutrex	20.0	0	0	0	1.3
Malathion 1%	31.0	0	0	0	0
Malathion 5%	24.0	0	0	0	0
Korlan (Dow ET-14) 7.3%	22.5	0	0	0	0
Part B. Percent mortality[b] (data obtained two hours after removal of flies from fog)					
Crag F-21		41.3	52.0	53.3	60.0
CFR-Mal		1.3	1.3	1.3	0
Crag LSO-30		77.3	94.7	94.7	97.3
Crag LSO-31		74.7	72.0	94.7	97.3
Tabutrex		0	0	0	2.7
Malathion 1%		84.0	61.3	42.7	66.7
Malathion 5%		93.3	93.3	93.3	93.3
Korlan (Dow ET-14) 7.3%		93.3	88.0	88.0	86.7

[a]All toxicity data corrected by mortality in untreated cages of flies.

[b]Data based on 75 flies (three cages of 25 flies each).

TABLE VI

CORRECTED[a] KNOCKDOWN AND MORTALITY OF STABLE FLIES RESULTING FROM EXPOSURE TO VARIOUS INSECTICIDE FOGS DISPERSED INTO A MILKING PARLOR FOR TWO MINUTES

Insecticide	Amount Emitted in 2 Minutes (ml.)	Additional Exposure to Fog after Its Introduction			
		None	1 Min.	3 Min.	5 Min.
Part A. Percent knockdown[b] (data obtained immediately after removal of flies from fog)					
Crag F-21	14.0	0	0	0	0
CFR-Mal	20.0	0	0	0	0
Crag LSO-30	108.0	4.0	53.3	73.3	89.3
Crag LSO-31	112.0	0	0	0	0
Tabutrex	80.0	0	2.7	1.3	1.3
Malathion 1%	124.0	0	1.3	1.3	0
Malathion 5%	96.0	0	0	0	6.7
Korlan (Dow ET-14) 7.3%	90.0	0	1.3	0	1.3
Part B. Percent mortality[b] (data obtained two hours after removal of flies from fog)					
Crag F-21		98.7	100.0	98.7	90.7
CFR-Mal		38.7	58.7	84.0	80.0
Crag LSO-30		97.3	97.3	97.3	97.3
Crag LSO-31		97.3	97.3	97.3	97.3
Tabutrex		0	2.7	0	1.3
Malathion 1%		98.7	98.7	100.0	96.0
Malathion 5%		93.3	93.3	93.3	93.3
Korlan (Dow ET-14) 7.3%		93.3	94.7	97.3	94.7

[a]All toxicity data corrected by mortality in untreated cages of flies.

[b]Data based on 75 flies (three cages of 25 flies each).

The Direct Application of Malathion to Dairy Cows

Fly populations on the cows. The daily fluctuation of the combined house fly and stable fly populations on the cows treated with the malathion formulations as compared with the untreated cows are presented in Figures 1 through 4. The fly populations which are presented graphically are the average number of flies on the four cows receiving each treatment. As stated previously, the fly populations were determined by counting the flies on the right side of the cows only. All of the population numbers presented here therefore represent approximately one-half of the total fly populations on the cows at the time of making the counts. The fly populations on the untreated cows for the entire project period are shown in each figure. The numbers on the treated cows are given only for the period during which the treatments were evaluated. The dates of application of the materials are given for each treatment in their respective figures. The atmosphere temperature for each morning during the project and the fly populations on the untreated cows for the same days are compared in Figure 5.

Effect on cholinesterase activity. The effects of the malathion treatments on the erythrocyte cholinesterase activity of the cows are presented in Figures 6 through 9. The data which are

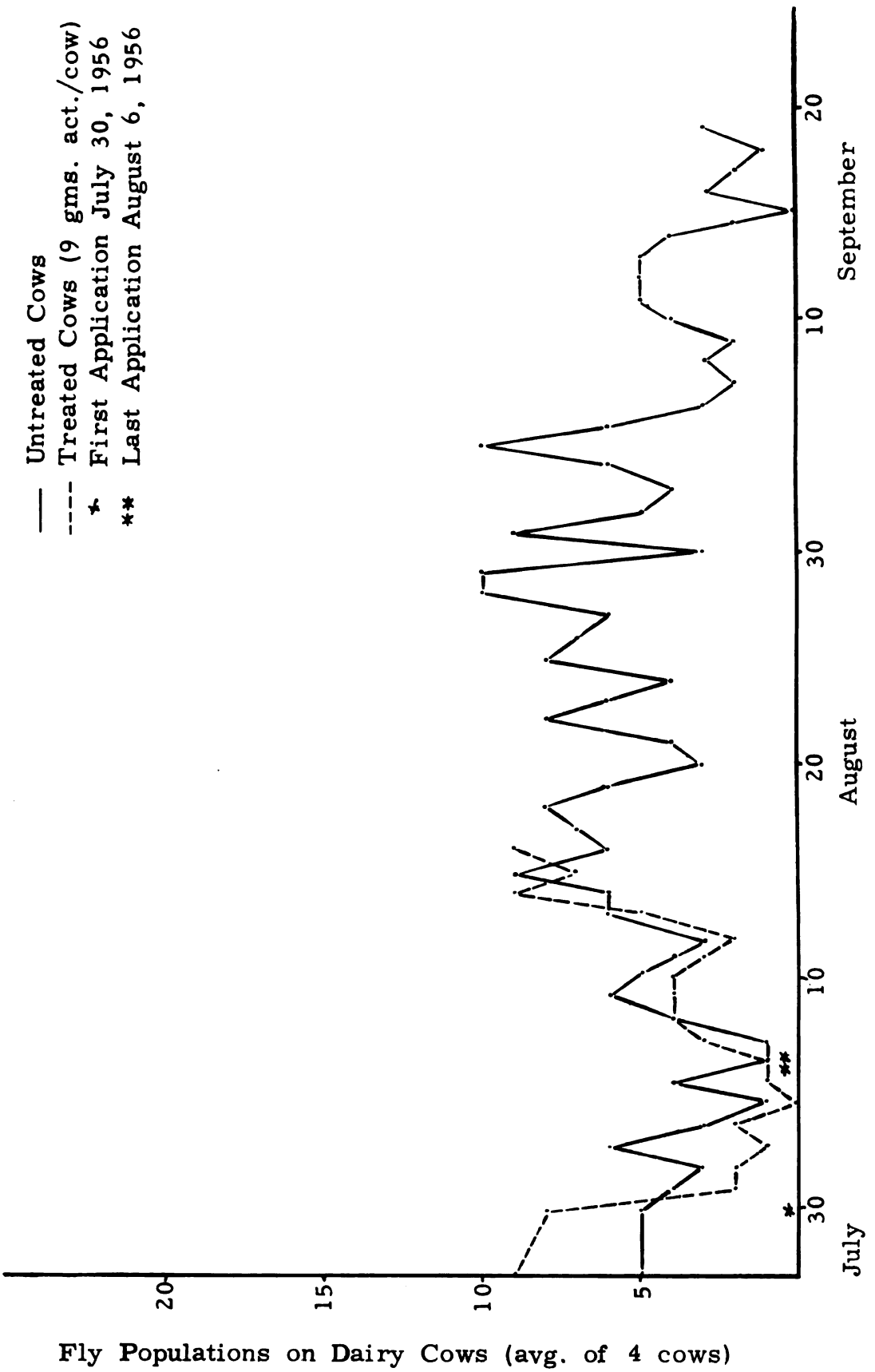


Figure 1. Combined populations of house flies and stable flies on untreated Holstein cows and on Holstein cows treated daily with 1 percent malathion emulsion.

Figure 2. Combined populations of house flies and stable flies on untreated Holstein cows and on Holstein cows treated every third day with 1 percent malathion emulsion.

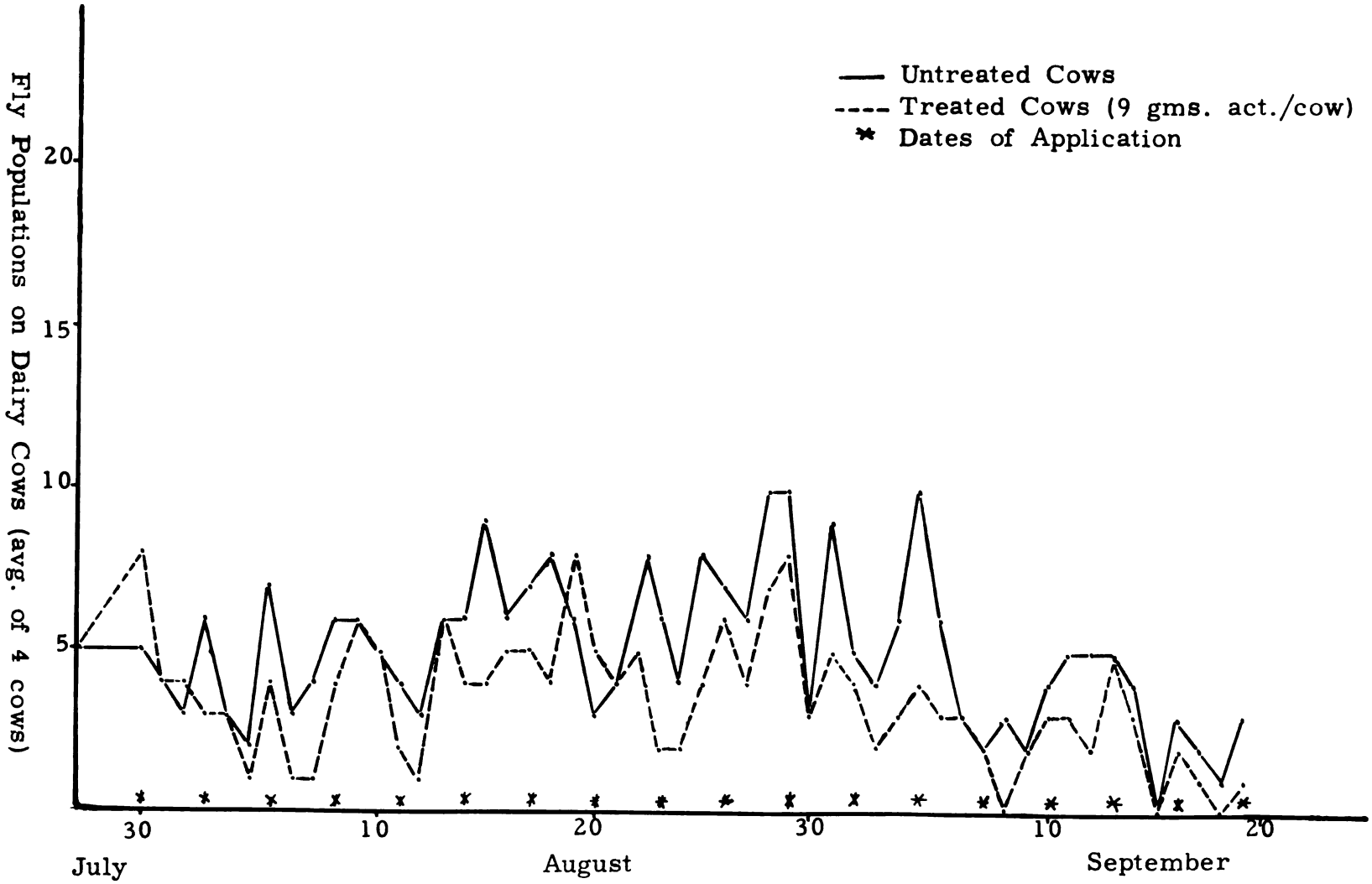


Figure 3. Combined populations of house flies and stable flies on untreated Holstein cows and on Holstein cows treated daily with 0.5 percent malathion emulsion.

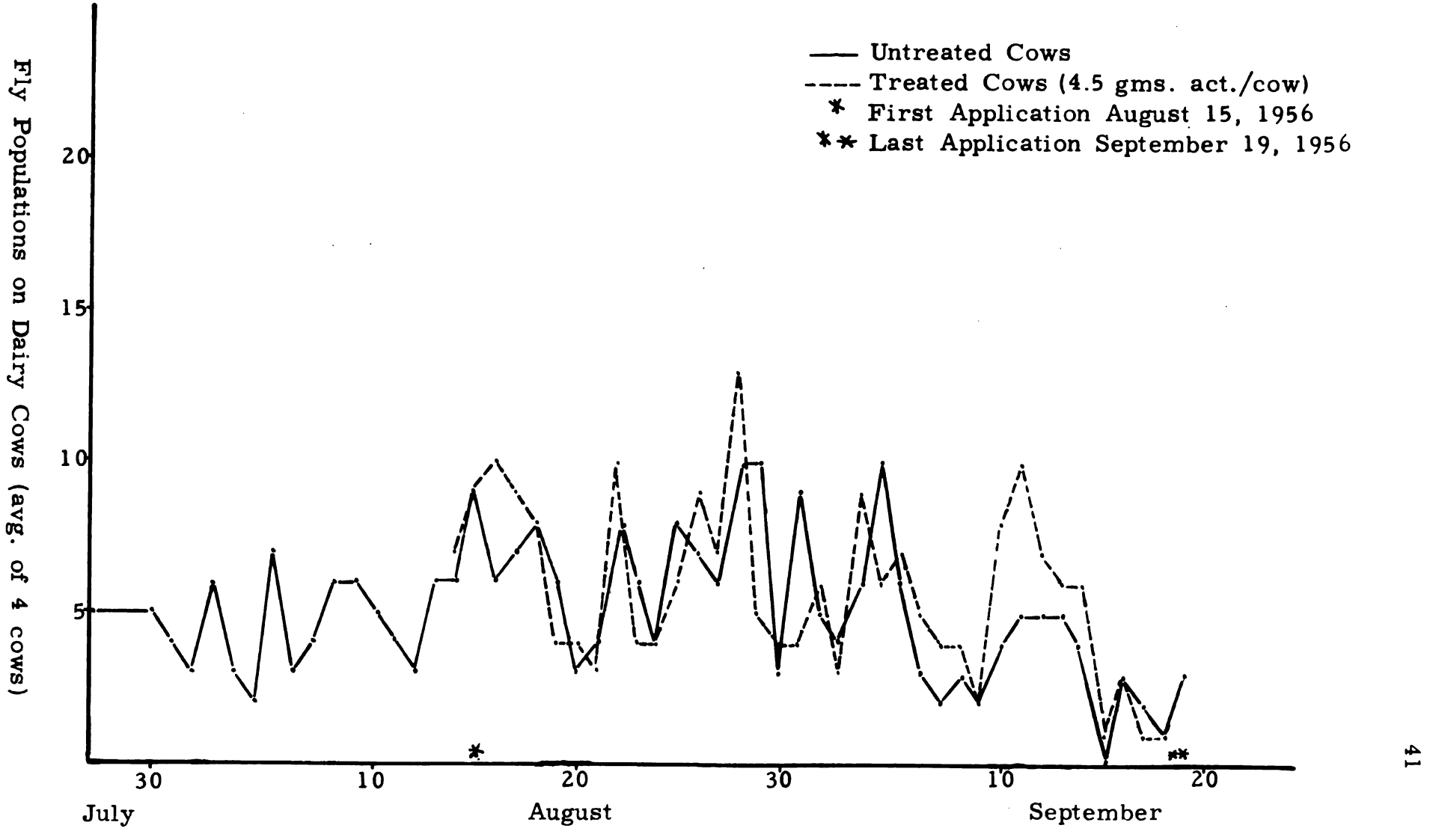
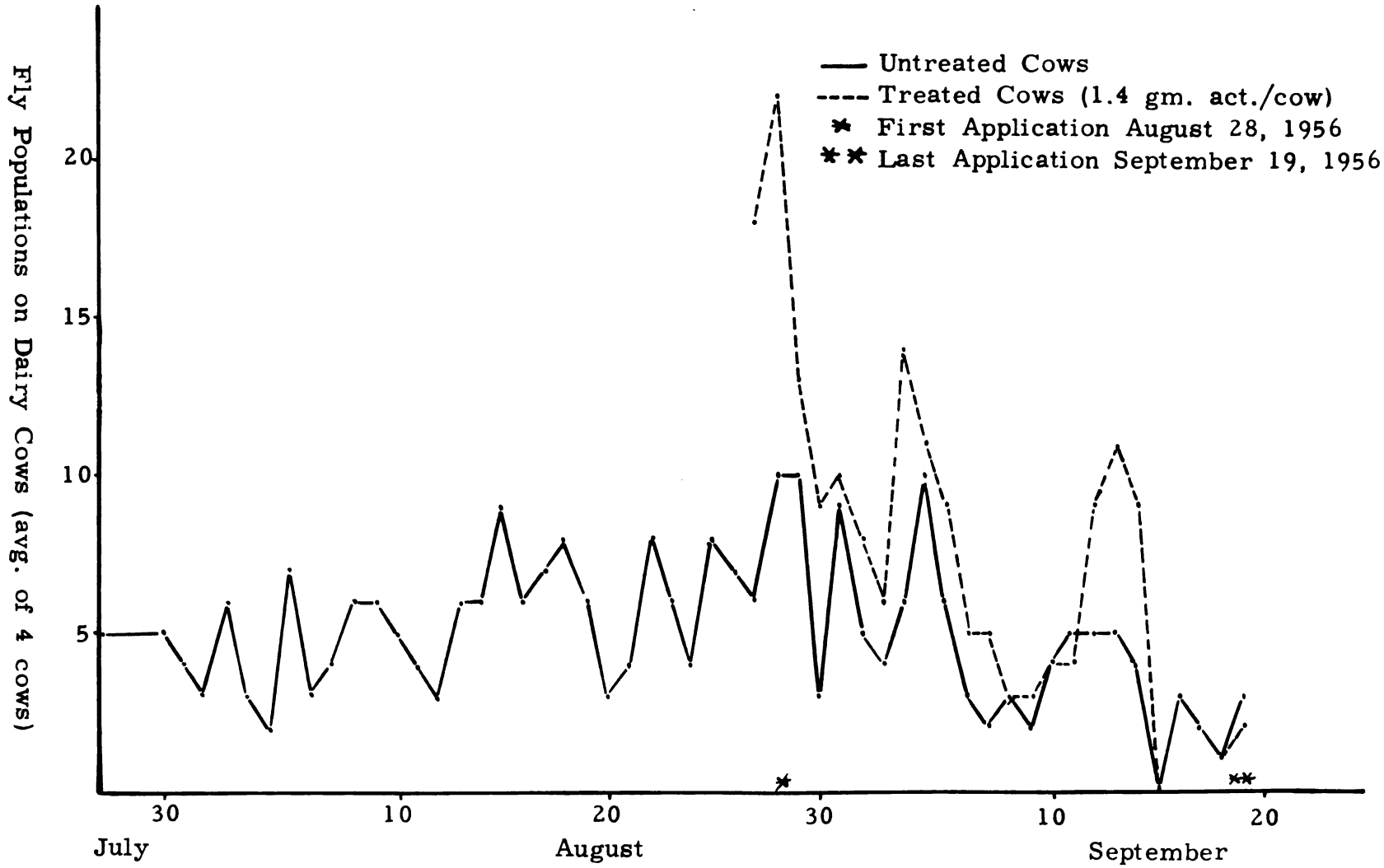


Figure 4. Combined populations of house flies and stable flies on untreated Holstein cows and on Holstein cows treated daily with 4 percent malathion dust.



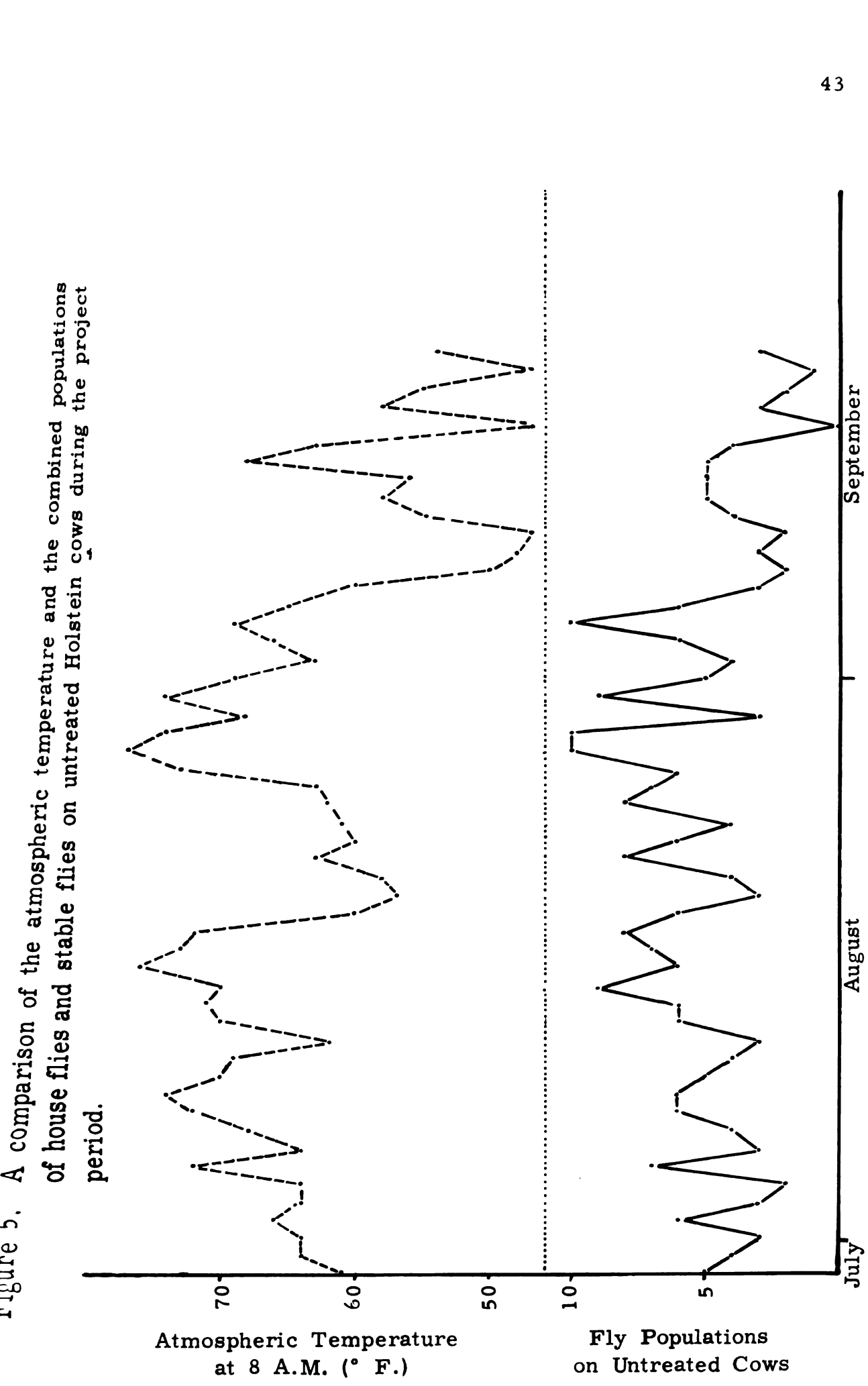
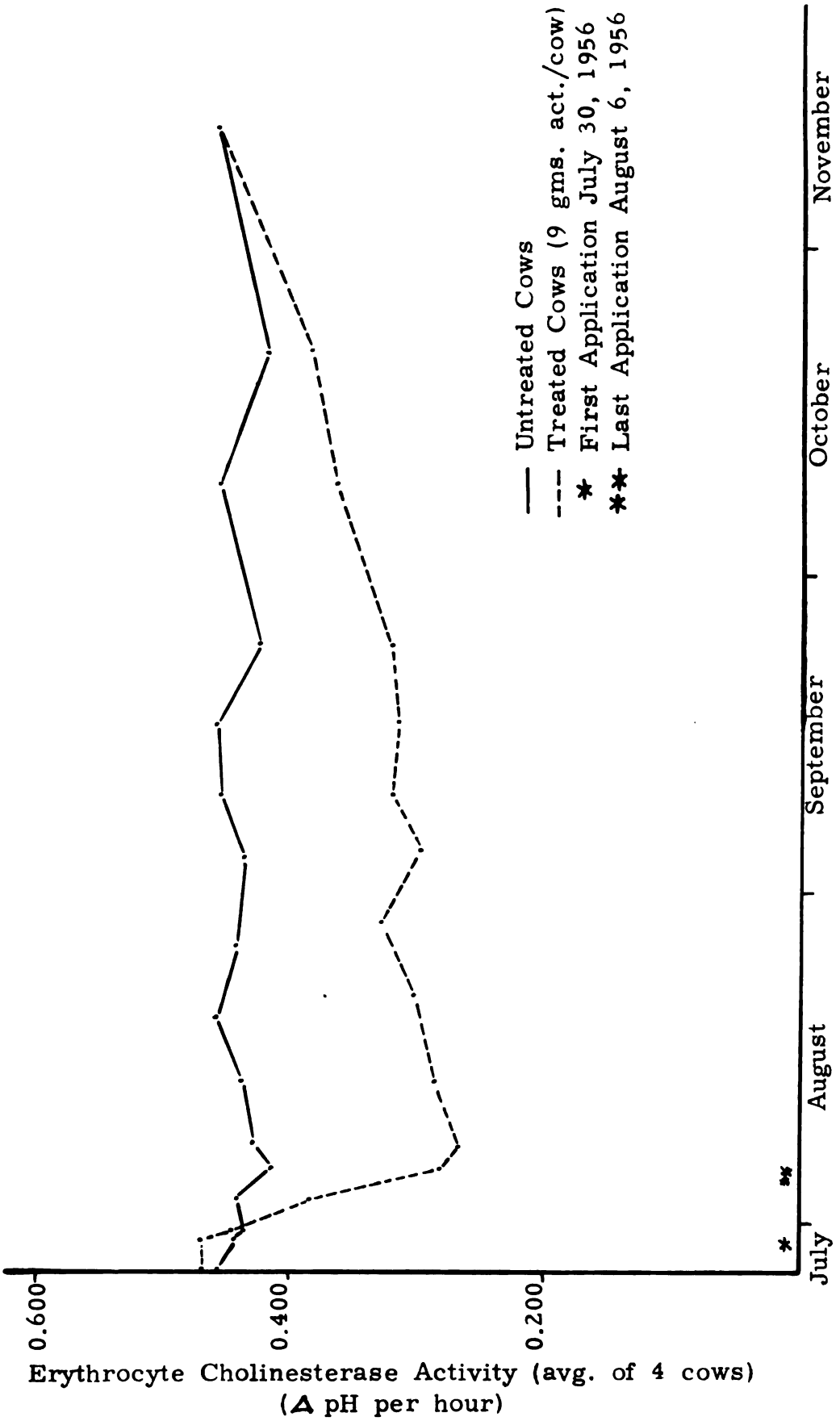


Figure 5. A comparison of the atmospheric temperature and the combined populations of house flies and stable flies on untreated Holstein cows during the project period.

Figure 6. Erythrocyte cholinesterase activity of untreated Holstein cows and of Holstein cows treated daily with 1 percent malathion emulsion.



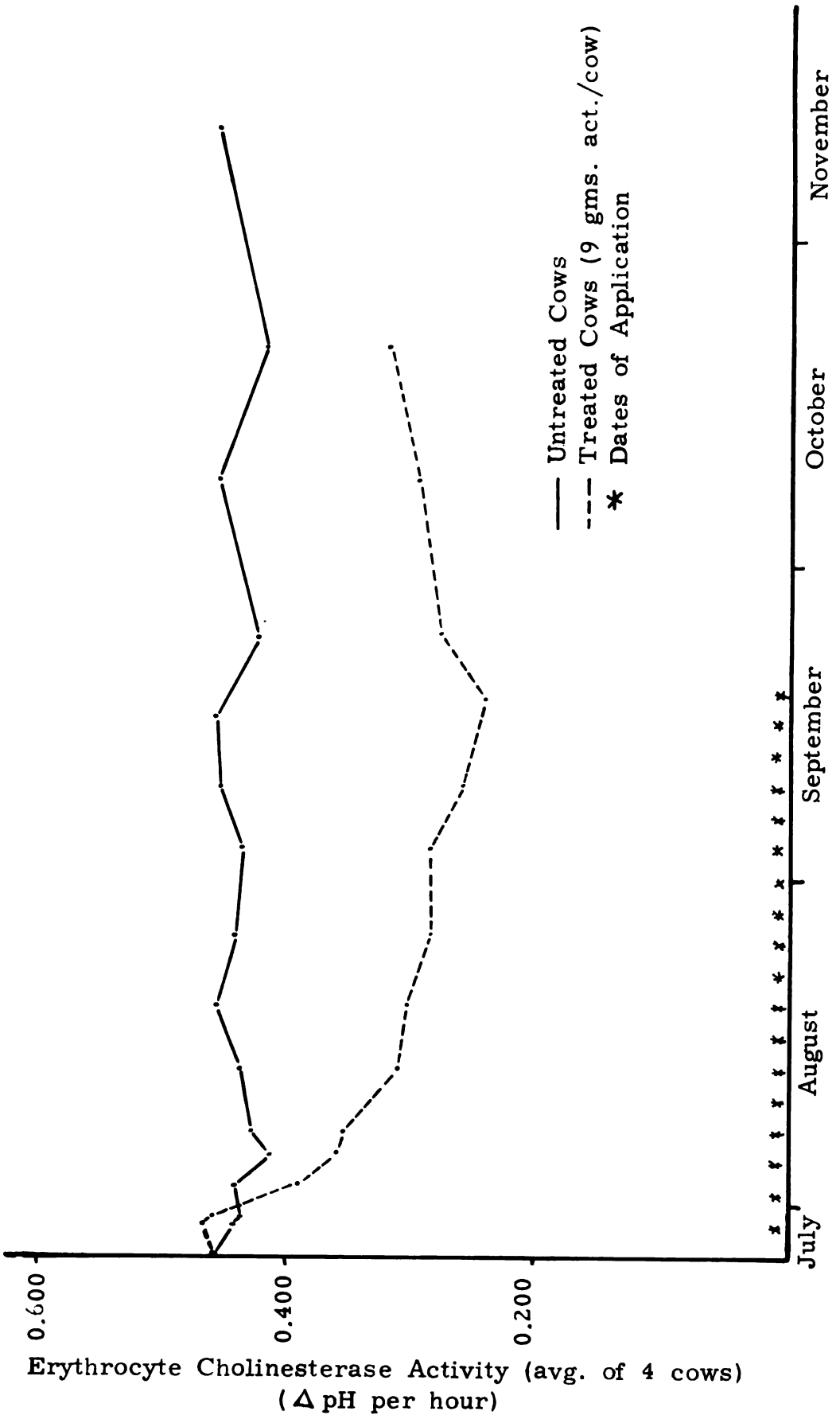


Figure 7. Erythrocyte cholinesterase activity of untreated Holstein cows and of Holstein cows treated every third day with 1 percent malathion emulsion.

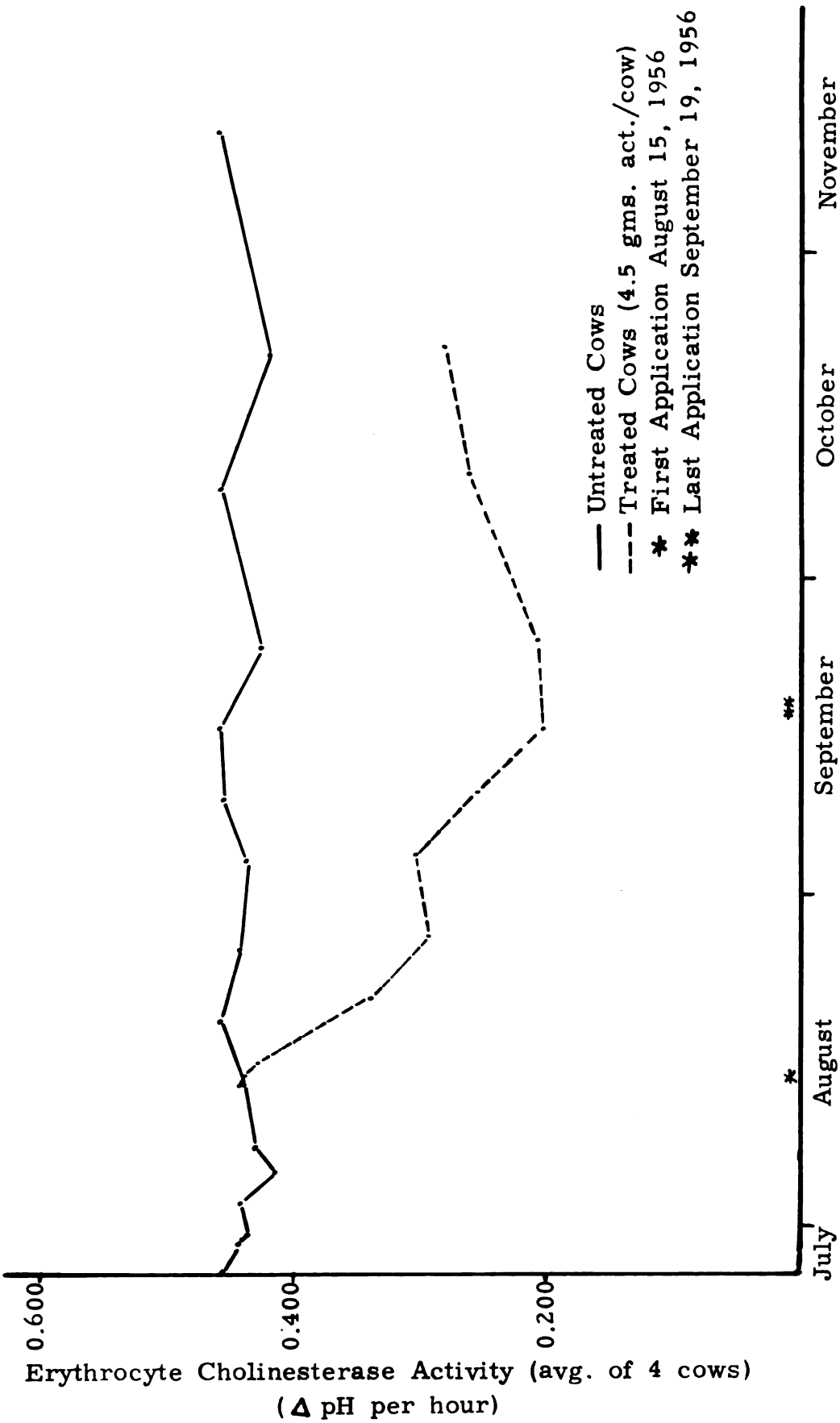
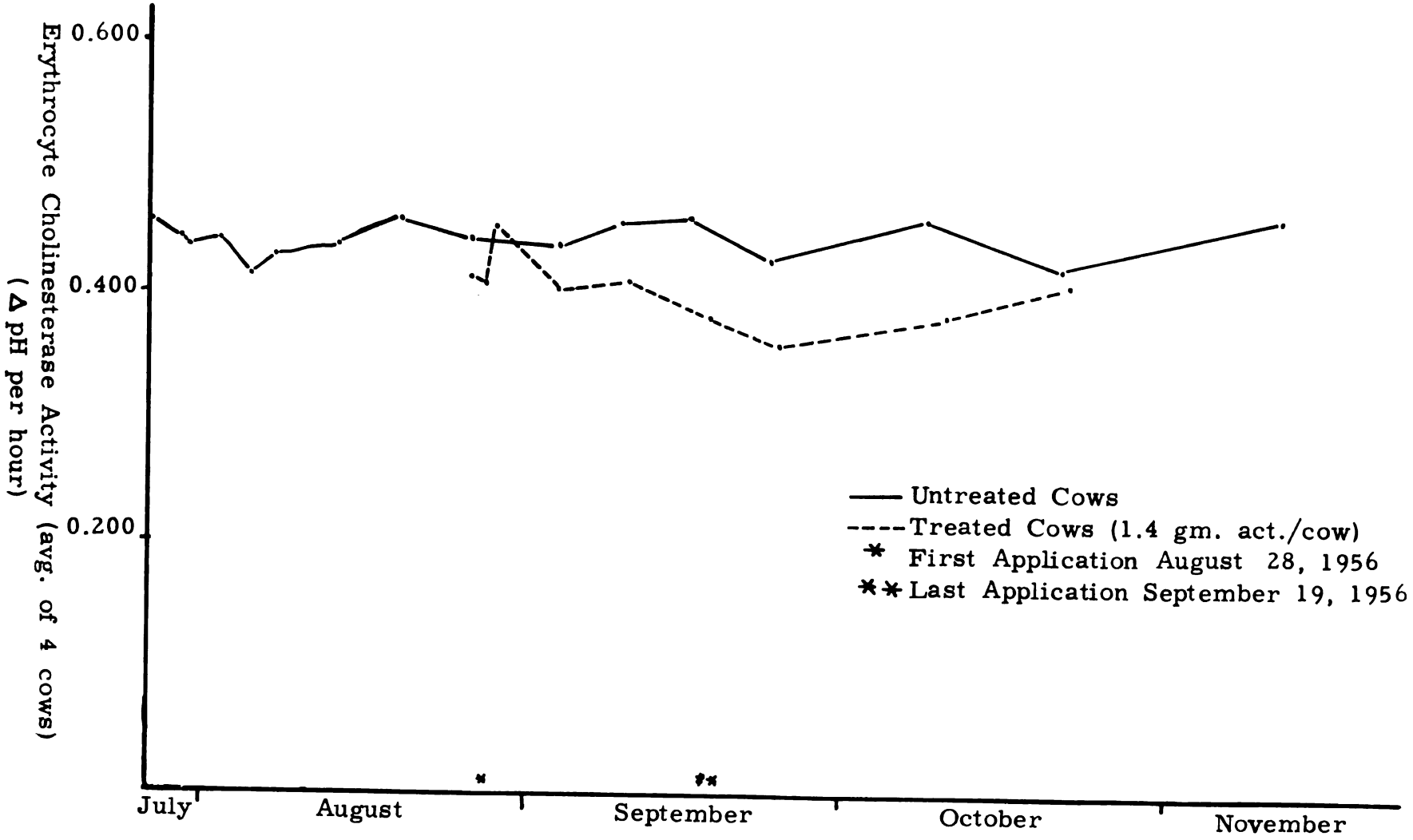


Figure 8. Erythrocyte cholinesterase activity of untreated Holstein cows and of Holstein cows treated daily with 0.5 percent malathion emulsion.

Figure 9. Erythrocyte cholinesterase activity of untreated Holstein cows and of Holstein cows treated daily with 4 percent malathion dust.



presented in each figure are the averages of the enzyme activity of the four cows receiving each treatment. These were obtained by the analytical determination of the erythrocyte cholinesterase activity of blood samples from the cows, and are expressed as the change in pH per hour recorded in the analytical analyses. The dates of application of the malathion to the cows are given for each treatment in its respective figure. The cholinesterase activity of the untreated cows are presented for comparison with the treated cows.

Malathion residue in the milk. The malathion residue analyses of the raw milk samples from the treated cows are presented in Figures 10 through 13. The data presented are the averages of the samples obtained from the four cows receiving each treatment. The residues are expressed in parts per million of the raw whole milk samples as obtained from the Holstein cows during the project. The dates of application of the malathion to the cows are given for each treatment in its respective figure. Residue analyses of milk samples from the untreated cows were made. Since no evidence of malathion was found in the samples, the data are not shown in the figures.

Figure 10. Malathion residue in raw milk from Holstein cows treated daily with 1 percent malathion emulsion.

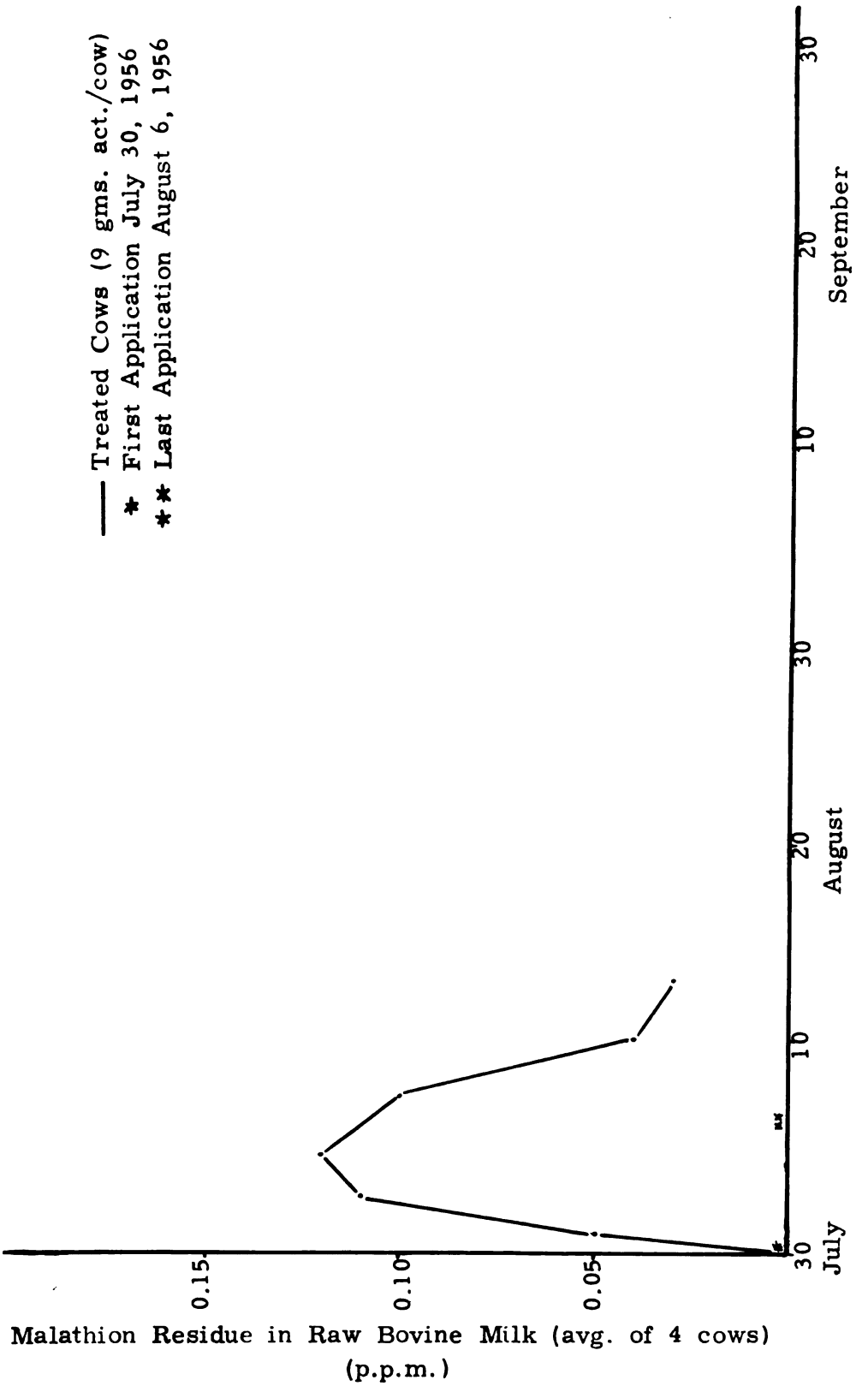


Figure 11. Malathion residue in raw milk from Holstein cows treated every third day with 1 percent malathion emulsion.

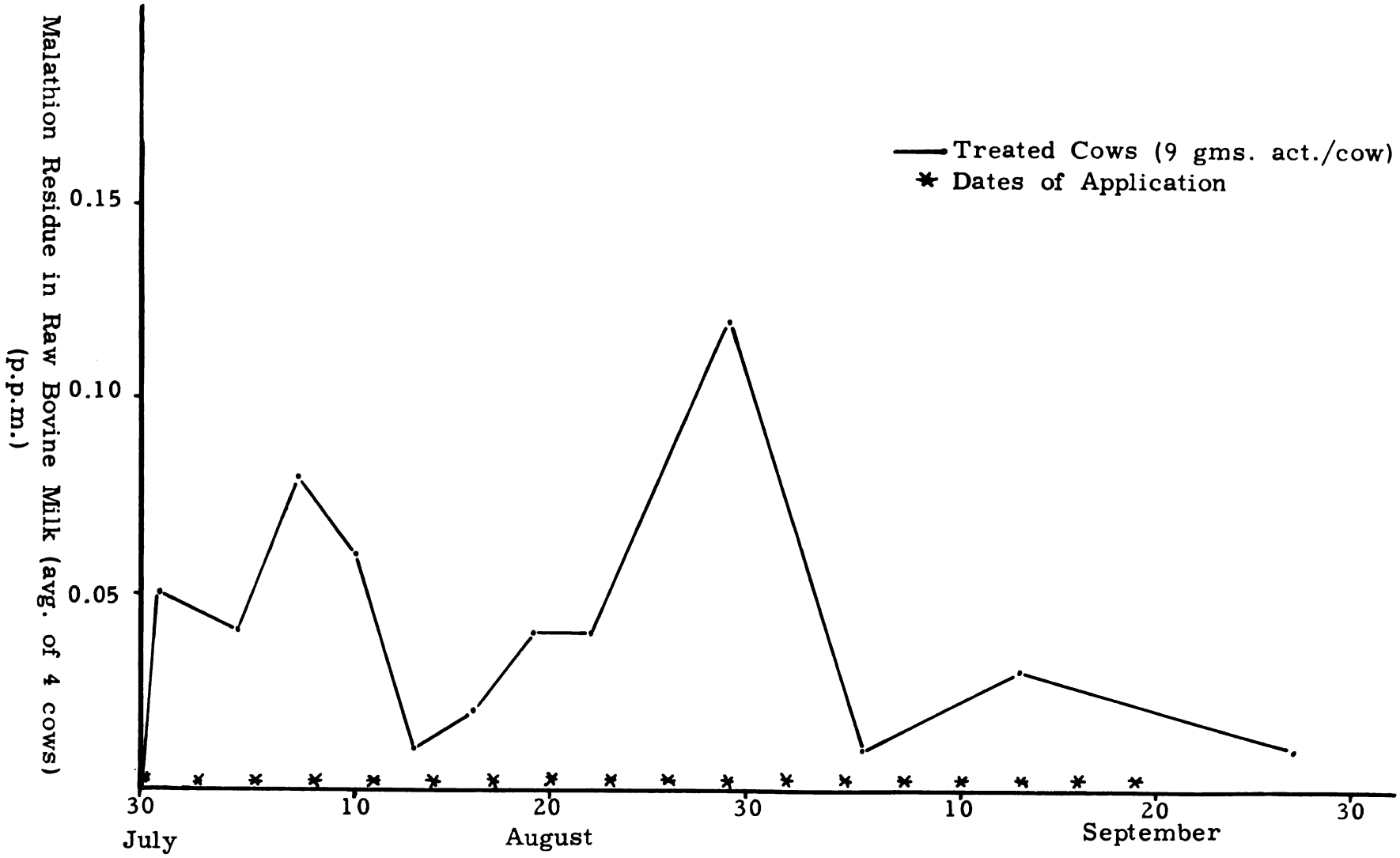
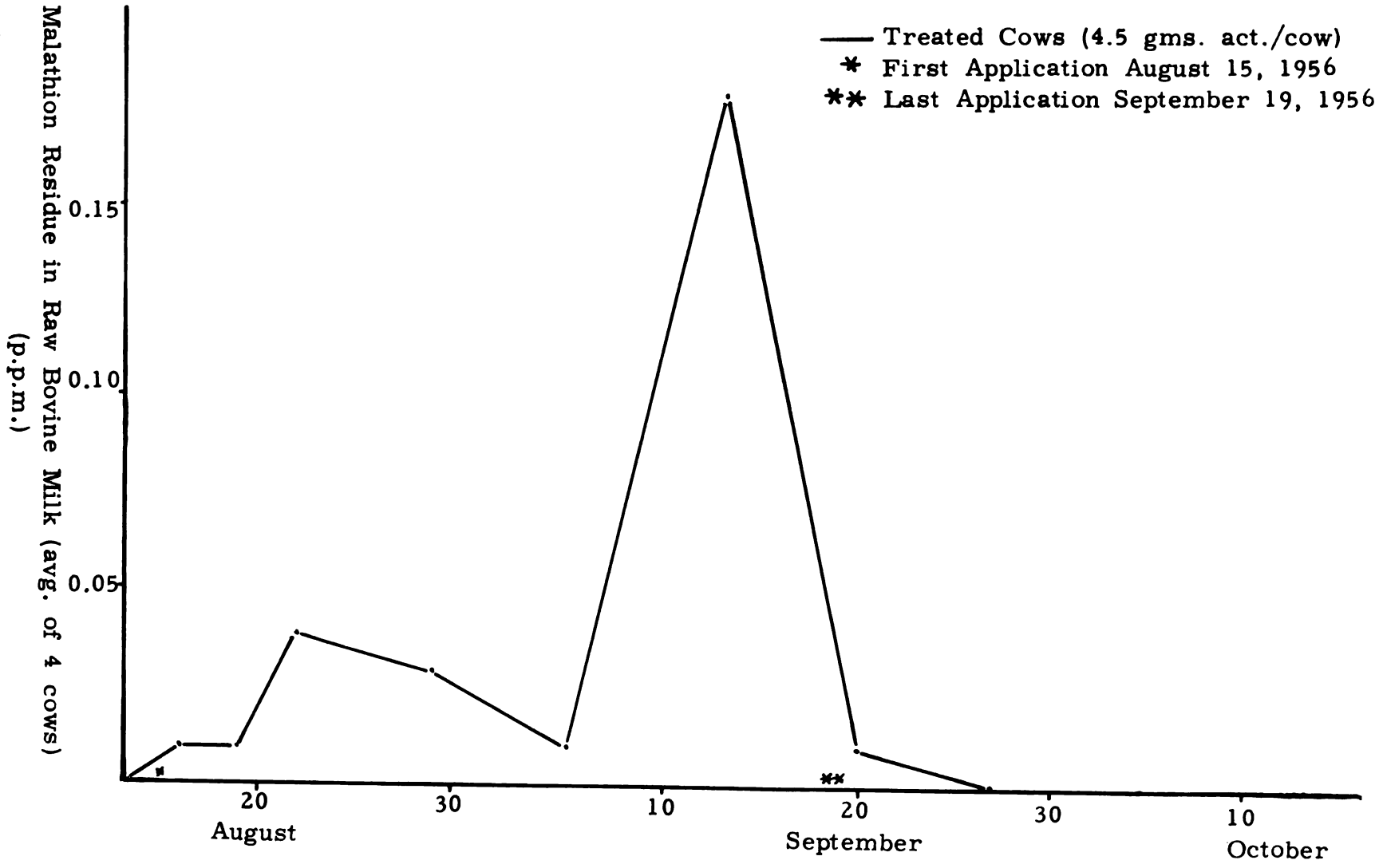


Figure 12. Malathion residue in raw milk from Holstein cows treated daily with 0.5 percent malathion emulsion.



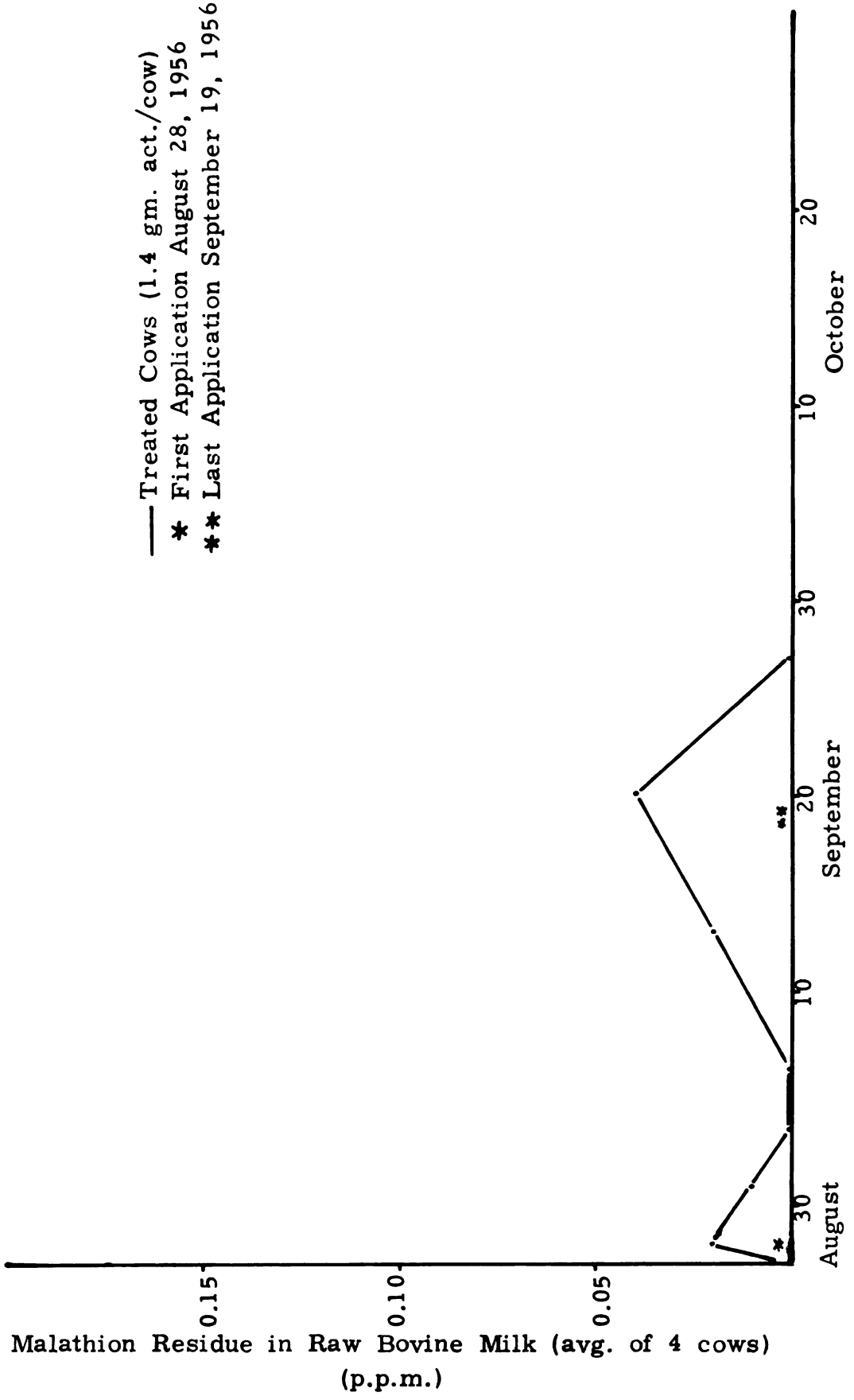


Figure 13. Malathion residue in raw milk from Holstein cows treated daily with 4 percent malation dust.

DISCUSSION

The Effects of Insecticide Fogs on Flies

The insecticides varied greatly in their action against the two species of flies. The house flies were less susceptible than were the stable flies to most of the materials. This difference could be attributed in part to the strains of flies used, the house flies having come from a strain which probably had been exposed to insecticides in the past and the stable flies from a laboratory culture. There could also be a physiological difference between the two species in their susceptibility to the toxicants.

The period of dispersion of the materials was constant in each test, but the rate of dispersion varied; therefore, the amount of each toxicant dispersed into the milking parlor was different. This difference must be considered when comparing their toxicities to the flies. All of the insecticides were tested at less than the 55 ml. per 1000 cu. ft. which is used in the Peet-Grady method (Shepard, 1951). All of the materials except the Crag F-21 were dispersed evenly around the room while the compressor was in operation. The small amount of fog from this material did not

disperse evenly in the one-half minute test; therefore, the cages were passed through the mist as they were taken out of the room when the compressor was shut off.

The insecticides exhibited little knockdown action against the house flies. Even the synergized pyrethrins had little knockdown effect on the flies. There was evidence of acute toxicity from some of the materials two hours after exposing the flies to the fogs. Exposure to the Korlan fog resulted in the highest mortality to the house flies at all lengths of exposure. The 5 percent malathion caused a high mortality to the flies after exposure to the higher concentration in the room. The other materials had very little, if any, toxic effect on the flies.

The LSO-30 was the only material to exhibit a knockdown of the stable flies. The 5 percent malathion, although containing a higher percentage of pyrethrins, did not compare with the LSO-30 in knockdown. This difference could be attributed in part to the action of the methoxychlor or butoxypolypropylene glycol in the LSO-30 or to the antagonistic effect between malathion and piperonyl butoxide which has been reported by Rai et al. (1956). If there was an antagonistic effect between these two materials, the synergism of the piperonyl butoxide to the pyrethrins could have been affected. The one-, three-, and five-minute additional exposure to the higher

concentration of LSO-30 fog caused a proportional increase in knockdown to the stable flies.

The 5 percent malathion resulted in the highest mortality after exposing the stable flies to the fog for one-half minute. The LSO-30 and LSO-31, upon additional exposure, also caused high mortality to the flies. The Korlan did not cause as high a mortality to the stable flies as it did to the house flies. The CFR-Mal, although containing a higher percentage of malathion than the 1 percent malathion, did not give as high a mortality. This is probably due to the lesser amount of CFR-Mal dispersed into the milking parlor.

The exposure of the stable flies to most of the fogs at the higher concentration in the room resulted in a high mortality. Generally, additional exposure to the fogs after the compressor was shut off did not increase the mortality. The only exception to this was the CFR-Mal. The one-, three-, and five-minute additional exposure increased the toxic effects proportionately. The Tabutrex showed no evidence of toxicity to the stable flies.

The Effects of Malathion When Applied Directly to Dairy Cows

Effect on flies. There was a great variation in the fly populations on the individual cows receiving each treatment. When the

mean numbers of flies on the cows treated with 1 percent spray daily and the controls for corresponding days are compared graphically, there appears to be a difference between them. Statistically, the evidence of this difference was not significant at the 5 percent level (Snedecor, 1950). The fly populations on the treated cows decreased after the first application from a point greater than the controls to a point less than the controls. They remained low for the duration of the spraying period. The counts on the treated cows tended to increase after the effects of the last treatment subsided.

The flies on the cows treated with 1 percent spray every third day varied in numbers from day to day depending on the date of application. The populations generally decreased or remained stable for a day, after which they tended to fluctuate until another treatment was applied. There was little difference between the populations on the second and third days after treatment when the totals on the individual cows for the testing period are summarized. Statistically there was no evidence of a difference between the populations on the first, second, or third day following treatment when compared to the controls.

The fly populations on the cows treated with 0.5 percent spray daily have a larger standard deviation from the mean than do the controls for the corresponding days. The numbers varied

from day to day even with a daily application of 4.5 grams of actual malathion. There were more flies on the treated cows during the project than on the controls, but this difference was not statistically significant.

The malathion dust appeared to have no effect on the fly populations on the treated cows after the second application. There is no way of knowing what the populations would have been on this group of cows if the treatments had not been applied. Although the numbers dropped after the first treatment was applied, they remained higher than the controls during the testing period until the climatic conditions changed abruptly on September 15. The counts were almost identical on the treated and untreated cows after this date.

Several factors are involved in analyzing the data obtained during this study. Probably the most important factor is the extreme variability in the number of flies on the cows. This fact is exemplified in the counts obtained from the four untreated cows serving as controls. One of them had only a maximum of five flies on her at one time during the mornings when the counts were being made. Other cows in the group had as many as twenty at one time. On some days the fly populations on a treated cow would decrease

from the previous day's count, while there would tend to be a similar increase on other cows receiving the same treatment.

Outline drawings of the markings on each of the cows were made to determine if the percentage of black or white hair had any effect on the fly populations on the cows. There appeared to be no correlation between the color and the number of flies on the cows. The control cows which had the highest and lowest total flies during the period had an almost identical amount of black markings. This was further shown by the fact that two of the cows treated with the dust were identical twins and had dissimilar fly populations.

The daily fluctuation in temperature seemed to affect the fly populations more than any of the treatments. When comparing the data in Figure 5, there appears to be a close correlation between the numbers of flies on the untreated cows and the daily fluctuation in atmospheric temperature. The flies increased in numbers when the temperature increased, and decreased when the temperature dropped. This daily fluctuation was apparent throughout the testing period. It was climaxed on September 15 when the temperature dropped 16° F. during the night and a cold rain occurred. Only four flies were counted on all of the cows the following morning. There was a similar correlation between the fly populations on the treated cows and the temperature. Whether this was due to the

variable action of the malathion or to the activity of the flies in the barn cannot be determined. There appeared to be no correlation between the relative humidity and the fly populations on the cows.

Effect on erythrocyte cholinesterase activity. The effect of malathion on the cholinesterase activity of the cows varied with the different formulations and rates of application. Enough malathion was absorbed through the skin of all of the treated animals to affect their blood. Although the data presented here are based on the averages of the four cows in each group, there was a variation between the cows.

The erythrocyte cholinesterase activity was depressed at the fastest rate in the cows receiving the 1 percent spray (9 grams of actual malathion) daily. The determination dropped from a change in pH of 0.471 per hour before any treatments had been applied to a reading of 0.270 after only six applications of spray. The treatments were discontinued at this time since the accumulative effect of malathion in this type of application was uncertain, although none of the cows exhibited any apparent symptoms of phosphate poisoning. The regeneration of the cholinesterase began about three days after the final application and continued slowly back to normal. The rate of regeneration was determined by sampling the blood periodically.

The cholinesterase activity returned to pretreatment level about 100 days after the final treatment.

The cholinesterase activity of the cows receiving the 1 percent spray every third day was depressed sharply after each of the first three treatments, after which it decreased more slowly until the last treatment was applied. At the rate of regeneration indicated by the three posttreatment samples, the cholinesterase activity should have compared with the controls about 104 days after the final treatment.

The daily application of the 0.5 percent spray depressed the cholinesterase activity of the cows continually during the 36 days of treatment. The indication of regeneration between August 28 and September 4 is probably caused by variations between the individual blood samples. The cholinesterase activity should have been back to normal in about 100 days after the last application of spray.

The applications of dust affected the cholinesterase activity the least of any of the treatments. This was probably due to the smaller amount of malathion applied per treatment, and to the formulation. Only 1.4 grams of actual malathion in this formulation was applied per treatment. It is possible that the dust applied to the ends of the hair was not absorbed as fast as the emulsions which were held in the hair until evaporation or absorption took place.

The cholinesterase activity appears to have been stimulated after the first treatment, since there was an increase in its activity the following morning. At the rate of regeneration indicated by the three posttreatment samples, they should have been back to the pretreatment level within 40 days after the final application.

Effect on milk production. Except for normal fluctuations, there appeared to be no changes in the milk production or butterfat tests of the treated cows during the period of treatment. The fly populations were so low that they probably had no effect on the milk production. Since the cows were on different feed rations at the time of the project, any variation in production due to the feed probably outweighed any effect the treatments might have had.

The lactation periods of some of the cows were completed shortly after the application of the treatments was terminated; thus the production of these cows was low during the project. Some of the cows freshened during the summer; thus their milk and butterfat production were affected. Their weekly weights would have been affected by these same factors.

Malathion residue in the milk. Malathion, like the chlorinated hydrocarbons, is soluble in the butterfat of milk. Most studies on pesticide residues in milk are made on whole milk and the data are

then converted to 4 percent fat-corrected milk. All of the residue analyses in this study were made on the raw whole milk, and the results are reported as found. Since the variations in the butterfat tests were so small in the Holstein milk, there was believed to be little need of converting the findings to fat-corrected milk for discussion here.

No malathion was found in any of the milk samples before the treatments were applied, and likewise none was found in any of the samples from the untreated cows throughout the project. This indicates that all of the unchanged malathion found in the milk entered the body by absorption through the skin. Malathion from all of the formulations was excreted in the milk in varying minute amounts depending upon the type of application and the length of interval between treatment and sampling.

An average of 0.05 parts per million of malathion was found in the milk from the cows receiving the 1 percent spray daily within 24 hours after the first treatment. The continuous applications caused an accumulative amount of malathion to be excreted in the milk. The treatments were terminated when the cholinesterase activity became depressed by the malathion. The residues diminished sharply after the last treatment until there was only 0.03 p.p.m. in the milk one week later.

The residues in the milk from cows receiving the 1 percent spray every third day was less consistent than from the daily applications. The first samples taken after the treatments began were the same in both groups of cows, but they differed from then on, depending on the time of application. No accumulative action was indicated from this type of application. Most of the malathion had been excreted each time before the following application was made. This coincides with the immediate passing of malathion metabolites in the urine of treated calves as reported by March et al. (1956). The relatively large residue in the milk on August 29, eight hours after a treatment, did not reoccur on September 13 in a similar situation. This phenomenon is unexplainable since the cows' rations were not changed and the climatic conditions were practically the same on both days. The malathion had been almost completely eliminated or metabolized one week after the final treatment, since only a trace was found in the milk.

The 0.5 percent spray and 4 percent dust brought about only traces of malathion in the milk except for one sampling from the cows receiving the 0.5 percent spray. This relatively high residue occurred on September 13. This cannot be attributed to any external factor that was evident. All traces of malathion were absent in the milk one week after the final applications of both formulations.

These residue analyses are in sharp contrast to the chlorinated hydrocarbon residues in milk following direct application to dairy cows. The chlorinated hydrocarbons are excreted in larger quantities and over a longer period than malathion after comparable rates of application.

SUMMARY

Insecticides were evaluated when dispersed as fogs in a milking parlor for their effects on house flies and stable flies. The physiological effects of dermal applications of malathion on dairy cows were studied. The results indicate:

1. Malathion and Korlan, when dispersed as fogs in a milking parlor, caused a higher mortality to house flies than did other synthetic fly toxicants.

2. The synergized pyrethrin sprays did not result in as great a knockdown on the house flies as they did on the stable flies.

3. There appears to be a possibility that there is an antagonistic effect on flies between malathion and piperonyl butoxide when formulated in the same compound.

4. Tabutrex did not exhibit a toxic effect on house flies or stable flies.

5. Increasing the length of exposure of the stable flies to the nonphosphate fogs except the Tabutrex resulted in a proportional increase in mortality. The Korlan and malathion formulations did not exhibit this activity.

6. Daily application of 0.5 and 1 percent malathion emulsions and 4 percent dust or applications of 1 percent emulsions every third day did not have a significant effect on the house fly and stable fly populations on Holstein cows.

7. There was a wide variation in the daily fly populations on all of the cows used in the project. This variation in susceptibility to fly attack seems to be due to a physiological difference between cows. Black cows were not found to consistently attract more flies than cows with a large amount of white markings.

8. There was a close correlation between the fluctuation of daily temperatures and the fly populations on the cows.

9. The erythrocyte cholinesterase activity in the Holstein cows was depressed for three months following eight daily applications of 1 percent malathion emulsion sprays.

10. Applications of 1 percent malathion emulsions every third day for a period of 52 days depressed the cholinesterase activity of the cows for about three months following treatment.

11. Daily applications of 0.5 percent emulsion and 4 percent dust affected the cholinesterase activity less than the 1 percent sprays.

12. Malathion is excreted in the milk in small amounts following dermal applications of 1 percent emulsions daily and every third day.

13. Daily applications of 0.5 percent emulsion and 4 percent dust resulted in less residue in the milk and for a shorter period following treatments than did the 1 percent applications.

14. The malathion treatments did not affect the milk or butterfat production or the weights of the cows.

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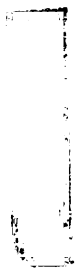

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